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Research Paper

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ANTIOXIDANT, REPELLENT, AND INSECTICIDAL ACTIVITIES OF *Azadirachta indica* LEAF CRUDE EXTRACTS AGAINST *Dinoderus minutus*

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ARTICLE HIGLIGHTS

- Neem crude leaf extracts exhibit strong antioxidant and insecticidal potential.
- Water-ethanol (1:1) mixture extracts more bioactive compounds than absolute ethanol or water.
- Extracts show repellent effect and mortality against powder-post beetles.
- Research confirms the potential of neem extract as a natural alternative as pest control for bamboo.
- Provides a sustainable alternative to synthetic insecticides

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ABSTRACT

Azadirachta indica, widely known as "neem plant," is recognized for its pesticidal and insecticidal applications due to its anti-feedant, repellent, and insect growth-regulating properties. Although various studies have reported the effectiveness of its botanical extracts in controlling insect pests, limited research has focused on their use in bamboo protection, especially against wood-boring beetles like *Dinoderus minutus* or powder post beetle. This study determined the total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), DPPH radical scavenging activity, repellency, and insecticidal properties against *D. minutus* of water, water-ethanol (1:1), and ethanol crude leaf extracts (CLEs) of *A. indica*. The water–ethanol (1:1) extract recorded the highest total phenolic content (TPC) at 139.67 mg GAE/g and the highest total antioxidant capacity (TAC) at 161.14 mg AAE/g. In contrast, the ethanol extract showed the highest total flavonoid content (TFC) at 72.08 mg QE/g. Notably, the strong antioxidant performance of the water–ethanol extract is consistent with its DPPH median inhibitory concentration (IC_{50}) of 51.16 µg/mL. After 24 hours of exposure, the water–ethanol (1:1) extract demonstrated promising repellency, falling within Class IV–V (fair to very repellent). In a 7-day insecticidal bioassay against *D. minutus*, the same extract exhibited the highest toxicity, with a median lethal concentration (LC_{50}) of 2.74%.

Keywords: *Azadirachta indica*, bamboo protection, botanical insecticide, crude leaf extract, *Dinoderus minutus*, repellency

INTRODUCTION

Bamboo is an essential renewable resource commonly used in traditional applications like construction, furniture-making, and handicrafts (Caparida *et al.* 2021). Many communities rely on bamboo as a primary material for housing and agricultural tools because of its strength, flexibility, and fast growth, making it valuable both economically and environmentally (Adier *et al.* 2023).

However, bamboo has low natural durability and is particularly vulnerable to pest damage (Liese & Kumar 2003). One of the most destructive pests is the powder post beetle (*Dinoderus minutus* Fabricius), which infests dried bamboo and even finished bamboo products. In the Philippines, *D. minutus*, locally known as "bokbok", is a major post-harvest pest that affects the quality, market value, and lifespan of bamboo materials (Caparida *et al.* 2021). The susceptibility of bamboo to such pest infestations highlights the need for sustainable protection and preservation strategies for bamboo materials and products. This supports Senate Bill No. 294, or the Philippine Bamboo Industry Development Act which reflects the government's recognition of bamboo's potential, pointing to the timeliness of pursuing bamboo-based innovations in the country (Senate of the Philippines 2026).

Conventional control and prevention methods for addressing pest infestations typically involve the direct application of inorganic or synthetic chemicals to freshly cut bamboo materials (Garcia 2005). However, these chemically-based treatments pose several drawbacks, particularly environmental contamination and health risks to humans, as synthetically-derived chemicals are non-biodegradable, which may result in long-term negative impacts on the ecosystems and biodiversity (Jyotsna *et al.* 2024). As a result, there is a growing interest in the development of sustainable alternative insecticides from plant-derived products.

Botanical insecticides are plant-derived, often biodegradable, target-specific, and generally less toxic to non-target organisms compared to synthetic pesticides. Studies have reported plant-derived extracts to have potential in pest control due to their natural bioactive properties and more selective mode of action that contributes to a low risk of pest/insect resistance (Souto *et al.* 2021). These attributes make them a promising, economical, and sustainable alternative approach to pest management, especially in the forestry and agricultural sectors, where ecological balance is critical (Dougoud *et al.* 2019).

Azadirachta indica, widely known as "neem plant", is recognized for its wide spectrum of biological activity and its promising properties, where its plant parts are valued for medicinal potential (Khanal 2021). The neem plant demonstrated potential for pesticidal and insecticidal use due to its anti-feedant, repellent, and insect growth-regulating properties that interfere with pest physiology and development (Ogbuewu *et al.* 2011). Previous studies have primarily focused on the use of neem seed oil as biopesticide for wood preservation and agricultural crop protection (Adusei & Azupio 2022; Ukoroije & Otayor 2020). Although several studies have demonstrated the effectiveness of neem botanical extracts in controlling insect pests, there is limited data on the application of *A. indica* leaf extract for bamboo protection, particularly against *D. minutus*. This research gap underscores the need to investigate and assess the potential of neem-based extracts as natural, sustainable alternatives for bamboo preservation.

Acknowledging the bioactive potential of *A. indica*, this study generally evaluated the potential of *A. indica* leaf extracts for insecticidal actions against powder post beetles in bamboos. Specifically, this study aimed to: 1) compare the effectiveness of three solvents in extracting bioactive compounds from neem leaves; 2) determine antioxidant activity; and (3) assess repellent and insecticidal effects against *D. minutus*.

MATERIALS AND METHODS

Sample Collection and Preparation

Leaves of *A. indica* were collected from Central Mindanao University, Musuan, Maramag, Bukidnon. These were placed in labeled polyethylene bags and transported to the Center for Natural Products Research, Development and Extension (CNPRDE) in campus. The collected leaves were washed thoroughly with tap water, rinsed with distilled water, and dried in an oven at 40 °C until a constant weight was achieved. Percent moisture content was calculated using Equation 1.

$$\% \text{ Moisture content} = \frac{\text{initial fresh weight} - \text{final fresh weight}}{\text{initial fresh weight of sample}} \times 100 \quad (1)$$

The dried samples were ground into a fine powder using an Osterizer® blender and stored in resealable cellophane containers at room temperature and protected from light until further use. The percent mass recovery was calculated using Equation 2.

$$\% \text{ Mass recovery} = \frac{\text{weight of dried powdered sample}}{\text{initial fresh weight of sample}} \times 100 \quad (2)$$

Sample Extraction and Concentration

The maceration method employed 1:4 (sample:solvent) ratio using distilled water, water-ethanol (1:1), and absolute ethanol solvents for 72 hours. Water extracts were freeze-dried, absolute ethanol extracts were concentrated using a rotary evaporator at 40 °C, and water-ethanol (1:1) extracts were first concentrated with a rotary evaporator, then freeze-dried. All concentrated extracts were kept in vials at 4°C. Percent extraction yields were calculated using Equation 3.

$$\% \text{ Extraction yield} = \frac{\text{weight of extract, g}}{\text{weight of air-dried sample, g}} \times 100 \quad (3)$$

Calibration and Calculation

Calibration curves were prepared using appropriate standards (0-100 ppm) for each assay: gallic acid (GA) for total phenolic content, quercetin (Q) for total flavonoid content, and ascorbic acid (AA) for total antioxidant capacity. All Extract concentrations were determined from the linear regression equation of the respective calibration curve (y = mx + c).

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu method (Ainsworth & Gillespie 2007) in a 96-well microplate at room temperature. Each 300 µL sample and GA standard was mixed with 300 µL of 10% Folin-Ciocalteu reagent, followed by 1200 µL of Na_2CO_3 solution. The mixtures were incubated in the dark for 2 hours, centrifuged for 5 minutes, and the absorbance was measured at 695 nm using a microplate spectrophotometer.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the aluminum chloride colorimetric assay (Chang *et al.* 2002) in a 96-well microplate at room temperature. Each 400 µL sample and Q standard was mixed with 120 µL of 10% $AlCl_3 \cdot 6H_2O$ and 1 M CH_3COONa, followed by 780 µL of solvent. The mixtures were vortexed, incubated in the dark for 40 minutes, and the absorbance was measured at 415 nm using a microplate spectrophotometer.

Evaluation of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using the phosphomolybdenum method (Prieto *et al.* 1999) in a 96-well microplate at room temperature. Each 400 µL sample and AA standard was mixed with 1200 µL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixtures were vortexed, incubated at 95°C for 90 minutes, cooled to room temperature, and centrifuged for 5 minutes. The absorbance was measured at 695 nm using a microplate spectrophotometer.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay

The DPPH assay was performed following the method of Ang & Uy (2023) with modifications in sample concentrations. Sample solutions at different concentrations (0-200 ppm) and a series of ascorbic acid solutions (0 – 10 ppm), used as a positive control, were prepared. Each solution was mixed with an equal volume (1:1 ratio) of 0.1 mM DPPH solution in a 96-well microplate. The mixtures were incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a microplate spectrophotometer. The percent inhibition of DPPH radicals was calculated using Equation 4, and the IC_{50} value, the concentration required to scavenge 50% of the radicals, was determined using regression equations of concentration-response curves.

$$\% \text{ DPPH Inhibition} = \frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample or standard}}}{\text{Absorbance}_{\text{blank}}} \times 100 \quad (4)$$

Toxicity of Neem Leaf Extracts against *Dinoderus minutus*

The toxicity of plant extracts against *D. minutus* was assessed using repellency and mortality assays following the method of Hasan *et al.* (2014) and Tabassum *et al.* (2023), with modifications in the test insect species used. D minutus insects were collected from naturally infested bamboo in the field located in Sinuda, Kitaotao, Bukidnon. The species was certified by a taxonomist and entomologist in CMU prior to analysis.

Repellency Test

Petri dishes were lined with filter paper, with one half treated with plant extracts at various concentrations (0 – 20% w/v) and the other half with acetone as the negative control. Papers were air-dried, and ten powder post beetles (PPB) were released per dish at room temperature. Counts on each side were recorded hourly for 24 hours, and percent repellency was calculated using Equation 5.

$$\% \text{ Repellency} = \frac{\text{No. of insects on control surface} - \text{No. of insects on treated surface}}{\text{Total number of insects released on surface}} \times 100 \quad (5)$$

Mortality Test

Plant extracts at various concentrations (0 – 30% w/v) and positive control, boric acid, were applied to filter paper in petri dishes and air-dried to ensure complete solvent evaporation at room temperature. Ten PPB were placed per dish. Mortality was recorded at 24-hour intervals for one week, and percent mortality was calculated using Equation 6.

$$\% \text{ Mortality} = \frac{\text{No. of dead PPB}}{\text{Total no. of PPB}} \times 100 \quad (6)$$

For probit analysis, the raw data were expressed as percent mortality values following the method of Finney (1952). For those with > 0% but < 100% mortality, the percent mortality was calculated using Equation 6. However, for those with 0% and 100% mortality, the percent mortality was calculated using Equations 7 and 8:

$$\% \text{ Mortality} = \left(\frac{0.25}{\text{initial no. of live PPB}}\right) \times 100 \quad (7)$$

$$\% \text{ Mortality} = \left(\frac{\text{initial no. of live PPB} - 0.25}{\text{initial no. of live PPB}}\right) \times 100 \quad (8)$$

Statistical Analyses

Treatment differences were analyzed using one-way analysis of variance (ANOVA) under a completely randomized design (CRD) at a significance level of $\alpha = 0.05$, followed by Tukey's Honest Significant Difference (HSD) post hoc test. TAC, TPC, and TFC were measured in four replicates, and IC_{50}, repellency, and mortality in three replicates. Pearson's Product-Moment-Correlation coefficients were computed to determine the relationships among the measured parameters.

RESULTS AND DISCUSSION

Mass Recovery, Moisture Content, and Extraction Yield

Leaf samples of *A. indica* exhibited high moisture content (73.91%), resulting in low mass recovery after drying (25.88%). Since water contributes to fresh sample weight, its removal during drying directly reduces recovered mass (Rocha *et al.* 2011), confirming the critical influence of moisture on sample mass determination.

Extraction yield was assessed to evaluate solvent efficiency in extracting phytoconstituents from *A. indica*. Water yielded the highest recovery (4.34%), followed by water-ethanol (1:1) (2.34%) and ethanol (2.03%). This is attributed to solvent polarity and selectivity: water, being non-selective, extracts a broad range of hydrophilic compounds including saponins, phenolics, and polysaccharides (Dai & Mumper 2010; Kumar *et al.* 2023).

Total Phenolic Content

TPC of neem CLEs was quantified using a GA calibration curve and expressed as mg GAE/g dry extract (Table 1). The water-ethanol (1:1) extract yielded the highest TPC (139.67 ± 0.45 mg GAE/g), followed by the ethanol extracts (59.65 ± 0.28), and water extracts (51.07 ± 0.90) extracts ($P < 0.05$), consistent with previous reports on the effectiveness of aqueous ethanol in extracting phenolic compounds from neem leaves (Singh *et al.* 2014; Puttongsiri *et al.* 2025). This variation reflects the influence of solvent polarity on phenolic extractability: acidic phenols favor highly polar solvents, while less acidic or neutral phenolics are better extracted by mid-polarity solvents (Routray & Orsat 2013). The predominance of water-ethanol (1:1) in TPC yield suggests that neem leaf phenolics are largely of intermediate polarity, in agreement with Sultana *et al.* (2009), who reported that mid-polar solvents generally extract higher phenolic content than pure water or ethanol.

Table 1 Total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) of *A. indica* crude leaf extracts

Crude Extract	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg AAE/g)
Water	51.07 ± 0.92^{c}	5.58 ± 0.09^{c}	52.64 ± 0.36^{c}
Water-Ethanol (1:1)	139.67 ± 0.45^{a}	34.98 ± 2.37^{b}	161.14 ± 3.70^{a}
Ethanol	59.65 ± 0.28^{b}	72.08 ± 1.04^{a}	100.74 ± 2.24^{b}

Notes: Results are expressed as mean ± standard deviation (n = 4). Different superscript letters within the same column indicate statistically significant differences at $P < 0.05$.

Table 2 Median inhibitory concentration (IC_{50}) of the *A. indica* crude leaf extracts obtained from DPPH assay

Crude extract	IC_{50} (ug/mL)	Antioxidant strength*
Water	243.65 ± 4.33^{d}	Medium
Water-Ethanol (1:1)	51.16 ± 3.70^{b}	Active
Ethanol	195.86 ± 2.34^{c}	Medium
Ascorbic Acid (positive control)	6.24 ± 0.15^{a}	Very Active

Notes: Results are expressed as mean ± standard deviation (n = 3). Different superscript letters indicate statistically significant differences at $P < 0.05$. *Antioxidant strength was classified as very active (< 50 µg/mL), active (50 – 100 µg/mL), or medium (101 – 250 µg/mL) (Fatmawaty *et al.* 2019).

Total Flavonoid Content

TFC of neem CLEs was quantified using a Q calibration curve and expressed as mg QE/g dry extract (Table 1). Leaf extracts of *A. indica* are consistently reported to have higher flavonoid content than other plant parts (Kiranmai *et al.* 2012; Naseer *et al.* 2014; Abdulkadir *et al.* 2017). In this study, the ethanol extract yielded the highest TFC (72.08 ± 1.04 mg QE/g), followed by the water-ethanol (1:1) extract (34.98 ± 2.37) and the water extract (5.58 ± 0.09) ($P < 0.05$). The higher yield in ethanol likely reflects the solubility of free flavonoids in organic solvents over water (Zhao *et al.* 2016). The result suggests that flavonoids predominant in neem leaves are most likely free flavonoids, consistent with the findings of Puttongsiri *et al.* (2025), who also reported higher TFC values in ethanol extracts.

Total Antioxidant Capacity

TAC of *A. indica* CLEs was quantified using a standard ascorbic acid (AA) calibration curve and expressed as mg AAE/g (Table 1). Neem extracts from various plant parts are known for their strong antioxidant potential (Sithisarn *et al.* 2005; Ghimeray *et al.* 2009). Consistent with this, the water–ethanol (1:1) extract yielded the highest TAC (161.14 ± 3.70 mg AAE/g), followed by the ethanol (100.74 ± 2.24) and water (52.64 ± 0.36) extracts ($P < 0.05$) (Table 1). As with other assays, the choice of solvent significantly influenced TAC, likely due to the extraction of different bioactive compounds (Bentoulla *et al.* 2024). These compounds may exert antioxidant effects through several mechanisms, such as inhibiting chain initiation, scavenging free radicals, enhancing reducing power, and decomposing peroxides (Yildirim *et al.* 2000). A strong correlation was observed between TAC and TPC in the water–ethanol (1:1) CLEs ($r = 0.84$), supporting the direct association between TPC and antioxidant activity in hydro-ethanolic neem leaf extracts as reported by Yadav *et al.* 2021. In contrast, the correlations between TPC and TAC in ethanol and water CLEs were weak ($r = 0.39$ and $r = 0.33$, respectively).

DPPH Radical Scavenging Assay

A. indica CLEs demonstrated antioxidant activity via DPPH radical scavenging, consistent with previous reports of high antioxidant potential in neem leaf extracts (Abdulkadir *et al.* 2017; Abdelhady *et al.* 2019). The water-ethanol (1:1) extract was the most potent, with the lowest IC_{50} (51.16 ± 3.70; active), followed by ethanol (195.86 ± 2.34; medium) and water (243.65 ± 4.33; medium) extracts ($P < 0.05$) (Table 2). These results align with TPC and TAC findings, further supporting water-ethanol (1:1) as the most effective solvent for extracting bioactive compounds from neem leaves.

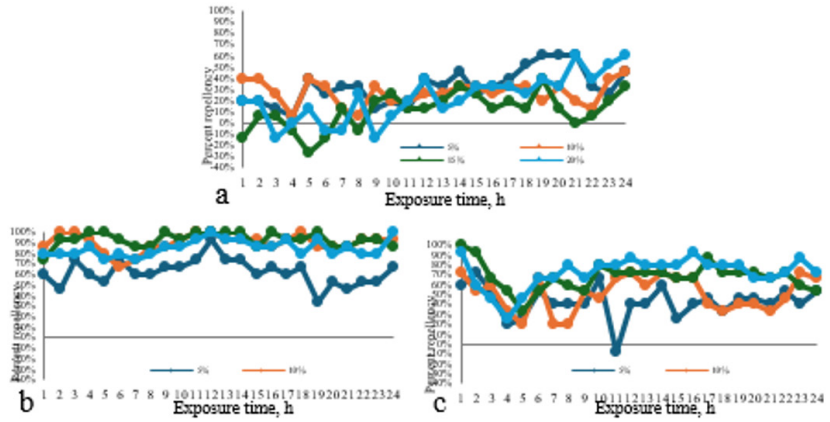


Figure 1 Repellent effect of *A. indica*
Notes: (a) = water; (b) = water-ethanol (1:1); (c) = ethanol crude leaf extracts for 24-hour period

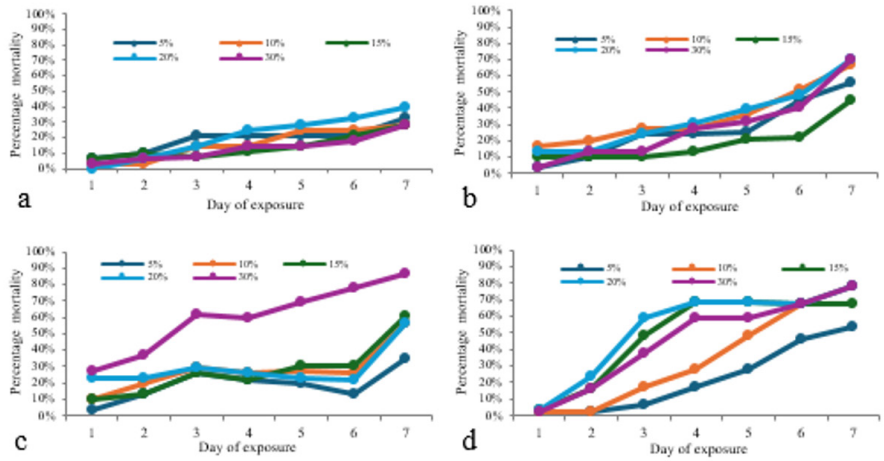


Figure 2 Mortality of *D. minutus* exposed to *A. indica*
Notes: (a) = water; (b) = water-ethanol (1:1); (c) = ethanol crude leaf extracts, (d) = boric acid for 7-day period

Table 3 Median lethal concentration (LC_{50}) of *A. indica* crude leaf extracts against *D. minutus* after 7-day exposure

Crude extracts	LC_{50}, (% w/v)*
Water**	>1000
Water-Ethanol	2.74
Ethanol	8.58
Boric Acid (positive control)	2.57

Notes: *Lower LC_{50} values indicate greater insecticidal potency of extract;

Repellent Effect of *A. indica* Leaf Extracts Against *D. minutus*

The assessment of the hourly repellent effect of varying concentrations (5%, 10%, 15%, and 20%) of neem CLEs showed that water-ethanol (1:1) extracts consistently showed the highest and most significant repellent effect across all concentrations (Fig. 1b). In contrast, the ethanol extract demonstrated moderate repellency (Fig. 1c), while the water extract exhibited low repellency values at all concentrations tested (Fig. 1a). The limited effectiveness of the water extract may be due to its limited ability to extract insect repellent constituents such as azadirachtin, an insect repelling compound from *A. indica* seeds (National Research Council (US) Panel on Neem 1992).

Although, results illustrated a decline in activity at specific time points, the overall repellency data revealed no loss of activity over time. This observation is likely due to biological factors, including the circadian rhythms, mobility, behavioral patterns, and other uncontrolled variability among test insects, which can influence

individual behavioral responses and sensitivity towards the repellents over time (Hooven *et al.* 2009). Moreover, classification of the overall repellency of the CLEs indicated that water-ethanol (1:1) extract ranged from Class IV to V (62.2 – 85.6) (fairly repellent to very repellent), while ethanol extract ranged from Class III to IV (43.1 – 72.5) (averagely repellent to fairly repellent), and water extract remained within Classes I to II (11.4 – 33.3) (very low to moderately repellent).

Insecticidal Effect of *A. indica* Leaf Extracts Against *D. minutus*

A general increase in mortality of *D. minutus* exposed to varying concentrations (5%, 10%, 15%, 20%, and 30% w/v) of neem CLEs over a 7-day period was observed, similar to that of the positive control, boric acid (Fig. 2). Analysis revealed that water extract exhibited no substantial toxic effect, as none of the tested concentrations achieved 50% mortality (Fig. 2a). In contrast, water-ethanol (1:1) extract resulted in 70.37% mortality at 15% and 20% concentrations (Fig. 2b), while the ethanol extract showed the highest mortality of 86.96% at 30% concentration (Fig. 2c). Accordingly, these were considered the most effective concentrations for the respective extracts.

Similar to the repellency results, mortality data showed no consistent concentration-dependent trend. This can be explained by the Individual Tolerance (IT) model of the Toxicokinetic–Toxicodynamic framework, which accounts for individual variability in sensitivity to toxicants, where each organism possesses a unique tolerance threshold (Ashauer *et al.* 2007). Mortality occurs when the damage induced by chemical stress exceeds this threshold, making death an individual-specific response rather than a probabilistic process (Sowa *et al.* 2024).

The median lethal concentration (LC50) obtained via Probit analysis showed that water-ethanol (1:1) extract had the lowest LC_{50} (2.74% w/v), followed by ethanol extract (8.58% w/v), while water extract exhibited an LC_{50} greater than 1000% w/v (Table 3), indicating it was essentially nontoxic. These results suggest that among the CLEs *A. indica*, the water-ethanol (1:1) extract showed the highest toxicity against *D. minutus* and was the most comparable to the positive control, boric acid, which has an LC_{50} value of 2.57%. This trend is consistent with the repellency results, further supporting the strong bioactive potential of the water-ethanol (1:1) extract.

The present findings align with previous research highlighting the insecticidal potential of *A. indica*, especially its seed and leaf extracts. Tulashie *et al.* (2021) reported strong insecticidal activity against fall armyworm, while Okolo & Oyewole (2019) observed improved pest control and crop yield. Study by Rosemary *et al.* (2018) also confirm that *A. indica* is a promising botanical pesticide with antifeedant, deterrent, and toxic effects against various insect pests. The insecticidal activity observed in this study may be due to various phytochemicals present in *A. indica* leaf which includes limonoids, saponins, azadirachtin, and flavonoids (Michel *et al.* 2023). Azadirachtin is a potent antioxidant known for its insecticidal properties which could be extracted using hydroethanolic solvent from *A. indica* leaves (Yadav *et al.* 2016; Martínez-Castro *et al.* 2024). Furthermore, high phenolic content has been linked to enhanced antioxidant activity (Hossain *et al.* 2014). Based on the study of Baruah *et al.* (2023), strong larvicidal activity against Aedes aegypti larvae was observed, which was attributed to the combined action of phenolic compounds and antioxidant molecules. This is further supported by the present study, which found a strong negative correlation between LC50 and total phenolic content (TPC) values of water-ethanol (1:1) CLEs ($r = -0.96$), reinforcing the role of phenolic compounds in the insecticidal efficacy of *A. indica* CLEs.

CONCLUSION

This study provided initial evidence that *A. indica* crude leaf extracts exhibit repellent and insecticidal activity against *D. minutus*, supporting its potential as a bio-insecticide for bamboo protection and preservation. Among the solvent systems evaluated, the water-ethanol (1:1) extract proved most promising under laboratory conditions, yielding the highest phenolic content, antioxidant activity, and bioactivity. As findings are limited to laboratory-scale bioassays, future studies should validate results on actual bamboo materials, assess formulation stability, and identify the active compounds responsible for the observed repellent and insecticidal effects.

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