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Efficiency of Niang shell *Archidendron pauciflorum* (Benth.) extracts in the control of adult *Aedes aegypti* (L.) (Diptera: Culicidae)

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Abstract

Niang shells are abundant agricultural waste; this research applies the Zero Waste concept to add value by transforming this biomass into a safe and environmentally friendly biopesticide. To investigate the efficacy of the *Archidendron pauciflorum* shell extract against adult *Aedes aegypti* using ATSB method compared with CDC bottle bioassay. The extraction of active compounds was performed using the maceration method with ethanol as solvent and analyzed using FT-IR and UV-Vis to characterize the functional groups and rotenone-related phytochemical. The extract was then evaluated through an ingestion-based delivery system using the ATSB method and external surface contact of mosquitoes using the CDC bottle bioassay method. The efficacy test using the ATSB method has the highest efficiency in mosquito elimination, demonstrating a maximum mortality rate of 100% and a half-lethal concentration (LC50) of 2.54% (v/v) and a 95% lethal concentration (LC95) of 6.15% (v/v). The ATSB method provides significantly better efficiency in eliminating adult *Ae. aegypti* mosquitoes compared to the CDC bottle bioassay method. This study indicates that *A. pauciflorum* shell extract has high efficacy and can be applied to enhance innovation, serving as a new alternative for using natural toxic baits to replace synthetic chemicals sustainably.

Keywords: *Aedes aegypti*; *Archidendron pauciflorum*; Attractive Toxic Sugar Baits (ATSB); CDC bottle bioassay; Zero waste

Introduction

Mosquitoes are insects found worldwide, especially in tropical and subtropical regions. Currently, more than 4,000 species of mosquitoes have been identified globally, with 450 species reported in Thailand (Burkett-Cadena, 2013; Eisen & Moore, 2013). Entomology can classify mosquitoes into three main subfamilies: *Toxorhynchitinae*, *Anophelinae*, and *Culicinae*. This study focuses on the *Aedes* genus, which belongs to the *Culicinae* subfamily, as *Ae. aegypti* mosquitoes play a crucial role as vectors for various infectious diseases. Notably, *Ae. aegypti* mosquito is the primary vector for spreading viruses that can cause emerging and re-emerging diseases in tropical areas, such as dengue fever (Sukhralia et al., 2019), Zika virus, yellow fever, and chikungunya. These diseases continue to be significant public health issues both in Thailand and internationally. Although there are currently various disease control measures, such as vaccination, eliminating mosquito breeding sites, using long-lasting insecticidal nets (LLINs) (Tungu, 2024; Yang et al., 2018), and employing synthetic and natural insecticides. Currently, there is still no specific treatment for dengue fever. Particularly, the *Ae. aegypti* mosquito lays its eggs in clean water containers, which are often found near human living areas. This can lead to rapid and widespread disease outbreaks, especially during the rainy season when natural breeding sites increase. Therefore, controlling the disease vector is crucial in managing the mosquito population. Thailand is rich in natural resources and high biodiversity, especially in terms of native plants in botany, pharmacology, and economics. This research focuses on *A. pauciflorum*, which belongs to the Leguminosae-Mimosoideae family. This perennial tree is found in the southern region of Thailand (Barceloux, 2009; Bamrungrak, 1986). The cultivation of *A. pauciflorum* is important to the local economy. Typically, people enjoy consuming it as a local food. The seeds can be eaten as fresh vegetables and desserts. Additionally, the seeds of *A. pauciflorum* contain phytochemicals with medicinal properties, such as lowering blood pressure and antioxidant qualities. However, excessive consumption may adversely affect health and induce toxicity due to the over-ingestion of compounds present in Niang seeds (Shukri et al., 2011). The shells of *A. pauciflorum* are often discarded as agricultural by products in large quantities; the accumulation of this waste can lead to environmental pollution if effective management systems are lacking. Aligning with the Sustainable Development Goals (SDGs), this research applies the Zero Waste concept to maximize the utilization of biological resource. Specifically, the discarded Niang shells are upcycled into a high-value bio-extract, offering a sustainable approach to reducing agricultural waste and developing eco-friendly innovations for vector

control. Currently, synthetic insecticides are the predominant method utilized for mosquito control, owing to their high effectiveness and operational convenience. However, the continuous and prolonged use of the same chemical classes has led to insecticide resistance in mosquito populations, thereby reducing their operational efficacy. Furthermore, this practice may disrupt ecosystems and pose risks to human health due to the accumulation of chemical residues (Jones et al., 2021; Dusfour & Chaney, 2022). Therefore, finding novel, eco-friendly, and sustainable alternatives to replace synthetic chemical insecticides is of paramount importance (Ghosh et al., 2012; Priya et al., 2023). This study focuses on evaluating the potential of botanical extracts, specifically the *A. pauciflorum* shell, using distinct control mechanisms. We integrated the ATSB technique, which targets mosquitoes through a sugar solution combined with bioactive compounds (Kanaphot, 2017) alongside the CDC bottle bioassay to assess susceptibility. This dual approach evaluates the specific modes of action via ingestion versus contact, ultimately determining the most effective route of exposure for the extract (Centers for Disease Control and Prevention, 2024).

Experimental

Materials

Plant material and reagents

The primary materials and reagents utilized in this study included the *A. pauciflorum* shell, collected from Hat Yai District, Songkhla Province. Chemicals for extraction and bioassays consisted of 96% ethanol and distilled water. A sugar solution was prepared as the bait base for the ATSB technique. The test mosquitoes were laboratory-reared *Ae. aegypti*, maintained through an artificial feeding system using blood for oviposition stimulation.

Preparation of A. pauciflorum

For sample preparation, *A. pauciflorum* shells were thoroughly washed with tap water to remove debris and then sectioned into small pieces (approximately 1–2 cm) to increase the surface area for accelerated drying. The sectioned shells were arranged in trays and covered with a fine mesh to prevent contamination from dust and microorganisms. Subsequently, the

sample was air-dried under direct sunlight for 2–3 days, or until completely dehydrated, to prevent microbial growth and spoilage. Once dried, the shells of *A. pauciflorum* were stored in airtight plastic bags or sealed containers kept in a cool, dry place to minimize air exposure and prevent the degradation of key bioactive compounds. Finally, the dehydrated shells were pulverized into a coarse powder using a laboratory blender to maximize the surface area accessible for the subsequent maceration extraction.

Methods

Extraction of A. pauciflorum shell

After completing the process of preparing the *A. pauciflorum* shell sample, weigh 10 g of the sample powder and place it in a 250 ml Duran bottle. Then, add 100 ml of ethanol as the solvent. The samples are then soaked using the maceration method for 7 days. Following the maceration period, the extract was filtered using Whatman No.3 filter paper to separate the plant residue from the solution. Then, the filtrate was subjected to solvent evaporation using a water bath at a temperature of 50°C, which is suitable for removing ethanol without affecting the stability of the bioactive compounds. Although the Rotenoid compounds are sensitive to heat and most stable at temperatures not exceeding 40°C (Zubairi et al., 2015), operating at this lower thermal exposure could induce greater degradation of the core bioactive constituents than a brief exposure at a higher temperature. The evaporation process was continued until most of the solvent was removed, resulting in a crude extract.

Test against mosquitoes with attractive toxic sugar baits

Preparation of sugar solution for mosquitoes feeding

To prepare the energy source for adult mosquitoes under laboratory conditions, 10% (w/v) sucrose solution was formulated. Initially, 10 g of sucrose was dissolved in 100 mL of distilled water. Ascorbic acid (Vitamin C) was added to the solution to maintain optimal physiological conditions and support the natural mosquitoes behaviors, and the mixture was stirred until completely homogenous. Finally, cotton-swabbed wooden applicator sticks were prepared, immersed in the prepare sugar solution, and place inside the mosquito cages to ensure uniform access for energy replenishment. The solution was replaced every 2 days to prevent

contamination and microbial growth. Any remaining prepared solution was stored in a tightly sealed container under controlled conditions at 4°C to preserve its quality for subsequent use.

Preparation of adult Aedes aegypti mosquitoes for use in testing

For mosquito preparation, a laboratory strain of *Aedes aegypti* (L.) was utilized in this study. The initial mosquito colony was kindly provided by the Department of Disease Control, Ministry of Public Health, Thailand. The mosquitoes were reared under controlled laboratory conditions at a temperature of 25±5°C and a relative humidity of 80±10%RH. A photoperiod of 12:12 hours (light: dark) photoperiod maintained to simulate natural environmental cycles (Kanaphot, 2017). The egg-hatching process was initiated using chlorine evaporation. This water was mixed with warm purified water to achieve a temperature of approximately 40°C, a thermal condition that effectively stimulates rapid and synchronized egg hatching, thereby optimizing hatching efficiency.

Preparation of ATSB for testing with adult Aedes aegypti mosquitoes

The formulation of the ATSB for efficiency testing against *Ae. aegypti* commenced with the extraction of *A. pauciflorum* shells via the maceration method. The filtrate from section 2.3 was designated as the 100% stock solution. To prepare the toxic sugar baits for the bioassays, this stock solution was subsequently diluted on a volume-per-volume (v/v) basis with a 10% sugar solution, which served as the standard laboratory rearing diet to achieve five distinct concentrations of the ATSB: 2, 5, 7, 10 and 15% (v/v). For the preparation of the bioassay apparatus, cylindrical cotton applicators (approximately 1 cm in diameter) were constructed to optimize absorption efficiency. Then, each cotton swab was immersed in (approximately 1 cm in diameter) were utilized to optimize absorption efficiency. Each cotton applicator was thoroughly immersed in 100 ml of the prepared toxic sugar bait at the specified concentrations, transferred into a fitted glass vial, and placed inside the experimental mosquito cages. Mortality data were then recorded at 24, 48, and 72 hours post-exposure to evaluate the efficacy of the extract.

Methods for preparing ATSB for testing with Aedes mosquitoes

For the adulticidal bioassay, adult female *Aedes aegypti* were collected from standard rearing cages using a mechanical aspirator. The mosquitoes were then transferred into experimental cages (15x15x15 cm). A total of 100 female mosquitoes were utilized per concentration, divided into four replicates with 25 mosquitoes per cage. Additionally, two

control groups were established: an ethanol control and a sugar solution control. Both control groups, Ethanol and sugar solution (Negative control) excluded the *A. pauciflorum* extract and utilized an identical number of mosquitoes as the treatment groups. Following transfer, the mosquitoes were acclimatized for 1 h to mitigate transfer-induced stress and monitor overall fitness; any injured or weak individuals were immediately replaced with healthy ones. Subsequently, the toxic sugar bait at each specified concentration was introduced into the cages, and the initial exposure time (T_0) was recorded. The bioassay was conducted under controlled conditions at $27\pm 2^\circ\text{C}$, $75\pm 10\%$ RH, and a 12:12 h (light: dark) photoperiod. Cumulative mortality was recorded at 24, 48, and 72 h post-exposure.

Test against mosquitoes with CDC bottle bioassay

Material

A total of 20 Duran bottles (250 ml) were employed for the experiments. The experimental design was organized into sets based on extract concentrations, where each set utilized five bottles: four replicates for the treatment group and one for the negative control group. The Additional equipment included a manual insect aspirator for mosquito transfer, a micropipette for the precise delivery of 1 ml solutions, standard stock solution storage bottles, and digital timers to monitor exposure periods.

Preparation of mosquitoes for bioassay

To evaluate susceptibility to the botanical extract, only female *Ae. aegypti* were selected for the bioassay, as females exhibit blood-feeding behavior and serve as the primary vectors for pathogen transmission. The mosquitoes utilized were derived from a standard susceptible strain (USDA strain), colonized under controlled laboratory conditions to standardize age and physiological status, thereby ensuring experimental uniformity and reproducibility. Selected adult mosquitoes were 2 to 5 days old and non-blood-fed to guarantee a consistent physiological response. 24 h prior to the bioassay, the mosquitoes were deprived of the sugar solution to standardize their physical condition. For each treatment group, a total of 125 mosquitoes were utilized across five replicates (25 mosquitoes per bottle). A negative control group, consisting of bottles coated exclusively with ethanol, was included to determine baseline mortality. Furthermore, the data were analyzed to compare the efficacy with international standards insecticides in the pyrethroid group according to the CDC global bottle bioassay guidelines (Centers for Disease Control and Prevention, 2024). Specifically, Deltamethrin at a

diagnostic dose concentration of 10 µg/bottle was planned to be used as a positive control standard for the evaluation of the experimental results.

Bottle coating and bioassay procedure

Prior to the main experiment, a pilot study was conducted to optimize the solvent system and dilution ratios to reduce the viscosity of the extract before the actual test, ensuring a uniform coating of the bottles. Prior to the assay, Duran bottles and caps were thoroughly inspected to ensure cleanliness and dryness. Dedicated pipettes were utilized for the solvent controls and plant extract treatments to prevent potential cross-contamination. For the control group, 1 ml of ethanol solvent was added to the bottle, which was then tightly capped. For the treatment groups, 1 ml of the botanical stock solution at each designated concentration was dispensed into the four respective test bottles. Subsequently, the bottom of each bottle was gently swirled. Following this, each bottle was inverted and swirled to ensure the inner cap was thoroughly coated and then rolled horizontally to evenly distribute the solution across the entire interior surface area. Once completely coated, the caps were removed, and the bottles continuously rolled horizontally until the solvent had completely evaporated. Following the coating procedure, the bottles were kept uncapped and positioned horizontally in a light-restricted area to avoid the photodegradation of bioactive constituents. These prepared bottles were conditioned in the dark overnight prior to bioassay testing. Following the introduction of mosquitoes into the coated bottles, the bioassay commenced by capping the bottles and initiating a timer. Knockdown status was recorded at 30 minutes (Diagnostic time). Immediately thereafter, the mosquitoes were transferred from the Duran bottles into holding cups, provide with a water via cotton pad, and maintained under laboratory conditions. Final mortality was recorded 24 h post-exposure. Although CDC guidelines focus on the diagnostic time for resistance monitoring, the 24-hour observation period was used to establish a standard for assessing mortality rates.

Statistical Analysis

The mosquito mortality data obtained from both the ATSB and CDC bottle bioassays at each specified concentration were subjected to Probit analysis using Microsoft Excel. The concentration data were log-transformed, and the dose response relationship was fitted to a Probit regression model to determine the LC₅₀. In the case where the control group (negative

control group) has a mortality rate, the data were adjusted using Abbott's formula before being analyzed using the Probit analysis. This analysis was performed to determine the median lethal concentration (LC₅₀), the 95% lethal concentration (LC₉₅), and the slope of the regression line. The χ^2 test was utilized to evaluate the goodness-of-fit of the regression model, where $p > 0.05$ indicates that the model provides a good fit for the experimental data (Finney, 1971; Robertson et al., 2007). These parameters constitute critical components for validating the reliability of the bioassay data (Robertson et al., 1984) and serve as a baseline to evaluate the most promising extracts for future application or development in vector control strategies.

Chemical characterization of A. pauciflorum shell extract

Fourier Transform Infrared spectroscopy analysis (FT-IR)

Functional group analysis of the *A. pauciflorum* shell extract was performed using Fourier-transform infrared spectroscopy (FT-IR) (Nicolet iS5, Thermo Fisher Scientific, USA). The instrument was equipped with an attenuated total reflection (ATR) accessory featuring a diamond crystal contact surface. During analysis, a droplet of the crude extract was placed directly onto the diamond crystal surface without further sample preparation. The spectrum was then recorded over a wavenumber range of 4000 to 500 cm⁻¹.

Ultraviolet-Visible spectrophotometric analysis (UV-Vis)

Qualitative analysis and structural confirmation of rotenone group bioactive compounds from the *A. pauciflorum* shell were performed using a UV-Visible spectrometer (Model UV-1800, Shimadzu, Kyoto, Japan). Prior to measurement, the crude extract was filtered to remove particulate matter and then diluted with ethanol to prevent turbidity, ensuring accurate analysis of the target compounds. The sample was scanned over a wavelength range of 200–400 nm to determine the maximum absorbance (λ_{max}) of the extract.

Ethics statements

Ethical approval

All experimental procedures were conducted in accordance with the institutional biosafety and animal welfare guidelines. The research protocol was approved by the Institutional Biosafety Committee (Approval no. 2025-ENV01-051) and the Institutional

Animal Care and Use Committee (IACUC) of Prince of Songkla University (Approval no. Ref.AR083/2025).

Results

Extraction and Phytochemical Characteristic

The shells of *A. pauciflorum* were ground into a coarse, dark brown powder. This powder was macerated in ethanol for 7 days. Following the maceration period, the mixture was filtered using vacuum filtration, which provided a dark brown transparent liquid. Then, the obtained solution was evaporated using a water bath until the volume was reduced to approximately half of its original volume. The concentrated extract appeared as a turbid, dark brown liquid, which was collected for subsequent bioassays and chemical analyses.

Adulticidal activity of ATSB

Mortality rates

As shown in Fig. 1, the efficacy testing of the *A. pauciflorum* shell extract using the ATSB method indicated that the extract concentration was directly and significantly correlated with mosquito mortality ($p<0.05$). Specifically, a clear dose-dependent trend was observed, where increasing concentrations were directly associated with higher mosquito mortality rates. The graph demonstrates that at an extract concentration of 2%, the average mosquito mortality rate was approximately 33%. This rate increased sharply at concentrations of 5% and 7%. This resulted in mortality rates of 91 and 93%, respectively. In contrast, concentrations of 10 and 15% resulted in complete mosquito mortality (100%). When tested with the control group receiving a 10% sugar solution and ethanol via the ATSB method, the mortality rate of mosquitoes was found to be extremely low and non-significant ($p>0.05$). This confirms that the experimental conditions and the solvent did not adversely affect the physiological state of the mosquitoes. The treatment groups exhibited higher mortality rates that correlated with the increasing extract concentrations, which indicates a dose-dependent response characteristic of the bioactive compounds present in the *A. pauciflorum* shell. These findings align with the principles of the ATSB technique, which relies on attracting mosquitoes with a sugar bait to

ensure the extract directly enters their digestive system, subsequently leading to effective mortality.

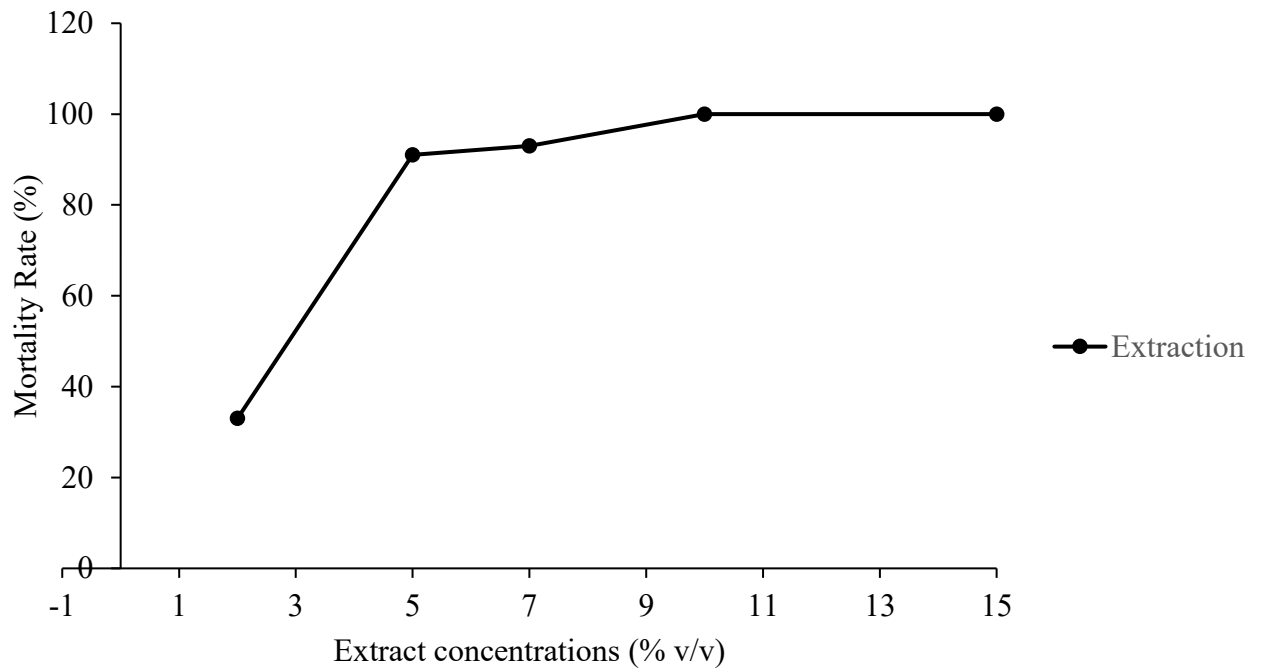


Fig. 1. Dose-response relationship of *Ae. aegypti* mortality after exposure to *A. pauciflorum* shell extract using ATSB at 72 h. Data points represent the mean mortality rate observed at various concentrations (2, 5, 7, 10 and 15% v/v). As shown, the mortality rate 100% at concentration of 10 and 15%

Adulticidal activity of CDC bottle bioassay

Mortality rates

The evaluation of the *A. pauciflorum* shell extract using the CDC bottle bioassay revealed a fluctuating trend in mosquito mortality rates. Specifically, extract concentrations of 25 and 75% resulted in mortality rates of 25 and 50%, respectively. Conversely, at concentrations of 50 and 100%, the mortality rates unexpectedly decreased. This demonstrates an inconsistent dose-response relationship in contact-based mortality compared to the ATSB method. This variation could be attributed to the behavioral responses of the mosquitoes, which may have prevented uniform contact with the extract due to potential uneven coating inside the bottles. Because the *A. pauciflorum* shell extract is highly concentrated and viscous, the rotation technique for bottle coating may result in incomplete and non-uniform distribution.

Furthermore, upon drying, the natural extract exhibits a sticky texture compared to synthetic chemicals, which likely contributed to the inconsistent exposure.

Probit analysis of the extract against Aedes aegypti

Determination of Lethal Concentrations (LC₅₀ and LC₉₅)

The mortality rates of adult *Aedes aegypti* tested with the *A. pauciflorum* shell extract via ATSB method were subjected to Probit analysis to determine the LC₅₀ and LC₉₅ values. The results of this analysis indicate a statistically significant dose-dependent relationship regarding the efficacy of the extract, where the adulticidal activity increased with the extract concentration. Based on the Probit analysis, the LC₅₀ value of extract evaluated via the ATSB method was 2.54% (v/v), while the LC₉₅ value was determined to be 6.15% (v/v). In contrast, for the CDC bottle bioassay, LC₅₀ and LC₉₅ values could not be reliably determined due to the inconsistent mortality data and poor fit of the regression model. For the ATSB method, the regression equation was $Y = -1.735 + 4.2809 \log_{10}(C)$, with a slope of 4.2809. The goodness-of-fit was evaluated using the χ^2 test, which confirmed that the model provides a good fit for the experimental data ($p > 0.05$) (Finney, 1971; Robertson et al., 2007). The dose-response curve shows that the mosquito mortality rate increases rapidly in the concentration range of 2 to 5%, before stabilizing and reaching 100% mortality at a concentration of 10% and above.

Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

FT-IR spectroscopy analysis was conducted to characterize the functional groups present in the *A. pauciflorum* shell extract, with measurements recorded over a wavenumber range of 4000 to 500 cm⁻¹. The resulting spectrum shown in Fig. 2 displays several significant peaks indicative of the chemical composition and structural characteristics of the compounds in the extract. Specifically, a prominent absorption band was observed at 3228 cm⁻¹, which is a broad peak indicating the vibration of the O-H bond in phenolic or alcohol groups. The peak at 1696 cm⁻¹ indicates the presence of a carbonyl group (C=O), which is characteristic of phenolic-based secondary metabolites. The detailed peak positions and corresponding functional group assignments are summarized in Table 1.

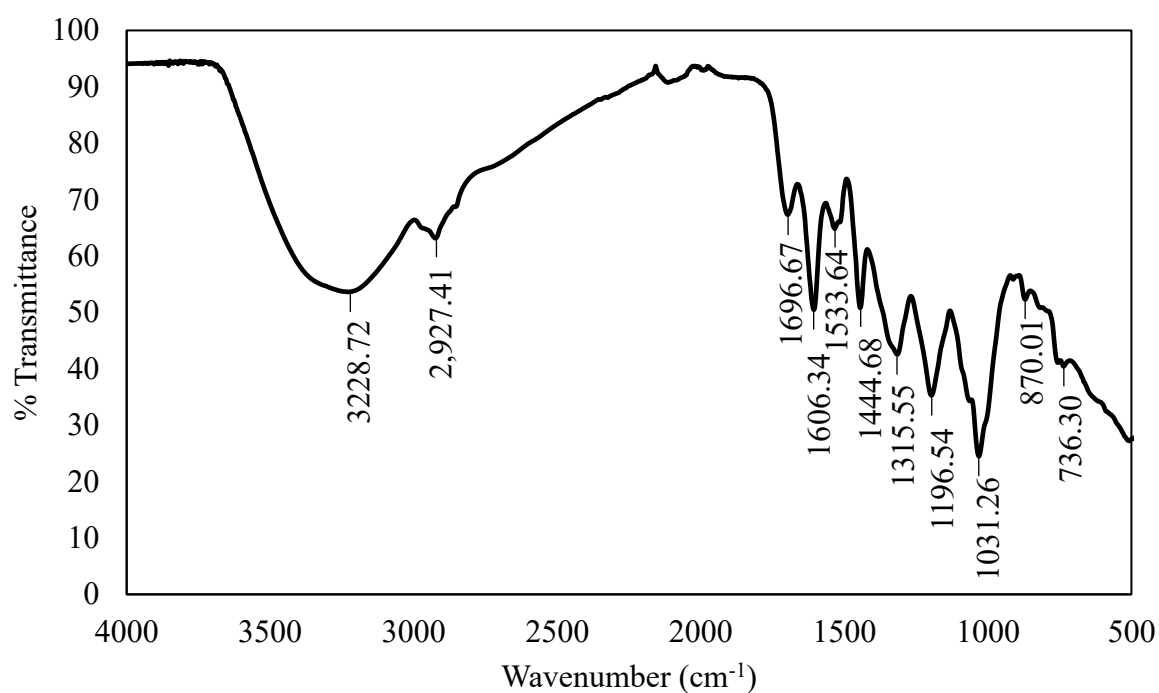


Fig. 2. The FT-IR spectrum of the crude extract from *A. pauciflorum* shell in the wavenumber range of 4000–500 cm⁻¹, showing the absorption positions of functional groups that indicate the presence of bioactive chemical components

Table 1 Fourier-Transform infrared peak assignments and functional groups identified in the crude of *A. pauciflorum* shell extract

Wavenumber (cm ⁻¹)	Bond	Function
3228.72	O-H stretching	Alcohol, Phenol
2924.43	C-H stretching	CH ₂ , CH ₃
1696.68	C=O stretching	Carbonyl, Carboxylic acid, or ketone/ Aldehyde
1606.34	C=C stretching (Aromatic)	Aromatic compounds
1533.64	N-H bending (Amide II) or C=C stretching (Aromatic)	Amide or Aromatic
1444.66	C-H stretching (Scissoring)	CH ₂ , CH ₃
1315.55	C-O stretching or O-H bending	Alcohol, Phenol, Ester, or Carboxylic
1196.54	C-O stretching	Ester or Ether
1031.26	C-O stretching	Primary Alcohol
870.01	C-H out of plane bending	Substituted Aromatic
736.30	C-H out of plane bending or rocking	Aromatic or Long-chain Alkyl

FT-IR= Fourier Transform Infrared

Ultraviolet-Visible Spectrophotometric Analysis

UV-Visible spectroscopy revealed, the absorption spectrum of the extract from the *A. pauciflorum* shell can be shown in the wavelength range of 200–400 nm. As shown in Fig.3, the maximum absorption spectrum is found in the range of 210–240 nm, with a prominent peak in the wavelength range of 270–290 nm. With the maximum absorbance ($\lambda_{\max}$) at approximately 280 nm, which is consistent with reported profiles of phenolic-based insecticides, plant extracts with insecticidal properties, particularly substances in the rotenoid group (Rotenoids) such as Rotenone, which exhibit $\lambda_{\max}$ values in the range of approximately 286–293 nm. From the characteristic of the spectrum, it is expected that *A. pauciflorum* shell extract suggests the presence of compounds with spectral characteristics resembling the

rotenoid group. These compounds have a chemical structure consisting of rings connected between a benzene ring and a tetrahydropyran ring, also known as chromane. These spectral features are indicative of a structure that may act as the core or phenolic compound that acts on the nervous system of insects similarly to the action of rotenone.

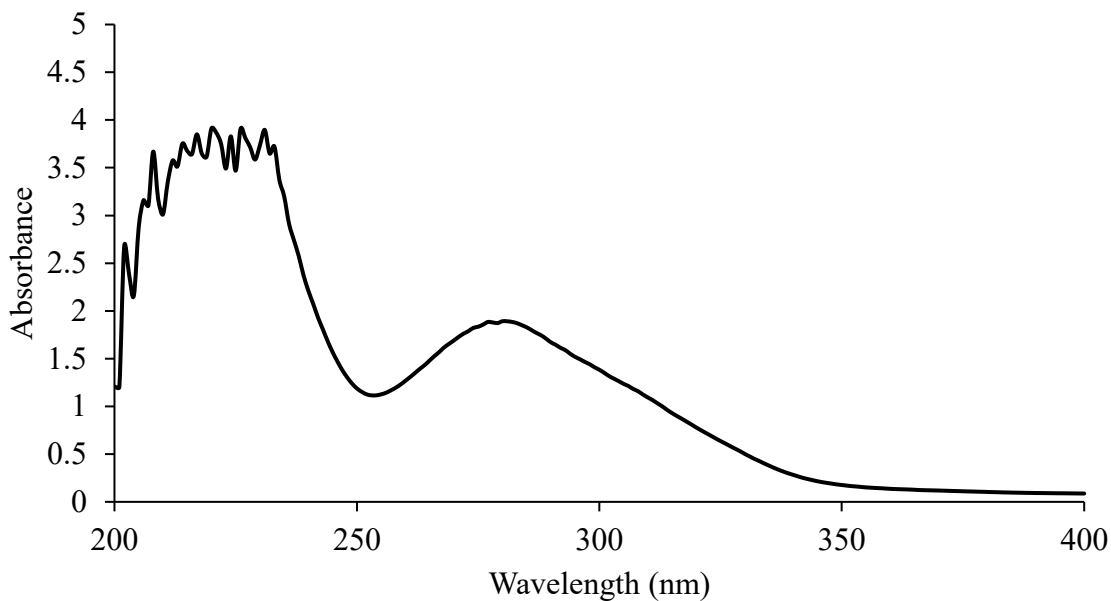


Fig. 3 UV-Visible absorption spectrum of *A. pauciflorum* shell extract, showing absorption peaks that indicate the presence of bioactive compounds within the range 200–400 nm

Discussion

A study of the efficacy of *A. pauciflorum* extracts against adult *Aedes aegypti* mosquitoes demonstrated the ATSB method achieved significantly higher elimination efficiency than the CDC bottle bioassay. The ATSB showed a high and consistent linear dose-dependent mortality in mosquitoes. The extract achieved 100% mortality at concentrations of 10% and 15%. The calculated LC_{50} and LC_{95} values were 2.54% (v/v) and 6.15% (v/v), respectively. When compared to Kanaphot research (Kanaphot, 2017) on the efficacy of Niang shell extract against adult *Ae. aegypti* using ATSB method, both studies achieved a maximum mortality rate of 100% with 72 h. Notably, the aforementioned research require a concentration level up to 20%, whereas the crude extract from this study was able to achieve 100% mortality rate at a lower

concentration level. From the LC_{50} values indicate that the *A. pauciflorum* extract possesses high acute toxicity and efficacy, with a low concentration ($LC_{50}=2.54\%$) being sufficient to reduce the adult mosquito population by 50%, demonstrating high acute toxicity and efficacy. Conversely, the CDC bottle bioassay method resulted in an inconsistent dose-response relationship that did not correlate with the extract concentration, making it impossible to calculate the LC_{50} and LC_{95} values. This clearly highlights the variations in efficacy driven by the specific delivery methods of the active substance.

The inconsistent dose-response relationship and variations in efficacy can be attributed to the route of exposure and the natural physiology of mosquitoes. Specifically, the ATSB method relies on an ingestion-based delivery system, with sugar serving as a natural attractant. Normally both male and female mosquitoes obligately consume environmental sugar sources, such as floral nectar, as an essential energy reserve for survival. This inherent fluid-feeding behavior, sugar acts as a stimulus that automatically attracts mosquitoes to feed on nectar thereby delivering the active compounds from the *A. pauciflorum* shell extract directly into the ingestion-based delivery system, resulting in rapid mortality (Allan, 2011). In contrast, the CDC bottle bioassay method relies on the absorption of substances through the external skin contact of mosquitoes. This method is typically designed for testing highly pure synthetic chemicals that can be evenly and smoothly coated on glass bottles (Centers for Disease Control and Prevention [CDC], 2024). However, *A. pauciflorum* shell crude extract exhibits high viscosity, leading to inconsistent and uneven coating efficacy. This unevenness likely alters mosquito behavior within the bottle, causing the duration and extent of exposure to deviate from controlled conditions, ultimately resulting in slower knockdown effects compared to direct ingestion. To overcome these limitations, future methodological optimization is required. Conducting a pilot study to evaluate different solvent systems or optimal dilution ratios could effectively reduce viscosity, thereby improving the uniformity of the glass coating and ensure a precise evaluation of contact efficacy. Furthermore, integrating a standard synthetic insecticide, such as deltamethrin, as a positive control is highly recommended to establish baseline diagnostic concentrations and exposure times in accordance with international guidelines (CDC, 2024).

Based on the effectiveness in controlling and eliminating *Aedes* mosquitoes, the *A. pauciflorum* shell extract was analyzed for composition and structural confirmation. The extract was analyzed using FT-IR and UV-Vis techniques for composition analysis and structural confirmation. The obtained FT-IR spectrum was subsequently compared with the spectrum of rotenone from the National Center for Biotechnology Information (NCBI, 2025).

The results revealed distinct signals consistent with rotenone, confirming the presence of core flavonoid frameworks within the extract. Specifically, the FT-IR spectrum exhibited a characteristic carbonyl peak (C=O stretching) at 1696.68 cm^{-1} and an aromatic ring vibration at 1606.34 cm^{-1} (which precisely aligns with the standard $1600\text{--}1610\text{ cm}^{-1}$ aromatic framework), and additional band at 1533.64 cm^{-1} corresponding to N-H bending or aromatic compounds. This corresponds to the UV-Vis results, showing a prominent absorption band in the wavelength range of $270\text{--}290\text{ nm}$, which is close to the $280\text{--}300\text{ nm}$ range reported in previous studies for the specific detection and classification of rotenoid compounds (Zeng et al., 2002; Wongthep, 2007). However, in the study of analysis using FT-IR and UV-Vis techniques, the comparison of the extracts spectral data with historical reference databases was made without comparing it with the standard rotenone under the same experimental conditions. This is because the purity of the crude extract may contain other components that interfere with the signal and obscure the specific peak of the standard substance. Therefore, to confirm the type and quantity of the extract more accurately and precisely, future studies should employ advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) to clearly identify the type and quantity of the substance. This chemical data confirms that *A. pauciflorum* shell contains a group of rotenoids, a sub-class of isoflavonoids, as the main active ingredient. The mechanism of action at the cellular level is highly specific in nature, as it inhibits the function of Mitochondrial Complex I (NADH:ubiquinone oxidoreductase). This inhibition disrupts the electron transport chain, leading to triggering cellular disruption and oxidative stress driven by the excessive accumulation of reactive oxygen species (ROS) (Sun et al., 2021). This abnormality significantly disrupts the balance of the cell autophagy process (Mader et al., 2012), leading to tissue failure and ultimately triggering cellular apoptosis (Zhang et al., 2024). The mechanism that disrupts cellular energy metabolism is a different mode of action compared to synthetic chemicals in the Pyrethroids group, such as Deltamethrin, which acts directly on the central nervous system of insects by binding to voltage-gated sodium channels on the surface of nerve cells (Soderlund, 2012). The difference in the target sites for destruction is a key factor that helps reduce the likelihood of resistance in *Aedes* mosquitoes. Therefore, the mechanism of internal physiological destruction through the ATSB extraction method demonstrates clear and acute efficacy in mosquito elimination.

This research not only fosters public health innovation but also aligns with the Zero Waste concept toward sustainable development. By transforming agricultural waste materials such as the *A. pauciflorum* shell, which are often left to decompose naturally and can produce

greenhouse gases affecting ecosystem sustainability, into a value-added biopesticide. This approach represents a strategic resource valorization aligned with circular economy principles, significantly mitigating the carbon footprint within the agriculture sector toward the Sustainable Development Goals (SDGs). In line with SDG 3 (Ensuring healthy lives and promoting well-being) and SDG 12 (Sustainable consumption and Production), upgrading agriculture waste to environmentally friendly biocontrol agents is a key factor in driving the circular economy from community foundations to tangible global sustainable. Future studies should develop of extracts in the form of Emulsifiable Concentrate (EC), which enhances absorption efficiency suitable for controlling disease-carrying insects or using surfactants as spreading agents to improve contact efficiency. This approach paves the way for practical bio-based innovations aligned with sustainable development goals.

Conclusion

In conclusion, this study shows that *A. pauciflorum* shell extract has high potential in eliminating and controlling adult *Aedes* mosquitoes. In comparing the mechanism of action between the ATSB method, where mosquitoes receive the extract through an ingestion-based delivery system, and the CDC bottle bioassay, where mosquitoes receive the extract through external contact, it can be demonstrated that the ATSB method is the most effective and has higher efficacy than the CDC bottle bioassay method. By the ATSB method, the extract acts directly on mosquitoes, capable of disrupting internal physiological processes of the mosquito. In this research, the concept of Zero Waste can be applied by utilizing local agriculture waste to its fullest potential and increasing the value of *A. pauciflorum* shell as a highly effective extract. This serves as a new, environmentally friendly option that is safe for users and effectively controls adult *Ae. aegypti* mosquito populations.

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