Larvicidal Efficacy Against Dengue Vector Aedes aegypti and Antibacterial Activity of Leaf Ethyl Acetate Extract of Clerodendrum indicum (L.) Kuntze

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Abstract

Clerodendrum indicum, a traditionally used medicinal plant in South and Southeast Asia, was evaluated for the bioactivity of its ethyl acetate leaf extract (EAECI). The extract showed strong larvicidal and ovicidal effects against *Aedes aegypti*, producing dose-dependent larval mortality with LC_{50} values of 187.427 µg/mL at 12 hours and 106.019 µg/mL at 24 hours. Complete ovicidal activity occurred at 350 µg/mL. EAECI exposure induced oxidative stress in larvae, evidenced by elevated levels of CAT, SOD, and MDA. The extract also exhibited notable antibacterial activity against *Bacillus megaterium* and *B. cereus*, with MICs between 100 and 500 µg/mL, and SEM imaging confirmed membrane deformation in treated bacteria. Phytochemical and FT-IR analyses verified the presence of hydroxyl, alkene, ester, and carboxylic acid groups. GC-MS profiling identified major components, particularly tetracosanoic acid methyl ester (36.17%) and 3,3-dimethylcyclohexene. Overall, the findings demonstrate that EAECI contains potent bioactive compounds with significant insecticidal and antimicrobial potential, supporting its use as an eco-friendly, plant-based agent for integrated vector and microbial disease control.

1. Introduction

Clerodendrum indicum (L.) Kuntze, belonging to the family Verbenaceae, is an annual shrub that can reach heights up to 2 to 3 meters. The lanceolate leaves can grow to be 23 cm long. The plant has big flower clusters with tubular white or yellow blooms. Its fruits are spherical and pulpy, and they change colour from green to blue-black or reddish-black when they are ripe (Manandhar and Manandhar, 2002). This plant is native to Sri Lanka and the Andaman Islands, and is widely distributed across tropical Asia. The plant holds a prominent place in traditional medicinal systems, particularly in Northeast India, where it is frequently used for its therapeutic properties (Somwong and Suttisri, 2018; Wang et al., 2018). Traditionally, *C. indicum* has been used for treating a broad spectrum of ailments, including respiratory disorders (asthma, bronchitis, colds), gastrointestinal issues (gastric tumors, intestinal worms), neurological conditions (epilepsy, hysteria, febrile convulsions), urogenital disorders (hematuria, painful urination, impotence), and musculoskeletal problems (arthritis, rheumatism) (Khare, 2007; DeFilipps and Krupnick, 2018). Phytochemical investigations have revealed that the plant contains a variety of bioactive compounds, including flavonoids, phenolics, terpenoids, saponins, and alkaloids, which contribute to its pharmacological effects such as antioxidant (Kar et al., 2014; Arokiyaraj et al., 2012), anti-inflammatory (Wang et al., 2018; Wahba et al., 2011; Saha et al., 2025), and antimicrobial activities (Pal et al., 2012). Despite its well-documented traditional uses and preliminary pharmacological evidence, the vector control potential of *C. indicum* remains underexplored. Only a few studies have been reported the bioactivities of its extracts such as the analgesic effect of crude ethanolic leaf extracts (Raihan et al., 2012; Das et al., 2025a; Das et al., 2025b; Mandal et al., 2023) and the *in vitro* antibacterial activity of root and stem extracts (Rahman et al., 2000). Mosquitoes (Diptera: Culicidae) are vectors of some of the deadliest human diseases, including malaria, dengue hemorrhagic fever, filariasis, Japanese encephalitis and yellow fever, making them one of the leading causes of global mortality (Mittal, 2003). According to the World Health Organization (2012), mosquito-borne diseases are responsible for

approximately one million deaths annually. In this context, vector control emerges as the most effective strategy for reducing mortality. The primary methods of mosquito control include the use of larvicidal chemicals and the application of repellents or insecticide-treated bed nets to deter adult mosquitoes. Arbovirus transmission to humans primarily occurs through the bites of infected female *Aedes* mosquitoes, which are known for their daytime feeding behaviour. The efficiency of *Ae. aegypti* as a dengue virus vector is partly due to the extended time infected females take to obtain a blood meal compared to uninfected ones (Hardy, 2020; Almadiy, 2020). Given the absence of vaccines or targeted therapies for many mosquito-borne diseases, pesticide use remains a cornerstone of control efforts. However, the indiscriminate use of chemical pesticides raises serious concerns about environmental harm, risks to non-target organisms, and human health (Yeguerman et al., 2022). Additionally, the overuse of conventional insecticides has led to the development of resistance in mosquito populations and negative environmental impacts. In recent years, researchers have increasingly focused on developing eco-friendly and cost-effective alternatives, with particular emphasis on plant-derived mosquitocidal compounds (Chatterjee et al., 2023a; Adhikary et al., 2024; Bag et al., 2025a). An ideal pesticide should be efficient, precise, durable, environmentally sustainable, minimally harmful to mammals, and economically viable (Bag and Chatterjee, 2025). Among these alternatives, *Bacillus thuringiensis*, a soil bacterium, has gained prominence for its entomopathogenic activity against mosquito larvae (Roy et al., 2021; Bag et al., 2022a; Bag et al., 2022b; Bag et al., 2025b; Saha et al., 2024). Furthermore, botanicals have attracted significant attention as natural insecticides against arthropod pests, including mosquitoes. Numerous studies have shown that plant extracts can act as larvicides, adulticides, pupicides, ovicides, repellents, and growth inhibitors (Fouda et al., 2022; Panneerselvam&Murugan, 2013; Chatterjee et al., 2023a; Chatterjee et al., 2023b).However, systematic evaluations focusing on its efficacy as a mosquito larvicide or ovicide, particularly against key disease vectors like *Ae. aegypti*, are notably lacking. Moreover, its antimicrobial spectrum has not been comprehensively studied using organic solvent extracts, which are known to improve the recovery of non-polar bioactive compounds. Given the global demand for eco-friendly, plant-based biocontrol agents, *C. indicum* represents a promising candidate for further investigation. Assessing its larvicidal, ovicidal, and antimicrobial potential could not only validate traditional knowledge but also contribute to the development of novel botanical formulations for integrated vector management and antimicrobial therapy.

2. Material methods

2.1. Plant material collection and preparation of extracts

Fresh green leaves of *C. indicum*, were collected from Joypur forest, West Bengal, India, and were taxonomically authenticated by the Botanical Survey of India (BSI), Kolkata. The voucher specimen (SR/REN/BWN/2018/01) is preserved at the Department of Zoology, The University of Burdwan. The freshly collected leaves were thoroughly washed with distilled water and air-dried for 10 days at room temperature in a well-ventilated space. Once dried, the leaves were pulverized using an electronic grinder

(Philips Mixture Grinder HL 1605, Kolkata, India), then stored in an airtight container for future use (Dutta and Ray, 2015; Dhibar et al., 2025). A powdered sample (20 g) of *C. indicum* leaves were properly packed within the soxhlet apparatus and add 500 mL ethyl acetate solvent (SA3F7005, MERCK) for the preparation of EAECI. The extract was coded as EAECI and kept in the refrigerator at 4°C for future use (Chipiti et al., 2015; Barman et al., 2020).

2.2. Mosquito rearing

Mosquito larvae of *Ae. aegypti* were collected from the outskirts of Burdwan, West Bengal, India. The larvae were cultured in plastic bowls under laboratory conditions, maintained at 29 ± 3°C and 75–85% relative humidity (RH). The rearing process was designed to mimic natural conditions and support optimal larval development. Adult mosquitoes were reared in wooden cages measuring 30×30×30 inches. They were provided with cotton pads soaked in a 10% glucose solution, while adult females were periodically blood-fed to sustain their reproductive cycle. Petri dishes lined with damp cotton were placed at the bottom of each cage to facilitate egg-laying. The development of mosquito larvae was managed according to the standard protocol established by the World Health Organization (WHO, 2014). All experimental trials were conducted using third instar larvae, as recommended by WHO guidelines.

2.3. Larvicidal bioassay

Laboratory bioassay experiments were conducted on third instar larvae of laboratory-reared mosquitoes under aseptic conditions, following WHO guidelines (WHO, 2005) with minor modifications. Twenty-five early instar larvae were placed in 50 mL disposable plastic cups and treated with plant extracts prepared in a suitable solvent and diluted with 0.01% DMSO as an emulsifier. EAECI extracts were tested at various concentrations (100, 200, 300, 400, 500and 600 µg/mL). For comparison, an equal number of larvae were maintained as controls, treated with 0.01% DMSO. The larvae were not fed during the experiment, and all other growth conditions were kept constant. Larval mortality was observed and recorded at 12 and 24 hours after treatment. Nonresponsive larvae were considered dead. Mortality data were converted into percentage mortality using the following formula:

$$\text{Larval mortality } (\%) = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100$$

The mortality was calculated, determined the lethal concentrations (LC_{50} and LC_{90}) and their 95% upper and lower confidence limit.

2.4. Ovicidal activity

Ovicidal activity was assessed following Su and Mulla's (1998) method, with minor modifications. Mosquito eggs or egg rafts were collected from a breeding cage (30 × 30 × 30 inches) containing an established mosquito colony. The eggs were then exposed to ten concentrations of EAECI (100, 150, 200, 250, 300, 350, 400, 450, 500 and 1000 µg/mL) in 100 mL plastic containers for a duration of 120 hours. After treatment, individual eggs or egg rafts from each concentration were transferred to glasses of distilled water for hatching evaluation. The eggs were counted under a microscope before

transferring. Each experiment was conducted six times on different days, with six replicates, using the appropriate solvent as a control. Following the 120 hours treatment period, hatch rates were calculated using the following formula:

$$\text{Egg mortality} \quad (\%) = \frac{\text{No of hatched egg}}{\text{Total No of eggs}} \times 100$$

2.5. Oxidative stress biomarkers

Mosquito larvae from each treatment group were homogenized individually in 200 µL of phosphate buffer (0.1M, pH 7.6) at 4°C. The homogenates were centrifuged using a HERMLE Labortechnik centrifuge at 10,000 rpm for 10 minutes at 4°C. The resulting supernatant was collected and stored at −20°C for use as an enzyme extract. The supernatant was immediately utilized as the enzyme source for biochemical assays. Each biochemical experiment was conducted in triplicate, with 25 larvae used for each replicate. Protein content was quantified using the Bradford method (1976), with bovine serum albumin (BSA) as the standard reference. Catalase (CAT) activity was determined by measuring the absorbance of residual H_2O_2 (Beers & Sizer, 1952), while superoxide dismutase (SOD) activity was assessed based on the inhibition of nitrobluetetrazolium (NBT) photoreduction (Beauchamp and Fridovich, 1971). Malondialdehyde (MDA) levels, indicative of lipid peroxidation, were measured by determining thiobarbituric acid-reactive substances (TBARS) (Ohkawa et al., 1979). SOD and CAT activities were expressed as U/mg protein, and MDA levels were reported as nMol TBARS/min/mg protein. All parameters were measured at room temperature (29°C) using a UV-visible spectrophotometer (Cecil Aquarius CE 7400). Detailed information regarding analytical instrument models, detection limits, and operational parameters for the detected biomarkers is provided in the supplementary materials.

2.6. Assays to determine the antibacterial activity

2.6.1. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MICs) of the EAECI were determined using the agar dilution method (Wiegand et al. 2008). Briefly, stock solutions of 1000 µg/mL were prepared for each extract, which were then serially diluted to produce concentrations of 0,100, 200, 300, 400, 500 µg/mL. These diluted extracts were poured into sterile petri dishes and allowed to solidify. A 2 µL drop of the prepared test and control organisms was then inoculated onto the surface of each plate using a template. After incubation at 35°C for 24 hours, the plates were examined, and the MIC was defined as the lowest concentration of the extract that prevented visible growth of the organism. For positive control tetracycline (30 µg) was used.

2.6.2. Scanning Electron Microscopy (SEM) study

According to Hayat (1981), The most susceptible bacterial strain to the EAECI was selected for scanning electron microscopy (SEM) analysis. Bacterial cells were collected from the inhibition zone after 24

hours of treatment and fixed in 2.5% glutaraldehyde solution for 45 min at room temperature. The fixed cells were dehydrated through a graded ethanol series (50% for 7–5 min, 70% for 10 min, 90% for 5 min, and absolute ethanol for 5 min), followed by transfer to a 1:1 isoamyl alcohol:absolute ethanol mixture for 5 min and subsequently to pure isoamyl alcohol for 5 min. The samples were then air-dried, mounted onto aluminum stubs, sputter-coated with gold, and examined under a Hitachi S-530 SEM to observe morphological alterations induced by the EAECI.

2.7. Functional group analysis by FTIR (Fourier transform infrared)

We used FT-IR spectroscopy to find the functional groups, various bonds, and chemical makeup of the substance in the EAECI. FT-IR spectroscopy was performed using the positive response band compound in an FT-IR spectrophotometer (JASCO FT-IR Model-4700) at room temperature (25 ± 5°C), with a scanning range of 400 to 4500 cm^{-1}, to identify the functional groups of the EAECI. The peaks have been analysed using KnowItAll software (serial no. 107733-00001F44). The functional groups were identified by comparing the characteristic peak frequencies with those reported in the reference literature (Stuart, 2021; Socrates, 2004).

2.9. GC-MS analysis

The EAECI was analyzed using GC-MS (Model: Clarus 680 GC) and the software used in the system is TurboMass Version 6.4.2 to identify and characterize bioactive components. The capillary column used is 'Elite- 5MS' having dimensions length 60 m, ID 0.25 mm and film thickness 0.25 µm and the stationary phase is 5% diphenyl 95% dimethyl polysiloxane. At a flow rate of 1 mL/min, helium gas (99.99%) was utilised as the mobile phase. The run time was around 40 min. An 8 min solvent delay was maintained. Data analysis library (NIST-2014) was used to identify the peak.

2.10. Statistical analysis

Analysis of the obtained data was performed by using Graph pad-prism 9 software. All data presented are mean ± SEM values of triplicates (n = 3) and obtained from separate experiments. Probit analysis was used to calculate the 12, 24 and 48 hours LC_{50} and LC_{90} values (Finney, 1971). Student's t-test were used to compare means of control and the oxidative stress biomarkers, namely CAT (Catalase), SOD (Superoxide dismutase), and MDA (Malondialdehyde).

3. Results

3.1. Larvicidal activity

The EAECI demonstrated dose-dependent larvicidal activity against *Ae. aegypti* larvae after 24 hours of treatment. The data were analyzed, and statistical values including LC_{50}, LC_{90}, lower confidence limit (LCL), upper confidence limit (UCL), were recorded. The larvicidal efficacy varied across different extracts of *C. indicum*, with distinct LC_{50} and LC_{90} values. For the 12-hour treatment, the EAECI showed

an LC_{50} of 187.427 (125.078- 280.856) µg/mL and an LC_{90} of 857.438 (572.204 -1284.857) µg/mL. In the 24-hour treatment, the LC_{50} was 106.019 (66.604-168.759) µg/mL and the LC_{90} was 539.890 (339.173-859.388) µg/mL. These results indicate that *Ae. aegypti* larvae were susceptible to the EAECI treatment (Fig. 1).

3.2. Ovicidal activity

The egg hatchability of dengue vector mosquitoes was tested across different concentrations of the extract. The hatchability percentage was found to be directly proportional to the number of eggs and inversely proportional to the concentration of the toxicant. The ovicidal activity of EAECI was observed against *Ae. aegypti*, where 100% egg mortality was recorded at a concentration of 350 µg/mL. In the control experiment, the eggs exhibited 100% hatchability.

3.3. Oxidative stress enzymes

Exposure of *Ae. aegypti* mosquito larvae to lethal concentrations of EAECI resulted in significant changes in total protein concentration and oxidative stress enzyme levels when compared to the control group. The total protein concentration in the control group was measured at 137.27 µg/mL, while in the EAECI-treated group, it increased to 220.02 µg/mL. Additionally, the levels of CAT, SOD, and MDA were elevated upon exposure to lethal concentrations of EAECI, with measurements of 57.04 µg/mL protein, 91.51 µg/mL protein, and 1.65 nMol TBARS/min/mg protein, respectively. In comparison, the control group showed values of 16.42 µg/mL protein, 14.36 µg/mL protein, and 0.92 nMol TBARS/min/mg protein. Statistical analysis revealed significant changes in oxidative stress parameters, with t-test results showing CAT ($P < 0.0001$), SOD ($P < 0.0001$), and MDA ($P = 0.6311$) levels significantly different from the control group.

3.4. Antibacterial activity

The antimicrobial activity of EAECI was assessed against *B. megaterium* (MTCC 2412) and *B. cereus* (MTCC 1272). EAECI demonstrated moderate inhibitory effects, with both bacterial strains showing a minimum inhibitory concentration (MIC) of 200 µg/mL (Fig. 2). However, their susceptibility varied slightly: *B. megaterium* exhibited a zone of inhibition (ZOI) of 10 mm, whereas *B. cereus* was more sensitive, showing the largest ZOI among tested strains at 12 mm. Scanning electron microscopy (SEM) further confirmed the bactericidal action of EAECI displayed significant structural damage: *B. megaterium* exposed to EAECI (Fig. 3a) exhibited clear membrane disintegration and cell surface roughening, while *B. cereus* cells (Fig. 3b) showed pronounced shrinkage, irregular surfaces, and partial lysis. These ultrastructural alterations indicate that EAECI compromises bacterial cell envelope integrity, ultimately leading to cell death. The combined MIC, ZOI, and SEM findings validate the bactericidal mechanism of EAECI against these pathogenic *Bacillus* species.

3.5. FTIR analysis

FTIR (Fourier-transform infrared) spectroscopy was employed to analyze the EAECI, aiming to identify the functional groups potentially responsible for their bioactivity. In the EAECI spectrum, the

characteristic absorption peaks were observed at 3432.67 cm^{-1}, corresponding to O–H stretching. Peaks at 2975.62 cm^{-1} indicated C–H stretching, the peak at 1642.09 cm^{-1} indicated C = C stretching, while the peak at 1046.19 cm^{-1} suggested the presence of an ester group (Fig. 4). These FTIR spectra confirm the presence of various functional groups, including hydroxyl, carboxylic acid, alkane, alkene, ester, aromatic, and ether groups, as outlined by (Socrates, 2004; Stuart, 2005).

3.6. GC-MS analysis

The chemical composition of the EAECI was analyzed using GC-MS, revealing the presence of main two compounds 3,3 dimethylcyclohexene (Fig. 5) and tetracosanoic acid, methyl ester (Fig. 6) (Table 1).

Table 1
GC-MS Spectral Analysis of EAECI

No.	RT (min)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	26.586	3,3-dimethylcyclohexene	C_8H_{14}	110.1094	1.74
2	36.98	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.6633	36.173

4. Discussion

Recently, plant-derived bio-pesticides have gained significant attention as safer alternatives to traditional synthetic insecticides, particularly in the control of disease-carrying vectors. These plant-based chemicals are favoured for their safety profile, as they are non-toxic to humans and animals, lack phototoxic effects, and do not leave harmful residues in the environment (Schmutterer, 1990; Chatterjee et al., 2023b). As the development of resistance to conventional synthetic insecticides becomes an increasing challenge in vector mosquito management, there is a pressing need for innovative solutions or novel insecticides (Chandre et al., 1998). The emergence of insecticide-resistant mosquito populations calls for the exploration of alternative control methods, such as the use of plant-derived bio-pesticides, which could play a key role in integrated vector management strategies. Bowers et al. (1995), emphasized that screening locally available medicinal plants for their mosquito control potential could stimulate local economies, reduce dependence on costly imported chemicals, and support community-driven public health initiatives. Plants contain a wide range of bioactive compounds with diverse biological activities, which are believed to be responsible for their pesticidal properties. These compounds, such as toxins and secondary metabolites, act as effective mosquito control agents, contributing to the larvicidal and adulticidal effects observed in many plant extracts (Niraimathi et al., 2010). This is particularly important for managing disease vectors, where reducing the population of mosquitoes is crucial in preventing the transmission of diseases like dengue, malaria, and Zika virus. Our study supports these findings, as it demonstrated that an increase in the concentration of EAECI significantly contributed to the mortality of *Ae. aegypti* larvae. While the medicinal properties of *C. indicum* have been well-documented (Shrivastava and Patel, 2007), the mosquito larvicidal effects of its

leaves have not been extensively explored. Our results provide valuable new insights into the potential of *C. indicum* as an effective natural bio-pesticide. Similar studies, such as the work by Mathew et al. (2009), who investigated the methanol extract of *C. ternatea* seeds, found notable larvicidal activity against *Ae. aegypti* larvae with an LC_{50} of 154.5 ppm. Likewise, Ansari et al. (2005), demonstrated that *Pinus longifolia* oil exhibited larvicidal efficacy against *Ae. aegypti* mosquitoes, with an LC_{50} value of 82.1 ppm. These findings align with our results, highlighting the potential of plant extracts for mosquito control. In addition, numerous other recent studies have corroborated our findings regarding the larvicidal activity of various plant extracts against *Ae. aegypti* larvae (Onah et al., 2022; Ajaegbu et al., 2022; Bakar et al., 2018). Mechanistic insights into its larvicidal effect were provided by biochemical assays, which revealed significant elevations in catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels in treated larvae, suggesting that EAECI induces oxidative stress that disrupts normal metabolic functions and damages cellular components, a mode of action consistent with earlier observations that phytochemicals trigger redox imbalance leading to larval death (Govindarajan and Benelli, 2016). This dual activity direct larvicidal toxicity along with oxidative stress induction indicates that EAECI may exert synergistic pressure on mosquito larvae, thereby reducing the likelihood of resistance development that is common with conventional synthetic insecticides (Chandre et al., 1998).

In parallel to its mosquitocidal efficacy, EAECI also displayed moderate antibacterial activity against two Gram-positive pathogens, *B. megaterium* and *B. cereus*, with a minimum inhibitory concentration (MIC) of (200 µg/mL) for both strains. The bactericidal nature of EAECI was further confirmed through scanning electron microscopy, where untreated control cells maintained smooth, intact rod-shaped morphologies, while treated cells showed dramatic ultrastructural alterations, including membrane disintegration, surface shrinkage, and partial lysis, thereby suggesting that EAECI compromises bacterial envelope integrity and leads to cell death. These observations align with earlier studies demonstrating that plant-derived secondary metabolites such as alkaloids, tannins, and saponins disrupt bacterial cell walls and interfere with critical physiological processes (Hassan et al., 2004). The moderate antimicrobial activity observed in our study is particularly noteworthy as bacterial pathogens like *B. cereus* are associated with food borne diseases, and plant-derived agents with membrane-disruptive properties offer promising natural alternatives to chemical antibiotics, especially in the context of increasing antimicrobial resistance. The phytochemical basis of EAECI's bio efficacy was further elucidated by FT-IR and GC-MS analyses. FT-IR spectra revealed functional groups including hydroxyl, ester, alkene and carboxylic acids, which are well known for contributing to biological activity by enabling hydrogen bonding, disrupting microbial proteins and enhancing lipophilicity to facilitate cell penetration (Sofowara, 1993; Stuart, 2005). GC-MS analysis identified two major compounds, 3,3-dimethylcyclohexene and tetracosanoic acid, methyl ester, the latter constituting 36.17% of the total peak area. Fatty acid esters like tetracosanoic acid derivatives are widely recognized for their antimicrobial and surface-active properties, and their high abundance in EAECI likely contributes substantially to the bactericidal and larvicidal activities observed in the present study (Rahman et al., 2000; Raihan et al., 2012). Taken together, these results demonstrate that EAECI exerts its bioactivity

through a combination of biochemical, structural and molecular mechanisms, including oxidative stress induction, membrane disruption, and the action of fatty acid derivatives and other phytochemicals. Importantly, the multifunctionality of EAECI highlights its potential for integration into eco-friendly vector and microbial control strategies, offering a sustainable alternative to chemical pesticides and antibiotics, which often face challenges of resistance, environmental persistence and non-target toxicity (Schmutterer, 1990; Bowers et al., 1995). Moreover, the dual larvicidal and antibacterial activities of EAECI underscore its relevance in tropical and subtropical regions where mosquito-borne diseases and bacterial infections frequently coexist as public health burdens, thereby providing a holistic approach to disease management. By validating the ethnomedicinal applications of *C. indicum* with modern scientific evidence, the present study not only supports its traditional therapeutic use (Shrivastava and Patel, 2007) but also contributes to the growing framework of plant-based integrated pest and pathogen management. Ultimately, the findings suggest that *C. indicum* EAECI, with its demonstrated efficacy against both insect vectors and bacterial pathogens, may serve as a promising natural bioagent capable of addressing urgent challenges in public health, including vector-borne disease transmission and antimicrobial resistance, while minimizing ecological risks and fostering sustainable disease control practices.

5. Conclusion

The present investigation highlights the ethyl acetate leaf extract of *C. indicum* (EAECI) as a potent multifunctional biocontrol agent with promising applications in integrated vector and microbial disease management. The extract exhibited strong larvicidal activity against *Ae. aegypti*, with complete ovicidal efficacy at relatively low concentrations, and induced significant oxidative stress in larvae, suggesting a dual mechanism of direct toxicity and redox imbalance. Alongside its mosquitocidal potential, EAECI demonstrated moderate antibacterial activity against *B. megaterium* and *B. cereus*, with a minimum inhibitory concentration (MIC) of 200 µg/mL, while scanning electron microscopy confirmed its bactericidal nature through observable ultrastructural damage to bacterial cell membranes. Phytochemical characterization by FTIR revealed the presence of hydroxyl, ester, alkene, and carboxylic acid groups, while GC-MS identified 3,3-dimethylcyclohexene and tetracosanoic acid, methyl ester the latter representing a major component likely responsible for its bioactivity. These findings collectively validate the traditional medicinal uses of *C. indicum* and provide mechanistic evidence supporting its efficacy as an eco-friendly alternative to synthetic insecticides and antibiotics, which are increasingly challenged by resistance and environmental concerns. By combining larvicidal, ovicidal, oxidative stress-inducing, and antimicrobial properties within a single botanical extract, *C. indicum* demonstrates multifunctionality that is particularly valuable in tropical regions burdened with vector-borne diseases and bacterial infections. Future research should focus on formulation development, field trials, and safety evaluations to facilitate its translation into practical, sustainable biocontrol solutions.

Declarations

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Declaration of Competing Interest

The authors declare no conflict of interest.

Author Contribution

G.D.: Investigation, Methodology, Antimicrobial Analysis, Writing – Original Draft.G.D., S.B., and D.S.: Scanning Electron Microscopy Analysis.S.B. and D.S.: Larvicidal Analysis, Writing – Review & Editing.B.G., R.C., and R.D.: Investigation, Writing – Review & Editing.S.R. and S.C.: Conceptualization, Investigation, Formal Analysis, Supervision, Writing – Review & Editing.All authors reviewed and approved the final manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. All the FTIR and GC-MS data are represented with Figure 4,5,6 and 7 in supplementary file attached.

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