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Original Article

Larvicidal Activity of Ethanolic Leaf Extract of *Cymbopogon citratus* Against Third-Instar Anopheles spp. Larvae

Christiana Jesumirhewe[1], Covenant N. Anyanwu[2*]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria; [2]Department of Pharmaceutical Microbiology, Prof. Dora Akunyili College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria.
**Correspondence: christiana.jesumirhewe@uniben.edu Tel: +2348034648066*

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Abstract

Resistance development and environmental toxicity limit the use of synthetic insecticides. Under controlled laboratory conditions, this study assessed the larvicidal activity of the ethanolic leaf extract of lemongrass (*Cymbopogon citratus Stapf, Poaceae*) against third-instar Anopheles spp. mosquito larvae. Fresh lemongrass leaves were collected, authenticated, shade-dried, and macerated in ethanol for 72 h to yield a crude extract. Larvae were raised to the third instar and subjected to extract concentrations of 62.5, 125, 250, and 500 ppm in triplicate bioassays. Mortality rate was observed at 1, 2, 24, and 48 hours after exposure. The extract was subjected to Fourier Transform Infrared (FTIR) spectroscopy and proximate analysis. Results indicated that the highest concentration (500 ppm) produced 94.67±2.31% mortality after 48 h, while 62.5 ppm yielded only 13.33± 2.31% mortality. The lethal doses (LC_{50} and LC_{90}) that resulted in 50% and 90% mortality at 48 h were 204.3 ppm and 612.1 ppm, respectively. FTIR analysis identified absorption bands consistent with functional groups of phenolics, flavonoids, terpenoids, aldehydes, and essential oil constituents. Proximate analysis revealed a carbohydrate-dominant profile (27.01±1.58%), with considerable crude fat (24.41±1.25%), moisture (21.49±1.09%), ash (14.63±1.87%), and crude protein (12.47±0.36%) levels; crude fiber was undetected. These findings demonstrate that *C. citratus* possesses meaningful concentration- and time-dependent larvicidal activity against Anopheles larvae, supporting its development as a potential locally available, biodegradable, and environmentally friendly botanical larvicide for integrated mosquito vector management.

Keywords: Anopheles spp., botanical larvicide, *Cymbopogon citratus*, integrated vector management, larvicidal activity

Introduction

Mosquito-borne illness is one of the most significant and persistent threats to public health worldwide, especially in tropical and subtropical parts of sub-Saharan Africa. Malaria, dengue fever, yellow fever, chikungunya, Zika virus disease, and lymphatic filariasis are among the vector-borne illnesses that significantly increase morbidity and death and disproportionately affect vulnerable groups, including pregnant women and children under five [1]. Malaria alone—which is mostly transmitted by female Anopheles mosquitoes—remains a significant cause of preventable mortality in Africa, with a projected 249 million cases and over 600,000 deaths globally in 2022 [1].

One of the main strategies to stop disease spread is vector management, particularly reducing mosquito population densities. Targeting the larval stage is a particularly strategic intervention among the existing control methods since mosquito larvae are restricted to aquatic breeding habitats, cannot escape treatments, and can be continuously exposed to bioactive compounds due to their filter-feeding behavior [2,3]. Early larval removal prevents adult, disease-carrying mosquitoes from emerging, and the limited number of larval breeding sites allows targeted larvicide application with minimal environmental impact [4,5]. During their aquatic development, mosquito larvae progress through four instars. Because of their robust physiological state and adaptability to experimental handling, researchers most often target the third and fourth instars in larvicidal bioassays.

Synthetic pesticides from the organophosphate, pyrethroid, and carbamate chemical classes have been the mainstay of conventional mosquito control. However, pesticide resistance in

mosquito populations has emerged and spread, significantly reducing the effectiveness of these agents and posing a growing public health problem [6,7]. Beyond resistance, synthetic insecticides are linked to several well-established negative outcomes, including aquatic ecosystem contamination, toxicity to non-target organisms, bioaccumulation in the food chain, and health risks from prolonged human exposure [6]. These limitations have generated substantial interest in creating safer, more sustainable, and biodegradable options for controlling mosquito larvae [8,9].

Plant-based larvicides have emerged as a particularly promising category of alternative vector control agents. Alkaloids, flavonoids, terpenoids, saponins, and essential oils are among the secondary metabolites produced by many medicinal plants and, through various mechanisms of action, have toxic, repellent, growth-inhibitory, or anti-feedant effects on mosquito larvae [8, 10-12]. Compared with single-molecule synthetic pesticides, the multi-component nature of plant extracts reduces the likelihood of resistance development, and their biodegradability reduces long-term environmental accumulation [8,9].

C. citratus(DC.) Stapf (Family: *Poaceae*), also known as lemongrass, is a fragrant perennial grass widely cultivated in tropical and subtropical nations, including Nigeria. It is native to tropical Asia. The plant is valued for its abundant essential oil content, which consists mainly of citral, a mixture of the geometric isomers' geranial (citral a) and neral (citral b), along with limonene, linalool, and other oxygenated terpenoids. Together, these compounds give the plant its distinctive lemon-like scent and wide range of biological activities [13,14]. Previous studies have documented the larvicidal activity of *C. citratus* essential oils and extracts against several mosquito species, including *Aedes aegypti, Culex quinquefasciatus,* and Anopheles spp. [15-18]. However, standardized, locally conducted research on the larvicidal activity of *C. citratus* is important to validate eco-friendly vector control methods, account for geographical variations in active phytochemicals, and establish consistent, reproducible dosages against localized mosquito vectors. This is necessary as findings from one region cannot reliably translate into effective public health interventions in another.

Therefore, using established WHO bioassay protocols, the current study assessed the larvicidal activity of the ethanol crude leaf extract of *C. citratus* against third-instar Anopheles spp. mosquito larvae under controlled laboratory conditions. To provide phytochemical and nutritional evidence for the reported biological activity, proximate compositional analysis and complementary FTIR spectroscopic characterization of the extract were carried out. The results contribute to the emerging evidence base validating the use of locally produced botanical larvicides in sustainable integrated vector management programs in Nigeria and other tropical regions.

Materials and Methods

Study area and ethical considerations

All laboratory work was conducted in the Department of Pharmaceutical Microbiology, Prof. Dora Akunyili College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria. The study area is located in the tropical rainforest zone, characterized by high humidity and high annual rainfall. Mosquito larvae were collected from discarded automobile tyres on the university campus. Standard entomological protocols were followed in all procedures involving mosquito larvae collection [4]. This study did not include any vertebrate animals or human volunteers.

Experimental design

The study used a completely randomized design (CRD). The study used three replicates of extracts at varying concentrations (62.5, 125, 250, and 500 ppm) applied to twenty-five Anopheles spp. larvae in their third instar.

Plant collection, authentication, and extraction

Fresh lemongrass (*C. citratus*) leaves were collected from the Okada Campus of Igbinedion University's Crown Estate. Dr Adebayo Muritala, Department of Pharmacognosy, Prof. Dora Akunyili College of Pharmacy, Igbinedion University, Okada, identified and authenticated the plant using a voucher specimen deposited under accession number IUO/10/004. To prevent thermal deterioration of heat-sensitive phytochemicals, the leaves were air-dried for 16 days at room temperature after being cleaned with clean water to remove surface impurities. A clean mechanical grinder was used to grind the dried material into a fine, uniform powder. Extraction was carried out by maceration: 200 g of the powdered plant material was immersed in 900 mL of absolute ethanol in a sealed glass container and allowed to stand at room temperature for 72 h with intermittent shaking to enhance solvent penetration and dissolution of bioactive constituents. The mixture was filtered through Whatman No. 1 filter paper to remove solid residues, and the resulting filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The semi-solid crude ethanolic extract was stored at 4 °C in an airtight amber glass container until use [19]. The percentage yield was calculated as given by **Equation 1**:

$$\%\,Yield = \frac{Weight\ of\ extract}{Weight\ pf\ original\ sample} * 100 \qquad (1)$$

Mosquito larvae collection and rearing

At specific outdoor locations on the Igbinedion University campus—well-documented artificial breeding environments for container-breeding mosquitoes, including Anopheles spp.—mosquito larvae were collected from abandoned car tyres that contained stagnant water and accumulated organic waste [20]. After carefully scraping the inner submerged walls of the tyres, the water and organic debris containing eggs were transferred into sanitized plastic buckets and transported to the laboratory. A leaf infusion medium was prepared by combining stagnant water, dry sand, and small quantities of organic matter from the same tyres with distilled water, then allowed it to stand for one hour to promote microbial growth as a larval food source. The containers were covered with fine, untreated mesh netting and positioned above the water surface to allow gas exchange while excluding extraneous debris and insects. Larvae were reared under ambient laboratory conditions (27°C and 82% relative humidity) until they reached the third instar stage. An Anopheles larva was determined to have successfully transitioned into the 3rd instar state by observing key morphological changes, size shifts, and developmental markers [21]. The identification of the Anopheles species was achieved in comparison with previously identified species using specific morphological keys which include their fan-like hair tufts dorsally on several abdominal segments, absence of a breathing siphon, presence of a pair of flat, dark spiracular openings on a dorsal plate on the 8th abdominal segment, and their resting position on water surface [21, 22].

Preparation of test solutions

The crude ethanolic extract was dissolved in acetone to prepare a stock concentration of 50,000 ppm (1 g extract dissolved in 20

mL acetone). Serial dilutions in acetone yielded intermediate working stock concentrations of 25,000 ppm, 12,500 ppm, and 6,250 ppm. Appropriate volumes were added from each working stock to 100 mL of sterile distilled water to obtain final test concentrations of 62.5, 125, 250, and 500 ppm. Acetone served as the carrier solvent because it is highly volatile and can emulsify hydrophobic essential oil constituents in the aqueous test medium [23]. The stock solution was stored in amber vials to prevent photodegradation.

Larvicidal bioassay

Larvicidal bioassays were conducted following WHO-described standard guidelines [4]. For each concentration, 25 third instar larvae were introduced into clear plastic cups containing 100 mL of the test solution. Each concentration was tested in triplicate. Larvae were not fed during exposure. The negative control consisted of 1 mL acetone diluted in 99 mL sterile distilled water with no plant extract. The positive control was a 2.5 ppm aqueous solution of Sniper® insecticide (dichlorvos, 1000 EC; NOSPEST Nigeria). Larval mortality was recorded at 1, 2, 24 and 48 post-exposure. A larva was considered dead if it showed no visible movement and did not respond to gentle mechanical probing with a fine glass rod. Mean percentage mortality was calculated as given by **Equation 2**:

$$\%\ Mortality = \frac{Number\ of\ dead\ larvae}{Initial\ number\ of\ larvaee} * 100 \qquad (2)$$

Experiments were arranged in a completely randomized design. All bioassays were performed in triplicate under uniform laboratory conditions to ensure reproducibility and minimize bias.

Proximate analysis of *C. citratus* ethanol leaves extract

The proximate analysis of the ethanol leaves extract of *C. citratus* was carried out using standard methods previously described [24-28].

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed using an Agilent Cary 630 FTIR spectrometer fitted with a diamond ATR accessory at a resolution of 8 cm^{-1} to identify potential functional groups in the plant sample. After cleaning the ATR crystal with ethanol, a background spectrum was recorded before placing approximately 10–15 mg of dried extract on the crystal and compressing it for adequate contact. A spectrum over the mid-infrared region (4000–650 cm^{-1}) using Agilent MicroLab software, and assigned absorption bands to functional groups based on standard infrared spectral correlation data and published literature was recorded.

Statistical analysis

Larval mortality was determined at 1, 2, 24 and 48 hours after larvae were exposed to 4 concentrations (62.5, 125, 250 and 500 ppm) of ethanolic *C. citratus* leaf extract. Each concentration was run in triplicate with 25 third instar Anopheles spp. Larvae in each replicate. In each assay, negative controls and positive controls (2.5 ppm DDVP) were included. Percent mortality for each replicate of the negative and each concentration of treatment was calculated at each sampling interval and means standard deviation (SD) of 3 replicates was determined at each sampling interval. Since there was no negative control larval mortality, correction of treatment mortality for negative control larval mortality using Abbott's formula was not necessary.

To compare concentration-response curves, 48-h mortality data for each replicate were converted into a probit scale. The observed mortality proportions were first corrected using the Finney correction, $p = (r + 0.25)/(n + 0.5)$, where r was the number of dead larvae and n was the number of exposed larvae (25), in order to avoid undefined probits with observations of 0 or 1. The corrected proportions were transformed to the z + 5 scale of the probit, and the mean standard error (SE) were estimated for each concentration.

For each time, median and 90% lethal concentrations (LC_{50} and LC_{90}) were obtained by probit regression of mortality at the replicate level (dead/25) against log_{10} concentration (4 concentrations × 3 replicates = 12 observations) and replicated at each time point (24 and 48 h) [29]. Models were fitted by maximum likelihood estimation and slopes with standard errors (SE) are shown. 95% confidence intervals (CI) for LC_{50} and LC_{90} are presented according to Fieller's theorem [30].

The goodness of fit was tested with Pearson's $\chi 2$ test ($\chi 2$:10 DF (12 data points minus two parameters)and when the p-value of the test was less than 0.15, there was heterogeneity among the replicates, and a heterogeneity factor, $\chi 2/df$, scaled the variances. Additionally, to compute confidence intervals, a t-distribution (df = 10, as the samples were independent between the two levels of the concentrations of interest) rather than normal distribution was used so that the interval was wider and more conservative. Lethal concentrations with estimate above the largest tested concentration (500 ppm) were considered extrapolation, but used for reference. All analyses were performed with IBM SPSS Statistics version 26.

Results

Extraction yield

A 6.0% (w/w) extraction yield of 12 g of crude semi-solid extract was obtained by macerating 200 g of dried *C. citratus* leaf powder in 900 mL of ethanol for 72 hours.

Table 1. Proximate composition of the ethanolic leaf extract of *C. citratus*

Parameter	Percentage composition (%)
Moisture content (%)	21.49±1.09
Ash content (%)	14.63± 1.87
Crude fat (%)	24.41±1.25
Crude fibre (%)	0.00
Crude protein (%)	12.47±0.36
Carbohydrate (%)	27.01±1.58

Values are presented as mean ± standard deviation (n = 3)

Proximate composition of the extract

Table 1 shows the proximate composition of the ethanol leaf extract of *C. citratus*. The extract was primarily composed of carbohydrates (27.01±1.58%), crude fat (24.41±1.25%), moisture

content (21.49±1.09%), ash content (14.63±1.87%), and crude protein (12.47±0.36%). None of the replicates had crude fiber (0.00%).

Larvicidal activity

The larvicidal activity of the ethanolic leaf extract of *C. citratus* against third-instar Anopheles spp. larvae at concentrations of 62.5, 125, 250, and 500 ppm, recorded over 1–48 h of exposure, is presented in **Table 2**. Larval mortality clearly showed a dose- and time-dependent relationship, increasing gradually with both concentration and exposure duration.

At the lowest concentration tested (62.5 ppm), mortality after 48 h was 13.33±2.31% indicating insufficient bioactive concentration for substantial larvicidal effect within the observation period. At 125 ppm, mortality increased to 26.67 ± 4.62% at 48 h, suggesting moderate but appreciable larvicidal activity. Mortality increased more markedly at 250 ppm, reaching 44.00 ± 4.00% at 48 h and demonstrating greater toxicity than at lower concentrations. The highest concentration (500 ppm) exhibited the greatest larvicidal activity with 94.67±2.31 % mortality at 48 h. The notably accelerated mortality between 2 h and 24 h at 500 ppm suggests a concentration threshold effect above which the bioactive phytochemical load becomes acutely toxic to larval physiological systems.

The negative control (acetone in sterile distilled water) produced 0% larval mortality at all-time points, confirming that neither the carrier solvent nor the experimental conditions independently contributed to larval death. The positive control (Sniper® insecticide, 2.5 ppm) produced 100% mortality at 1 h and remained 100% throughout the 48-h observation period, validating larval susceptibility and confirming the bioassay's reliability.

Table 2. Mean % mortality response of third instar Anopheles spp. larvae exposed to ethanolic *C. citratus* leaf extract at different concentrations and time points

Conc (ppm)	1 h (%)	2 h (%)	24 h (%)	48 h (%)	Probit (48 h)
62.5	5.33 ± 2.31	12.00 ± 0.00	13.33 ± 2.31	13.33 ± 2.31	3.92 ± 0.10
125	12.00 ± 8.00	21.33 ± 2.31	26.67 ± 4.62	26.67 ± 4.62	4.39 ± 0.13
250	24.00 ± 17.44	37.33 ± 2.31	42.67 ± 2.31	44.00 ± 4.00	4.85 ± 0.10
500	29.33 ± 18.48	49.33 ± 12.86	93.33 ± 2.31	94.67 ± 2.31	6.55 ± 0.17
Negative control	0	0	0	0	–
Positive control (2.5 ppm DDVP)	100	100	100	100	–

Values are mean ± SD of three replicates (25 larvae per replicate) for each concentration and time point. Probit values were calculated for each replicate from 48-hour mortality using the Finney correction, $p = (r + 0.25)/(n + 0.5)$, where r is the number of dead larvae and n = 25, and expressed on the $z + 5$ probit scale (mean ± SD).

Table 3. Probit-regression estimates of LC_{50} and LC_{90} of ethanolic *C. citratus* leaf extract against third instar Anopheles spp. larvae at 24 and 48 h.

Time	LC_{50} ppm (95% CI)	LC_{90} ppm (95% CI)	Slope ± SE	χ^2 (df = 10)	p	Het. factor
24 h	209.2 (170.1–261.5)	647.2 (465.5–1129.0)	2.61 ± 0.34	15.69	0.109	1.57
48 h	204.3 (165.8–255.2)	612.1 (443.1–1057.4)	2.69 ± 0.36	16.60	0.084	1.66

Probit regression of replicate-level mortality (dead/25) on log_{10} concentration (4 concentrations × 3 replicates = 12 observations), fitted by maximum likelihood. 95% confidence intervals were obtained by Fieller's theorem. Goodness of fit was assessed with Pearson's χ^2; because $p < 0.15$, a heterogeneity factor (χ^2/df) was applied to the variances and the t-distribution (df = 10) was used for the intervals. LC_{90} values exceed the highest tested concentration (500 ppm).

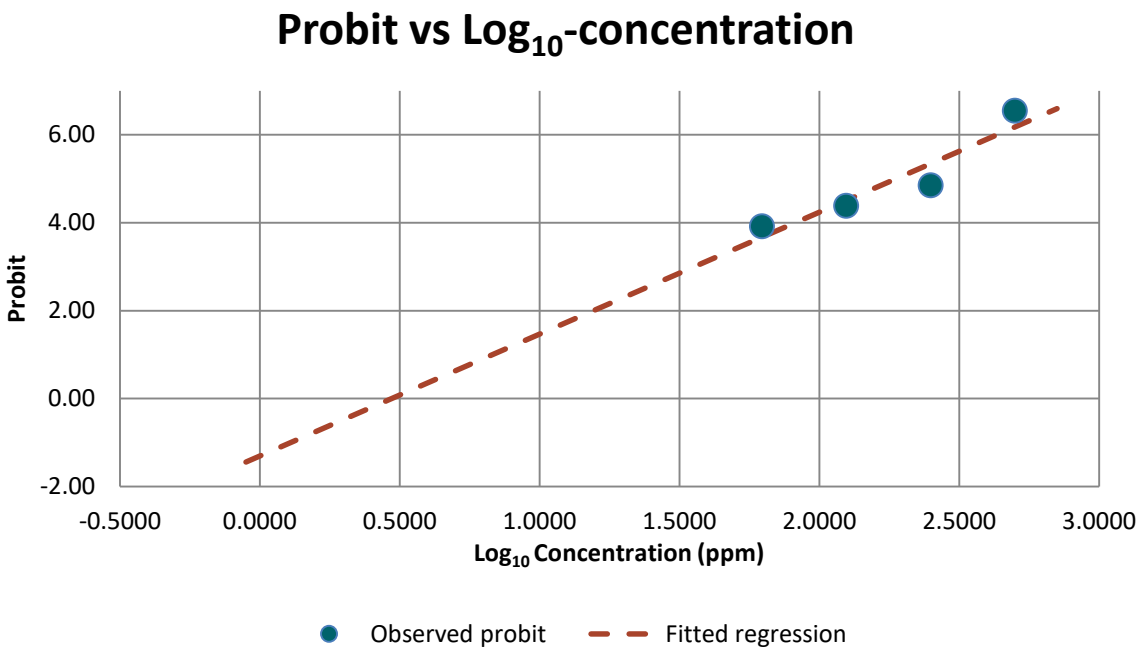


Figure 1: Probit vs. Log_{10}-concentration.

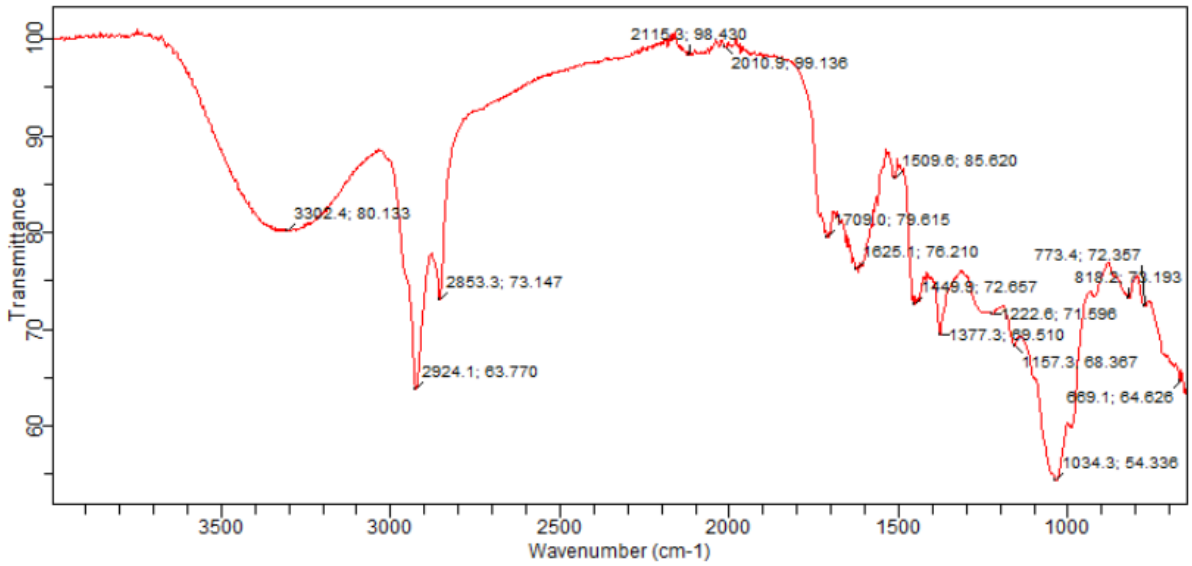


Figure 2. FTIR spectrum of the ethanolic leaf extract of *C. citratus* (4000–650 cm $^{-1}$, ATR accessory, Agilent Cary 630 FTIR spectrometer).

Probit against log-concentration of the extract

Table 3. shows the Probit-regression estimates of LC_{50} and LC_{90} of ethanolic *C. citratus* leaf extract against third instar Anopheles spp. larvae at 24 and 48 h. The lethal concentrations (LC_{50} and LC_{90}) that resulted in 50% and 90% death at 48 h were 204.3 ppm and 612.1 ppm, respectively, based on the probit-on-log-concentration regression (**Figure 1**).

FTIR spectroscopic characterization of the extract

Figure 2 shows the FTIR spectrum of the ethanolic leaf extract of *C. citratus*. Several distinctive absorption bands were identified. O–H stretching vibrations were assigned to a broad, strong absorption band at 3302 cm^{-1}. Aliphatic C–H stretching vibrations of methylene and methyl groups produced strong absorption peaks at 2924 cm^{-1} and 2853 cm^{-1}. Overtone or combination bands from complex molecular environments within the extract likely correspond to minor peaks near 2115 cm^{-1} and 2010 cm^{-1}. A characteristic absorption band was assigned at 1709 cm^{-1} and attributed to C=O stretching. The peak at 1377 cm^{-1} was attributed to symmetric methyl group bending, while absorption bands in the 1509–1450 cm^{-1} region were assigned to aromatic C=C ring stretching and C–H bending vibrations. The bands at 1249–1157 cm^{-1} and a strong peak at 1034 cm^{-1} were attributed to C–O stretching vibrations. Out-of-plane C–H bending in alkene systems and substituted aromatic rings were assigned to peaks in the 989–689 cm^{-1} fingerprint region.

Discussion

Our investigation showed that the *C. citratus* leaf extract had notable larvicidal efficacy against third-instar larvae of Anopheles spp. This study provides evidence that the crude ethanol leaf extract of *C. citratus* has meaningful larvicidal activity against third-instar Anopheles spp. mosquito larvae, with effects that are clearly concentration- and time-dependent. The results align with and extend a growing body of published literature supporting this plant species' mosquito larvicidal potential [10,19,31-33], while providing regionally relevant data generated under standardized WHO bioassay conditions. The lethal concentrations causing 50% and 90% mortality (LC_{50} and LC_{90}) at 48 h were 204.3 ppm and 612.1 ppm, respectively, based on a probit regression of log concentration. An LC_{50} of less than 200 ppm for any plant extract or volatile oil generally indicates high activity [34]. In a previous study to access the effect of lemongrass leaves and stalks extracts using methanol as the eco-friendly larvicides on fourth instar *Aedes aegypti* Larvae Lethal Concentration (LC_{50}) and LC_{90} were achieved at 2.5% (25,000 ppm) and 4.1% (41,000 ppm) of extract, respectively [32]. Another comparative study on the larvicidal effect of ethanol leaf extracts of *C. citratus* (Lemongrass) and *Ximenia americana* (Sea Lemon) on Anopheles and Culex Larva, the result of probit analysis on Anopheles larvae to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the larvae (LC_{50}) after 24 and 48 hours were 5.095 mg/ml (5,095 ppm) and 3.731 mg/ml (3,731 ppm) and LC_{90} were 16.241 mg/ml (16,24 1ppm) and 9.391 mg/ml (9.391 ppm) respectively [35]. The LC_{50} (Lethal Concentration 50%) and LC_{90} (Lethal Concentration 90%) values of lemongrass (*C. citratus*) leaf extract vary significantly across studies because they depend on the target organism, the extraction solvent, and the exposure duration.

The concentration-dependent increase in larval mortality across the four tested concentrations (62.5–500 ppm) aligns with the general principle that higher phytochemical concentrations in the test medium increase exposure and uptake of bioactive compounds by filter-feeding larvae, leading to greater physiological disruption. The mortality of Anopheles larvae exposed to extracts of *C. citratus* was both concentration and time-dependent which agrees with the findings of Saeed et al. [36], who reported the larvicidal effects of ethanol leaf extracts of *Artemisia annua*, *C. citratus* and *Mentha piperita* against Anopheles and Culex mosquito larvae and with the observations of Ani et al. [33] and Deekshith et al. [17], who demonstrated that larvicidal potentials of ethanolic leaf extracts of *C. citratus* consistently exhibit dose-dependent larvicidal responses attributable to the cumulative action of their bioactive phytochemical profiles. Prolonged exposure to sub-lethal concentrations may disrupt larval enzyme systems, inhibit acetylcholinesterase, or interfere with hormonal regulation of molting, all of which have been documented as mechanisms of action for terpenoid and flavonoid compounds in insecticidal plant extracts [8,37]. The lower mortality rate at 62.5 and 125 ppm throughout the 48 h exposure period (13.33±2.31% and 26.67±4.62%, respectively) indicates that, while bioactive compounds were present, their concentrations were insufficient to produce substantial acute toxicity within the test window. These lower concentrations may still act as sublethal stressors that impair larval growth, molting, or development into adult mosquitoes—effects that future mechanistic studies should investigate.

Several distinctive absorption bands indicate the presence of a wide variety of phytochemical functional groups. O–H stretching vibrations, which are suggestive of alcoholic and phenolic hydroxyl groups showed a band breadth compatible with the substantial hydrogen bonding typical of polyphenolic substances [38]. Aliphatic C–H stretching vibrations of methylene and methyl groups indicated the presence of hydrocarbon chains linked to fatty acid and essential oil content [39]. A characteristic absorption band at 1709 cm^{-1} indicated the presence of aldehyde, ketone, or ester functional groups which correlates with citral and related aldehyde terpenoids, the main bioactive components of lemongrass essential oil [39]. The bands at 1249–1157 cm^{-1} and a strong peak at 1034 cm^{-1} indicated the presence of alcohols, esters, ethers, and oxygenated phytochemicals such as glycosides and polysaccharides [40]. The diverse array of phytochemical classes in the extract, with functional groups suggested by the FTIR spectroscopic analysis corresponds to phenolics, flavonoids, terpenoids, aldehydes, alcohols, and carbohydrate-linked compounds. These findings align with the known phytochemical composition of *C. citratus* as reported by Kiani et al. [41] and Beena et al. [42], and provide chemical evidence supporting the observed biological activity. Phenolic compounds and flavonoids—evidenced by the O–H stretching band at 3302 cm^{-1} and aromatic C=C bands at 1509–1450 cm^{-1}—have been reported to contribute to insecticidal activity by disrupting cellular membranes, inhibiting enzyme function, and inducing oxidative stress in target organisms [43]. The C=O band at 1709 cm^{-1}, specifically consistent with the aldehyde functional group of citral constituents, corroborates the established role of this compound class as the primary larvicidal agent in lemongrass [44,45]. Specifically, the essential oil constituents of *C. citratus* suggested in the present study by the characteristic C=O aldehyde band at 1709 cm^{-1} and aliphatic C–H bands at 2924 and 2853 cm^{-1} — have been reported to interfere with mosquito larval nervous system function, respiratory processes, feeding behavior, and membrane integrity, ultimately leading to paralysis and death [8,44,46]. Slight differences in band positions relative to published spectra for *C. citratus* from other geographical origins are consistent with known

influences of geographic provenance, harvesting season, drying conditions, and extraction solvent polarity on phytochemical composition [8,43].

The relatively high crude fat content detected in the proximate analysis is noteworthy, as lipid components may serve as carriers for essential oil terpenoids and facilitate their penetration into biological membranes, potentially enhancing larvicidal efficacy [47]. The absence of detectable crude fiber in our study contrasts with previous studies that detected crude fiber in *C. citratus* samples. Manna et al. [48] reported crude fiber (16.7 ± 0.4%) in lemongrass powder, indicating its suitability as a dietary fiber source. Yusuf et al. [27] detected crude fiber 1.01% in aqueous crude extracts of *C. citratus* (lemon grass). The absence of detectable crude fiber is consistent with the ethanolic extraction process, which selectively dissolves non-polar and semi-polar bioactive compounds while excluding insoluble fibrous cell wall material. These compositional findings agree with previously reported nutritional profiles for *C. citratus* extracts [27,48] with quantitative differences attributable to plant developmental stage, geographic origin, and extraction methodology.

The zero mortality in the negative control throughout the experiment confirms that the carrier solvent (acetone) and experimental conditions did not independently contribute to larval mortality, ensuring that all observed mortality in the treatment groups was attributable to the plant extract. The positive control (Sniper®, 2.5 ppm) produced 100% mortality from 1 h onward, validating larval susceptibility to larvicidal agents and confirming the reliability and sensitivity of the bioassay method [4].

Limitations of the study

The present study has several limitations that should be acknowledged. First, mosquito larvae were not identified to species level because of limited morphological identification resources; species-level identification would enable more targeted comparisons with published species-specific LC_{50} values. Second, the study was conducted under laboratory conditions that may not fully replicate the variable temperature, pH, organic load, and UV irradiation of natural larval habitats, where phytochemical degradation may alter effective concentrations. Future studies should address these limitations by including field evaluations of *C. citratus* extract efficacy and safety, isolation of specific active compounds, and ecotoxicological assessments of non-target aquatic organisms.

Conclusion

The ethanol crude leaf extract of *C. citratus* demonstrated notable concentration- and time-dependent larvicidal activity against third instar Anopheles spp. mosquito larvae under standardized laboratory conditions. These findings provide scientific evidence supporting the potential of lemongrass as a locally available, biodegradable, and environmentally safe botanical larvicide for use in integrated mosquito vector management programs. Further studies incorporating species-level larval identification, active compound isolation, mechanism-of-action studies, field evaluation, and eco-toxicological safety assessment are recommended to advance the translational potential of these findings.

Declarations

Ethical approval
Ethical approval was not required for this study.

Informed consent
Not applicable

Consent for Publication
Not Applicable

Availability of data and materials
Supplementary data are available upon request from the corresponding author.

Competing interests/Conflict of interests
The authors declare that they have no competing interests.

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Authors' contributions
CJ: Conceptualization, methodology, supervision of the work, data analysis, writing the original draft, reviewing and editing the manuscript. CAN: Laboratory investigations and validation; manuscript review and editing.

Acknowledgements
Not applicable

Artificial Intelligence (AI) Disclosure
Authors hereby declare that generative AI technologies such as ChatGPT have been used during writing or editing of this manuscript. The authors remain solely responsible for the study design, data generation, analysis, interpretation of findings, and final manuscript content. Details of AI use: ChatGPT was used for minor editing, limited support, text restructuring, and language refinement—source: OpenAI (ChatGPT). The authors reviewed and take full responsibility for the final content.

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