



Molecular Synergy of GC-Identified Volatile Compounds from *Chromolaena Odorata*, *Hyptis Suaveolens* and *Cymbopogon Citratus* against *Anopheles Gambiae*

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(Received: 16 February 2026

Revised: 25 March 2026

Accepted: 10 April 2026)

KEYWORDS

Asteraceae,
Boefficiency,
Citronella.

ABSTRACT:

Introduction: Malaria, a life-threatening disease, is one of the major deadly infectious diseases worldwide. The disease is caused by the protozoan Plasmodium and it is transmitted in humans by an effective bite of an infected adult female *Anopheles* mosquito. In various parts of the world, malaria has been widespread for many decades, yet it still remains a major public health burden in affected areas, predominantly the tropical and subtropical areas in Africa.

Objectives: This research aimed to explore the synergistic potential of plant-derived volatile compounds from *Chromolaena odorata*, *Hyptis suaveolens* and *Cymbopogon citratus* as novel mosquito control agents, specifically targeting *Anopheles gambiae*, a major vector of malaria in Africa.

Methods: The chemical compositions of the leaf extracts were analyzed using Gas Chromatography (GC-) to identify bioactive compounds responsible for their bioactivity. The combined extract was tested at five different concentrations (0.125, 0.25, 0.50, 1.0 and 2.0mg/l) by exposing laboratory reared *Anopheles gambiae* mosquitoes and observing the time and effects on the mosquitoes. The Effective dose (ED₅₀) was also determined.

Results: Results showed a dose-dependent increase in mosquito control efficacy. At the lowest concentration (0.125mg/l), 20% of mosquitoes were killed within 5min, while 100% mortality was achieved with 0.50 to 2.0mg/l concentrations within 20min and 5min respectively, showing a quicker action. Also the ED₅₀ for the combined extract was significant 0.002mg/l, (P<0.05) which competed with N,N-diethyl-meta-toluamide (DEET) control (0.0009mg/l), whereas the ED₅₀ for the single extracts were; *C. odorata* (0.70mg/l), *H. suaveolens* (0.50mg/l) and *Cymbopogon citratus* (0.50mg/l) which were not significant (P>0.05). The high insecticidal activity was attributed to compounds such as citral, citronella, geraniol, beta- caryophyllene, and flavonoids found in the plant extracts.

Conclusions: The study concluded that the combined extracts provided a quick, effective, and eco-friendly solution for mosquito control. The superior efficacy can be attributed to high synergistic effect of the essential oils, affirming that plant-based volatile compounds are viable alternatives to synthetic repellents, offering environmental, affordability, sustainability and reduced health risks.

1. Introduction

Mosquito-borne diseases, including malaria, dengue fever, and Zika virus, are among the most prevalent vector-borne diseases globally, causing significant morbidity and mortality, especially in tropical and subtropical regions.¹ The World Health Organization (WHO) estimates that malaria alone affects over 200 million people each year, with over 400,000 fatalities annually.¹ Similarly, dengue fever and Zika virus have been linked to significant outbreaks and public health emergencies, particularly in regions of

Southeast Asia, Africa, and the Americas (Centers for Disease Control and Prevention).² These diseases are primarily transmitted through the bite of *Aedes* and *Anopheles* mosquitoes, creating a critical need for effective vector control strategies. Chemical control methods have been the traditional approach for managing mosquito populations and preventing the spread of mosquito-borne diseases. Synthetic insecticides, such as pyrethroids, organophosphates, and carbamates, are commonly used to kill adult mosquitoes or larvae in water bodies where mosquitoes



breed. Additionally, repellents such as DEET (N,N-diethyl-metoluamide) are widely used by individuals to protect themselves from mosquito bites. These chemicals are effective in reducing mosquito populations and minimizing the transmission of diseases.² However, over-reliance on chemical control methods has led to several challenges. One of the major concerns with chemical control is the development of insecticide resistance. Mosquitoes, like other pests, can evolve to withstand chemical treatments, reducing the efficacy of traditional insecticides over time. Resistance to common insecticides, such as pyrethroids, has been documented in various regions, making it more difficult to control mosquito populations and prevent the spread of diseases.³ Additionally, chemical insecticides can have detrimental effects on non-target organisms, including beneficial insects such as bees and aquatic life, and they may contribute to environmental pollution.⁴ Human health risks, such as skin irritation, neurotoxicity, and potential long-term effects on the nervous system, further underscore the need for alternative control methods.⁴ As a result of these challenges, there has been a growing interest in exploring alternative, environmentally friendly mosquito control methods. Plant-based repellents have emerged as a promising solution due to their natural origin, safety for humans and animals, and lower environmental impact. Given the rising concerns over the safety and sustainability of chemical insecticides, the exploration of plant-based mosquito control strategies offers a promising approach to reducing the global burden of mosquito-borne diseases.

Many plants produce bioactive compounds, including essential oils, terpenoids, flavonoids, and alkaloids, have demonstrated insect repellent, insecticidal, or larvicidal properties. These compounds are often non-toxic, biodegradable, and less likely to contribute to the development of resistance among mosquito populations.^{5,6} The use of plant-based repellents offers several advantages over synthetic repellents, including: biodegradable, non-toxic, and environmentally friendly have lower human health risks compared to synthetic repellents, more cost-effective than synthetic repellents, particularly in resource-poor settings, more culturally acceptable, especially in communities where traditional medicine is prevalent.⁷ Plant-based repellents also offer a diverse range of repellent compounds, reducing reliance on single chemicals, sourced locally, promoting self-sufficiency and reducing reliance on imported chemicals, contain novel repellent compounds with unique modes of action, addressing the development of pesticide-resistant mosquito populations, can be integrated into comprehensive pest management strategies, combining physical, chemical, and biological controls.⁵

Plants like *Chromolaena odorata* (Independence leaf), *Hyptis suaveolens* (Bush tea), and *Cymbopogon citratus* (Lemongrass) have been used traditionally in various cultures as mosquito repellents. Their essential oils and extracts contain compounds like eugenol, citronella, and eucalyptol, which have been found to effectively repel mosquitoes and disrupt their ability to locate hosts.⁸ Unlike synthetic chemicals, plant-based repellents are often biodegradable and pose fewer risks to non-target organisms and the environment. Additionally, these natural repellents have the

potential to be produced sustainably, offering an eco-friendly alternative to conventional chemical control methods.⁶ Research into the chemical properties, efficacy, and potential applications of these plants is crucial to developing effective, environmentally safe alternatives to synthetic repellents and insecticides.

Chromolaena odorata (L) King and Robinson, formerly known as *Euparotum odoratum* (L) belongs to the family Asteraceae. Its common names include “Awolowo”, Akintola weed, “Independence weed”, Siam weed, Triffid weed, Bitter bush or Jack in the bush, Devil weed, French weed, Communist weed.⁹ This obnoxious weed has been known to have high medicinal values on account of its phytochemical constituents. For instance, the plant has been reported to have antispasmodic, antiprotozoal, antitrypanosomal, antibacterial and antihypertensive activities.¹⁰ The presence of secondary metabolites such as alkaloids, saponins, tannins, flavonoid attest to its medicinal values. Usunobun and Ewere,¹⁰ had identified compounds such as steroids, triterpenes, alkaloids, flavonoids, tannins, diterpenes, and saponins and glycosides from the petroleum ether, methanol and chloroform extracts of *C. odorata* leaves.

The essential oil of *C. odorata* has been found to contain several bioactive compounds that are responsible for its repellent properties using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy.¹¹ The main constituents of *C. odorata* essential oil have been found to be β -caryophyllene, α -humulene, and caryophyllene oxide.¹² β -Caryophyllene, a sesquiterpene, has been found to be the major component of *C. odorata* essential oil.¹¹ These compounds are responsible for insecticidal and repellent properties against several mosquito species.¹³

Several studies have been conducted by different individuals to analyze the chemical composition of *C. odorata*. Analysis of the essential oil of *C. odorata* using gas chromatography-mass spectrometry (GC-MS) identified 23 compounds, including β -caryophyllene (23.4%), α -humulene (17.3%), and caryophyllene oxide (12.5%).¹⁴

These studies demonstrate that the chemical composition of *C. odorata* essential oil can vary depending on factors such as the plant's geographical location, climate, and soil type. However, β -caryophyllene, α -humulene, and caryophyllene oxide are consistently reported as the major compounds present in the essential oil.

The chemical composition of *Hyptis suaveolens*, a plant species commonly known as bush tea, has been extensively studied due to its insecticidal and repellent properties against mosquitoes.¹¹ The essential oil of *H. suaveolens* has been found to contain several bioactive compounds, including terpenes, phenolic compounds, and alkaloids. The major components responsible for the repellent properties of *H. suaveolens* essential oil are limonene, α -pinene, and β -pinene.¹⁵ Limonene, a monoterpene, has been found to possess insecticidal and repellent properties against several mosquito species.¹³ The repellent properties of *H. suaveolens* essential oil are attributed to the synergistic effect of its major



components.¹⁶ Limonene, α -pinene, and β -pinene have been found to interact with the mosquito's olfactory system, disrupting their ability to detect attractants such as carbon dioxide and lactic acid.⁵ The chemical composition of *H. suaveolens* essential oil can equally vary depending on factors such as the plant's geographical location, climate, and soil type. However, limonene, α -pinene, and β -pinene are consistently reported as the major compounds present in the essential oil.

Citronella grass or lemongrass is the common name for *Cymbopogon citratus*. This species is a member of the Gramineae family. The metabolites such as the monoterpenes like camphor, eugenol, citronellol, terpinolene, α -pinene, thymol, limonene, and cineole are the usual components in several essential oils showing mosquito repellent behavior. The chemical composition of the essential oil of *C. citratus* varies according to the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered. Chemical structure of the major constituents of lemongrass essential oil consists of luteolin and its 6-C and 7-O-glycosides, isoorientin 2'-O-rhamnoside, kaempferol and apiginin from the aerial parts.¹⁷ The phenolic compounds elimicin, catecol, chlorogenic acid, caffeic acid and hydroquinone are also isolated from the plant. The important constituents of the oil of *C. citratus* are citral and citronella. Citral has a toxic effect on the sensory nervous system in insect antennae that affect the behavior and physiology of insects.¹⁰ Citronella oil is a renowned plant-based insect repellent and has been registered for this use in the United States since 1948. The bioactive components of lemongrass powder have been evaluated using GC-MS.¹⁹ GC/MS analysis of methanolic extract of lemongrass leaves revealed the existence of Pentane, 2,4-Dimethyl, Dodecanoic acid tert-butyl ester, 2,6 Bis (1,1-dimethylethyl)- 4-[(4-chloro-6-(3,5, bis (1,1-dimethylethyl)-4-hydroxyanilino)-1,3,5-triazin-2-yl)amino]phenol and 3-Formyl-4,5-dimethyl-pyrrole. The presence of these compounds in the plant extract may at least be responsible for the pharmacological properties of *C. citratus* and thus recommended as plant of phytopharmaceutical importance. Research by Asadollahi et al,²⁰ also indicated that essential oils from plants such as lavender, camphor, catnip, geranium, jasmine, broad-leaved eucalyptus, lemongrass, lemon-scented eucalyptus, amyris, narrow-leaved eucalyptus, carotin, cedarwood, chamomile, cinnamon oil, juniper, cajeput, soya bean, rosemary, niaouli, olive, tage tes, violet, sandalwood, litsea, galbanum, and Curcuma longa, showed good repellent with 8 h complete repellent against different species of Anopheles.

Ali et al,²¹ indicated that lemon grass contains high contents of citral and its cis-isomer (neral), beta myrecene and other aldehydic compounds, it also contained mono terpenes esquiterpens which are included among them oxygenated and non- oxygenated compounds as well as it contained phenolic compounds. Citral "neral and geranial isomers", have been approved by the U.S Food and Drug administration as being safe, so its use as natural preservative and flavoring agent due to its antibacterial activity against gram-negative and positive bacteria such as *Escherichia*

coli and *Staphylococcus aureus*, respectively.²² The extracted lemongrass essential oil according to Ali et al.²¹ also indicated a high sensitivity of bactericidal and fungicidal, showing that lemon grass oil is a potentially valuable antifungal and anti-inflammatory agent for the prevention and treatment of acute inflammatory skin conditions.

According to Kimutai et al.¹⁷, overall, percentage repellent increased with increasing doses of the essential oils while biting rates decreased with increasing concentrations of the oils. The research carried out by Baldacchino et al,¹⁸ on "The repellent of lemongrass oil against stable flies, tested using video tracking" showed that *S. calcitrans* EAG amplitudes increased significantly in a dose-dependent fashion with increasing doses of lemongrass oil in the stimulus pipette. These results indicate that lemongrass oil is a strong stimulant for the olfactory receptor cells of *S. calcitrans* and thus a suitable candidate for behavioral tests. These findings suggest that lemongrass oil could be used as a repellent against stable flies.

Blended oils, ointments and cream formulations of the oil of *C. nardus* in different bases were also evaluated. At 10 % and 20 % concentrations, all the oils showed a minimum of 90 % and 95 % relative protection, respectively, soon after application. These were not significantly different in efficacy from N,N-diethyl-metaltoluamide (DEET), *C. citratus* was reported to be the second best repellent oil which provided above 70 % relative protection for as long as 3 h which declined afterwards. However, DEET provided above 75 % relative protection up to 7 h after its application.

Potential plant-based repellents stratified by protection time with at least 4 h protection time were reported by Asadollahi et al.²⁰ The highest repellency effect was identified from *Ligusticum sinense* extract, followed by *citronella*, *pine*, *Dalbergia sissoo*, *peppermint* and *Rhizophora mucronata* oils with complete protection time ranging from 9.1 to 11.5 h. Ethanolic 25% extract of *L. sinense* was able to completely repel *Anopheles minimus* for 11.5 h.

Another investigation on "The Effectiveness of Lemon Grass as a Natural Mosquito Repellent" looking at the anti-agents adequacy of lemon grass concentrate to the DEET (N,N-diethyl-m-toluamide) based anti-agents or the main business brands for creepy crawly anti-agents has discovered that lemon grass concentrate could be utilized as an option in contrast to substance bug repellent insecticides.²³

According to the study of Baldacchino et al,¹⁸ *C. citratus* was a powerful anti-agent against mosquitoes and houseflies. In this examination, its adequacy was surveyed on stable flies in research center conditions. In the first place, the researchers showed that lemongrass oil was a functioning substance for antennal olfactory receptor cells of *Stomoxys calcitrans* as shown by a huge increment in the electroantennogram reactions to expanding dosages of lemongrass oil. Encouraging decision tests in a flight confine with stable flies approaching two blood-splashed clean cushions, one of which was treated with lemongrass oil, demonstrated that stable flies invested fundamentally more energy in the untreated zone than in the treated zone. No stable flies



benefited from the treated cushion, while nine benefited from the untreated cushion. These outcomes propose that lemongrass oil could be utilized as a powerful anti-agent against stable flies.

The repellent effect of citronella was investigated in several studies. Mgbemena et al,²³ reported that citronella could repel *Anopheles stephensi* and *Anopheles dirus* for 8 and 6 h, respectively. Moreover, 100 µl and 0.1 ml of citronella grass essential oil showed 2.16 and 0.8 h complete protection time against *An. Minimus* and *An. dirus* respectively. In general, the mosquitocidal activity and percentage protection of plant extract increase with increasing concentration of the extracts in different formulations.²⁴ This study establishes and reaffirms the potential of applying indigenous Nigerian plants' oil extracts with insecticidal properties for *Anopheles gambiae* control.

Plant-based mosquito repellents offer a promising alternative to synthetic repellents. Further research is needed to fully explore the potential of these repellents and to develop effective and sustainable methods of mosquito control.

2. Objectives

This research aimed to explore the synergistic potential of plant-derived volatile compounds from *Chromolaena odorata*, *Hyptis suaveolens* and *Cymbopogon citratus* as novel mosquito control agents, specifically targeting *Anopheles gambiae*, a major vector of malaria in Africa. To compare knockdown period/ time offered by each plant species. To determine the optimal concentration and formulation (e.g., spray) of each plant-based repellent for effective mosquito repellency.

3. Methods

Sample Collection

The plant samples (*C. odorata*, *H. suaveolens*, and *C. citratus*) were collected from locations within Federal University of Technology Owerri, Nigeria, ensuring that the collection sites are ecologically diverse to capture possible variations in phytochemical composition due to environmental factors such as soil type, climate, and altitude, and authenticated by a botanist in the department of biology. All the plant used has herbarium name as authenticated by International Association for Plant Taxonomy (IAPT).

Preparation of Plant Materials

The leaves of *C. odorata*, *H. suaveolens* and *C. citratus* were washed, dried, and shredded. The dried plant materials were ground into fine powder using a grinding machine. Each container holding the powdered plant material was labeled using masking tape and a marker for proper identification.

Extraction Process

Approximately 250g of the powdered form of each

plant was measured and placed into

separate labeled containers. 20% of Ethanol was added to each container, and the contents were thoroughly mixed using a spatula. The mixture was filtered using a net sieve folded into four layers to ensure proper separation. The filtrate was allowed to stand for 7 days to facilitate the complete evaporation of ethanol, leaving a dried residue. **Gas Chromatography (GC)**

Gas Chromatography (GC-) was carried out to determine the phytochemical compositions of the three plants under study. An advanced analytical technique (GC) was to separate, identify, and quantify chemical compounds in complex mixtures according to standard procedure. GC separates volatile compounds based on their boiling points. This technique provides high sensitivity, accuracy, and specificity in chemical analysis.

Analysis

One (1ml) of pure standard TPH (Total Petroleum Hydrocarbon) was first injected into the GC- to obtain a standard chromatogram and Peak Area. This was used to calibrate the GC- for the test samples. Then 1ml of the test sample -was injected into the GC= as well to obtain equivalent chromatogram and Peak Area. Then the peak area of the test sample is compared to that of the standard with respect to the concentration of the standard to get the concentration of the test sample;

Conc of the test sample = $\frac{\text{Sum peak area of sample} \times \text{conc of standard}}{\text{Sum peak area of the standard}}$

Sum peak area of the standard

Preparation of plant extract as insecticide

About 10-20g of the combined plant extract was obtained. Then ethanol (20g) is mixed with water in the ratio of 2:1 and stir until is homogeneous, and combined with extract until well combined. Vineger (1g) is added as emulsifier to ensure complete mixing and vitamin E is also added as stabilizer. Fill pump spray bottle with emulsion and ensure is properly sealed.

Test on mosquitoes

The study utilized three to five-day-old *Anopheles gambiae* mosquitoes that were reared in a controlled laboratory environment. A one-square-meter plastic cage was constructed and sealed on top with mosquito net, and small tape created beneath the plastic, Stagnant water containing mosquito eggs and larva was poured into the plastic and left for 3 to 5 days for development of adult mosquito which were identified by the presence of plain antenna and unique mouth part to be female anopheles. After development, the water was removed, and the number of mosquitoes present were recorded. The prepared plant-insecticide was applied using a spray



pump. Observations were made to record the response of the mosquitoes, such as the number knocked down, weakened, or killed after exposure at different concentrations and time intervals. The procedure was repeated with varying concentrations of the insecticide to assess its efficiency over time. DEET insecticide (control) and each of the plant extract were all tested against *Anopheles gambiae* mosquito.



Plate 1: A one-square-meter plastic cage

Determination of dose response

For each test, 20 female mosquitoes were withdrawn from a stock cage and placed into each plastic cage using an aspirator. The application dosage for the repellent was 10 % of extract, ethanol 20%, water 20%, emulsifier (vinegar) 1% and stabilizer (vit E) 0.5%. After mixing together for 2 min, was sprayed on the cage using spray pump and stop watch to determine time. This was repeated with increasing doses (0.125, 0.25, 0.50, 1.0 and 2.0mg/l) of the extract and checking time until no mosquito was recorded moving at a given concentration and time.

Determination of knock down time and percentage mortality/mosquito control action

After identifying the concentration of the extract that prevented complete flying during the 30-sec period, the extracts were tested for percentage of the mosquito control action at 0.125, 0.25, 0.50, 1.0 and 2.0mg/l concentrations.

Percentage mosquito control action and mortality were calculated using the following equations:

$$\% \text{ Control action} = (C - T/C) \times 100$$

where: C is the number of mosquitoes exposed

and T is the number of mosquitoes knocked-down or killed from the test.

$$\% \text{ Mortality} = \frac{\text{Number of dead mosquitoes}}{\text{Number of mosquitoes exposed}} \times 100$$

The evaluations were conducted under controlled conditions, with temperatures ranging between 20–25°C and relative humidity maintained within 60–70%.

Data analysis

All experiments were replicated three times. Data on mosquito control, and time, were recorded using the Microsoft Excel programme. Control groups in the experimental bioassays with >20% repellent were repeated. The dose-mortality data was analysed by log-probit method of Finney²⁵ and effective concentrations for 50% (ED₅₀) mortality determined. Statistical significance of the recorded mosquito control action of the various test concentrations and the controls were analyzed using one-way analysis of variance (ANOVA) at P < 0.05.

Results and Discussion

Results

The gas chromatography (GC) analysis of *C. citratus* (table 1, fig 1) provided valuable insights into its chemical composition, highlighting the bioactive compounds responsible for its mosquito repellent efficacy. The retention time, peak area, peak height, and external units were key indicators of the concentration and effectiveness of each compound present in the extract. Retention time represents the duration a compound takes to pass through the GC column, which is essential in determining the volatility and stability of each component. Lower retention times indicate compounds that evaporate quickly, providing immediate repellent action, while higher retention times suggest more stable, longer-lasting compounds that contribute to prolonged efficacy. The peak area is a critical factor in evaluating the relative abundance of each compound in *C. citratus*. A larger peak area signifies a higher concentration of the respective compound, making it more influential in the repellent activity. The most dominant compounds exhibited substantial peak areas, confirming their significant presence in the extract. Additionally, the peak height, which measures the intensity of the signal generated by each compound, further supports the findings from the peak area. Compounds with greater peak heights indicate stronger bioactivity, reinforcing their importance in the overall effectiveness of the mosquito repellent formulation.

External units provide quantitative data on certain key compounds, particularly those with well-documented bioactivity. The presence of bioactive components such as citronellal, geraniol, linalool, quercetin, and caffeic acid, each with varying retention times, peak areas, and peak heights, underscores the efficacy of *C. citratus* as a



mosquito repellent. Citronellal, with a retention time of 12.350 minutes, exhibited one of the highest peak areas (1591.549) and peak heights (83.703), confirming its dominance in the extract. This compound is widely recognized for its strong mosquito repellent properties, as it interferes with mosquito olfactory receptors, making it difficult for them to detect human hosts. Geraniol, identified at a retention time of 14.233 minutes, showed a peak area of 1981.716 and peak height of 86.092, further reinforcing its role as a potent mosquito deterrent. Linalool, with a retention time of 13.416 minutes, displayed a peak area of 1456.970 and a peak height of 66.066, contributing to both repellent and insecticidal activity. Other flavonoids not only enhance the repellent activity but also provide antioxidant and antimicrobial properties, which contribute to the extract's overall stability and efficacy.

The efficacy of *C. citratus* in repelling mosquitoes can be attributed to the synergy between its major bioactive constituents. The combination of fast-evaporating monoterpenes and longer-lasting flavonoids ensures both immediate and sustained protection against mosquitoes. The compounds with lower retention times, such as caffeic acid, provide an initial burst of repellent action, while those with higher retention times, like quercetin and geraniol, contribute to prolonged effectiveness. Furthermore, the high peak areas and peak heights of key compounds suggest that *C. citratus* contains sufficient concentrations of these active ingredients to serve as a potent natural repellent.

The external unit values further validate the strong bioactivity of the identified compounds, supporting their role in mosquito deterrence. The analysis confirms that *C. citratus* is highly effective as a natural mosquito control due to its rich composition of volatile oils and flavonoids. The high concentrations of citronellal, geraniol, and linalool, combined with the presence of flavonoids such as quercetin and orientin, contribute to its efficacy. The retention time data indicate that these compounds are well-balanced in terms of volatility and stability, ensuring immediate action and prolonged protection. The substantial peak areas and peak heights further reinforce the

effectiveness of the extract, as they indicate the dominant key mosquito control compounds in the formulation. These findings highlight the potential of *C. citratus* as an environmentally friendly and sustainable alternative to synthetic mosquito repellents.

The gas chromatography (GC) analysis of *H. suaveolens* (table 2, fig 2), revealed a diverse range of bioactive compounds contributing to its mosquito control efficacy. The retention time, peak area, and peak height provided

insights into the concentration and potency of each compound. Among the key constituents, beta-caryophyllene, 1,8-cineole (eucalyptol), and rosmarinic acid stood out due to their high peak areas and peak heights, indicating their significant presence and bioactivity. Beta-caryophyllene, with a retention time of 3.616 minutes, exhibited strong insecticidal properties, interfering with mosquito sensory perception. 1,8-Cineole, identified at 10.933 minutes, displayed a peak area of 1119.892, reinforcing its role as a neurotoxic agent for insects. Additionally, rosmarinic acid, with a peak area of 1041.4755, contributed to the mosquito control activity by offering both antimicrobial and insect-deterrent effects. Ursolic acid and betulinic acid, appearing at retention times of 12.600 minutes and 12.116 minutes, respectively, further enhanced the mosquito control potential due to their anti-inflammatory and bioactive properties. The flavonoids quercetin and kaempferol, identified at 14.550 minutes and 15.150 minutes, respectively, played a role in the extract's long-term stability and effectiveness. The overall high peak areas and peak heights of these compounds confirm that *H. suaveolens* possesses strong mosquito-control properties. The presence of fast-acting monoterpenes like 1,8-cineole ensures immediate control, while the flavonoids and phenolic acids contribute to prolonged efficacy. These findings highlight *H. suaveolens* as a valuable natural mosquito control with potential applications in sustainable insect control.

The gas chromatography (GC) analysis of *C. odorata* (table 3, fig 3), identified a range of bioactive compounds that contribute to its mosquito control properties. The retention time, peak area, and peak height of these compounds provided insight into their concentration and efficacy. Among the major components, beta-caryophyllene, limonene, and 1,8-cineole (eucalyptol) were prominent due to their high peak areas and peak heights, confirming their strong insecticidal activity. Beta-caryophyllene, with a retention time of 3.183 minutes, exhibited moderate peak area and height, indicating its potential role in disrupting mosquito olfactory receptors. Limonene, found at 9.350 minutes, had a peak area of 855.135, reinforcing its effectiveness as a neurotoxic agent against mosquitoes. 1,8-Cineole, detected at 12.100 minutes, further contributed to mosquito deterrence by affecting their sensory perception. Additionally, quercetin and kaempferol, identified at retention times of 12.583 minutes and 13.400 minutes, respectively, played a role in enhancing the stability and longevity of the repellent effect. P-hydroxybenzoic acid, with a peak area of



447.6400, offered antimicrobial benefits, supporting the overall bioactivity of the extract.

The high concentration of these compounds, reflected in their significant peak areas and peak heights, confirms the efficacy of *C. odorata* as a mosquito control agent. The presence of both fast-acting monoterpenes, such as limonene, and longer-lasting flavonoids, like quercetin, ensures both immediate and sustained protection against mosquitoes. These findings validate *C. odorata* as a potential natural alternative to synthetic insect repellents, offering an environmentally friendly approach to mosquito control.

The bio-efficacy of the combination of *C. odorata*, *H. suaveolens*, and *C. citratus* at 0.125mg/l concentration against *Anopheles gambiae* table (4) demonstrated mosquito control potential. Within the first 5 min of exposure, 4 mosquitoes were knocked down and killed instantly. The data increased at 20 mins, further confirming the rapid control action even at a low concentration. The presence of volatile compounds such as citronellal, geraniol, and beta-caryophyllene played a crucial role in the knockdown effect. Citronellal and geraniol, both known for their strong insecticidal activity, interfere with the mosquito's nervous system, causing immediate paralysis and death. Some of the mosquitoes that initially resisted in the first 5mins succumbed when the time was extended to 10-20mins, demonstrating the potent and immediate effect of the extract. The absence of weakened mosquitoes suggests that the combination either killed the mosquitoes outright or left them unaffected, with no intermediate state observed. The bio-efficacy at 0.25mg/l concentration exhibited even more immediate effects, with more number of mosquitoes killed within the first 5 mins. Like the 0.125mg/l concentration, there were no cases of weakened mosquitoes, indicating that increasing the concentration led to faster and more efficient mortality. The rapid insecticidal action at this level suggests a heightened impact of the bioactive compounds, ensuring that the

mosquitoes did not have time to recover before succumbing to the effects. Also in table (5), the ED₅₀ for the combined extract was significant 0.002mg/l, (P<0.05) which competed with N,N-diethyl-meta-toluamide (DEET) control (0.0009mg/l), whereas the ED₅₀ for the single extracts were; *C. odorata* (0.70mg/l), *H. suaveolens* (0.50mg/l) and *Cymbopogon citratus* (0.50mg/l) which were not significant (P>0.05). The key mosquito control components in *C. citratus*, such as citronellal and linalool, along with beta-caryophyllene from *H. suaveolens*, worked synergistically to produce an overwhelming toxic effect. This confirms that at a 0.25mg/l concentration, the formulation is sufficient to achieve reasonable (60, 70, and 80%) mosquito mortality within a matter of mins, making it a highly effective natural alternative to synthetic agents (table 6, fig 4). At the highest concentration of 2.0mg/l, the results mirrored those observed at 1.0mg/ml, with complete mortality recorded within 5 mins of exposure. The immediate elimination of all mosquitoes without any observed weakening reaffirms the potency of the combination. This suggests that once a critical concentration threshold is reached, increasing the concentration further does not necessarily enhance efficacy beyond immediate kill rates. However, the 2.0mg/ml concentration may provide extended residual protection, ensuring that mosquitoes remain repelled for a longer duration after application. The consistency in results between 0.25mg/l and 2.0mg/l concentrations highlights the efficiency of the bioactive compounds in disrupting the neurological function of mosquitoes. Also, table (6) demonstrated the comparative action of the extract and control (DEET), with the extract having the same 100% mortality at 0.50 concentration within 20min of exposure as the control. The high concentration of citronellal, geraniol, and limonene likely intensified the control effect, making it impossible for the mosquitoes to survive exposure.

TABLES AND FIGURES SHOWING THE RESULTS

Table 1: Phytochemical components obtained from the extract of *Cymbopogon citratus* using

GC- analysis

Component	Retention	Area	Height	External Units
Solvent	0.466	92543.9245	5643.390	0.0000



Chlorogenic Acid	2.100	925.3485	17.473	0.0000 %
Hydroquinone	3.316	302.6690	11.674	30.2669 ppm
Caffeic Acid	3.966	368.1715	11.984	36.8171 ppm
Catechol	7.016	954.4870	17.284	0.0000
Orientin	9.133	1469.1890	34.888	0.0000
Delphinidine	9.816	271.1630	21.874	0.0000
Lycopene	10.983	925.1270	34.683	0.0000
Citronellal	12.350	1591.5490	33.703	0.0000
Swertiajaponin	12.650	313.9115	31.544	0.0000
Linalool	13.416	1456.9700	56.066	0.0000
Geranoil	14.233	1981.7160	36.092	0.0000
Quercetin	15.216	2059.5840	96.641	0.0000
Cymbopogon	15.866	5317.9710	434.904	0.0000
Cymbopogonol	16.366	579.0555	34.541	0.0000
Apiginin	16.816	183.4015	19.835	0.0000
Luteolin	17.150	1212.1165	94.906	0.0000
Ferulic Acid	17.433	107.0235	13.715	0.0000
TOTAL			112563.3780	57.0840

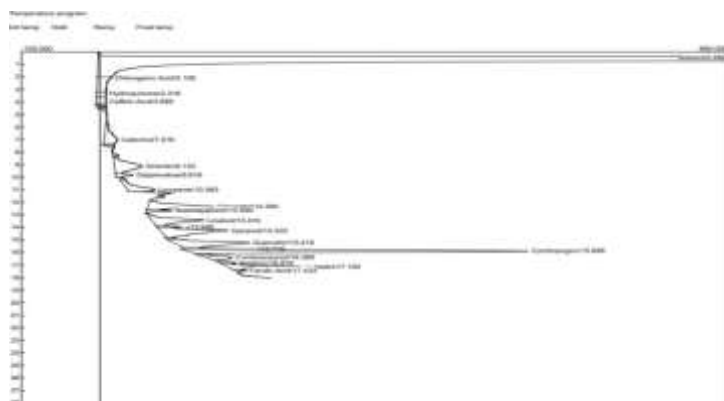


Fig 1: Chromatogram of the components of *C. citratus*



Table 2: Phytochemical components obtained from the extract of *Hyptis suaveolens* using GC-analysis

Component	Retention	Area	Height	External	Units
Solvent	0.35	67004.9195	5666.812	0	
Chlorogenic Acid	3.033	436.792	9.847	0	
Beta-Caryophyllene	3.616	361.045	10.891	0	
P-Hydroxybenzoic Acid	4.4	959.034	45.92	0	
Gallic Acid	5.016	291.11	15.82	0	
Catechin	8.166	688.81	54.373	0	
Protocatechuic Acid	9.366	695.29	25.803	0	
Rosemarinic Acid	9.75	1041.476	110.208	0	
1_8-Cineole	10.933	1119.892	65.176	0	
Beta-Sitosterol	11.216	2141.666	192.297	0	
Betulinic Acid	12.116	854.225	60.981	0	
Ursolic Acid	12.6	2100.368	164.801	0	
Chicoric Acid	12.95	171.063	18.111	0	
Suaveolic Acid	13.416	1192.192	49.338	0	
Suaveolol	13.9	1493.186	141.84	0	
Quercetin	14.55	1018.019	67.658	0	
Kaempferol	15.15	2000.448	135.765	0	
Rutin	15.783	745.705	39.625	0	
Apigenin	16.3	2266.912	123.67	0	
Naringenin	17.033	306.6645	36.712	0	
Hesperidin	17.383	880.0725	91.616	0	
Bergamotol	18.083	181.351	10.942	0	
Spathulenol	18.433	961.646	83.274	0	
TOTAL		90172.32		0	

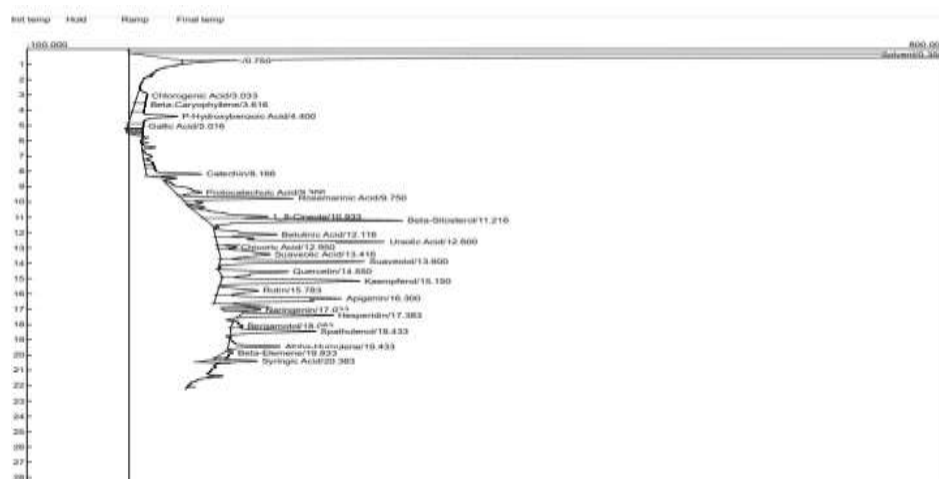


Fig 2: Chromatogram of the components of *H. suaveolens*

Table 3: GC Phytochemicals composition of *Chromolaena odorata*

Components	Retention	Area	Height	External	Units
Solvent	0.350	55691.1730	5673.622	0.0000	
B-caryophyllene	3.183	232.5670	10.218	0.0000	%
P-Hydrobenzioc Acid	4.383	447.6400	31.391	44.7640	ppm
Curcumin	8.150	352.3210	21.946	35.2321	ppm
Alpha-Pinene	8.416	192.8335	13.436	0.0000	
Limonene	9.350	355.1350	22.633	0.0000	
Resveratrol	9.733	482.7100	44.525	0.0000	
P-Coumaric Acid	10.916	582.6550	29.192	0.0000	
Aromadendrin	11.200	946.1670	55.612	0.0000	
1_8-Cineole	12.100	190.3270	18.366	0.0000	
Quercetin	12.583	530.0140	54.523	0.0000	
Kaempferol	13.400	357.7120	20.210	0.0000	



Quercetagetin	13.883	443.1460	40.474	0.0000
Germacrene D	14.533	137.1390	15.729	0.0000
Apigenin	15.116	545.4980	35.986	0.0000
Naringenin	16.266	273.5065	28.784	0.0000
Alpha-Humulene	17.016	104.6110	10.149	0.0000
Myrtenol	17.350	412.1730	27.089	0.0000
Cadalene	18.433	528.8650	27.159	0.0000
Chrysoeriol	19.416	143.3550	10.833	0.0000
TOTAL		73649.5480		79.9961

Temperature program:

Init temp Hold Ramp Final temp

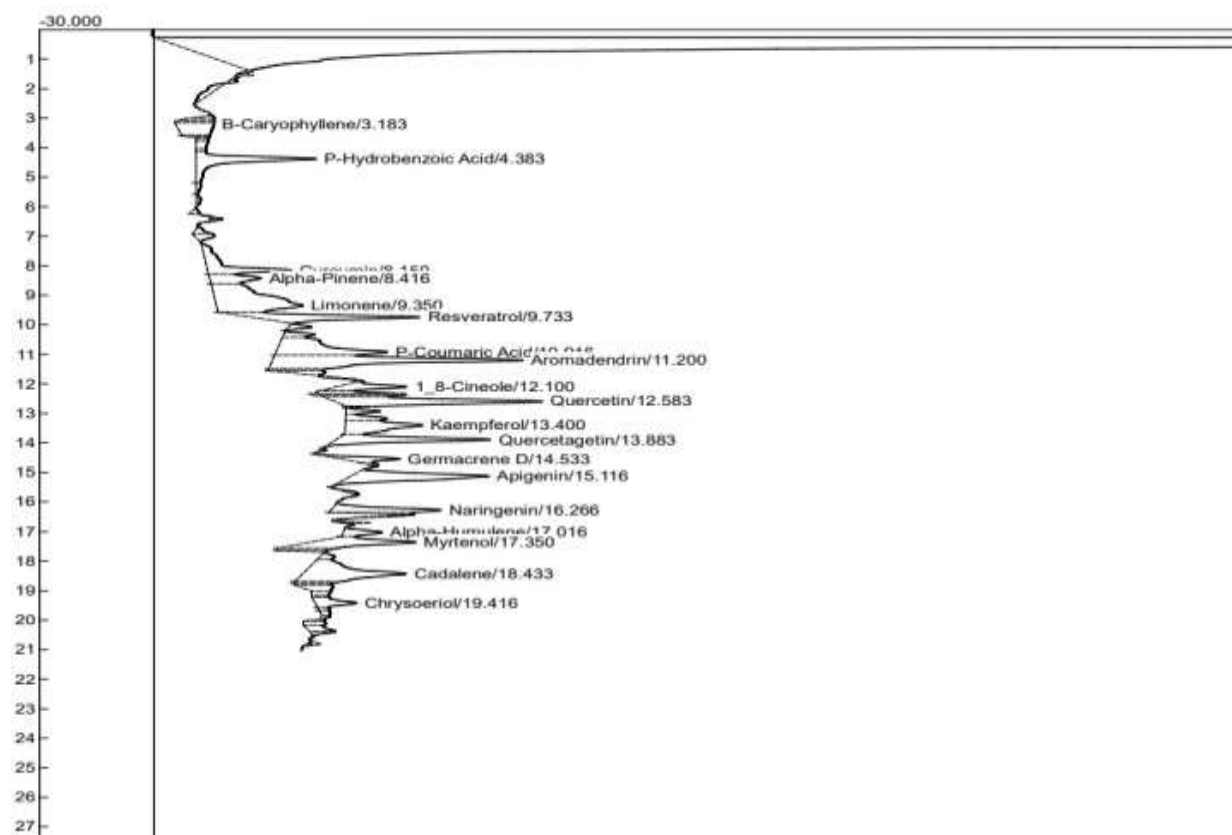


Fig 3: Chromatogram of the components of *C. odorata*



Table 4: The bioefficiency of the combination at different concentrations and time against

Anopheles gambiae

Substance	No of mosquitoes exposed	Substance conc (mg/l)	Time interval	No of mosquito knockeddown/killed
Plant extract Combination of <i>C. odorata</i> , <i>H. suaveolens</i> , and <i>C. citratus</i>	20	0.125	5mins	4
	20		10mins	5
	20		20mins	10
	20	0.25	5mins	12
	20		10mins	14
	20		20mins	16
	20	0.50	5min	16
	20		10min	18
	20		20min	20
20	1.0	5mins	20	
20		10mins	20	
20		20mins	20	
20	2.0	5mins	20	
20		10mins	20	
20		20mins	20	



DEET (n,n-diethyl- β-methylbenzamide)	20	0.196	5mins	20
	20		10mins	20
(Control)	20		20mins	20

Table 5: ED₅₀ of the different extracts at 0.50mg/l concentration against *Anopheles gambiae*

REPELLENT	No of mosquitoes	ED ₅₀ (mg/ml)	95% CL (mg/ml)	Significance levels
<i>C. odorata</i>	20	0.70	0.70±0.07	P>0.05
<i>H. suaveolens</i>	20	0.50	0.50±0.07	P>0.05
<i>Cymbopogon citratus</i>	20	0.50	0.50±0.06	P>0.05
Combination of <i>C. odorata</i> , <i>H. suaveolens</i> , and <i>C. citratus</i>	20	0.002	0.002±0.005	P<0.05
DEET (control)	20	0.0009	0.0009±0.0001	P<0.05

ED₅₀= Effective dose that causes 50% of knockdown, mean dosage are significantly different (P<0.05) from each other if 95% confidence limits do not overlap.



Table 6: Comparative mortality rate in relation to time at different concentrations of extract and control against *Anopheles gambiae*

Substance	Concentration (mg/ml)	% Mortality /Time		
		5mins	10mins	20mins
Extract combination of <i>C. odorata</i> , <i>H. suaveolens</i> , and <i>C. citratus</i>	0.125	20	30	50
	0.25	60	70	80
	0.50	80	90	100
	1.0	100	100	100
	2.0	100	100	100
DEET (n,n-diethyl-3-methylbenzamide) (Control)	0.196	100	100	100

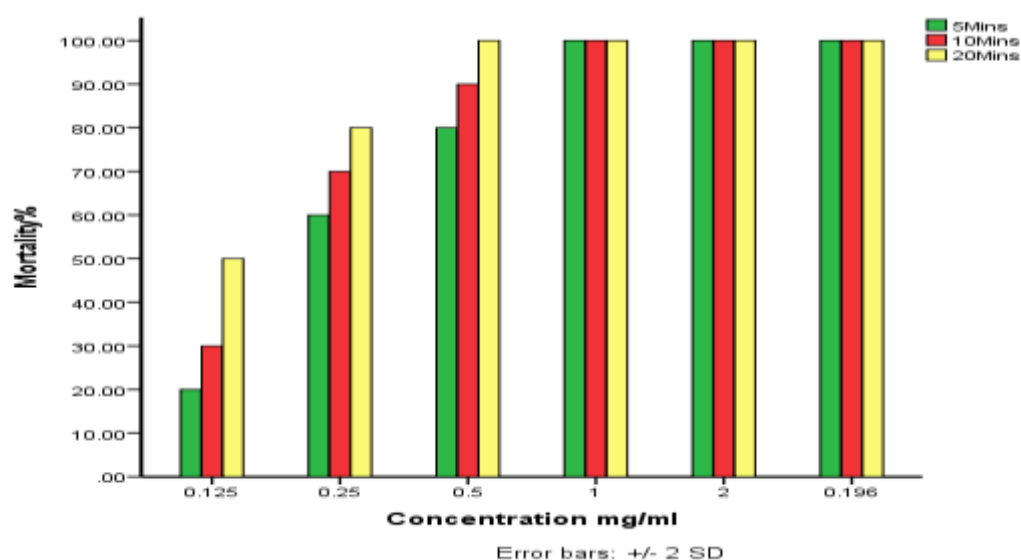


Fig 4: Mortality/Dose-response of mosquitoes in relation to concentration and time



DISCUSSION

Arthropod repellents can be an effective way to deter mosquitoes, ticks, and other blood-sucking arthropods from biting humans and transmitting pathogens. Vector control, achieved through indoor residual spraying (IRS) and large-scale distribution of long-lasting insecticidal nets (LLINs), is one of the currently implemented malaria control strategies. This method, along with other malaria control strategies, has reduced malaria-related illness and mortality in the country. Despite reports stating the efficacy of the vector control methods (IRS and LLINs), insecticide resistance in malaria vectors poses a serious threat to the success of malaria vector control programs in Africa.¹ In addition, various studies have pointed out that IRS and LLINs have a limited effect on malaria vectors that are active during the early hours of the night and have an exophagic feeding behavior. Furthermore, the chemical insecticides used to prepare the IRS and LLINs may pose a serious threat to human health and the environment.¹

Consequently, to overcome the aforementioned limitations, there is a need to develop new vector control methods that complement the currently used vector control approaches. An alternative to mosquitoes control could be plant-based natural materials like plant oils that could prevent the adverse effects of synthetic repellents. In comparison with synthetic mosquito control, they are deemed safe and good for the environment.

However, the efficacy and duration of different mosquito control products from plants can vary dramatically.⁶ The type and concentration of active ingredient(s) in a repellent product is critical to the product's efficacy at deterring mosquito and tick bites. Insect control of plant origin have been used to protect humans from the bites of host-seeking mosquitoes. Such mosquito controls may have a fundamental role in areas where malaria vectors are active during the early hours of the night, as the inhabitants are often outdoors at these times. Thus, they may serve as a complementary method to the indoor-based vector control method.⁸ This study evaluated the molecular synergy of GC-identified volatile compounds from *Chromolaena odorata*, *Hyptis suaveolens* and *Cymbopogon citratus* against *Anopheles gambiae*. The GC results of *C. citratus* (Table

1, fig 1) revealed a high concentration of monoterpenes and aldehydes, which are well-known for their mosquito repellent effects. The major components identified were Citral (Geraniol and Neral). Citral is a strong mosquito repellent and is responsible for the characteristic lemon-like aroma of lemongrass. It disrupts the mosquito's olfactory receptors, reducing its ability to locate hosts. Geraniol, A potent mosquito repellent with antimicrobial properties. Linalool – Known for its sedative effects on insects, interfering with their nervous system. Citronellal – has strong insecticidal properties and contributes to mosquito knockdown effects. Myrcene – enhances the efficacy of other compounds by acting as a synergist. These findings align with previous study by Bossou,¹⁹ who reported similar chemical compositions in *C. citratus* essential oil. The essential oil composition of *H. suaveolens* (table 2, fig 2) was characterized by high levels of terpenes and phenolic compounds, including: Beta-Caryophyllene – A sesquiterpene with strong insecticidal and anti-inflammatory properties. It disrupts mosquito sensory perception, making it harder for them to detect humans. 1,8-Cineole (Eucalyptol) – A monoterpene ether that has insecticidal properties and acts as an irritant to mosquitoes, P-Hydroxybenzoic Acid – A phenolic compound with antimicrobial and mosquito deterrent effects, Rosmarinic Acid – A polyphenol known for its antioxidant and insect repellent properties, Limonene – A monoterpene that contributes to mosquito repellency by interfering with their olfactory receptors. These compounds confirm earlier findings by Nebie *et al.*¹⁶, who reported that *H. suaveolens* essential oil is effective in repelling mosquitoes due to its high beta-caryophyllene and cineole content. The GC-analysis of *C. odorata* (table 3, fig 3) showed that it contained a variety of flavonoids, alkaloids, and terpenoids, which contribute to its insecticidal properties. The key compounds identified were: Flavonoids – These include quercetin, kaempferol, and aromadendrin, which have antioxidant and repellent effects. Alkaloids – These contribute to the plant's bitter taste and insecticidal properties. Limonene – A well-known mosquito repellent that works by masking human odor. Germacrene D – A sesquiterpene with strong insecticidal activity, Alpha-Humulene – has antimicrobial and insect repellent properties. 1,8-Cineole – acts as an irritant and repellent to mosquitoes. These findings are in agreement with Afolabi *et al.*¹⁴ and Uyi *et al.*¹² who also reported the



presence of these bioactive compounds in *C. odorata* essential oil. However, the concentration of essential oils in *C. odorata* was lower compared to *C. citratus* and *H. suaveolens*, which explains its relatively moderate mosquito repellent activity.

The bioefficacy tests demonstrated that the combination of these plant extracts significantly enhanced mosquito knockdown and death. At a 0.125mg/l concentration, (table 4) 20% of mosquitoes were killed within 10mins. At 0.25 and 0.50mg/l concentrations, reasonable mortality were achieved within 10 to 20 mins. This rapid knockdown/ control effect confirms that blending these plant extracts enhances their bioactivity, likely due to the synergistic effects of their essential oils. The ED₅₀ for the combined extract of *C. citratus*, *H. suaveolens* and *C. odorata*, (table 5) showed that these plant part may be good substitution for conventional DEET as efficient and affordable mosquito control. The effectiveness of the combination extract (table 5), was significance (P<0.05).

The strong activity and insecticidal effects observed in this study supported previous researchers,^{21,23} who found that *C. citratus* provided the longest-lasting mosquito control effect due to its high citral and geraniol content. Similarly, Akinkulore *et al.*¹³ reported that *C. odorata* demonstrated moderate control activity, which can be improved when combined with other plants. Research by Duniya *et al.*⁶, supported the idea that essential oil-based repellents can be just as effective as synthetic ones when properly formulated.

Synthetic mosquito repellents, such as DEET, have been widely used but come with drawbacks, including toxicity, skin irritation, and environmental concerns.^{2,5} The results from this study suggested that plant-based repellents, particularly when combined, offered safer alternative with comparable or superior efficacy.

Conclusion

This study evaluated the mosquito control efficacy of the combination of *C. citratus*, *H. suaveolens*, and *C. odorata*, focusing on their chemical composition and bioactivity against *Anopheles gambiae*. The results demonstrated that this extract showed significant control efficiency against *A. gambiae*. The superior efficacy can be attributed to the high synergistic effects of the essential oils. The findings confirm that plant-based

repellents are viable alternatives to synthetic repellents, offering environmental sustainability and reduced health risks. Given the increasing concerns about synthetic repellent toxicity and resistance development in mosquitoes, these plant extracts provide a promising natural solution for mosquito control. However, further research is needed to optimize their formulation and assess their long-term efficacy in field conditions.

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