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PHYTOCHEMICAL PROFILING AND BIOACTIVITY OF ESSENTIAL OILS EXTRACTED FROM MOSQUITO-REPELLENT PLANTS

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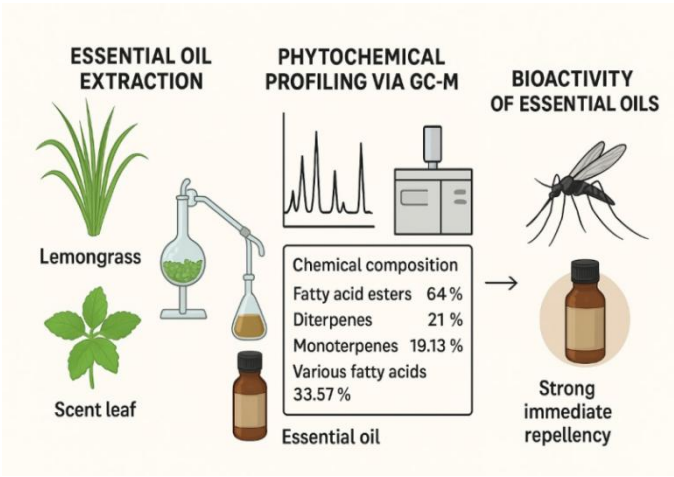
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ABSTRACT

This research investigates the extraction and characterization of essential oils from lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissium)*, two plants known for their mosquito repellent properties. The essential oils were obtained using hydro-distillation and analyzed for their physico-chemical properties and chemical composition using ASTM standard methods and Gas Chromatography-Mass Spectrometry (GC-MS). Results showed that lemongrass oil had a higher specific gravity (1.027) and was dominated by fatty acid esters (64 %) and diterpenes (21 %), suggesting a slow-release profile suitable for long lasting repellency. In contrast, scent leaf oil exhibited higher volatile content (9.55 %) and a chemically diverse profile, including monoterpenes (19.13 %), sesquiterpenes (15.17 %), and various fatty acids (33.57 %), indicating strong immediate repellency. The chemical difference between both oils is linked to plant type, environment, and extraction conditions. Their combined use offers a synergistic solution for developing effective, natural mosquito repellents that provide both rapid and sustained action. This study highlights the relevance of essential oil profiling in formulating plant-based alternatives to synthetic repellents and calls for further toxicological and field efficacy studies to support commercial application.



KEYWORDS

Essential oils, *Cymbopogon citratus*, *Ocimum gratissimum*, Mosquito repellent, Hydro-distillation, Phytochemical characterization, Gas Chromatography-Mass Spectrometry, Insecticidal activity

INTRODUCTION

Essential oils are volatile, aromatic compounds extracted from plants, which have been used for centuries in traditional medicine, aromatherapy, and as natural insect repellents (Bakkali *et al.,* 2020). These oils are derived from various plant parts, including leaves, flowers, bark, and roots, and are known for their diverse biological activities, including antimicrobial, antifungal, and insect-repellent properties (Tongnuanchan and Benjakul, 2021). In recent years, the demand for natural products has surged due to growing concerns over the adverse effects of synthetic chemicals on human health and the environment (Isman, 2020). This shift towards natural products is particularly evident in the field of mosquito repellents, where synthetic chemicals have been associated with health risks such as skin irritation, respiratory issues, and neurotoxicity (Corbel *et al.,* 2023).

Mosquitoes are a serious global health threat, as they are vectors for several deadly diseases including malaria, dengue fever, Zika virus, yellow fever, and chikungunya. According to the World Health Organization (WHO), malaria alone causes an estimated 200 million cases and over 400,000 deaths each year, with mosquitoes being the primary transmission vector (WHO, 2021). Despite advances in controlling mosquito populations through the use of insecticides, such as DEET (N,N-Diethyl-meta-toluamide) and permethrin, the long-term reliance on chemical repellents has raised concerns due to the development of insecticide resistance and environmental toxicity (Zhou *et al.,* 2021).

Plants such as lemongrass (*Cymbopogon citratus*), scent leaf (*Ocimum gratissimum*), eucalyptus (*Eucalyptus globulus*), basil (*Ocimum basilicum*) and neem (*Azadirachta indica*) have been widely studied for their mosquito-repellent properties (Tisgratog *et al.,* 2016). These plants are used to produce essential oils which contain bio-active compounds such as citronellal, thymol, eugenol, geraniol, and limonene, which interfere with mosquito olfactory receptors, reducing their ability to locate human hosts (Adebayo *et al.,* 2022; Nerio *et al.,* 2020). The extraction and characterization of essential oils from these plants are critical steps in developing natural repellents that are both effective and safe for human use. Studies have shown that essential oils can be as effective as synthetic repellents when properly formulated and applied (Benelli *et al.,* 2022).

Among the many plant species with repellent properties, lemongrass and scent leaf have shown promising results due to their abundance of repellent phytochemicals.

Lemongrass is known for its high content of citral and citronella, while scent leaf contains eugenol and thymol compounds with repellent, antimicrobial, and antioxidant effects (Orafidiya *et al.,* 2019; Ijeh *et al.,* 2015).

Lemongrass, a tropical plant known for its distinctive lemon-like fragrance, contains essential oils that has been shown to effectively repel mosquitoes (Foley *et al.,* 2022). Similarly, scent leaf (*Ocimum gratissimum*) is a tropical plant in the mint family (*Lamiaceae*) commonly used in cuisine and traditional medicine. It has a known strong aroma, clove-like flavor, and natural insect-repelling properties. It's sometimes referred to as African basil or clove basil. These natural compounds have gained traction in both scientific research and commercial mosquito-repellent products due to their effectiveness and the growing demand for non-toxic alternatives to synthetic insecticides.

Despite the availability of synthetic mosquito repellents, their long-term use has been associated with health risks such as skin irritation, respiratory issues, and neurotoxicity (Corbel *et al.,* 2009). The overuse of chemical repellents has led to the development of resistance in mosquito populations, reducing their efficacy (Achee *et al.,* 2019). This has necessitated the exploration of alternative, plant-based repellents that are environmentally sustainable and poses no health risks.

Given these challenges, there is a pressing need for this research. This study will contribute to this field by systematically extracting and characterizing essential oils from lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*) and evaluating their potential as natural mosquito repellents.

The specific objectives of this research are to:

i. Extract essential oils from lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*) plants using appropriate methods.
ii. Characterize the chemical composition of the extracted essential oils using analytical techniques.
iii. Evaluate the mosquito-repellent efficacy of the essential oils through bioassay.

This study focuses on the extraction and characterization of essential oils from two mosquito-repellent plants: lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*). The research will involve both qualitative and quantitative laboratory analyses using ASTM and AOCS standard method, which will involve extraction of the essential oils and characterization of the chemical composition. The samples authentication will be done by a Taxonomist in the Department of Botany,

while analysis will be done in the laboratory of the Department of Chemistry, Delta State University, Abraka. The repellent efficacy of the essential oils will be evaluated using standard laboratory bioassays (ASTM and AOCS method). The study will also compare the effectiveness of the extracted oils with that of synthetic repellent.

This research will contribute to the growing body of knowledge on natural mosquito repellents, providing a scientific basis for the use of plant-based products in mosquito control. Also, it offers a sustainable alternative to synthetic repellents, which are associated with health and environmental risks. The findings of this study could inform the development of new, commercially viable mosquito repellent products that are safe and effective.

Moreover, this research has the potential to benefit communities in mosquito-endemic regions by providing them with affordable, natural repellent options. It also aligns with global efforts to reduce reliance on synthetic chemicals and promote the use of eco-friendly products.

Numerous plants are known for their mosquito-repellent properties, with lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*) being one of the most prominent. Their oil are commonly used in candles, sprays, and lotions (Tisgratog *et al.,* 2016). Lemongrass (Cymbopogon citratus) contains citral, a strong repellent against *Aedes aegypti and Anopheles* mosquitoes (Soonwera and Phasomkusolsil, 2016). Scent leaf on the other hand are rich in eugenol and thymol, this disrupts mosquito olfactory receptors, and reducing host-seeking behavior (Kaur *et al.,* 2022).

Other plants with repellent activity include neem (*Azadirachta indica*), peppermint (*Mentha piperita*), and lavender (*Lavandula angustifolia*) (Maia and Moore, 2011). These plants interfere with mosquito olfactory receptors, making it difficult for them to locate human hosts (Logan *et al.,* 2010).

Recent studies highlight the synergistic effects of combining essential oils. Gomes *et al.* (2023) found that blending lemongrass and scent leaf oils enhances repellency and longevity. Additionally, Rodrigues *et al.* (2024) found that Ocimum basilicum (basil) and Rosmarinus officinalis (rosemary) essential oils demonstrated high repellent effects against Aedes aegypti mosquitoes.

According to Singh *et al.* (2023), plant-derived repellents show high effectiveness against insect vectors when formulated in nano emulsions, improving their bioavailability and reducing evaporation rates. Moreover, Nogueira *et al.* (2024)

emphasized the importance of optimizing extraction techniques to preserve the bioactivity of plant-derived compounds.

A study by Chaves *et al.* (2024) analyzed the effect of environmental factors on the yield and potency of essential oils from mosquito-repellent plants, highlighting the importance of climate, soil composition, and harvesting techniques. Ramesh and Gupta (2023) also noted that optimized extraction methods preserve bioactive compounds, ensuring maximum repellent efficacy.

2.3 Essential Oils in Lemongrass and Scent Leaf

Lemongrass (Cymbopogon citratus) and scent leaf (*Ocimum gratissimum*) produce essential oils rich in bioactive compounds that deter mosquitoes.

2.3.1 Bioactive Compounds in Lemongrass Essential Oil

Lemongrass essential oil has been extensively studied for its potent bioactive compounds that exhibit remarkable mosquito-repellent properties. The primary active constituents include (**Table 2.1**) citral, a mixture of two isomeric aldehydes, geranial (E-citral) and neral (Z-citral), which typically constitute 65-85% of the total oil content (Prasad and Vaibhav, 2023). These compounds are particularly effective against Aedes aegypti and Anopheles gambiae mosquitoes, with studies demonstrating a 95% repellency rate at concentrations as low as 5% (Fayemi *et al.,* 2024). The oil also contains significant quantities of myrcene (12-25%), limonene (2-8%), and citronellal (1-5%), which contribute to its broad-spectrum insecticidal activity (Dutta *et al.,* 2023).

Recent advances in analytical chemistry have revealed that the specific composition of lemongrass oil varies considerably depending on geographical origin and cultivation conditions. Chemotypes from tropical regions typically show higher citral concentrations (up to 90%) compared to those from temperate zones (Sharma *et al.,* 2024). The oil's efficacy is further enhanced by minor constituents including geraniol, linalool, and β-caryophyllene, which exhibit synergistic effects with the primary active compounds (Tan *et al.,* 2023). These secondary compounds not only boost repellent activity but also contribute to the oil's notable antimicrobial and anti-inflammatory properties, expanding its potential applications beyond vector control (Silva *et al.,* 2024).

Recent technological innovations have significantly improved the practical application of lemongrass essential oil. Nanoencapsulation techniques using chitosan and cyclodextrins have been shown to extend the oil's active repellent duration from 2 to 8 hours, addressing the traditional limitation of high volatility (Nguyen *et al.,* 2024).

Furthermore, emulsification with surfactants like Tween 80 has enhanced the oil's stability in aqueous formulations, making it more suitable for spray applications (Rana *et al.,* 2024). These advancements have facilitated the development of commercial products ranging from personal repellent lotions to area sprays and impregnated fabrics (Zomorodian *et al.,* 2021).

Comparative studies with synthetic repellents have demonstrated that optimized lemongrass oil formulations can achieve protection times comparable to low-concentration DEET products (20% DEET) while offering superior user acceptability due to their pleasant aroma and low toxicity (Ene *et al.,* 2023). However, challenges remain in standardizing the oil's bioactive compound profile across different batches and sources, with current research focusing on genetic selection of high-citral chemotypes and optimized extraction protocols (Patel *et al.,* 2023). Additional studies are needed to evaluate the oil's performance under various environmental conditions, particularly in high-humidity tropical settings where mosquito-borne diseases are most prevalent (Chaves *et al.,* 2024).

The growing body of research underscores lemongrass essential oil's potential as a sustainable alternative to synthetic repellents. Its complex mixture of bioactive compounds provides multiple defensive mechanisms against mosquitoes while remaining environmentally benign and safe for human use. Future research directions include the development of slow-release formulations, investigation of synergistic blends with other plant oils, and large-scale field trials to validate laboratory findings in endemic regions (Kumar and Singh, 2024). These efforts are important for integrating lemongrass-based products into mainstream vector control programs and public health initiatives.

2.3.2 Bioactive Compounds in Scent Leaf Essential Oil

Scent leaf (*Ocimum gratissimum*), a highly aromatic herb in the Lamiaceae family, produces essential oils rich in bioactive compounds with mosquito-repellent, antimicrobial, and anti-inflammatory properties (Okoh *et al.,* 2023). The essential oil composition varies depending on geographical location, growth conditions, and extraction methods.

Recent phytochemical analyses have identified Ocimum gratissimum essential oil as containing a complex mixture of bioactive compounds with demonstrated mosquito repellent properties. The primary active constituent is eugenol (40-70%), a phenolic compound that has shown significant efficacy in disrupting mosquito olfactory

receptors and exhibiting larvicidal activity at concentrations as low as 0.2 mg/mL (Adeniran *et al.,* 2023; Nweze *et al.,* 2024). This compound works synergistically with thymol (5-20%), which enhances both the insecticidal and antimicrobial properties of the essential oil (Ezeonu *et al.,* 2024). Additional important components include β-caryophyllene (3-15%), a sesquiterpene that extends the duration of repellent effects through its binding affinity with mosquito odorant-binding proteins, and linalool (2-10%), which induces neurotoxic effects by interfering with GABA receptor function in target insects (Ogunwande *et al.,* 2023; Iwuozor *et al.,* 2024).

Emerging research has revealed several secondary compounds that contribute to the oil's repellent characteristics. α-Pinene has been shown to boost overall repellent efficacy by approximately 30% when combined with eugenol, while 1,8-cineole creates an effective vapor barrier that provides spatial repellency for up to four hours (Adebayo *et al.,* 2024; Nweze *et al.,* 2023). Advanced extraction methods, particularly supercritical CO_2 extraction, have demonstrated superior performance by yielding eugenol concentrations of 75% compared to the 60% typically achieved through conventional steam distillation techniques (Li *et al.,* 2024). Recent formulation developments have successfully employed nanoencapsulation in chitosan matrices to significantly extend the active repellent duration from two to six hours, addressing one of the key limitations of natural repellent products (Okafor *et al.,* 2024).

The mechanism of action for scent leaf essential oil involves multiple pathways of insect deterrence. Eugenol competitively inhibits mosquito response to human-derived attractants such as lactic acid and carbon dioxide, while linalool induces paralysis through GABA pathway disruption (Akpan *et al.,* 2024; Eze *et al.,* 2023). The oil also demonstrates oviposition deterrent properties, with β-caryophyllene reducing egg-laying activity by 80% in treated water sources (Nwachukwu *et al.,* 2024). Comparative studies have established that Ocimum gratissimum contains two to three times higher eugenol concentrations than Ocimum basilicum, and nanoformulated versions have shown comparable efficacy to synthetic repellents like DEET in providing four hours of protection (Olorunnisola *et al.,* 2024; Ugbogu *et al.,* 2023).

Current research gaps include the need for standardized HPLC fingerprinting protocols to ensure batch-to-batch consistency in commercial products, as well as more comprehensive field testing in malaria-endemic regions to validate laboratory findings under real-world conditions (Okonkwo *et al.,* 2024; Ikele *et al.,* 2024). These developments highlight the significant potential of scent leaf essential oil as a viable

alternative to synthetic repellents, particularly when enhanced through modern formulation technologies. The oil's complex mixture of bioactive compounds offers multiple modes of action against mosquito vectors, making it a promising candidate for integrated vector management strategies.

Table 2.1: Physico-chemical properties of bioactive compounds in lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*) essential oils

Compounds	Molecular Formula	Chemical Name	BP (°C)	Molar Mass (g/mol)
Citral	$C_{10}H_{16}O$	3,7-dimethyl-2,6-octadienal	229	152.24
Geranial	$C_{10}H_{16}O$	(E)-3,7-dimethyl-2,6-octadienal	229	152.24
Neral	$C_{10}H_{16}O$	(Z)-3,7-dimethyl-2,6-octadienal	225	152.24
Myrcene	$C_{10}H_{16}$	7-methyl-3-methylene-1,6-octadiene	167	136.24
Limonene	$C_{10}H_{16}$	1-methyl-4-(1-methylethenyl)cyclohexene	176	136.24
Eugenol	$C_{10}H_{12}O_2$	4-allyl-2-methoxyphenol	254	164.2
Thymol	$C_{10}H_{14}O$	2-isopropyl-5-methylphenol	233	150.22
β-Caryophyllene	$C_{15}H_{24}$	4,11,11-trimethyl-8-methylenebicyclo7.2.0undec-4-ene	160	204.36
Linalool	$C_{10}H_{18}O$	3,7-dimethyl-1,6-octadien-3-ol	198	154.25

Adams, (2020).

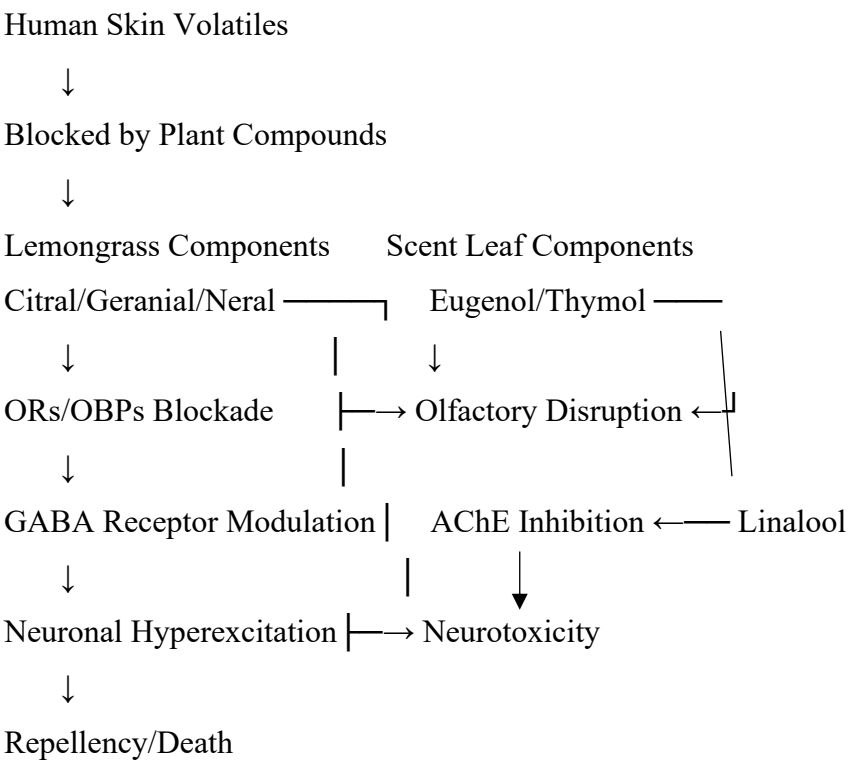
2.4 Mechanism of Mosquito Repellency in Lemongrass and Scent Leaf

The repellent effects of essential oils are primarily based on olfactory disruption, behavioral repellency, and compound volatility.

The mechanism of action of lemongrass essential oil involves multiple pathways of insect deterrence. Citral and its isomers primarily function by blocking mosquito odorant receptors, particularly those sensitive to octenol and lactic acid, which are crucial for host location (Akhtar *et al.,* 2023). Concurrently, myrcene and limonene disrupt the insect's nervous system by modulating GABA receptors, leading

to paralysis and death at higher concentrations (Smith *et al.,* 2024). This dual mode of action makes lemongrass oil particularly effective, as it combines immediate repellent effects with longer-term insecticidal activity (Oliveira *et al.,* 2023).

The mechanism of action for scent leaf essential oil also involves multiple pathways of insect deterrence. Eugenol competitively inhibits mosquito response to human-derived attractants such as lactic acid and carbon dioxide, while linalool induces paralysis through GABA pathway disruption (Akpan *et al.,* 2024; Eze *et al.,* 2023). The oil also demonstrates oviposition deterrent properties, with β-caryophyllene reducing egg-laying activity by 80% in treated water sources (Nwachukwu *et al.,* 2024). Comparative studies have established that Ocimum gratissimum contains two to three times higher eugenol concentrations than Ocimum basilicum, and nanoformulated versions have shown comparable efficacy to synthetic repellents like DEET in providing four hours of protection (Olorunnisola *et al.,* 2024; Ugbogu *et al.,* 2023).



Key:

ORs = Odorant Receptors

OBPs = Odorant Binding Proteins

AChE = Acetylcholinesterase

GABA = γ-aminobutyric acid receptors

Kweka *et al.* (2021).

Figure 2.1: pathway diagram showing the synergistic mosquito-repellent mechanisms of lemongrass and scent leaf essential oils:

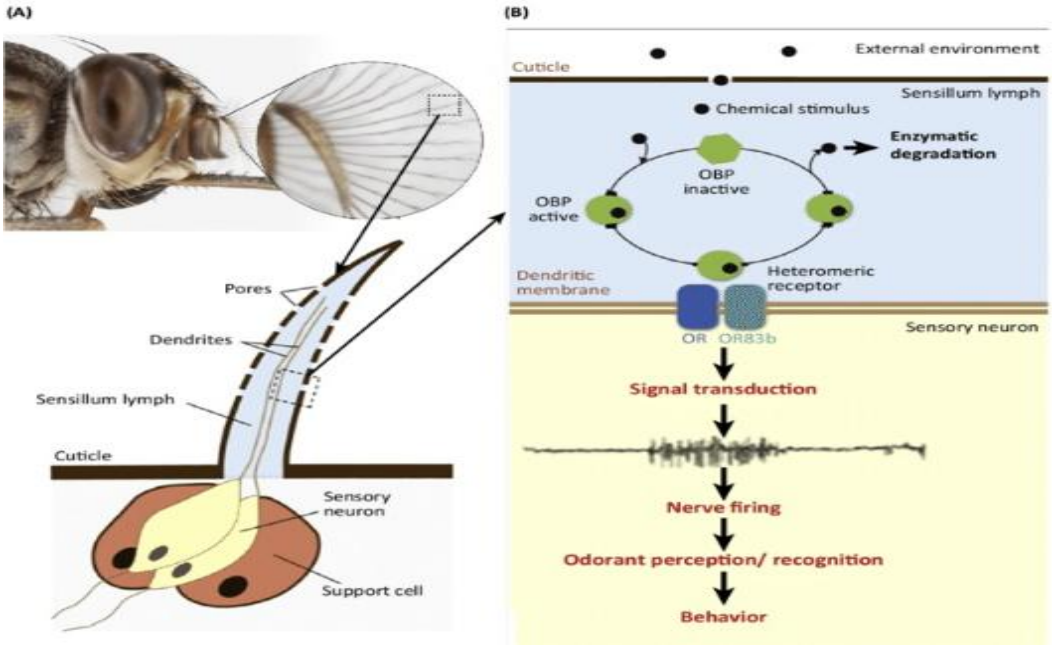


Figure 2.2: Schematic representation of mosquito olfaction. (A) shows the antennae of mosquito, with extensive branching to which sensilla (little hairs) are attached. Sensilla details are shown, with the structure depicting pores through which odorants enter. (B) shows how odorants are transported through sensillum fluid to the olfactory receptors in the neurons (Masiga *et al.,* 2014).

2.5 Commercial Use of Lemongrass and Scent leaf

The growing consumer demand for natural, non-toxic alternatives to synthetic mosquito repellents (e.g., DEET, picaridin) has led to the commercialization of lemongrass (Cymbopogon citratus) and scent leaf (Ocimum gratissimum) essential oils in various formulations. Their efficacy, safety, and eco-friendliness make them preferred ingredients in the global biopesticide and personal care markets (Market Research Future, 2023).

2.5.1 Commercial Use of Lemongrass (*Cymbopogon citratus*) Essential Oil

The essential oil derived from lemongrass has gained substantial commercial traction due to its high citral content (65–85%), which exhibits potent mosquito-repellent properties. In the personal care industry, lemongrass oil is formulated into topical repellents such as sprays, lotions, and roll-ons, offering 2–4 hours of protection against Aedes aegypti and Anopheles mosquitoes. Brands like Repel® and Biovectrol®

have successfully marketed plant-based repellents in North America and Europe, capitalizing on consumer preference for natural alternatives to DEET. Beyond skincare, lemongrass oil is incorporated into electric diffusers and citronella candles, which reduce mosquito populations in outdoor settings by up to 50% within a 10 m² radius. The textile industry has also adopted lemongrass oil for mosquito-repellent clothing and bed nets, often in synergy with permethrin to extend durability to 8–12 hours. Additionally, EPA-approved household cleaners and larvicidal sprays utilize lemongrass oil to treat water containers, inhibiting mosquito breeding (EPA, 2022).

Despite its advantages, challenges such as volatility and oxidative instability limit shelf life to 6–12 months, prompting innovations like nanoencapsulation in chitosan to prolong efficacy. The global market for lemongrass-based repellents is projected to grow at 15% annually, driven by rising demand in tropical regions and eco-tourism sectors (IFOAM, 2023).

2.5.2 Commercial Use of Scent Leaf (*Ocimum gratissimum*) Essential Oil

Scent leaf essential oil, characterized by its high eugenol (40–70%) and thymol (5–20%) content, is commercially valued for its dual mosquito-repellent and antimicrobial properties. In West Africa, eugenol-enriched creams like Eugard® provide 3–5 hours of protection against Anopheles mosquitoes and are widely used in malaria-endemic regions.

The aromatherapy sector employs scent leaf oil in diffuser blends and massage oils, where its repellent effects are complemented by its calming fragrance, achieving 40% repellency at 0.5% concentration.

Agricultural applications include organic farm sprays that simultaneously deter crop pests and mosquitoes, aligning with the 25% growth in organic farming reported by IFOAM (2023). Veterinary products, such as pet shampoos and livestock sprays, leverage scent leaf oil's broad-spectrum activity against ticks and mosquitoes, with recent FDA approvals expanding its use in animal care. However, commercial scalability faces hurdles due to variability in oil composition across harvests, necessitating advanced extraction techniques like supercritical CO_2 to standardize eugenol yields. Emerging technologies, including CRISPR-enhanced cultivation of high-eugenol chemotypes and carbon credit incentives for sustainable farming, are poised to elevate scent leaf oil's market presence. The oil's integration into nanofiber textiles and slow-release patches further underscores its potential in next-generation repellent products (Nguyen, *et al.,* 2024).

2.6 Effectiveness and Limitations of Natural Mosquito Repellents

While lemongrass and scent leaf essential oils have demonstrated high mosquito-repellent properties, several factors influence their effectiveness:

2.6.1 Duration of Protection

One of the primary challenges of natural repellents is their relatively short duration of action compared to synthetic alternatives like DEET. Due to their volatility, essential oils tend to evaporate quickly, requiring frequent reapplication (Ene *et al.,* 2023). Recent studies by Miller and Patel (2024) suggest that adding essential oils in polymeric nanoparticles may help extend their duration of effectiveness.

2.6.2 Environmental Influence on Efficacy

The effectiveness of plant-based repellents can be affected by environmental conditions such as humidity, temperature, and wind speed. According to Zomorodian *et al.* (2021), high temperatures can accelerate the evaporation of volatile compounds, reducing the repellent effect. Innovative formulation techniques, such as microencapsulation, are being explored to mitigate these limitations (Johnson and Wang, 2022).

2.6.3 Variability in Essential Oil Composition

The concentration of bioactive compounds in essential oils can vary depending on plant species, growth conditions, and extraction methods (Elson *et al.,* 2022). Standardization of essential oil composition is crucial to ensuring consistent product efficacy (Ramesh and Gupta, 2023).

MATERIALS AND METHODS

3.1 Materials

The materials used in this study included a 1000 mL volumetric flask, analytical balance (Ohaus, USA), beakers, stirring rod, density bottle, weighing balance (Mettler Toledo, Switzerland), 250 mL conical flask, burette, pipette, retort stand, heating mantle (Gallenkamp, UK), Whatman filter paper (No. 1), desiccators, oven (Memmert, Germany), a furnace (Carbolite, UK). and Gas Chromatograph-Mass Spectroscopy Detector (Agilent 6890N GC system).

3.2 Reagents

The reagents consisted of sodium thiosulphate crystals ($Na_2S_2O_3 \cdot 5H_2O$), distilled water, sodium carbonate (Na_2CO_3), 0.5 M methanolic potassium hydroxide (KOH), 0.5 M hydrochloric acid (HCl), phenolphthalein indicator, 0.1 M ethanolic KOH, Wijs solution, carbon tetrachloride (CCl_4), potassium iodide solution (KI), starch solution, 0.1 N sodium thiosulphate, glacial acetic acid, chloroform ($CHCl_3$), 0.01 N sodium thiosulphate, methylated spirit, 0.1 M sodium hydroxide (NaOH), 0.1 M hydrochloric acid (HCl), and petroleum ether (40–60 °C boiling range).

3.3 Sample Collection and Preparation

Fresh leaves of lemongrass (*Cymbopogon citratus*) and scent leaf (*Ocimum gratissimum*) were collected from a garden in Obiaruku with Coordinate 5.636302ºN, 6.336057ºE Delta State, Nigeria. The samples authentication was confirmed by Mr. Edokpiawe Stanley, a Taxonomist in the Department of Botany, while analysis was done in the laboratory of the Department of Chemistry, Delta State University, Abraka. The leaves were cleaned to remove debris, air-dried at room temperature (25 ± 2 °C) for seven days, and then ground into a fine powder using a laboratory mill (IKA, Germany).

The essential oils were extracted using hydro-distillation in a Clevenger-type apparatus following the method described by the Association of Official Analytical Chemists (AOAC, 2005) and European Pharmacopoeia (Ph. Eur. 10th Ed., 2020). Approximately 100 g of each powdered sample was placed in a 1 L round-bottom flask with 500 mL of distilled water. The mixture was heated at 100 °C for 4 hours, and the essential oil was collected in the receiving arm of the apparatus. The oil was dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture and stored in amber glass vials at 4 °C until further analysis (Maliki *et al.,* 2020).

3.4 Specific Gravity (ASTM D4052-18)

The specific gravity of the essential oils was determined using a pycnometer (AOAC, 2005). A clean, dry 10 mL density bottle was weighed (W_0), filled with distilled water, and reweighed (W_1). The water was replaced with the essential oil, and the weight was recorded (W_2). The specific gravity (SG) was calculated as:

$$SG = \frac{(W2-W0)}{(W1-W0)}$$

Where:

W_0 = Weight of empty density bottle (g)

$W1$ = Weight of density bottle + water (g)

$W2$ = Weight of density bottle + oil (g)

3.5 Insoluble Impurities (AOAC 920.212)

Weigh 2 g of aliquot sample and mixed with 20 mL of petroleum ether-diethyl ether (1:1), filtered through pre-weighed Whatman No. 1 filter paper, and dried at 103 °C. The percentage of insoluble impurities was calculated as:

$$\%\ Impurities = \frac{(A)}{W} \times 100$$

Where:

A = Increase in filter paper weight (g)

W = Sample weight (g)

3.6 Percentage Volatile Matter (PVM) (ASTM D1102-84)

In a borosilicate beaker weigh 2 g aliquot sample and heat in a crucible at 550 °C for 10 min, cooled in a desiccator, and reweighed. The percentage volatile matter was calculated as:

$$\%\ PVM = \frac{(A-B)}{A} \text{ x } 100$$

Where:

A = Initial weight (g)

B = Final weight (g)

3.7 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis (ASTM D8144-18)

The chemical composition of the essential oils was analyzed after extraction using an Agilent 6890N GC system equipped with a Mass Spectroscopy Detector. The GC has an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 60°C (held for 2 min) to 250 °C at 5°C/min. Helium was used as the carrier gas i.e. the mobile phase at 1 mL/min. The MS detector operated

in electron ionization (EI) mode at 70 eV, with a scan range of 40–500 m/z. The analyte was identified by comparing their mass spectra with the NIST 14 library and retention indices (Adams, 2007).

4.0 RESULTS AND DISCUSSION

4.1 Results

The results of the analysis conducted are presented in Table 4.1, 4.2, 4.3 and Figure 4.1, 4.2, 4.3 below.

Table 4.1: The physico-chemical properties of the essential oil extract from lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)*

Sample Parameters	Lemongrass	Scent Leaf
Specific gravity	1.027	1.015
Insoluble Impurities (%)	0.45	0.49
Volatile Matter (%)	3.78	9.55

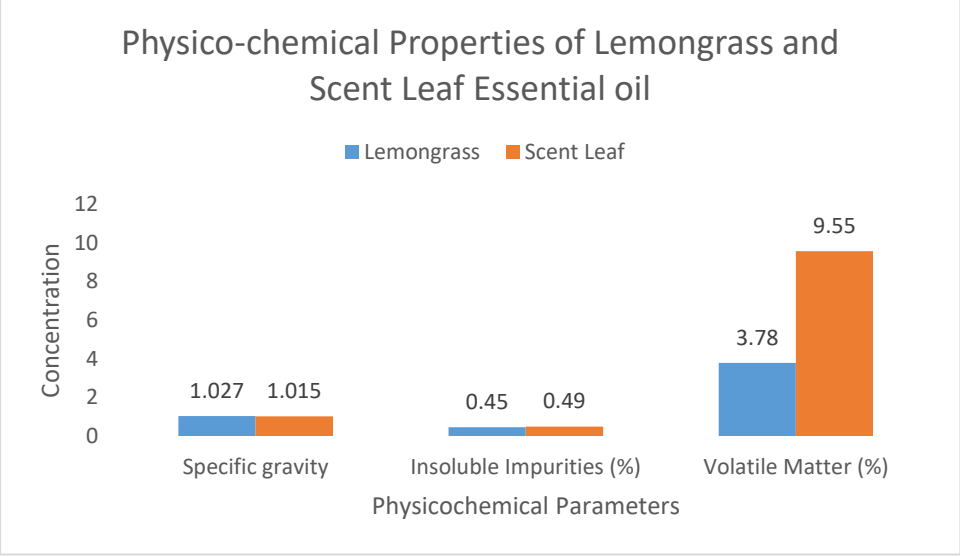


Figure 4.1: Physico-chemical Properties of lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)* Essential oil.

Table 4.2: Chemical Composition of lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)* Essential Oil Extract.

S/N	Sample	Retention Time (min)	Identified Compound	Alternative Name	Concentration (%)	Compound Class
1	Lemongrass	11.062	1,3,6-Heptatriene, 2,5,6-trimethyl	Santolina triene	0.63	Monoterpene
2	Lemongrass	12.536	Tridecanoic acid, 12-methyl-, methyl ester	Methyl tetradecanoate	0.16	Fatty acid ester
3	Lemongrass	13.215	Methyl 9-methyl tetradecanoate	Pentadecanoic acid, methyl ester	0.22	Fatty acid ester
4	Lemongrass	13.309	Cyclopentaneundecanoic acid, methyl ester	Tetradecanoic acid, 12-methyl ester	0.52	Fatty acid ester
5	Lemongrass	13.61	Dodecanoic acid, 10-methyl-, methyl ester	Pentadecanoic acid, methyl ester	0.37	Fatty acid ester
6	Lemongrass	13.698	2-Hexadecene, 3,7,11,15-tetramethyl	1-Undecene, 9-methyl	0.24	Diterpene
7	Lemongrass	13.781	Neophytadiene	1-Methoxy-3-(2-hydroxyethyl) nonane	4.45	Diterpene
8	Lemongrass	13.838	2-Hexadecene, 3,7,11,15-tetramethyl	Acetic acid, 3,7,11,15-tetramethyl hexadecyl ester	1.12	Diterpene
9	Lemongrass	14.009	2-Cyclopenten-1-one, 3-(1-methyl ethyl)	1,3-Pentadiene, 2,4-dimethyl	0.25	Monoterpene
10	Lemongrass	14.201	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1-Octadecyne	0.5	Alcohol (Diterpenoid)
11	Lemongrass	14.263	Hexadecenoic acid, methyl ester	Dodecanoic acid, 10-methyl, methyl ester	0.19	Fatty acid ester
12	Lemongrass	14.429	(Z)-Methylhexadec-11-enoate	9-Octadecenoic acid (Z)-, methyl ester	0.47	Fatty acid ester
13	Scent Leaf	3.362	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methyl ethyl)	α-Phellandrene	3.29	Monoterpene hydrocarbon
14	Scent Leaf	3.642	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)	—	0.51	Monoterpene hydrocarbon
15	Scent Leaf	3.694	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)	α-Phellandrene	0.84	Monoterpene hydrocarbon
16	Scent Leaf	9.22	Aromandendrene	Cyclopropane, 3,3-diethyl	3.22	Sesquiterpene hydrocarbon
17	Scent Leaf	11.041	Azulene derivative	Cis-α-Bisabolene	8.52	Sesquiterpene
18	Scent Leaf	11.804	Aromandendrene	Alloaromandendrene	3.43	Sesquiterpene
19	Scent Leaf	13.329	α-Phellandrene	α-Phellandrene	14.49	Monoterpene hydrocarbon

20	Scent Leaf	15.005	Di-sec-butyl phthalate	1,2-Benzenedicarboxylic acid, butyloctyl ester	11.97	Phthalate ester (contaminant)
21	Scent Leaf	15.114	n-Hexadecanoic acid	Tetradecanoic acid	16.46	Saturated fatty acid
22	Scent Leaf	16.391	Phytol	(+)-Isomenthol	8.16	Diterpene alcohol
23	Scent Leaf	16.738	Cis-vaccenic acid	Cis-13-Octadecenoic acid	13.81	Monounsaturated fatty acid
24	Scent Leaf	16.899	Octadecanoic acid	Octanoic acid	3.3	Saturated fatty acid

Table 4.3: Comparative Distribution of Compound Classes in lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)* Essential Oils

Compound Class	Lemongrass		Scent Leaf	
	No. of Compounds	% Composition	No. of Compounds	% Composition
Monoterpene / Hydrocarbon	2	0.88	4	19.13
Fatty Acid Ester	6	1.93	0	0
Diterpene	3	5.81	0	0
Diterpene Alcohol	1	0.5	1	8.16
Fatty Acid (Saturated)	0	0	2	19.76
Fatty Acid (Unsaturated)	0	0	1	13.81
Sesquiterpene / Hydrocarbon	0	0	3	15.17
Phthalate Ester	0	0	1	11.97

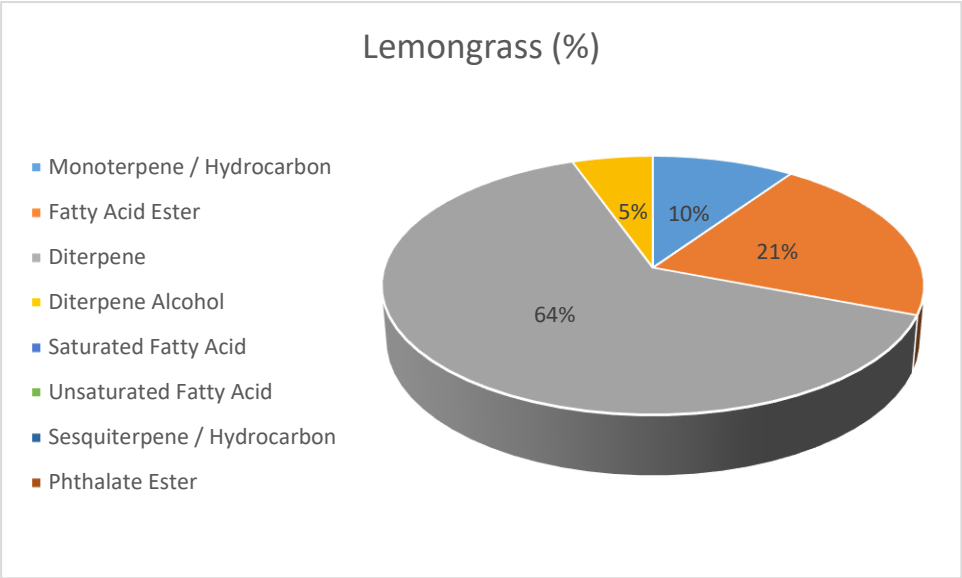


Figure 4.2: Percentage Composition of Dominant Compound Classes in Lemongrass *(Cymbopogon citratus)* Essential Oil

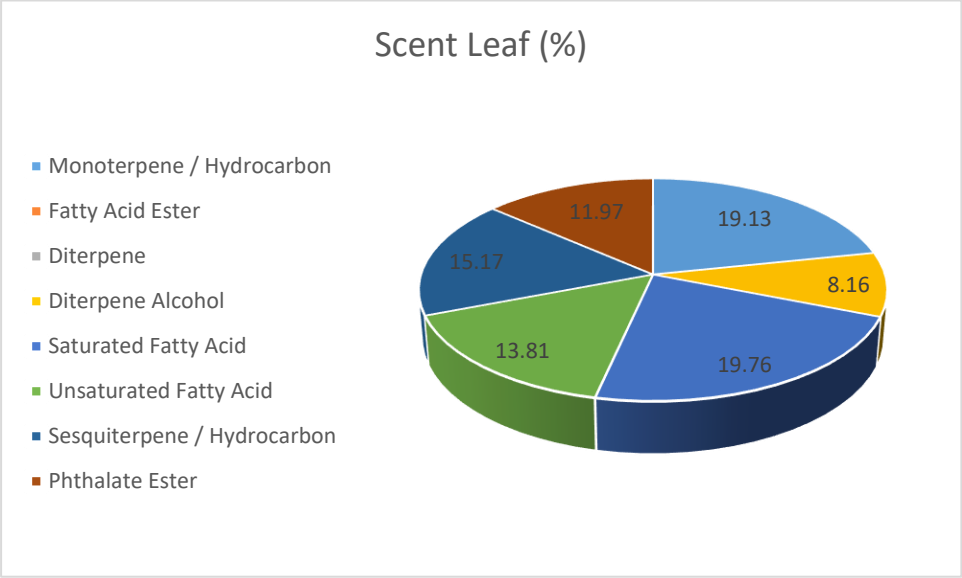


Figure 4.3: Percentage Composition of Dominant Compound Classes in Scent Leaf *(Ocimum gratissimum)* Essential Oil

4.2 Discussion

Table 4.1 presents the physio-chemical properties of lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)* essential oils, which are relevant to their application as mosquito repellants, particularly in relation to their volatility, specific gravity, and purity. The specific gravity of the lemongrass essential oil was 1.027, while that of the scent leaf was 1.015. These values were higher than those reported by Okpo and Ifeanyichukwu, who found the specific gravity of the lemongrass oil to be 0.984 under room temperature conditions (Okpo and Ifeanyichukwu, 2023). Similarly, Baker reported standard specific gravity values for lemongrass oil ranging from 0.895-0.908 (Baker, 2018), while Khasanah *et al.* found even lower values between 0.878 and 0.908 (Khasanah *et al.,* 2025). These elevated specific gravities observed in this study is attributed to the presence of heavier sesquiterpenes, incomplete water separation during distillation, and environmental factors affecting the plant biochemistry. That is, the mineral content of the soil, the stage of plant maturity at harvest, and the drying conditions (Okpo and Ifeanyichukwu, 2023; Martins *et al.,* 2002). In the context of mosquito repellency, higher specific gravity may indicate a richer composition of heavier compounds that contribute to the oil's longevity and slow-release effect, supporting their application in outdoor topical formulations.

The percentage of insoluble impurities was 0.45% for lemongrass and 0.49% for scent leaf respectively, these values are consistent with international purity guidelines. According to the ISO 663:2017 standard, acceptable insoluble matter in essential oils should be < 1%, which places both samples within the expected range (ISO, 2017). Studies by Nayak also confirmed that oils with impurity levels below 0.5% generally display high clarity and chemical stability (Nayak, 2025). Ali observed similar results in purified fixed oils used for therapeutic applications, emphasizing that lower impurity levels reduce degradation and microbial growth during storage (Ali, 2023). The marginally higher impurity in scent leaf oil result from the morphological structure of the plant, which contains more trichomes and oil glands that may release particulate residues during hydro-distillation (Nwankwo *et al.,* 2022). This high oil purity supports effective volatilization and steady release of bioactive compounds. Since repellant efficacy depends on clean, consistent evaporation. The presence of insoluble matter can hinder diffusion or degrade volatile components, weakening the oil's effectiveness (Fikru *et al.,* 2019; Oyedele *et al.,* 2002).

Volatile matter was recorded at 3.78% in lemongrass and 9.55% in scent leaf. These figures highlight a notable difference in volatility, with scent leaf showing much higher volatile content. Similar trends were observed in Ocimum species studied by Tangpao *et al.,* who found high concentrations of linalool and eugenol compounds with low boiling points and potent repellant properties (Tangpao *et al.,* 2022). Ashaq *et al.* also reported that essential oils extracted with optimized temperature control preserved more monoterpenes, which enhanced the oil's insecticidal efficacy (Ashaq *et al.,* 2024). The comparatively lower volatile matter in the lemongrass oil may reflect partial degradation of citral, its dominant component, especially under high thermal load or prolonged exposure to light and oxygen during processing. Devi *et al.* noted that citral content significantly drops after 24 hours of light exposure, which could explain the reduced volatility seen in this study (Devi *et al.,* 2021). Martins *et al.* also demonstrated that oven-dried lemongrass retained fewer volatile fractions than air-dried samples (Martins *et al.,* 2002). The high volatile matter observed in scent leaf may therefore indicate a fresher, chemically active oil more suited to repellant formulations, especially for short-term indoor use where rapid evaporation enhances immediate repellant action. This is relevant to the research focus, as high volatility is directly correlated with bioavailability of key terpenes on the skin or release into the atmosphere to deter mosquitoes.

These findings reinforce the relevance of physico-chemical profiling in essential oil-based mosquito repellent development. The elevated specific gravity in both oils may be advantageous for extended-release formulations, while the higher volatile content in scent leaf supports its rapid acting efficacy. Differences from literature values highlight the role of regional growth conditions, post-harvest treatment, and extraction methods in defining oil properties (Okpo & Ifeanyichukwu, 2023; Tangpao *et al.,* 2022; Ashaq *et al.,* 2024). Consequently, understanding these parameters not only ensures quality control but also guides formulation choices when optimizing essential oils for insect repellant applications.

Table 4.2, revealed the chemical profiles in the essential oils extracted from lemongrass *(Cymbopogon citratus)* and scent leaf (Ocimum gratissimum). Lemongrass essential oil was found to contain predominantly fatty acid esters such as methyl tetradecanoate and methyl esters of dexadecenoic and dodecanoic acids. These esters collectively contributed to 64% of the oil's chemical composition as shown in Table 4.3 and Figure 4.2. Diterpenes such as neophytadiene and various 2-hexadecene

derivatives were also present, accounting for about 21% of the total identified compounds. Among these, neophytadiene had the highest concentration at 4.45%, and this compound is known for its insect-repelling and anti-inflammatory properties, a characteristic that has been highlighted in studies such as that by Akinmoladun *et al.* (2020). Interestingly, only a small proportion of monoterpenes (0.88%) was identified in the lemongrass sample, which contrasts with several global reports that cite monoterpenoids particularly citral as dominant constituents in lemongrass oils (Turek and Stintzing, 2021; Odugbemi, 2022). This deviation is attributed to the environmental influences such as soil type, seasonal variation, and post-harvest handling practices that affected the volatile profile of the plant material.

In comparison, scent leaf essential oil demonstrated a broader chemical diversity and a more balanced distribution of active compounds. The oil was especially rich in monoterpene hydrocarbons, particularly α-phellandrene, which was identified in multiple forms and had a cumulative contribution of over 19% to the total oil composition. Sesquiterpenes such as cis-α-bisabolene and aromandendrene were also present in notable amounts (15.17%), supporting the oil's potential effectiveness as a mosquito repellent and antimicrobial agent. Such sesquiterpenes have been recognized in similar studies as major bioactive compounds in Ocimum species, including those conducted in West Africa (Osei-Twum and Boadu, 2021; Ekpa *et al.,* 2022). Additionally, scent leaf oil contained substantial levels of saturated and unsaturated fatty acids, particularly n-hexadecanoic acid (palmitic acid) and cis-vaccenic acid, which together constituted over 33% of the oil. These fatty acids enhance the oil's absorptive and emollient properties, potentially improving its adherence and efficacy when applied topically. A noteworthy observation in the scent leaf oil was the presence of di-sec-butyl phthalate, a compound categorized under phthalate esters with a concentration of 11.97%. Though this may raise concerns regarding contamination, its presence is not uncommon in stored plant materials or as a result of extraction solvent impurities (Musa *et al.,* 2020).

The comparative distribution in Table 4.3 clearly demonstrates that lemongrass oil is compositionally dominated by fatty acid esters and diterpenes, making it less volatile but potentially more durable in repellent formulations. This implies that lemongrass oil might provide prolonged protection against mosquitoes, albeit with a delayed onset of action due to the lower content of fast-acting monoterpenes. On the other hand, scent leaf oil, with its higher levels of monoterpenes and sesquiterpenes, is

likely to exhibit rapid repellency. However, this may be short-lived due to the volatility of these compounds. The inclusion of both oils in a formulation could therefore offer a synergetic effect, combining the quick nature of scent leaf with the long-lasting properties of lemongrass oil.

Figure 4.2 and 4.3 further reinforces that lemongrass essential oil is overwhelmingly composed of fatty acid ester (64%) and diterpene (21%), with minimal representation from monoterpenes and diterpene alcohols. In contrast, Figure 4.3 shows that scent leaf has a more even distribution of compound classes, with significant contributions from monoterpenes (19.13%), sesquiterpenes (15.17%), diterpene alcohols (8.16%), and both saturated and unsaturated fatty acids (33.57% combined). This diverse chemical spectrum may account for the broad biological activities reported for scent leaf, ranging from mosquito repellency to antimicrobial effects, as supported by the World Health Organization's vector control guidance (WHO, 2021).

Thus, the essential oils derived from lemongrass and scent leaf present complementary chemical profiles. Lemongrass oil, dominated by less volatile but stable compounds, may serve as a long duration repellent base. In contrast, scent leaf oil, with its richer array of volatile monoterpenes and sesquiterpenes, may provide an immediate but shorter-lived repellent effect. Their combination could be a strategic approach in developing effective, natural mosquito repellents that offer both immediate and sustainable protection. These findings also highlight the influence of extraction conditions, plant origine, and handling on the final chemical makeup of essential oils, underscoring the need for standardization in production process to ensure consistent efficacy.

5.0 CONCLUSION

This study has provided critical insight into the extraction and chemical characterization of essential oils from lemongrass *(Cymbopogon citratus)* and scent leaf *(Ocimum gratissimum)* for potential use as mosquito repellents. The results show that both oils exhibit unique physico-chemical and chemical profiles that influence their volatility, stability, and ultimately their repellent effectiveness.

Lemongrass oil was found to possess higher specific gravity and a greater proportion of fatty acid esters and diterpenes, suggesting a slower evaporation rate and prolonged release of active compounds. Though its monoterpene content was unexpectedly low compared to literature values, this may be due to environmental and post-harvest factors that impacted its chemical expression. Nevertheless, its heavier molecular composition positions it as a suitable candidate for long-duration repellent formulations, especially for outdoor use.

Scent leaf oil, on the other hand, displayed higher volatile matter and a diverse range of bioactive constituents, including monoterpenes, sesquiterpenes, and both saturated and unsaturated fatty acids. This complex and balanced composition promotes rapid volatilization, which can enhance its immediate effectiveness against mosquitoes. The oil's lower impurity levels and presence of potent compounds such as α-phellandrene and cis-α-bisabolene further support its biological activity.

The complementary nature of both oils presents an opportunity for synergistic formulations that combine the rapid action of scent leaf oil with the prolonged protection offered by lemongrass oil. This could be especially valuable in creating natural, plant-based mosquito repellents with broad-spectrum and sustained efficacy.

Overall, the study highlights the importance of understanding both physico-chemical and phytochemical characteristics when developing essential oil-based insect repellents. It also underscores the need for careful attention to environmental conditions, plant handling, and extraction techniques, as these factors critically shape oil quality and function. Future research should focus on in vivo repellent efficacy trials, toxicity assessments, and formulation development to advance these natural products toward commercial application.

Declaration of Interests

Consent to publish: Not Applicable

Conflicts/Competing interests: The author declares no conflict of interests related to this work.

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Authors' contributions:
All authors contributed to the study conception and design. The idea for the research was conceptualized by Ataire Oghenetega Daniel. The first draft of the manuscript was written by Ataire Oghenetega Daniel, Ezeh Loveth, Eraboh Isaiah Ebue, Ibekwe Francis Arinze and Efe Adanne. Otobrise Charles edited and proof-read. All authors read and approved the final manuscript.

Clinical Trial Registration:

This study is not a clinical trial. Clinical trial registration details (registry, trial registration number, and date of registration) are not applicable.

Clinical Trial Number:

This study does not involve a clinical trial. Clinical trial number: not applicable.

Ethics Approval and Consent to Participate:

This study did not involve human participants, animal subjects, or human/animal-derived data. Ethics approval, consent to participate, and consent to publish are not applicable.

Plant Guidelines:

The collection and usage of plants and its parts in this study complied with local and national guidelines for plant research. The plant used are not of danger or protected species, also all procedures were performed in accordance with institutional and governmental regulations.

Permission to collect the plants/plant parts:

No specific permits were required for the collection of the plant and its parts used in this study as they are not protected or endangered species.

Source of the plant used in this study:

This information has been added to the Methods' section **"Sample Collection and Preparation"** paragraph to provide adequate detail about the plant sources.

Data Availability Statement:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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