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RESEARCH ARTICLE

Mosquito Repellency, Physiochemical Properties and Chemical Composition of Volatiles of *Ocimum tenuiflorum* Growing in Different Agro-Ecological Zones in Kenya

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ABSTRACT

Concerns over insecticide resistance and environmental impact necessitate effective alternatives to synthetic repellents like DEET. *Ocimum tenuiflorum* (Holy Basil), traditionally used in Kenya by communities including the Kamba, Kikuyu, and Maasai for repelling insects, presents a promising candidate. However, its efficacy, chemical composition, and yield may vary with growing conditions. This study therefore assessed how different Kenyan agro-ecological zones (Kajiado, Kiambu, and Kitui Counties) affect the yield, chemical composition, and repellent efficacy of *Ocimum tenuiflorum* essential oils against *Anopheles gambiae*. Plants were harvested from these zones, their essential oils extracted via hydro-distillation, analyzed using GC-MS, and tested for repellency via the WHO arm-in-cage bioassay. Essential oil yield varied significantly ($F_{(2,12)} = 567.3$, $p<0.001$), with Kiambu yielding the highest (0.40% ± 0.0079), followed by Kajiado (0.30% ± 0.0079) and Kitui (0.26% ± 0.0035). Physicochemical properties were consistent across zones. GC-MS revealed linalyl anthranilate and terpinen-4-ol as major constituents, with preliminary trends showing quantitative differences. Repellent efficacy was concentration-dependent. At 10^{-1} g/ml, all EOs showed >95% PE, with Kitui essential oils achieving 100% protection. At lower concentrations, Kajiado and Kitui essential oils were significantly more potent ($p<0.0001$). MCPT for all essential oils (2.25–2.50 hours) was significantly shorter than DEET 20% (4.00 hours, ($p>0.05$), but no significant difference in MCPT was found among the essential oils themselves ($p>0.05$). A combination of essential oils did not enhance efficacy or duration. Soil properties, climate and topography significantly influence the yield and repellent potency of *Ocimum tenuiflorum* essential oils, with Kitui and Kajiado essential oils demonstrating superior efficacy at lower concentrations despite lower yields. These findings support the potential of regionally sourced *Ocimum tenuiflorum* as a natural short-term repellent and highlight the need for formulation strategies to extend protection time.

Keywords: *Ocimum tenuiflorum; Essential oil; Agro-ecological zone; Mosquito repellent; Anopheles gambiae*

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INTRODUCTION

Mosquito-borne diseases, particularly malaria, remain a major public health burden in tropical and subtropical regions, including Kenya (WHO report, 2022). While synthetic repellents like N, N-Diethyl-3-methylbenzamide (DEET) are effective, concerns over safety, environmental persistence, and emerging insecticide resistance have spurred the search for plant-based alternatives (Ranson et al.,2016; Nerio et al., 2010).

The genus Ocimum (Lamiaceae) is renowned for its aromatic species, many of which possess insect-repellent properties (Pandey et al., 2014). *Ocimum tenuiflorum* L. (holy basil), widely used in traditional medicine, has shown promising repellent activity against various mosquito species (Osei-Owusu et al.,2023; Kaushik et al.,2010). The efficacy of plant essential oils is primarily attributed to their complex mixture of volatile compounds, such as monoterpenes and sesquiterpenes (Isman, M. B., 2006). However, the composition and concentration of these bioactive compounds are highly influenced by environmental factors, including soil type, climate, and altitude (Gobbo-Neto et al., 2007)

In Kenya, diverse agro-ecological zones present a unique opportunity to study how geography affects the quality and bioactivity of medicinal plants. While *Ocimum tenuiflorum* is used locally, a systematic evaluation of the repellent efficacy of Kenyan varieties and the impact of growing conditions is lacking. This study aimed to bridge this gap by determining the physicochemical properties and chemical composition of *Ocimum tenuiflorum* essential oils from three different agro-ecological zones in Kenya, and evaluate their repellent efficacy against the primary malaria vector, *An. gambiae.*

METHODS

Study Design

This study adopted an experimental laboratory-based design. It involved the comparative phytochemical profiling and evaluation of the repellent activity of essential oils obtained from *Ocimum tenuiflorum* collected across three distinct agro-ecological zones in Kenya: Kiambu, Kajiado, and Kitui counties. This research design was adopted as it provides a controlled, reductionist framework essential for isolating and directly comparing region-specific plant extracted compounds and their associated bioactivities. It is convenient for research aiming to establish definitive, reproducible, and mechanistic insights into the chemical and functional properties of plant extracts. Standard methods such as extraction procedures, analytical parameters and bioassay conditions which would have otherwise be compromised in field studies were controlled thereby reducing observed variance in chemical composition and repellent efficacy exhibited by the essential oils of *Ocimum tenuiflorum*.

Chemicals and reagents

The specific materials used for each procedure included mature *Ocimum tenuiflorum* plants that were collected from the wild in Kitui, Kajiado, and Kiambu Counties, Ethanol AR grade (LOBA Chemie, India) for miscibility analysis and repellent activity negative control, n-Hexane (Analytical Grade, Sigma-Aldrich) for GC-MS analysis, and N, N-Diethyl-3-methylbenzamide (DEET) as repellent properties positive control. All other chemicals and reagents used in this study were of analytical grade.

Plant material collection and sampling

Aerial parts of *Ocimum tenuiflorum* were collected from wild populations in three counties representing distinct agro-ecological zones namely; Kajiado (semi-arid), Kiambu (highland, fertile), and Kitui (arid to semi-arid), following good agricultural and collection practices (GACP) as per WHO, 2003 guidelines. A stratified random sampling design was employed in the collection of *Ocimum tenuiflorum*, wherein the study area was first partitioned into the three target agro-ecological zones namely; Kiambu, Kajiado and Kitui counties as primary strata. Within each stratum, sampling coordinates were generated randomly using geographical information system (GIS). At each coordinate, a quadrat of 5 X 5meters square was established, and all mature *Ocimum tenuiflorum* individuals within were harvested. A minimum of 15 meters was maintained between quadrats to ensure spatial independence. Limuru (Kiambu county), Isinya and Karen (Kajiado County) and Mutito, Thome wa Klisto & Mui (Kitui County) were chosen as specific collection localities within each of the primary agro-ecological strata due to their dominant soil series that matched the agro-ecological zone, consistency in land-use practices and healthy, naturally regenerating population of *Ocimum tenuiflorum*.

Collected plant materials were placed in clean dust free well marked mesh bags and were transported in the open air to Kenyatta University. The raw plant materials were then subjected to preliminary processing where undesirable debris and contaminants were sorted out. Those plant materials showing any indication of fungi or insect attack were also discarded.

Samples collected from each agro-ecological stratum were evenly mixed and dried on drying racks at room temperature (22 ^{0}C) as a single homogeneous sample. Collection was done in 15 randomly selected sites from which about 15kgs of the fresh plant material was harvested. After the drying process, 10kgs of the dried plant sample was drawn to constitute a composite sample for each agro-ecological zone and essential oil independently extracted via hydro distillation.

The identification and authentication of the collected *Ocimum tenuiflorum* was done by a plant botanist from Kenyatta University, Botany Department and voucher specimens deposited at their departmental herbarium (voucher specimen Nos; Kiambu (KIM/MRC/OC/01), Kajiado (KAJ/MRC/OC/01), Kitui (KIT/MRC/OC/01).

Description of the three agro ecological zones

Kitui County is situated in the eastern region of Kenya, and lies between approximately Latitude 0°10' South and 3°0' South and Longitude 37°50' East and 39°0' East (Kitui County Government, 2018). It borders other counties like Machakos, Makueni, Tana River, Tharaka-Nithi, Meru and Embu. Kitui County soil types are majorly sandy red and black clay cotton soils (Jaetzold et al., 2007, Republic of Kenya, 2005). The soils are moderately acidic to alkaline with a pH range of 5.67-8.7 and are predominantly vulnerable to erosion, are poorly drained and are restricted in their ability to reserve moisture and nutrients. Kitui County has a characteristic arid and semi-arid climate with an annual rainfall average range of about 200-300mm (Kenya meteorological department).

Kajiado County is situated in the southern part of Kenya, within the rift valley region. It shares its borders with several Kenyan counties and one international border, Nairobi County and Kiambu County to the North and Machakos County and Makueni County to the Northeast It lies approximately between latitude 1^{0} 3' south and longitude 36^{0} 38' east (Kajiado County Government, 2018). Kajiado County soils are majorly quaternary volcanic soils, basement rock soils and the pleio-cene soils and are truly acidic having a pH of between 5.6 and 5.8. It has a climate that is predominantly semi-arid with an annual rainfall amount range of about 300 mm – 800 mm (Kenya County Climate Risk Profile Series, 2017).

Kiambu County is situated in the central highlands of Kenya, within the former Central Province. The county lies between approximately latitude 0° 45' and 1° 31' South and Longitude 36° 36' and 37° 10' East (Kiambu County Government, 2018). Kiambu County is predominantly covered by three broad categories of soils consisting of high-level upland soils, plateau soils and volcanic soils. These soils, especially the volcanic soils can be termed highly fertile with pH ranges of 4.4 - 6.0. It receives an annual precipitation range of about 600-1300 mm (Kenya County Climate Risk Profile Series, 2017).

Essential Oil Extraction

To isolate essential oils by hydro distillation, 2kg of dried *Ocimum tenuiflorum* plant material was packed in a hydro distiller and a sufficient amount of water was added, secured with a lid and brought to boil. The vapor mixture of water and oil was condensed by indirect cooling with cold tap running water. The condensed distillates were collected into a separator, where the essential oils separated automatically as per their density from the distillate water. Hydro distillation process for each sample lasted for six hours. The process was repeated 5 times for each plant material sample to achieve a substantial amount of the essential oil per sample. The essential oils were stored at -20 °C until analysis. The percentage yield was calculated based on the dry weight of the plant material.

Physicochemical and organoleptic analysis

The extracted essential oils were characterized for key organoleptic and physicochemical parameters to determine quality, purity, and authenticity. Specific gravity (relative density) was determined at 20 °C using a 10 mL Gay-Lussac type pycnometer according to ISO 279:1998 (Essential oils; Determination of relative density at 20 $_{0}$C), calibrated against the density of distilled water at 20 °C (0.9982 g/cm^{3}). Refractive index was measured at 20 °C using a refractometer (model no. Rfm 330, Bellingham + Stanely) as per ISO 280:1998 (Essential oils; Determination of refractive index), calibrated with certified reference standards of deionized water (n_D^{20} = 1.3330) and n-heptane (n_D^{20} = 1.3876).

The pH of essential oils was determined using a modified potentiometric method optimized for hydrophobic matrices. Due to the non-conductive and immiscible nature of essential oils in aqueous solutions, a standardized water extraction technique was employed to obtain a measurable aqueous phase. The procedure was adapted from principles outlined in Association of official analytical collaboration (AOAC) official Method 965.15 (pH of Oils) and ISO 1842:1991 (Fruit and vegetable products; Determination of pH), with modifications for essential oil chemistry. Measurements were performed using a bench top pH meter (Model: H12211 (Hanna instruments).

The miscibility of essential oils in ethanol was determined to assess purity and detect potential adulteration with non-volatile or hydrophobic substances. The procedure was conducted according to the standardized protocol specified in ISO/FDIS 210:2023 (Essential oils; Determination of solubility in ethanol) and the European Pharmacopoeia (Ph. Eur. 11th Ed., General Chapter 2.8.10: Solubility in Ethanol), with modifications for precise volumetric measurement.

Organoleptic evaluation for colour and odor was performed according to modified guidelines from the International Organization for Standardization (ISO 9235:2021) on aromatic natural raw materials and the general principles for sensory evaluation of volatile compounds as described in the European Pharmacopoeia (Ph. Eur. 11th Edition, Chapter 2.8.20).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The essential oils were diluted to 0.2% in n-Hexane prior to analysis. Analysis was performed using a gas chromatography-mass spectrometry (GC-MS) system (Shimadzu Model QP2020NX). To enhance accuracy in compound identification, retention indices (RIs) were calculated by co-injecting a standard solution of a homologous series of n-alkanes (C8–C40) with the samples under identical chromatographic conditions. The gas chromatography-mass spectrometry (GC-MS) system (Shimadzu Model QP2020NX) was calibrated daily prior to sample runs using the manufacturer's recommended PFTBA (perfluorotributylamine) tuning solution to ensure mass accuracy and optimal instrument sensitivity.The instrument was equipped with an SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The oven temperature was programmed from 50°C (hold 0 min) to 330 °C at 3°C/min. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. Mass spectra were obtained in electron impact mode (70 eV), scanning from 50 to 500 m/z. Constituents were identified by comparing their mass spectra and retention indices with those in the NIST and FFNSC-3 libraries. Gas chromatography-mass spectrometry (GC-MS) analysis was performed once per each sample due to the exploratory nature of the study. While this precludes statistical analysis of technical variation, the data are presented as preliminary findings to identify major compositional trends/differences. All samples were processed in a randomized order and analyzed with the same instrument method and internal standards, including quality control standards.

Mosquito Repellent Bioassay

The repellence activity was tested following a controlled laboratory "arm-in-cage" experiment protocol (WHO,1996) consisting of eight biological replicates. The volunteer panel consisted of healthy adult volunteers, comprising four males and four females, aged 18 to 35 years. They were all non-smokers, had no known allergies to essential oils or mosquito bites, and had refrained from using perfumes, scented lotions, or consuming caffeine and alcohol for 12 hours prior to testing. All participants provided written informed consent. The study protocol was approved by the KEMRI Scientific and Ethics Review Unit (Non-KEMRI Protocol No. 4520).

All bioassays were conducted in a controlled laboratory environment. The temperature and relative humidity were monitored and maintained at 27 ± 2 °C and 65 ± 5%, respectively, using air conditioning and a humidifier, in line with WHO guidelines to ensure consistent mosquito host-seeking behavior.

Experimental design employed repeated measures bioassay where the eight volunteers served as replicates, each volunteer testing for the three essential oils; Kiambu (KIM/MRC/OC/01), Kajiado (KAJ/MRC/OC/01), Kitui (KIT/MRC/OC/01), the essential oils combination and DEET reference. Both treated and control arms from each volunteer were used, alternating regularly to minimize bias.

The repellent activity was tested against 5-7-day-old, sugar-fed but starved female *An. gambiae* s.s. (Kisumu strain). For each replicate, 100 mosquitoes were transferred to a standardized test cage (30 × 30 × 30 cm) one hour prior to testing. Serial dilutions of each essential oil (10^{-1}, 10^{-3}, and 10^{-5} g/ml) were prepared in ethanol AR grade. A 1.5 g equivalent of oil per 600 cm^2 was applied to a volunteer's forearm. The opposite forearm, treated with ethanol AR grade, served as a negative control. DEET (20%) was used as a positive control. The treated and control arms were exposed alternately for 3 minutes to a cage containing 100 mosquitoes every 30 minutes for up to 4 hours. The number of landings/probings was recorded. The percentage protective efficacy (PE) was calculated as:

PE = [(C - T) / C] × 100

where C is the mean number of landings on the control arm and T is the mean number of landings on the treated arm during the same exposure period.

The Complete Protection Time (CPT) was recorded as the time until the first confirmed bite.

Statistical Analysis

Repellency data from eight replicates were subjected to a one-way analysis of variance (ANOVA) using R software. Means were separated using the Student-Newman-Keuls (SNK) test at a significance level of $p < 0.0001$. Essential oil yields and Mean Complete Protection Time (MCPT) were compared using one-way analysis of variance (ANOVA). Post-hoc pairwise comparisons were done using Tukey's Honestly Significant Difference (HSD) test.

RESULTS

Essential oil yield

The highest mean essential oil yield was obtained from the Kiambu sample (0.40% ± 0.0079), followed by Kajiado sample (0.30% ± 0.0079) and Kitui sample (0.26% ± 0.0035). The consistency within samples from each zone was high, as indicated by the low standard deviations, especially for Kitui (SD = 0.0035), suggesting minimal variability in the extraction process as shown in Table 1

Table 1:

Essential oils yield from three Agro-ecological zones

Essential oils	% Yield + SD	SE	Tukey Group
Kitui (KIT/MRC/OC/01)	0.26 ± 0.0035	0.0016	A
Kajiado (KAJ/MRC/OC/01)	0.3 ± 0.0079	0.0035	B
Kiambu (KIM/MRC/OC/01)	0.4 ± 0.00791	0.00354	C

Different letters indicate statistically significant differences ($p < 0.05$), n=5, (Tukey's Honestly Significant Difference (HSD) test)

Organoleptic and Physicochemical Properties

The oils were pale yellow liquids with a characteristic green, herbaceous, clove-like odor. The physicochemical properties (relative density, refractive index, pH) were consistent across all Samples. (Table 2).

Table 2:

Organoleptic and Physicochemical Properties of O. tenuiflorum essential oils from three agro-ecological zones.

Property	Kajiado	Kiambu	Kitui
Relative Density at 22 ^{0}C	0.9605± 0.0154	0.9535 ± 0.0146	0.9569 ± 0.0151
Refractive Index at 22 ^{0}C	1.523 ± 0.0228	1.516 ± 0.0211	1.528 ± 0.0234
pH value	5.76 ± 0.05	5.77 ± 0.03	5.77 ± 0.02
Miscibility with Ethanol (AR grade) at 22 ^{0}C	1:1	1:1	1:1
Odor	Green herbaceous clove like odor	Green herbaceous clove like odor	Green herbaceous clove like odor
Colour	Pale yellow	Pale yellow	Pale yellow
Phase	Liquid/Fluid	Liquid/Fluid	Liquid/Fluid

Comparative Composition of Essential Oils by GC-MS Screening

The relative chemical composition of essential oils from Kajiado, Kiambu, and Kitui, as determined by single-run GC-MS analysis, is presented in Table 3. The data reveal notable qualitative similarities and quantitative trends across the three samples. Linalyl anthranilate was the most abundant compound identified in all three oils, with its relative concentration appearing highest in the Kajiado sample (21.05%) compared to Kitui (18.17%) and Kiambu (16.78%). Similarly, terpinen-4-ol showed a trend of higher relative abundance in the Kajiado sample (16.34%) than in the Kitui (15.77%) and Kiambu (13.30%) samples. Other major monoterpenoids and sesquiterpenes, including terpineol, eucalyptol, terpinene, caryophyllene, and limonene, were present in all samples at comparable levels, with minor variations observed in their relative percentages. These data provide a preliminary compositional profile and suggest that the geographic origin may influence the relative proportions of key volatile constituents.

The overlay of gas chromatograms for the three essential oil samples (Kajiado, Kiambu, and

Kitui) revealed shared distinct compositional features, as shown in Figure 1. Visual inspection indicated a high degree of qualitative similarity across the samples, with numerous major peaks eluting at consistent retention times, suggesting a common set of core chemical constituents.

Table 3:

Relative composition of major chemical compounds in Ocimum tenuiflorum essential oils from three agro-ecological zones. (Single GC-MS Measurements)

Compounds	Kajiado essential oil (KAJ/MRC/OC/01)		Kiambu essential oil (KIM/MRC/OC/01)		Kitui essential oil(KIT/MRC/OC/01)	
	% conc	Retention index	% conc	Retention index	% conc.	Retention index
Linalyl Anthranilate	21.05	16.754	16.78	16.96	18.17	17.015
Terpinen-4-ol	16.34	20.562	13.30	20.731	15.77	20.784
Terpineol	8.73	21.187	7.42	21.323	7.11	21.373
Eucalyptol	3.84	13.576	3.68	13.631	3.60	13.667
Terpinene	4.25	14.75	4.16	14.812	4.12	14.848
Caryophyllene	2.83	31.404	3.01	31.461	3.02	31.507
Limonene	2.37	13.442	2.66	13.493	2.74	13.528

RIs = retention index relative to n-alkanes (C8–C40) on SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness), Calibration =PFTBA (perfluorotributylamine) tuning solution, MS =NIST and FFNSC-3 libraries.

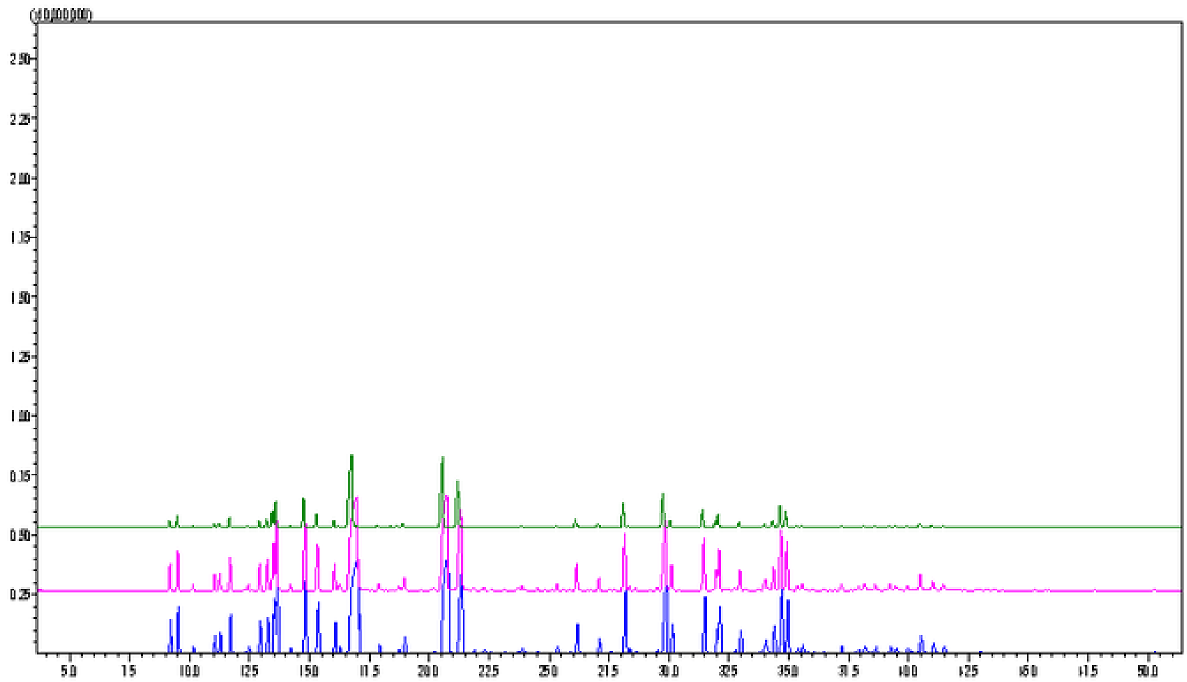

Figure 1:

Chromatogram overlay for the KAJ/MRC/OC/01 (Green), KIM/MRC/OC/01 (Pink) and KIT/MRC/OC/01 (Blue) samples

Mosquito Repellent Activity

Ocimum tenuiflorum essential oils extracted from different agro-ecological zones exhibited significant and concentration-dependent protective efficacy (PE) against *An. gambiae* as shown in Table 4. All tested essential oil samples and the combination showed excellent repellency at the highest concentration of 10^{-1} g/ml, with mean PE values exceeding 95%. Specifically, KIT/MRC/OC/01 achieved complete (100^{a}) protection, while KAJ/MRC/OC/01 and the Combination provided near-complete protection of 99.1 ± 1.8^{a} and 98.4 ± 3.2^{ab} respectively. The data revealed critical differences in repellent potency among the three essential oils, particularly at lower concentrations. At 10^{-3} g/ml, the PE ranged from 34.2% to 58.3%. KAJ/MRC/OC/01 (57.9 ± 1.7^{e}) and KIT/MRC/OC/01 (58.3 ± 0.4^{e}) were statistically similar and markedly more effective than KIM/MRC/OC/01 (34.2 ± 2.8^{jk}). This trend persisted at the lowest concentration observed for 10^{-5} g/ml as shown in Table 4.

Table 4:

Mean percent protective efficacy (MPE ± SE) of Ocimum tenuiflorum essential oils against An. gambiae.

Essential oils (EOs)	% Mean protective efficacy (MPE) ± SE		
	10^{-1} g/ml	10^{-3} g/ml	10^{-5} g/ml
KIM/MRC/OC/01	95.6 ± 2.5^{c}	34.2 ± 2.8^{jk}	22.3 ± 1.5^{m}
KAJ/MRC/OC/01	99.1 ± 1.8^{a}	57.9 ± 1.7^{e}	48.7 ± 2.4^{g}
KIT/MRC/OC/01	100^{a}	58.3 ± 0.4^{e}	33.2 ± 0.9^{jk}
Combination	98.4 ± 3.2^{ab}	42.7 ± 2.5^{h}	37.7 ± 1.9^{j}

Means with the same superscript are not significantly different at P = 0.0001. [a] Student–Newman– Kuels (SNK) test, R (2023)

Complete protection time (CPT)

Table 5 presents the duration of repellent efficacy for *Ocimum tenuiflorum* essential oils against *An. gambiae* mosquitoes, measured as Mean Complete Protection Time (MCPT). All tested *Ocimum tenuiflorum* essential oils and their combination provided a moderate MCPT ranging from 2.25 to 2.5 hours. The positive control, 20% DEET, demonstrated a significantly longer MCPT of 4.00 hours with no variation (SD ± 0.00), indicating complete and consistent protection for twice the duration of the tested essential oils. The Combination of essential oils yielded an MCPT of 2.31 hours, which falls within the range of the individual oils.

Table 5:

Mean Complete Protection Time (MCPT) by the essential oils against An.gambiae mosquitoes

Treatment	n	MCPT ± SD (hours)	Tukey Group
DEET 20%	8	4.00 ± 0.00	A
KAJ/MRC/OC/01	8	2.50 ± 0.44	B
KIT/MRC/OC/01	8	2.50 ± 0.38	B
KIM/MRC/OC/01	8	2.25 ± 0.71	B
COMBINATION	8	2.31 ± 0.46	B

Different letters indicate statistically significant differences ($p < 0.05$). (Tukey's Honestly Significant Difference (HSD) test)

DISCUSSION

Essential oil yields from *Ocimum tenuiflorum* harvested from three agro-ecological zones revealed significant differences among the agro-ecological zones ($F(2,12) = 567.3$, $p < 0.001$). Post-hoc pairwise comparisons (Tukey's Honestly Significant Difference (HSD) test) demonstrated that all agro-ecological zones differed significantly from each other ($p < 0.05$). Kiambu produced the highest yield (0.400 ± 0.0079%), followed by Kajiado (0.300 ± 0.0079%), with Kitui yielding the lowest (0.260 ± 0.0035%). This strong statistical evidence suggests that environmental or genetic factors associated with each agro-ecological zone substantially influence essential oil yield.

The higher oil yield from Kiambu County can be attributed to its more favorable growing conditions; higher rainfall, fertile volcanic soils, and moderate acidity, which are conducive for the plant's growth and secondary metabolite production (Sairam et al., 2006). Kajiado's intermediate yield may reflect semi-arid conditions that induce moderate stress, potentially balancing growth and oil biosynthesis. Kitui's lower yield might result from more pronounced arid conditions, limiting metabolic resources for essential oil synthesis.

All samples were pale yellow and liquid/fluid at room temperature, which is typical for many hydro-distilled essential oils and indicates similar extraction efficiency. Each oil was described as having a green herbaceous clove-like odor. The consistent description of a green herbaceous clove-like odor across all three essential oil samples strongly indicates a shared dominant chemical profile, characteristic of specific *Ocimum tenuiflorum* chemotypes. This sensory characteristic is primarily attributed to phenylpropanoids combined with sesquiterpenes like caryophyllene that contribute fresh green and woody-spicy undertones, respectively (Pandey et al., 2014; Verma et al., 2010).

Complete miscibility with ethanol at 22 ^{0}C (1:1) confirmed the lipophilic nature and purity, free from excessive water or polar impurities (Ravi et al., 2023). The consistent, slightly acidic pH (5.76–5.77) reflects the presence of acidic compounds e.g., phenolic components and is typical for many essential oils (Turek et al., 2013).)

Refractive index (RI) at 22 ^{0}C values showed a consistent trend across the three agro-ecological zone with Kitui (1.528 ± 0.0234), Kajiado (1.523 ± 0.0228), and Kiambu (1.516 ± 0.0211). The higher RI observed for Kitui may reflect a differing proportion of constituents with greater molecular density or polarizability, potentially attributable to genotypic differences or environmental stress conditions at the collection site (Hăncianu et al., 2021). Relative densities were very close ranging between 0.9535–0.9605, with the highest mean value recorded for Kajiado (0.9605± 0.0154).

Kitui oil which expressed lower yield but a higher RI shown to have a more concentrated or slightly distinct blend of active compounds, which could explain its high repellent efficacy (100% PE at 10^{-1} g/ml concentration) as seen in Table 4, despite having a lower extraction yield. The consistent organoleptic properties and miscibility are favorable for product formulation, ensuring batch-to-batch uniformity in appearance, aroma, and solubility in common carriers.

GC-MS screening (Table 3) indicated consistent qualitative profiles across the three essential oils, with linalyl anthranilate and terpinen-4-ol representing the major constituents. All essential oils were characterized by a high concentration of Linalyl anthranilate, an ester not commonly reported as a major constituent compared to eugenol or methyl eugenol in *Ocimum tenuiflorum* (Joshi, 2014). This trend merits further investigation on *Ocimum tenuiflorum* growing in Kenya involving a broader sampling and deeper genetic analysis.

It is important to note that this chemical composition data is derived from single GC-MS measurements, which limits ability to assess analytical precision or perform statistical comparisons between regions. Nevertheless, the variation in the concentration of key terpenes like terpinen-4-ol and caryophyllene across the zones underscores the influence of environmental stress, as higher plants often produce secondary metabolites as a defense mechanism (Gobbo-Neto et al., 2007).

The consistent detection of compounds like eucalyptol, limonene, and caryophyllene across all samples supports their role as characteristic markers. Future work should prioritize replicated GC-MS analysis, ideally with authentic standards for quantitative calibration, to confirm these observed trends and robustly evaluate the impact of geographical origin on oil composition.

Protective efficacy was strongly correlated with oil concentration. The superscript letters denoting statistical significance (e.g., a, c, jk, m) allow for a nuanced comparison across samples and concentrations (Table 4). At the highest concentration (10^{-1} g/ml), all samples performed exceptionally well. KIT/MRC/OC/01 (100^{a}) and KAJ/MRC/OC/01 (99.1 ± 1.8^{a}) share the statistically top-performing group, while the

Combination (98.4 ± 3.2[ab]) is in the overlapping group. KIM/MRC/OC/01, though still highly effective at 95.6 ± 2.5[c] is in a separate statistical group ('c'), indicating its mean PE is significantly lower than the top performers at this concentration. At intermediate concentration (10^{-3} g/ml), the performance gap between samples becomes more apparent. KIT/MRC/OC/01 and KAJ/MRC/OC/01 remain the most effective and are statistically equivalent (58.3 ± 0.4[e], 57.9 ± 1.7[e] respectively). The Combination shows intermediate efficacy (42.7 ± 2.5[h]), while KIM/MRC/OC/01 is the least effective at this dilution (34.2 ± 2.8[jk]).

At the lowest concentration (10^{-5} g/ml), a similar trend is maintained. KAJ/MRC/OC/01 retains the highest efficacy (48.7 ± 2.4[g]), followed by the Combination (37.7 ± 1.9[j]) and KIT/MRC/OC/01 (33.2 ± 0.9[jk]), with KIM/MRC/OC/01 being the least effective (22.3 ± 1.5[m]).

The steep decline in PE with dilution underscores that the bioactive components in these essential oils require a sufficient concentration to be effective. This has direct implications in formulating natural repellents. The essential oils must be present above a critical threshold to provide meaningful protection.

The significant differences in PE between the three geographically sourced essential oils (KIM, KAJ, KIT) highlight the impact of chemotypic variation in *Ocimum tenuiflorum* that could be attributed to factors such as soil composition, climate, topography and harvest time leading to varying biological potency. KAJ/MRC/OC/01 emerged as the most robust across all concentrations (Nararak et al., 2020; Maia et al., 2011).

All tested *Ocimum tenuiflorum* essential oils and their combinations provided a shorter MCPT, ranging from 2.25 to 2.5 hours, compared to 4 hours for 20% DEET. The results revealed statistically significant differences in Mean Complete Protection Time (MCPT) among the treatments ($F(4,35) = 20.22$, $p < 0.001$, one way ANOVA). Post-hoc analysis (Tukey's Honestly Significant Difference (HSD) test) demonstrated that DEET 20% (4.00 ± 0.00 hours) provided significantly longer protection than all essential oil formulations ($p < 0.001$). However, no statistically significant differences were observed among the essential oils from different geographical origins: Kitui (2.25 ± 0.71 hours), Kajiado (2.50 ± 0.44 hours), Kiambu (2.50 ± 0.38 hours), or their combination (2.31 ± 0.46 hours) (all $p > 0.05$).

Despite certain essential oils (e.g., KAJ/MRC/OC/01 and KIT/MRC/OC/01) showing high protective effectiveness (PE at 10^{-1} g/ml concentration), their effectiveness tends to dissipate over time. However, since the MCPT was comparable for the first 2.5 hours, these essential oils are viable natural alternatives. The lower efficacy of the Kiambu essential oil, despite a higher yield, indicates that oil quantity does not always equate to repellent quality, which is more dependent on the specific chemical profile. This duration is typical of many unformulated plant essential oils, which tend to evaporate rapidly from the skin surface.

Conclusion

The essential oils from Kajiado, Kiambu, and Kitui showed no significant differences in their physicochemical and organoleptic properties. The chemical profile of the essential oils varied notably based on their agro-ecological zone. The mosquito repellent activity was confirmed to be dosage-dependent, with higher concentrations providing superior protection. Oils sourced from Kitui and Kajiado demonstrated significantly higher repellent potency against *An. gambiae* compared to the oil from Kiambu. There were no improved potency and repellent properties observed in combination.

Recommendations

Based on the findings of this study, the following recommendations are made to enhance the quality and efficacy of plant-based repellents:

- Prioritize region-specific cultivation for optimal repellent efficacy. For commercial development of *Ocimum tenuiflorum*-based repellents, cultivation should be targeted in agro-ecological zones similar to Kitui and Kajiado Counties. Analysis of the repellency bioassay (ANOVA, SNK test at $p < 0.0001$) revealed that essential oils from Kitui and Kajiado provided significantly higher protective efficacy (PE) at intermediate (10^{-3} g/ml) concentration, 58.3 ± 0.4 % and 57.9 ± 1.7% respectively compared to Kiambu essential oils (34.2 ± 2.8%). At the highest concentration, Kitui and Kajiado EOs were statistically top-performing (100% and 99.1 ± 1.8% respectively).
- Product formulations must ensure the essential oil is present at a concentration equivalent to or greater than 10^{-1} g/ml to provide high-level protection (>95%). The repellent effect was strongly concentration-dependent. A significant drop in efficacy was observed at lower concentrations for all essential oils (e.g., Kiambu PE fell from 95.6% at 10^{-1} g/ml to 34.2% at 10^{-3} g/ml).

- Future research and quality control must employ replicated GC-MS analysis with quantitative standards to statistically confirm compositional differences and identify key efficacy markers.
- Research should focus on encapsulation or polymer-based formulations to extend the short evaporation time of the essential oils. The high initial efficacy demonstrates potential, but practical utility requires technologies to prolong the duration of action to match user expectations.
- To re-evaluate the strategy of combining oils from different regions as geographic blend of these essential oils did not enhance performance. The combination of essential oils did not result in statistically superior efficacy or protection time. At the critical intermediate concentration (10^{-3} g/ml), the combination's PE (42.7 ± 2.5 %) was significantly lower than that of the top-performing individual i.e. Kitui (58.3 ± 0.4 %) and Kajiado (57.9 ± 1.7%).

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Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

Data supporting the conclusions of this study are included in this published article and its supplementary information files.

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