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Research Article

Repellency Potential, Chemical Constituents of *Ocimum* Plant Essential Oils, and Their Headspace Volatiles against *Anopheles gambiae* s. s., Malaria Vector

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African malaria mosquitoes (*Anopheles gambiae* sensu stricto) transmit a malaria parasite (*Plasmodium falciparum*) to humans. The current control strategies for the vector have mainly focussed on synthetic products, which negatively impact the environment and human health. Given the potential use of environmentally friendly plant-derived volatiles as a control, this work aims to examine and compare the repellency potential of essential oils and headspace volatiles from *Ocimum gratissimum, Ocimum tenuiflorum,* and *Ocimum basilicum* and their chemical compositions. The repellency potential and chemical composition of the plants were achieved by using the protected arm-in-cage method and gas chromatography-mass spectrometry (GC-MS) analysis. Among the three *Ocimum* species, both the essential oils and the headspace volatiles from *O. tenuiflorum* achieved the longest repellency time lengths of 90–120 minutes. One hundred and one (101) chemical constituents were identified in the headspace volatiles of the three *Ocimum* spp. Nonetheless, (−)-camphor, (*E*)-γ-bisabolene, terpinolene, β-chamigrene, cubedol, (*E*)-farnesol, germacrene D-4-ol, viridiflorol, γ-eudesmol, tetracyclo [6.3.2.0 (2,5).0(1,8)] tridecan-9-ol, 4,4-dimethyl, α-eudesmol, iso-longifolol, and endo-borneol were unique only to *O. tenuiflorum* headspace volatiles. Either essential oils or headspace volatiles from *O. tenuiflorum* could offer longer protection time length to humans against *An. gambiae*. Though field studies are needed to assess the complementarity between the chemical constituents in the headspace volatiles of *O. tenuiflorum*, our observations provide a foundation for developing effective repellents against *An. gambiae*.

1. Introduction

Malaria remains one of the most critical public health problems in sub-Saharan Africa (SSA) [1, 2]. In 2021, 247 million malaria cases were recorded globally, with more than 95% of all the cases reported in SSA [3, 4]. In the case of Ghana, malaria accounts for 39% of outpatient attendance, 25% of all admissions to hospitals or other healthcare centers, and 4% of all death cases [5]. Among the culicids, *Anopheles gambiae* complex (i.e., the African malaria mosquitoes) vectors are two microparasites, namely, *Wuchereria bancrofti* and *Plasmodium falciparum* [6]. The former is a zooparasitic nematode that causes a filarial disease commonly known as elephantiasis [7], whereas the latter is a parasitic sporozoan that causes malaria in humans widely across SSA [3, 8–10]. In Ghana, the *Plasmodium* parasite accounts for more than 85% of all malaria cases [2]. Millions of people are infected daily by *P. falciparum* through mosquito bites [11]. Although progress has been made to combat malaria through many interventions such as prompt and accurate diagnosis, single and combination therapy with known antiplasmodial drugs, insecticide-treated nets, and malaria vaccines, there are still significant morbidity and mortality primarily due to the inherent difficulties associated with the vector control [12].

Unfortunately, none of the control methods have effectively reduced malaria transmission rates among people in SSA [13]. The majority of the control strategies to reduce malaria have largely been vector-based interventions which include reducing mosquito breeding sites, use of screens against mosquitoes, application of N, N-diethyl-3-methylbenzamide (DEET), a synthetic repellent, genetic control methods through the release of mosquitoes carrying a lethal gene to suppress target populations, application of human-derived volatiles as a trap, and application of indoor residue spray [2, 8, 12, 14]. However, there have been numerous reported limitations associated with the current control strategies [3, 15–17]. For example, the population control of the vector through reducing breeding sites presents a great challenge due to mosquitoes' ability to breed wherever stagnant water is available [18]. Although providing screens such as insecticide-treated nets are excellent means of personal and community protection against malaria disease [19, 20], there has been a decline in the use of treated nets mainly due to skin irritation and demand for sufficient indoor air circulation [2]. The DEET developed over 6 decades ago is still the most widely used synthetic mosquito repellent [15, 21]. Unfortunately, accessibility and undesirable properties such as an unpleasant odor and skin irritation have limited its use. Also, DEET has been observed to inhibit the function of ion channels and acetylcholinesterase in humans and other mammals [22]. The active ingredients of most indoor mosquito sprays belong to the pyrethroid, organochlorine, organophosphate, and carbamate classes of pesticides [9]. However, considering the environmental impact, health implications, and insecticide resistance development in mosquitoes to these synthetic sprays [23, 24], it suggests the need for study on (i) environmentally friendly vector management approaches and (ii) people's views on the use of these vector control tactics.

In Africa, traditional medicinal plants and/or their derived products continue to be vital against malaria vectors [25]. These plants are usually burnt to generate smoke or hung in houses to repel mosquitoes [13]. The most cited genera of plant species with promising mosquito repellent activities have been *Cymbopogon*, *Eucalyptus*, and *Ocimum* [25, 26].

Most insects, including mosquitoes, use their sense of smell to detect attractive or repellent compounds [15, 21]. In this case, the biology and behavior of insect pests generally offer opportunities to study plant volatile compounds (PVCs) to develop newer and safer mosquito repellents. This is because naturally occurring PVCs are semiochemicals that significantly reduce the host-seeking behavior of insects and/or act as a deterrent [27]. For example, (E)-β-farnesene, a plant-derived volatile, stops the movement of aphids (Homoptera: Aphididae) on crops [28]. Moreover, a blend of (E)-4,8-Dimethyl-1,3,7-nonatriene (DMNT), indole, n-hexyl acetate, (RS)-1-octen-3-ol, and (RS)-linalool was repellent to *Maruca vitrata* [27]. Plant volatile compounds such as α-pinene and oct-1-en-3-ol and linalool have demonstrated insecticidal properties against *Tribolium confusum*, *Tribolium castaneum*, *Sitophilus zeamais*, *Callosobruchus maculatus*, and *Rhyzopertha dominic* [29]. Furthermore, (E)-caryophyllene and α-humulene act as an oviposition deterrent to *Ae. aegypti* [30].

Ocimum gratissimum L., *Ocimum tenuiflorum* L., and *Ocimum basilicum* L. are aromatic medicinal plants, belonging to the family Lamiaceae, that are found in Africa, Asia, and Mediterranean countries [31]. These plants possess antimicrobial, antioxidant, repellent, larvicidal, and insecticidal properties [32, 33]. Traditionally in Ghana, these plants are burnt or placed in rooms to serve as mosquito repellents, though the practice is yet to be investigated. Most mosquito repellency studies on these plants have focused on plants' essential oils, usually obtained through hydrodistillation. However, the heat in the hydrodistillation process can decompose some constituents [34]. For a more realistic plant volatile profile, headspace volatile collection is appropriate, especially when it involves ecological application [35]. To the best of our knowledge, however, there has been no study on the headspace volatile composition of *O. gratissimum*, *O. tenuiflorum*, and *O. basilicum* isolated by dynamic headspace volatile techniques and their mosquito repellency activity. Given the potential use of these environmentally friendly plant-derived volatiles as *Anopheles* mosquito repellents, the aim of the current study was in two-folds: (1) compare the mosquito repellency activity of essential oils or headspace volatiles from the three *Ocimum* plants to commercially available, mosquito repellent used in Ghana; (2) investigate the chemical composition of the headspace volatiles from these *Ocimum* species.

2. Materials and Methods

2.1. Culture of Ocimum Plant Species. Seeds of *O. gratissimum*, *O. tenuiflorum*, and *O. basilicum* for cultivation were obtained from the Department of Pharmacognosy Physics Garden, Kwame Nkrumah University of Science and Technology-Kumasi (KNUST). The seeds were nursed for three weeks and transplanted onto heat-sterilized loamy soil in a greenhouse condition with ambient temperatures ranging from 23 to 36°C, 70 ± 5% relative humidity (RH) and a photoperiod of 12 D: 12L at the KNUST Chemical Ecology Laboratory. Leaves used to extract essential oils and headspace volatiles were obtained from 4-month-old plants of the three *Ocimum* species. The Ocimum plants were identified and authenticated with the help of a plant botanist at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. Vouchers specimens of the three Ocimum plants (*O. gratissimum* (UESD/OG/001/23), *O. tenuiflorum* (UESD/OT/002/23), and *O. basilicum* (UESD/OB/003/23)) were deposited in the herbarium of the Department of Biological Sciences, University of Environment and Sustainable Development.

2.1.1. Essential Oil Extraction from Ocimum Species. About 100 g of fresh *Ocimum* plant leaves were hydrodistilled for their essential oil using the Clevenger-type apparatus for 3 hrs. The extracted oil was dried over anhydrous sodium sulphate to eliminate hydrosols and then stored in a refrigerator at −4°C until used for repellency assay. The percentage oil yield was calculated using equation (1)

$$\text{percentage oil yield} = \frac{\text{volume (v) of oil obtained}}{\text{weight (w) of plant used}} \times 100. \quad (1)$$

(1) Organoleptic Tests for Essential Oils from Ocimum Plants. The smells of the essential oils were evaluated by randomly selected 30 people to grade the smell of the oil from 0 to 10, as previously described by Buckle [36]. Oils were graded as follows: 10 corresponding to excellent; 7 to 9 ranked as very good; 5 to 6 as good; 4 as acceptable; and ≤4 as offensive. Colour of the essential oil was done by physical examination using the eyes.

(2) Refractive Index of Essential Oils from Ocimum Species. Refractive index of the essential oil was evaluated using an Abber refractometer (A. KRÜSS Optronic GmBH–DR6300-TF, Hamburg USA) at 25°C.

2.1.2. Dynamic Headspace Volatile Collection from Ocimum Plants. Headspace volatile entrainment from the plant leaves was done according to the modified method described by Osei-Owusu et al. [27]. Fresh leaves (100 g) from each of the three *Ocimum* plants were individually subjected to a 24 hrs air entrainment using Pye volatile collection kits (Kings Walden, UK). Conditions for the air entrainment were an inflow air rate of 700 mL/min, which was then drawn through a Porapak Q trap (50 mg polymer load, 50/80 mesh, Supelco, Bellefonte, PA) at 600 mL/min under room temperature. The trapped volatiles were eluted from the polymer by washing with 750 μL of redistilled diethyl ether. An empty glass chamber served as a control.

2.2. Colonies of Anopheles gambiae (s.s.). Larvae of *An. gambiae* were obtained from a breeding colony at a rearing insectary unit of the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana; the number of generations was not considered since every filial level of female *An. gambiae* adults does vector and transmit *P. falciparum* to humans [6, 7]. Colonies of *An. gambiae* were reared in cages (sizes were similar to those used by [27]) under dark laboratory conditions (27°C; photoperiod = 12L: 12D; RH = 70 ± 5%), using the procedures in the report of Das et al. [37]. Five-day-old female *An. gambiae* adults from the colonies were used for repellency assays.

2.3. Repellency Assays

2.3.1. Dilution of Essential Oils in 70% Ethanol for Repellency Assays. Seventy percent ethanol was used to dilute essential oil from each *Ocimum* plant to adjust the ratios between the volume of essential oil and that of 70% ethanol to be 1 : 0, 1 : 1, and 1 : 9. Thus, the corresponding percentages of the amount of an essential oil present in the mixtures (i.e., oil + 70% ethanol) were undiluted at 50% and 10%, respectively. These mixtures were separately labelled and used for repellency assays.

2.3.2. Treatments with Essential Oils from Ocimum Plants against Anopheles gambiae (s.s.). The oil was tested as undiluted, 50%, and 10% ‘diluted in 70% ethanol for the essential oil repellency assays. A 0.1 mL of the undiluted essential oil was applied to protect the first author’s forearms (i.e., about 30 cm from wrist to elbow; plastic hand gloves were used to cover hands). A treated forearm was then exposed to 25 blood-starved 5-day-old female *An. gambiae* in a cage ($30 \times 30 \times 30\ cm^3$) under laboratory conditions (27°C; photoperiod = 12L: 12D; RH = 70 ± 5%). The time-length observed for the first mosquito bite during exposure of the treated forearm to the insects was recorded as the repellency time of the plant essential oil. Similar treatments to the author’s forearms were done for 50 and 10% diluted oils in 70% ethanol. For reference, 0.1 mL of commercially available mosquito repellent (i.e., Odomos; Dabur Limited, India) was used as a positive control, whereas 0.1 mL of 70% ethanol only was used as a negative control treatment to the section of the author’s forearm, which was exposed to 25 female *An. gambiae* in a cage. In each case of a treatment, three replicates were made.

2.3.3. Assays with Headspace Volatiles from Ocimum Plants against Anopheles gambiae (s.s.). The repellency activity of headspace volatiles from each *Ocimum* species was assessed according to the method described by Logan et al. [38]. A 0.1 mL of the eluted headspace volatile in 750 μL of diethyl ether was applied to protect the author’s forearms; the procedures and conditions applied in the present study were similar to those mentioned above in the case of the undiluted essential oils and the Odomos. However, only the redistilled diethyl ether was used as the negative control. The time (in minutes) for the first mosquito bite was used as the repellency time of the plant’s headspace volatiles.

2.3.4. Assay Data Analysis. The R software (R.v.4.1.1~Rstudio.v.1.4.1717) developed by the R Core Team [39] was used to analyze the repellency time lengths (RTLs) of the essential oils or the headspace volatiles. Having applied the Shapiro–Wilk test, normality in the data for treatments at 10 and 50% dilution levels was significant ($P < 0.05$) for lack of normal distribution, whereas data for treatments of essential oils or headspace volatiles were normally distributed. Moreover, all transformation techniques applied to data using formulae did not improve outputs. Therefore, we compared the P values of nonparametric analyses (Wilcoxon rank-sum, Kruskal–Wallis rank-sum, and Dunn's tests) to those of parametric tests (two-sample (or paired) t-tests, analysis of variance (ANOVA), and Tukey's Honestly Significant Difference (HSD)), respectively, similar to the analyses in the report of Heve et al. [40]. Because all the parametric statistics generated the lowest P values for better conclusions in this study [40], a paired t-test at P values ≤0.05 was used to compare the "mean ± standard error (SE)" values of two variables [41, 42]. On the other hand, Tukey's HSD test at P values ≤0.05 was used to compare "mean ± SE" values of RTLs among variables that were more than two [41].

2.4. GC/GC-MS Analysis

2.4.1. Chemicals. Authentical standards (>95%) used to confirm structures via coeluting were obtained from Sigma-Aldrich (in the USA), Alfa Aesar (in the UK), and Fluka (in Switzerland). (E)-4,8-Dimethylnona-1,3,7-triene (DMNT) and (E)-β-Farnesene were synthesized via the synthetic route reported by Osei-Owusu et al. [27]. Similarly, (E)-Ocimene was synthesized via the synthetic route previously reported by Hassemer et al. [43].

2.4.2. GC/GC-MS Analytical Procedures. Constituents of the essential oils hydrodistilled from the *Ocimum* species considered in this study have been reported [13, 33, 44–48]. As a result, we focused on the identification of compounds that are present in the headspace volatiles isolated from the three *Ocimum* spp. Analysis of the headspace plant volatile was done according to the method previously described by Osei-Owusu et al. [27]. Volatile extracts were analyzed on a GC (Agilent Technologies, 6890N, Stockport, UK), equipped with a flame ionization detector (FID) and an HP-1 capillary column (50 m × 0.32 mm i.d., 0.52 μm film thickness). The oven temperature was maintained at 30°C for 1 min and programmed at 5°C/min to 150°C, where it was held for 0.1 min, then at 10°C/min to 230°C and held for 27 min. The carrier gas was nitrogen. One (1) μL of the sample was injected into the injection port of the equipment manually. GC-MS analysis of eluted volatiles was performed using a Hewlett-Packard 5880A gas chromatograph (HP-5880A) with an HP-1 capillary column (50 m × 0.32 mm id, 0.52 μm film thickness). Ionization was by electron impact (70 eV, source temperature 250°C). Helium was the carrier gas. The oven temperature was maintained at 30°C for 5 min and then programmed at 5°C/min to 250°C. Tentative identifications were made by comparison of mass spectra with the NIST 2005 mass spectral database and Adam's library. Confirmation of peak identity was made by comparing their Kováts index (KI) values and GC peak enhancement with authentic compounds. The KIs of the compounds were calculated based on the homologue series of n-alkane (C7–C22) in the following equation:

$$I = 100 \times \left[n + \frac{tr\ (\text{unknown}) - tr\ (n)}{tr\,(N) - tr\,(n)} \right], \qquad (2)$$

where I = Kováts retention index, n = number of carbon atoms in the smaller n-alkane, N = number of carbon atoms in the larger n-alkane, and t_r = retention time.

3. Results

3.1. Yield of Essential Oils. The percentage yield, refractive index, colour, and smell of the hydrodistilled essential oil are presented in Table 1. *Ocimum gratissimum* had the highest essential oil content (1.16%), whereas *O. basilicum* had the lowest oil content of 0.98%. The colour of the distilled oil ranged from whitish to yellowish. The smell of *O. tenuiflorum* was ranked as very good, while that of *O. gratissimum* was ranked as offensive.

3.2. Essential Oils from Ocimum Plants against Anopheles gambiae. At each dilution level of the ethanol, the repellency time length (RTL values in each column) did not significantly vary between *O. gratissimum*, *O. tenuiflorum*, and *O. basilicum* (Table 2). However, a decrease in the dilution level of essential oil against *An. gambiae* was a significant source of variation ($F_{(2,18)} = 3.7$; $P = 0.04508$) in RTLs (in rows of Table 2). In the case of essential oil from *O. gratissimum* or *O. tenuiflorum*, the RTL decreased as the dilution level of the oil was decreased (Table 2). However, in the case of essential oil from *O. basilicum* against *An. gambiae*, a decrease in the dilution level of the oil caused an inconsistent trend in RTL values: 0.1 mL of 50% essential oil from *O. basilicum* significantly reduced its RTL value to 10 ± 10 minutes, whereas the 10% essential oil from the same *Ocimum* species rather caused an increase in the RTL value (Table 2).

Comparing the performance of each essential oil to that of Odomos, no significant difference was observed in RTLs between the undiluted oil from *O. tenuiflorum* and Odomos (Table 2). Similarly, the RTL values of 50% essential oil from *O. tenuiflorum* were also not significantly different from those observed for Odomos (Table 2).

3.3. Headspace Volatiles from Ocimum Species against Anopheles gambiae (s.s.). Although the RTL values for headspace volatile from *O. basilicum* was significantly lower than those of *O. gratissimum* or *O. tenuiflorum* (Table), no significant differences in RTL values were observed between the undiluted essential oils (Table 2) and headspace volatiles (Table 3). When compared to the RTL values of Odomos, only the headspace volatiles from *O. basilicum* achieved lower RTL values than those observed for Odomos (Table 3). The RTL values observed for the headspace volatiles from either *O. gratissimum* or *O. tenuiflorum* were not significantly different from those observed for Odomos (paired t-tests in Table 3).

Table 1: Average percentage yield, RI, colour, and smell of hydrodistilled essential oils.

Plant material	Percentage yield	RI	Colour	Smell (mean ± SD)[¶]
O. gratissimum	1.16	1.496	Whitish	2.30 ± 1.10
O. tenuiflorum	1.02	1.490	Whitish yellow	7.10 ± 1.13
O. basilicum	0.98	1.482	Yellowish	5.30 ± 1.60

Note: values were hedonically generated and then compared to ranges for their meanings, according to Buckle [36]. SD: standard deviation. RI: refractive index.

Table 2: Mean ± standard error values of repellency time-length (RTL in minutes) of essential oils extracted from species of *Ocimum* as compared with those of Odomos against *An. gambiae*.

Source of essential oil	Performance of essential oils at ethanol dilution levels, compared to that of Odomos against *An. gambiae*					
	Dilution levels of essential oil in 70% ethanol			*P* value (paired *t*-test) comparing RTLs at each ethanol dilution level to those of Odomos[¶]		
	Undiluted essential oil	50% essential oil	10% essential oil	Undiluted *vs.* Odomos	50% oil *vs.* Odomos	10% oil *vs.* Odomos
O. gratissimum	60 ± 17.32 a	20 ± 20 a	10 ± 10 a	0.05479*	0.02482*	0.03107*
O. tenuiflorum	90 ± 17.32 a	50 ± 36.06 a	40 ± 10 a	0.1045	0.06014	0.04694*
O. basilicum	30 ± 17.32 a	10 ± 10 a	20 ± 10 a	0.03156*	0.03107*	0.03539*

Tukey's HSD tests at *P* value ≤ 0.05: the same letter against the mean ± SE values in each column indicates no significant difference between the sources of the essential oils; mean ± standard error (SE) of RTL values observed for Odomos was 180 ± 34.64 minutes; using the paired *t*-tests at *P* value ≤ 0.05, RTL (mean ± SE) of Odomos was compared to RTL of each concentration of essential oil dissolved in 70% ethanol, which achieved RTL values < 1 minute; *: *P* value ≤ 0.05 denotes that the difference is significant.

Table 3: Mean ± standard errors of repellency time-length (RTL in minutes) of headspace volatiles extracted from species of *Ocimum* against *An. gambiae*: comparison to the performance of either essential oils (in Table 3) or Odomos.

Source of headspace volatile	RTL value of headspace volatile (eluted in 750 μL of diethyl ether) against *An. gambiae*	*P* value (paired *t*-test) for the comparisons	
		Headspace volatile *vs.* essential oil in Table 3	Headspace volatiles *vs.* Odomos[¶]
O. gratissimum	90 ± 17.32 a	0.2879	0.1045
O. tenuiflorum	110 ± 10 a	0.3868	0.1733
O. basilicum	10 ± 10 b	0.3868	0.03107*

Tukey's HSD tests at *P* value ≤ 0.05: the different letters against the RTL values (in column) indicate significant difference between the sources of headspace volatile; RTL values for 0.1 mL redistilled diethyl ether were <1 minute; mean ± standard error (SE) of RTL values observed for Odomos is in the footnote of Table 2;*: *P* value ≤ 0.05 suggests that the difference is significant.

3.4. Chemical Composition of Headspace Volatiles from Ocimum Species. GC/GC-MS analysis revealed varying chemical compositions (Table 4), occurring in the headspace volatiles from the three *Ocimum* species, with some constituents previously reported to have repellent and insecticidal activities. For the chemical composition of *O. tenuiflorum,* a total of 49 constituents were identified with *E*-Caryophyllene (45.223%), α-Selinene (10.779%), eugenol (10.395%), and α-Humulene (8.798%) as the major constituents. In the case of *O. basilicum*, 61 constituents were identified, with Terpinene-4-ol (26.897%), linalool (17.776%), *E*-α-bergamotene (17.699%), and *E*-citral (14.196%) as the major constituents. For *O. gratissimum*, cymene (25.487%), thymol (7.5642%), α-thujene (5.5245%), and thymol methyl ether (4.2625%) were identified as the major components of the headspace volatiles. DMNT was found in the headspace volatiles of *O. tenuiflorum* and *O. gratissimum* with a percentage composition of 1.1955 and 1.4954%, respectively. Caryophyllene oxide, Sabinene (+), α-phellandrene, α-cubebene, and α-copaene were identified in the headspace volatiles from *O. tenuiflorum* and *O. gratissimum* but not in *O. basilicum.* Structures of the major constituents found in the headspace volatiles from the *Ocimum* spp. are shown in Figure 1.

4. Discussion

The control strategy of mosquitoes using conventional synthetic insecticides has been challenging due to resistance, environmental impact, and health implications. Thus, more environmentally friendly vector management strategies are warranted. Using plant extracts provides a promising strategy for controlling the malaria vector [13, 49, 50]. Extracts from several plant species worldwide have been tested against different *Anopheles* spp. [51]. In field conditions, essential oils from *Pinus* spp., lemon grasses (i.e., citronella oil from *Cymbopogon* spp.), and *Dalbergia sissoo* (Roxburgh) have shown high repellency potential, thereby offering a longer effective protection time between 6 and 11 hours (hrs) against *Anopheles culicifacies* (Giles),

Table 4: The headspace chemical composition of the three *Ocimum* species.

Serial number	Compound	KI[P]	Percent composition in the *Ocimum* species			Identification method
			O. tenuiflorum	*O. basilicum*	*O. gratissimum*	
1	α-thujene	927	—	—	5.5245	b, d
2	α-pinene	935	0.7200	0.0036	1.1026	a, b, c, d
3	Camphene	949	0.3179	0.0033	0.2567	a, b, d
4	Cosmene	961	—	—	0.6591	d
5	1-octen-3-ol	966	0.3802	0.0157	2.6542	a, b, c, d
6	β- terpinene	970	—	0.0043	—	d
7	Sabinene (+)	971	0.3708	—	1.2353	a, b, d
8	β-pinene	975	0.3297	0.0025	4.7758	a, b, c, d
9	β-myrecene	984	—	0.0096	0.5417	a, b, c, d
10	6-Methyl-3-heptanone	985	0.2601	—	—	a, d
11	*Z*-3-hexenyl acetate	989	0.1001	0.0095	0.2911	a, b, c, d
12	δ-3-carene	1001	—	—	0.0564	d
13	α-phellandrene	1002	0.116	—	0.4614	b, d
14	*Z*-β-ocimene	1013	2.5447	0.0041	0.1448	b, d
15	Cymene	1015	—	0.0030	25.487	b, d
16	Limonene	1026	—	0.0147	5.1065	a, b, c, d
17	Eucalyptol	1028	0.8155	0.0371	—	a, b, d
18	*E*-β-ocimene	1040	0.052	0.0730	0.0454	a, b, c, d
19	γ-terpinene	1054	0.064	0.0112	2.1045	a, b
20	Cis-sabinene hydrate	1062	0.0582	0.0978	3.3181	a, b, d
21	Linalool oxide cis (furanoid)	1066	—	0.0035	0.2411	a, b, d
22	Trans-linalool oxide (furanoid)	1080	0.0673	0.0794	0.1247	a, b, d
23	Terpinolene	1085	0.1542	—	—	a, b, d
24	(*R* or *S*)-linalool	1089	0.2234	17.776	0.3686	a, b, d
25	Cis-p-menth-8-en-1-ol	1100	—	0.0406	0.1283	b, d
26	Unknown	1104	—	—	0.4293	
27	*E*-DMNT	1106	1.1955	—	1.4954	a, b, c, d
28	Fenchol	1108	—	0.0782	—	a, b
29	Cis-2-p-menthen-1-ol	1118	—	0.0711	—	d
30	1,3,8-p-menthatriene	1132	—	0.0419	0.7805	b, d
31	(−)-Camphor	1134	0.2552	—	—	a, b
32	Unknown	1141	—	—	1.5645	
33	3-Thujen-2-one	1159	—	—	0.4423	d
34	Endo-borneol	1161	2.3530	—	—	a, b, d
35	δ-terpineol	1157	—	0.0306	—	d
36	Terpinene-4-ol	1175	—	26.897	0.3104	a, b, d
37	L-α-terpineol	1181	0.1440	0.4479	—	a, b, d
38	Acetic acid, octyl ester	1194	—	0.1582	—	d
39	2-hydroxylcineole	1203	—	0.0277	—	d
40	Fenchyl acetate	1217	—	0.2033	—	a, b, d
41	Thymol methyl ether	1219	—	—	4.2625	d
42	Z-citral	1222	—	0.1123	—	b, d

Table 4: Continued.

Serial number	Compound	KI^P	Percent composition in the *Ocimum* species			Identification method
			O. tenuiflorum	*O. basilicum*	*O. gratissimum*	
43	α-terpinyl acetate	1227	—	0.2878	0.3886	a, b, d
44	Geraniol	1239	—	0.5556	—	a, b, d
45	Linalyl butyrate	1243	—	0.2845	—	a, d
46	*E*-citral	1250	—	14.196	—	a, b, d
47	Unknown	1262	0.032	—	—	
48	Thymol	1269	—	—	7.5642	a, b
49	(−)-Bornyl acetate	1280	0.066	0.0769	—	a, b
50	4-terpinenyl acetate	1292	—	0.0777	0.5474	d
51	Methyl geranate	1305	—	0.1970	—	d
52	Eugenol	1337	10.395	0.0873	—	a, b, c, d
53	Unknown	1339	—	—	0.1478	
54	α-cubebene	1362	0.0816	—	0.6548	b, d
55	Geranyl acetate	1364	—	0.0037	—	b, d
56	Methyl eugenol	1376	0.0076	0.2129	—	a, b, c, d
57	Cis-jasmone	1382	—	0.0513	—	b, d
58	α-copaene	1391	1.7136	0.3696	0.4503	a, b
59	β-cubebene	1401	—	0.2768	0.2159	a, b, d
60	β-copaene	1408	0.0023	0.1492	—	a, b
61	α-bergamotene	1423	—	0.1556	—	b, d
62	*E*-caryophyllene	1438	45.223	0.1861	7.5624	a, b, c, d
63	α-guaiene	1447	—	—	2.147	a, d
64	*E*-α-bergamotene	1452	—	17.699	—	b, d
65	*E*-β-farnesene	1463	—	1.2800	—	a, b, c, d
66	Calarene	1467	—	0.4021	—	d
67	α-humulene	1470	8.798	0.4315	0.7278	a, b, d
68	γ-cadinene	1481	—	—	0.2459	a, b
69	(+)-epi-bicylcosesquiphellandre	1487	—	0.9120	2.7354	d
70	γ- muurolene	1500	—	1.5490	—	a, b, d
71	β-selinene	1502	—	—	4.4975	a, b, d
72	α-selinene	1504	10.779	—	1.9591	a, b
73	Bicyclogermacrene	1511	—	1.5490	—	d
74	β-chamigrene	1512	0.021	—	—	a, b
75	δ-guajene	1523	—	1.0255	—	d
76	δ-cadinene	1529	0.7523	3.2697	0.2554	d
77	(*E*)-γ-bisabolene	1534	0.4662	—	—	a, b, d
78	Selina-3,7(11)-diene	1535	—	—	0.3692	b, d
79	Cubedol	1543	0.3424	—	—	b, d
80	α-murolene	1549	—	0.0812	0.3287	a, b, d
81	Elemol	1552	0.206	—	—	a, b, d
82	*E*-nerolidol	1557	0.7986	0.1848	—	a, b, c, d
83	Cubenol	1627	—	0.5606	—	b
84	Unknown	1566	0.6621	—	—	

Table 4: Continued.

Serial number	Compound	KI[P]	Percent composition in the *Ocimum* species			Identification method
			O. tenuiflorum	*O. basilicum*	*O. gratissimum*	
85	*E*-farnesol	1571	0.2393	—	—	b, d
86	Unknown	1582	0.1916	—	—	
87	Germacrene D-4-ol	1587	0.4726	—	—	a
88	Caryophyllene oxide	1597	2.998	—	0.9561	a, b, d
89	Viridiflorol	1608	0.5527	—	—	a, d
90	Unknown	1622	0.1177	—	—	
91	γ-eudesmol	1642	0.1474	—	—	a, b, d
92	Tetracyclo[6.3.2.0 (2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	1646	0.2586	—	—	b
93	(−)-β-cadinene	1650	—	2.8968	—	d
94	Unknown	1652	0.2681	—	—	
95	α-cadinol	1662	—	0.1659	—	d
96	α-eudesmol	1665	0.5379	—	—	a, d
97	Eudesm-7(11)-en-4-ol	1673	—	0.0237	—	d
98	Isolongifolol	1680	0.3284	—	—	a
99	α-bisabolol	1682	—	0.0271	—	d
100	Unknown	1691	0.0737	—	—	
101	Unknown	1731	—	0.085	—	

[P]On a nonpolar GC column (HP-1). Compound identity confirmed by (a) Adams library (2007), (b) KI, (c) coelution with authentic standard, and (d) NIST mass fragment. Dashes indicate the absence of a compound. Percentage composition represents mean, with $n = 3$.

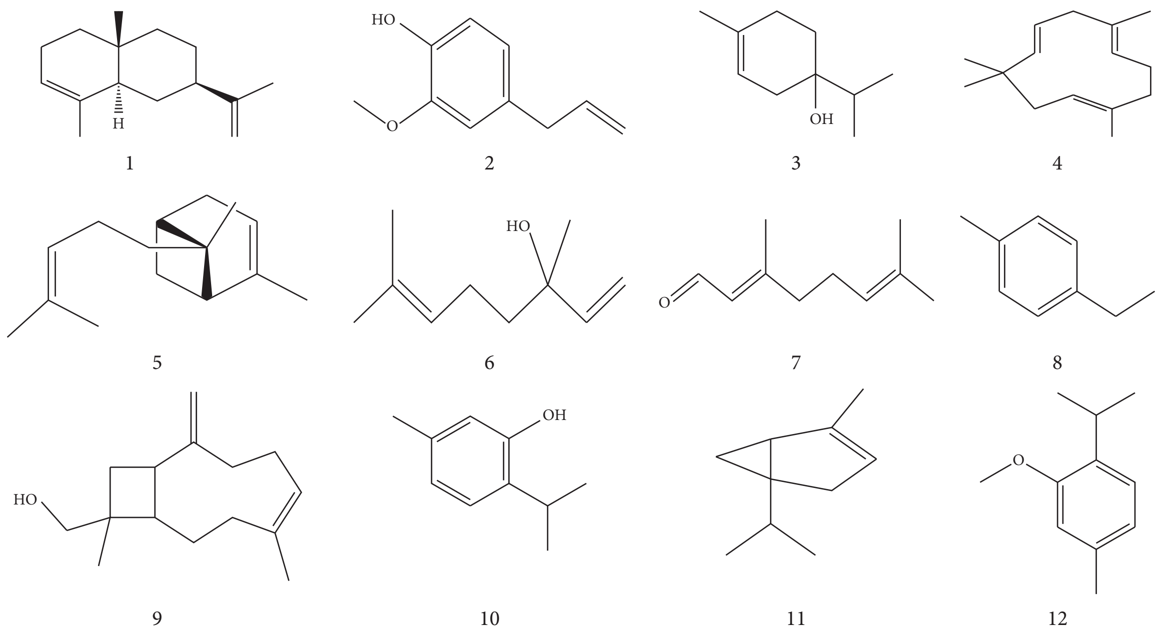

Figure 1: Structures of the major constituents found in the headspace volatiles from the *O. tenuiflorum*, *O. gratissimum*, or *O. basilicum*: (**1**) α-selinene, (**2**) eugenol, (**3**) terpinene-4-ol, (**4**) α-humulene, (**5**) *E*-α-bergamotene, (**6**) linalool, (**7**) *E*-citral, (**8**) cymene, (**9**) *E*-caryophyllene, (**10**) thymol, (**11**) α-thujene, and (**12**) thymol methyl ether. Structures were drawn using ChemDraw professional software.

Anopheles subpictus, and *Anopheles annularis* [51–53]. Laboratory trials have also revealed that essential oils from *Ligusticum sinense* (Umbell.) showed a remarkable repellency time over 11.5 hrs against *An. minimus* and *Ae. aegypti* (L.) [54], whereas essential oils from *Cymbopogon* spp., *Eucalyptus* spp., *Aniba rosaeodora* (Ducke), *Lavandula angustifolia* (Miller), *Nepeta cataria* (L.), *Pelargonium graveolent* (L'Héritier), *Thymus serpyllum* (L.), *Jasminum grandiflorum* (L.), *Amyris balsamifera* (L.), *Glycina soja* (Siebold and Zuccarini), *Juniperus virginiana* (L.), and *Citrus limon* (L.; Osbeck), among others, offered repellency (or protection) time lengths between 5 and 8.5 hrs against *An. stephensi* and *An. sundaicus* [51]. Nonetheless, essential oils from the majority of plants tested under laboratory conditions could only achieve a repellency time <5 hrs against *An. albimanus, An. dirus*, and *An. stephensi* [51]. In the case of laboratory trials involving essential oils from the majority of plant species against *An. arabiensis* and *An. gambiae*, the observed repellency time seems to be ≤1.5 hrs [51].

To the best of our knowledge, there are no previous reports on the headspace volatiles of *O. tenuiflorum*, *O. gratissimum*, and *O. basilicum* against mosquitoes. In the current study, we explored the mosquito repellency properties and chemical composition of the headspace volatile isolated from the three *Ocimum* species. We observed that the undiluted essential oils from the three *Ocimum* plants tested against *An. gambiae* achieved a repellency time of 90 ± 17 minutes. Moreover, our laboratory trials involving headspace volatiles from these *Ocimum* plants proved that the repellency time-lengths observed for the headspace volatiles were within the range of those observed for the essential oils. Thus, both the undiluted essential oils and the headspace volatiles achieved similar repellency time lengths, which were not significantly different from those of a commercially available repellent. Our observations in this study suggest that repellency time-lengths achieved by either essential oils or headspace volatiles from *Ocimum* plants against *An. gambiae* were similar to those observed for essential oils from *Ocimum* spp. tested against *Ae. aegypti* [48] or the list of several plant species tested on culicids in the report of Asadollahi et al. [51]. Although complementarity between oils and headspace volatiles may be more effective, essential oils or headspace volatiles from the *O. tenuiflorum* may be an alternative to protect humans against *An. gambiae*. This is because oils or headspace volatiles from *O. tenuiflorum* consistently outperformed the remaining *Ocimum* plants used in this study, possibly because of some unique constituents.

PVCs are chemicals that have been shown to significantly reduce the host-seeking behavior of insects and/or act as a deterrent [27, 55]. A previous study has shown that the chemical profiles of leaf volatile compounds from a plant genus are highly diverse [30]. For example, plants such as *Tephrosia vogelii* and *Lippia javanica* (Burm. f.) exhibited an extreme variation of bioactive principles [56]. Also, the chemical variability in the essential oil composition of the *T. vogelii* plant sampled from different locations in eastern Uganda has been reported [56]. The variation in the essential oils of the plants can be attributed to the extraction methods, seasonal variation, and chemotaxonomic factors [56, 57]. In the current study, the headspace volatiles of the three *Ocimum* plants were diverse, but mostly monoterpene and sesquiterpene hydrocarbons were common, as it has been in the case of many essential oil-bearing plants [29, 30, 32, 58]. Our GC-MS data revealed that 6-methyl-3-heptanone, terpinolene, (−)-camphor, endo-borneol, β-chamigrene, (*E*)-γ-bisabolene, elemol, *E*-farnesol, germacrene D-4-ol, viridiflorol, α-eudesmol, and isolongifolol were unique to *O. tenuiflorum*. In addition, we found that α-thujene, cosmene, δ-3-carene, 3-thujen-2-one, thymol methyl ether, thymol, α-guaiene, γ-cadinene, β-selinene, and selina-3,7 (11)-diene were present only in the headspace volatile of *O. gratissimum*. In the case of *O. basilicum*, β-terpinene, *E*-β-farnesene, fenchol, cis-2-p-menthen-1-ol, δ-terpineol, geraniol, and *Z*-citral were unique to the plant. Similar chemical constituents identified in the three *Ocimum* plants used in the present study have been previously reported in their leaf's essential oil [13, 33, 34, 44, 48, 58–60]. However, for the first time, we have identified additional constituents in the *Ocimum* plant headspace volatiles that have not been previously reported in its leaf's essential oils. These new constituents included *E*-DMNT, fenchol, cis-jasmone, and thymol methyl ether, among others.

Most of the identified compounds in this current work have been reported to have insect-repellency and/or insecticidal properties. For example, *E*-caryophyllene and α-humene showed significant oviposition deterrent effects against *Ae. aegypti* females [30]. Hao et al. [61] reported that geraniol and citral were effective at reducing the host-seeking ability of the Asian tiger mosquito (*Ae. albopictus*). β-caryophyllene oxide is an effective repellent against two mosquito strains (*Ae. albopictus* and *An. dirus*) under laboratory conditions [62]. Logan et al. [38] showed that a combination of plant-derived volatiles (6-methyl-5-hepten-2-one and geranyl acetate) effectively repelled *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. gambiae*. Also, eugenol, α-pinene, and β-caryophyllene, which are major essential oil constituents found in *Coleus barbatus* (syn. *Plectranthus barbatus*), have been effective against *An. subpictus* ($LC_{50} = 25.45–41.66\,\mu g{\cdot}mL^{-1}$), followed by *Ae. albopictus* ($LC_{50} = 28.14–44.77\,\mu g{\cdot}mL^{-1}$) and/or *Cx. tritaeniorhynchus* ($LC_{50} = 30.80–48.17\,\mu g{\cdot}mL^{-1}$). In another study, eugenol, which is a major component of the essential oils of basil, cinnamon, cloves, and other plants, caused significant mortality of *Ae. aegypti* and *An. darlingi* larvae [50]. Thymol, which is a major constituent of *Carum copticum* (L.) and *Semenovia tragioides* (Boiss.) Manden and *O. gratissimum*, provided a 1-hour repellency against *An. stephensi* adults at the dose of 25 mg $(mat)^{-1}$ [63]. Endo-borneol, caryophyllene oxide, and (−)-camphor are commonly used as insect repellents [62, 64, 65]. Meanwhile, in this study, (−)-camphor, (E)-γ-bisabolene, and endo-borneol were identified in the headspace volatiles from *O. tenuiflorum* only. Although the amounts of (−)-camphor and endo-borneol observed in *O. tenuiflorum* headspace volatiles were very low (i.e., 0.2–0.5%), these compounds could synergistically complement the repellency activities of the major constituents also identified in *O. tenuiflorum*, thereby causing the headspace volatiles from *O. tenuiflorum* outperforming those from *O. gratissimum* and *O. basilicum* against *An. gambiae* in the bioassays. In our view, research is required to investigate the complementarity of the chemical constituents observed in *O. tenuiflorum*.

5. Conclusions

When the performancesof essential oils from *O. gratissimum*, *O. basilicum*, and *O. tenuiflorum* against An. gambiae in assays, the latter achieved the longest repellency time comparable to the commercially sourced repellency. In similar assays involving headspace volatiles from the three *Ocimum* species against *An. gambiae*, *O. tenuiflorum* outperformed and again achieved the longest repellency time again. Using GC/GC-MS analysis, 101 chemical constituents were identified in the headspace volatiles from the three *Ocimum* species. However, (−)-camphor, (E)-γ-bisabolene, and endo-borneol were present in very low quantities in the headspace volatiles from *O. tenuiflorum* only, suggesting that longer repellency time lengths achieved by *O. tenuiflorum* could hypothetically be linked to these three unique chemical constituents. Thus, further studies are required to investigate the usefulness of the complementary roles of chemical compounds identified in the more repelling *O. tenuiflorum*.

Data Availability

The data used to support the findings of this study are made available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

J.O–O., W.K.H., O.F.A., M.J.O., J.A., K.N.D., B.Y.V., K.A.A.M., M.A., A.A., M.B., and A.H. conceptualized the study. J.O–O., W.K.H., O.F.A., M.J.O., J.A., K.N.D., B.Y.V., K.A.A.M., M.A., A.A., and A.H. investigated the study. J.O–O., W.K.H., K.A.A.M., O.F.A. performed data analysis. J.O–O., W.K.H., O.F.A., M.J.O., K.N.D., and M.A. wrote the original draft. J.O–O., W.K.H., O.F.A., M.J.O., A.P., K.N.D., K.A.A.M., M.A., and M.B. wrote, reviewed, and edited the article. J.O–O., W.K.V., O.F.A., K.N.D., M.B., and A.H. supervised the study. All authors have read and agreed to the published version of the manuscript.

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