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Phytochemical screening and repellence potencies of bioactive molecules of plant extracts derived from *Ocimum suave, Ocimum americanum* and *Eucalyptus citriodora* against *Anopheles gambiae*

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Abstract

Background Malaria poses a global threat to human health. It's a vector-borne disease of public health concern and affects the socio-economic status of people in developing countries. Malaria management faces many challenges namely, affordability, availability, and quality of drugs. Plants are considered a very significant resource in many parts of the world due to their variety of uses in treating diseases and ailments. Conventional drugs are expensive and not readily available. Repellents have been in use for the prevention of *Anopheles* bites, but all these have a myriad of negative effects to the user, such as allergy and dermatitis. This study sought to develop a plant-based *Anopheles gambiae* repellent for control of malaria, because it is eco-friendly and non-toxic.

Methods The plant leaf samples: *Ocimum americanum* and *Eucalyptus citriodora* were collected from Mugui village in Tharaka Nithi County, Kenya, while *Ocimum suave* was harvested at Gacuru village in Meru County, Kenya. The samples were hydro-distilled using a Clevenger apparatus to obtain the essential oils. The experimental tests were done in a repellent testing chamber. The values of repellency action were determined over control at a p-value of 0.05 and 0.01 by one-way ANOVA and separated using Student-Newman-Keels at $P \leq 0.05$ using Minitab software. The chemical analysis of the essential oils was done using a Gas Chromatography-Mass Selective detector instrument (GC-MSD). The human-bait method was used to assess the repellency efficacy of the essential oils and their blends against *An. gambiae*.

Results The GC-MSD results revealed that the plants are endowed with terpenoids, such as 1,8-Cineole. β-Bisabolene, β-Pinene, α-Terpineol, and Geranial as the most abundant compounds in the samples. The blend of *O. suave* and *O. americanum* in the ratio of 1:1 was the most potent (100.00 ± 0.00) and compared well with the positive control Ballet™ (100.00 ± 0.00). The observation that the blend of *O. suave* and *O. americanum* was comparable to Ballet™, suggests that this may be due to additive or synergistic effects of individual constituents.

Conclusion This study revealed that these plants are endowed with bioactive compounds such as terpenoids and flavonoids that possess potent repellency against *An. gambiae* mosquitoes.

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Keywords Repellent activities, *Ocimum suave*, *Ocimum americanum*, *Eucalyptus citriodora*, Essential oils, *Anopheles gambiae*

Background

Among the most significant public health issues and barriers to socio-economic development of developing nations, particularly in the tropics, are vector-borne diseases, among them malaria [1]. There are many species of mosquitoes that cause vector-borne diseases responsible for the death of many people in Kenya and Africa [2]. Avoiding *Anopheles* bites is one of the best ways to avoid the spread of malaria [3–5]; and the use of plant extracts can contribute to the reduction in malaria cases. Asadollahi et al. [6], demonstrated that the management of the adult *Anopheles gambiae* population is aided by the use of plant extracts. Plants are considered a very significant resource in many parts of the world due to their variety of uses in treating diseases and ailments [7]. Traditionally, *An. gambiae* repellent herbs in western Kenya have been the subject of ethnobotanical investigations, which revealed that the plant branches of *Ocimum suave, Ocimum americanum,* and *Eucalyptus citriodora* are efficient against malaria vectors by repelling *An. gambiae,* when burned or thermally ejected with household charcoal stoves [8]. They disrupt the olfactory receptors in mosquitoes, interfering with their ability to locate hosts for blood-feeding [9]. These disruptions in the mosquito's sensory perception contribute to the repelling of *An. gambiae* and reducing their biting behaviour. These findings underscore the importance of understanding the underlying mechanisms of action of plant-based repellents to develop more effective and long-lasting mosquito control strategies [10]. The essential oils from *O. suave, O. americanum*, and *E. citriodora* plants have insecticidal and repellent properties that can effectively deter mosquitoes, hence holding promise for repelling *An. gambiae* and could be potential alternative mosquito control measures [13]. The advantages of using plant extracts, such as essential oils, in repelling *An. gambiae* are that they are natural, biodegradable, and environmentally friendly, unlike chemical insecticides, which can harm the environment and non-target species [11, 12].

This study sought to determine the phytochemical screening and repellency potential of essential oils from *O. suave, O. americanum*, and *E. citriodora.* Therefore, the findings of this study will be applied in formulating a potent *An. gambiae* mosquito repellent.

Methods

Sample collection

The plant leaf samples of *O. americanum* and *E. citriodora* were collected from the natural habitat in Tharaka South Sub-County, Tharaka constituency in Tharaka Nithi County GPS location 0º3′4′42.7842″S, 37º51′58.75092″E, while *O. suave* was harvested at Gacuru village, Kiagu location, Meru central district, Meru County, Kenya. GPS coordinates were (19° 4′3″ N, 72° 52′40"E) and (0° 2′17"N, 37° 49′43′′E). The collection of these samples was done based on the ethnobotanical information availed by local herbalists in the area. The leaf samples were identified by a taxonomist from the National Museums of Kenya, and a voucher specimen was deposited at Tharaka University herbarium for future reference.

Sample preparation and extraction

The authors sought and obtained a permit from the National Commission for Science Technology and Innovation (NACOSTI) (NACOSTI/P/23/3959). This research was authorized by the ethical committee of Chuka University (CU/ERC/NACOSTICOSTI/1423). The leaf samples were collected and washed to remove any dust and other contaminants and finally air-dried at room temperature to prevent loss of volatile phytocompounds. They were then cut into smaller pieces to increase surface area during extraction. The extraction of essential oils was done using hydro-distillation apparatus. Five hundred grams of clean, dry and crushed plant leaves were weighed, packed in a round-bottomed flask and a sufficient quantity water was added. The distillate obtained made up of the aqueous layer and organic layer was collected separately, where the organic layer (essential oils) was allowed to dry over anhydrous sodium sulfate. The dry essential oils were weighed, put in a vial, and stored in a refrigerator at 4 °C for use in both chemical and experimental analysis.

Phytochemical screening

The essential oils of *O. suave, O. americanun, and E. citriodora* were analyzed using GC-MSD at the International Centre for Insect Physiology and Ecology (ICIPE). The gas chromatograph was operating at the following temperature set on the computer: 70 °C for 4 min, ramp at 4 °C/min to 220 °C for 5 min; carrier gas, N_2. The computer-based method of peak area normalization without any correction factors was used to estimate the relative concentrations of the various elements. Peaks found were compared to data from a GC–MS analysis.

Experimental insects and repellent test

The experimental mosquitoes were procured from the School of Biological Sciences Department of Biology, Insectary section at the University of Nairobi, Kenya. The human-bait technique, as shown in Fig. 1, was used to gauge the extracted oils'level of repellency. Evaluations were conducted in a 6 ×6×3 m room with a humidity level of 60–80% and a temperature range of 25–29 °C. Five human participants having a 3 by 10 cm area marked with a permanent marker on each forearm were used. For efficacy, the testing time lasted up to eight hours during the day and at night. *An. gambiae* repellency was examined between 0800 and 1600 h.

Blending *O. suave* and *O. americanum* essential oils in the ratio of 1:1 ratio. To prepare 3 ml of the repellent, 0.3 ml of the essential oil blend was measured carefully in a clean, dry container. Three drops of Tween 80 were added to the essential oil blend in the container, and then ethanol was gradually poured into the container while gently stirring the mixture until a total volume of 3 ml was attained. The formulated *An. gambiae* was labelled and stored in a cool and dark place using an airtight container.

Repellent test procedure

The test was conducted in a 6 ×6×3 m room with a humidity level of 60–80% and a temperature range of 25–29 °C. Five volunteers were used for each testing. The individuals had to clean their hands, including the arm, followed by drying and putting on a latex glove. Twenty female mosquitoes were released into the cage and left to acclimatize. The experiment was conducted in the dark and during the day; the number of counts of landing mosquitoes on the tester was scored and used in data analysis. The volunteer with blank control (nothing applied) was allowed to insert their arm covered with gloves into the cage after a consistent amount of repellent (1 mL per 600 cm^2 of skin) was uniformly applied on a designated area of the skin, such as the forearm. The repellent was allowed to dry up for 10 min to avoid the transfer or evaporation during testing. The repellent was applied onto the arm (1 ml), and each volunteer put the test forearm in *An. gambiae* cage that measured (40 ×40×40 cm), containing 50 *An. gambiae*, for the first three minutes of every half-hour exposure. The repellency test was continued until at least two *An. gambiae* mosquitoes landed on or bite the hand. The experiment was conducted in five replicates (Fig. 1).

Fig. 1 Repellent testing chamber

The following formula by [14] was used to determine the percentage repellency in the trials.

$$\%\ \text{Repellency} = \frac{C - T}{C} \times 100 \tag{1}$$

where T is total number of *An. gambiae* bites in the treated areas; C is total number of *An. gambiae* bites in the untreated (control) areas.

Data analysis and presentation

The mean % repellency data was normalized by logarithmic transformation before being subjected to analysis of variance (ANOVA). The means between treatments were separated using Student–Newman–Keuls at P ≤ 0.05 using Minitab software version 17 so as to determine the potency of the essential oils.

Results

GC–MS results

The GC-MSD analysis of the essential oil of *O. suave, O. americanun and E. citriodora* gave the mass spectra as shown in Figs. 2, 3, 4 and their chemical composition, retention time and relative abundance as shown in Tables 1, 2, 3.

The GC-MSD results revealed that *O. suave* plant leaves contained forty-nine compounds (Fig. 2 & Table 1). The major components in *O. americanum* plant leaves were β-Bisabolene, α-Pinene, Geranial and Neral.

The GC-MSD results revealed that *O. americanum* plant leaves contained forty-four compounds (Fig. 3 and Table 2). The major components in *O. americanum* plant leaves were 1,8-Cineole, α-Terpineol and Linalool.

The GC-MSD results revealed that *E. citriodora* plant leaves contained fifty-two compounds (Fig. 4 and

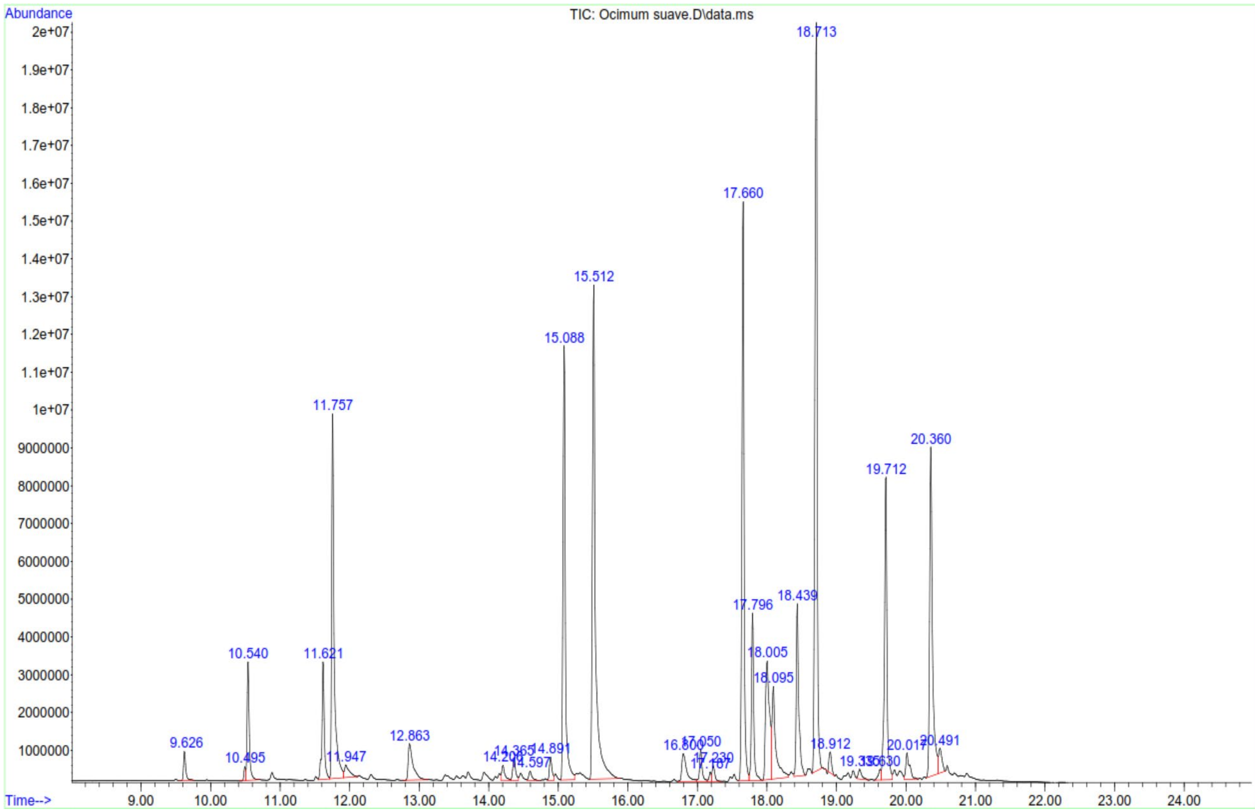

Fig. 2 The GC-MSD Total Ion chromatogram of essential oil of essential oil of O. suave

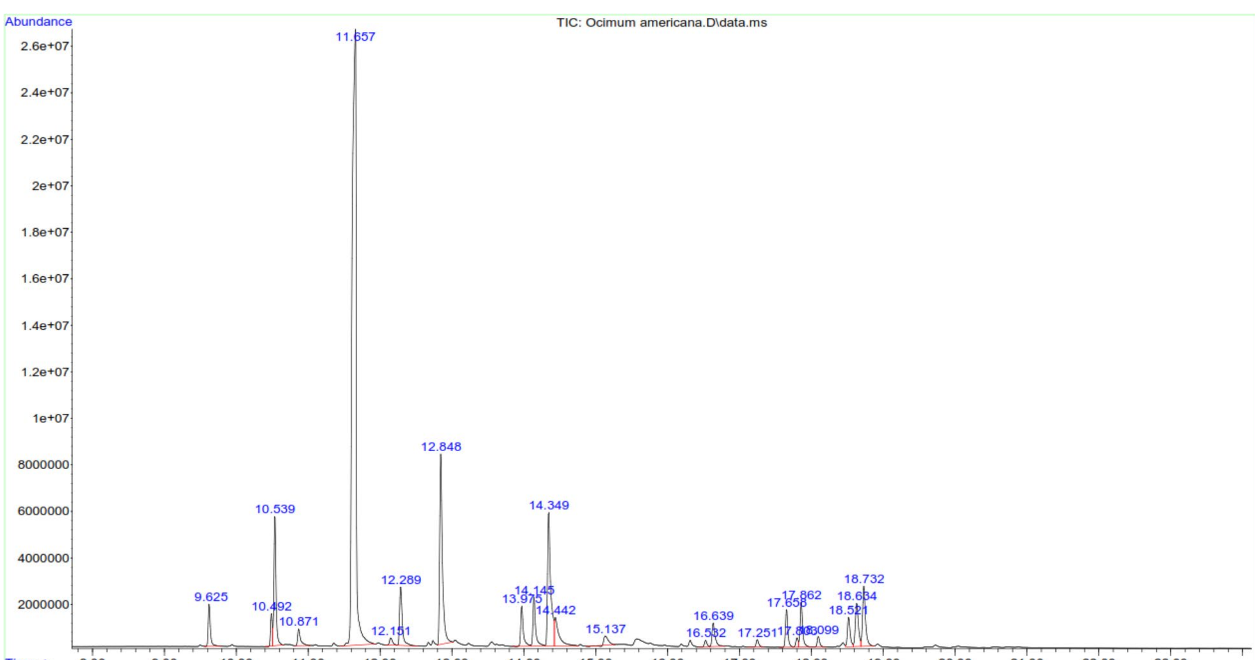

Fig. 3 The GC-MSD Total Ion Chromatogram (TIC) of essential oil of essential oil of *O. americanum*

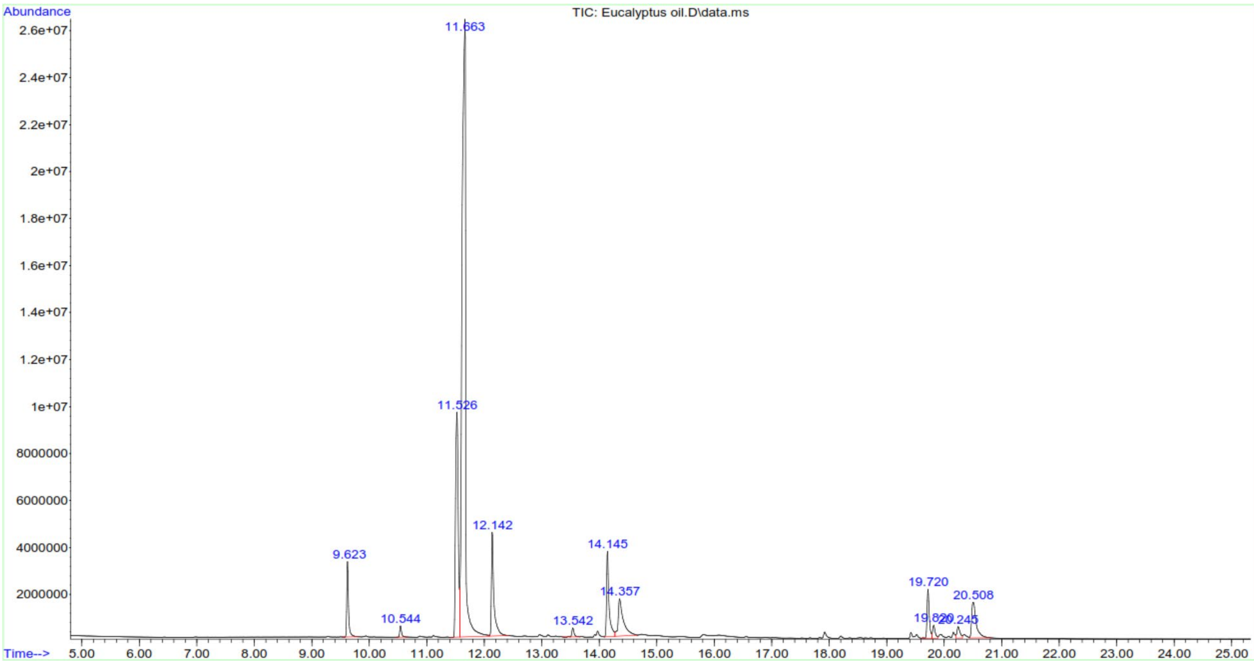

Fig. 4 GC-MSD Total Ion chromatogram of essential oil of *E. citriodora*

Table 3). The major components in *E. citriodora* plant leaves were 1,8-Cineole, o-Cymene, and γ-Terpinene.

Repellency activity test for the plants leaf extracts

Repellency of essential oils of *E. citriodora, O. suave, O. americanun*, the blends and the positive control, Ballet against *An. gambiae* are shown in Table 4 and Figs. 5, 6, 7.

Table 4 shows there is no significant difference in the repellency activities of the blend of *O. suave* and *O. americanum* in the ratio of 1:1 and the existing mosquito repellent in Kenyan shops, Ballet™ (SNK, $p \geq 0.05$, 95% CL).

Rankings of different essential oils against *An. gambiae* show that there was no significant difference in the repellency activities of different essential oils with time (SNK, $p \geq 0.05$, 95% CL). Figure 6 shows the mean repellency of essential oils of *E. citriodora, O. suave, O. americanun*, against *A. gambiae*.

The findings reveal that the blends had more repellency than single essential oils (Table 4 & Fig. 7). Among the plant essential oils, there was a significant ($p < 0.05$) increase in repellency between the exposure of the cohort replicate against *An. gambiae* when *O. suave* and *O. americanum* essential oils were blended and used in the ratio of 1:1 (Table 4 & Fig. 7). However, there was a drop in repellency with the exposure of the cohort of *An. gambiae* to blends with *E. citriodora*. Thus, the level of repellency from the essential oil of each plant appears to be negatively affected by the presence of other conspecific plants (Table 4 & Fig. 7).

For a given dose, there were varying degrees of dose-dependent responses. All of the individual essential oils tested had significant repellent effects against *An. gambiae*. However, a blend of these compounds had more repellent activities against *An. gambiae* as shown in Table 4 and Fig. 7. Among the tested essential oils assayed, the most repellent were *O. suave* and *O. americanum* with a repellence activity of 85.33% and 92.67% at a concentration of 0.75 g/mL. The blend of *O. suave,* and *O. americanum* in the ratio of 1:1 was the most potent repellent with a mean percentage repellency of 96% at a concentration of 0.75 g/mL (Table 4 and Fig. 7). Similarly, this study utilized a positive control, a mosquito repellent in Kenyan supermarkets/shops and chemists, Ballet™. There was no significant difference in the repellency of the mosquito repellent Ballet™ and that of the blend of *O. suave* and *O. americanum* in the ratio of 1:1 (Table 4 and Fig. 7). It is worth noting that *O. americanum* was the most potent single essential oil, hence the biggest contributor to the high potency of the blend (Table 4 & Fig. 7). The blends had more repellency than single essential oils, and the most potent blend was *O. suave* and *O. americanum* in the ratio of 1:1. However, there was a drop in repellency with the blends with *E. citriodora* against *An. gambiae*. Thus, the level of repellency from essential oils of each plant appears to be negatively affected by the presence of other

Table 1 GC-MSD results for *Ocimum suave*

NO	RT	Compound Name	CAS	Relative %
1	9.50	Thujene	000099-83-2	0.82
2	9.63	α-Pinene	000080-56-8	1.16
3	9.95	Camphene	000079–92-5	0.80
4	10.54	β-Pinene	000127-91-3	2.26
5	10.88	Myrcene	000123-35-3	1.02
6	11.36	δ–2-Carene	000554-61-0	0.79
7	11.52	p-Cymene	000099-87-6	0.81
8	11.62	1,8-Cineole	000470-82-6	2.03
9	11.76	α-Pinene	000080-56-8	4.53
10	11.95	9.42 (E)-β-Ocimene	003779-61-1	1.26
11	12.14	γ-Terpinene	000099-85-4	0.93
12	12.31	Sabinene hydrate	017699-16-0	0.96
13	12.69	Terpinolene	000586-62-9	0.81
14	12.87	Linalool	000078-70-6	1.91
15	13.38	allo-Ocimene	007216–56-0	1.01
16	13.54	epiphotocitral A	1000365-93-8	0.86
17	13.63	Citral	005392-40-5	0.85
18	13.70	3,3-Dimethyl-hepta-4,5-dien-2-one	1000190-54-1	1.00
19	13.94	(Z)- Isocitral	072203-97-5	1.00
20	14.21	(E)-Isocitral	055722-59-3	1.23
21	14.37	α-Terpineol	000098-55-5	1.05
22	14.47	(1R)-(-)-Myrtenal	018486-69-6	0.88
23	14.82	(Z)-Neral	000106-26-3	0.80
24	14.89	Nerol	000106-25-2	1.21
25	15.09	Neral	000106-26-3	5.77
26	15.31	Geraniol	000106-24-1	1.12
27	15.51	Geranial	000141-27-5	9.40
28	16.38	2,4-Dioxaspiro[5.5]undec-8-ene, 7,11,11-trimethyl-	069745-74-0	0.78
29	16.67	α-Cubebene	017699-14-8	0.79
30	16.81	Eugenol	000097-53-0	1.45
31	17.05	α-Copaene	003856-25-5	1.17
32	17.23	β-Cubebene	013744-15-5	1.10
33	17.53	(Z)-α-Bergamotene	018252–46-5	0.91
34	17.66	(E)-Caryophyllene	000087-44-5	5.86
35	17.79	(E)-β-Bergamotene	018252-46-5	2.53
36	18.00	(Z)-Isoeugenol	005912-86-7	3.05
37	18.09	α-Humulene	006753-98-6	2.76
38	18.44	Germacrene D	023986-74-5	3.14
39	18.60	γ-Muurolene	030021-74-0	1.07
40	18.71	β-Bisabolene	000495-61-4	11.20
41	18.91	α-Copaene	003856-25-5	1.42
42	19.24	Elemicin	000487-11-6	0.93
43	19.34	Nerolidol 2	1000285-43-6	1.08
44	19.71	Caryophyllene oxide	001139-30-6	4.75
45	19.84	2-(1-Hydroxycycloheptyl)-furan	115754-89-7	0.91
46	20.02	Humulene epoxide II	019888-34-7	1.31
47	20.20	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	016728-99-7	0.80
48	20.36	Isoelemicin	000487-12-7	5.65
49	20.60	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	000515-17-3	1.07

Table 2 GC-MSD results for *Ocimum americanum*

NO	RT	Compound name	CAS	Relative %
1	9.50	Thujene	000099-83-2	1.03
2	9.63	α-Pinene	000080-56-8	2.06
3	9.94	Camphene	000079–92-5	1.06
4	10.54	β-Pinene	000127-91-3	4.28
5	10.87	Myrcene	000123-35-3	1.55
6	11.10	p-Mentha-1(7),8-diene	000499-97-8	1.09
7	11.24	Octanal	000124-13-0	0.99
8	11.36	δ–2-Carene	000554-61-0	1.09
9	11.65	1,8-Cineole	000470-82-6	25.96
10	11.98	(E)-β-Ocimene	003779-61-1	1.26
11	12.15	γ-Terpinene	000099-85-4	1.30
12	12.29	Sabinene hydrate	017699-16-0	2.77
13	12.67	Terpinolene	000586-62-9	1.08
14	12.85	Linalool	000078-70-6	6.54
15	13.05	1-Octen-1-ol, acetate	077149-68-9	1.44
16	13.56	(E)- Epoxy-ocimene	028977-57-3	1.26
17	14.14	Terpinen-4-ol	000562-74-3	2.14
18	14.35	α-Terpineol	000098-55-5	5.91
19	14.79	Fenchyl acetate	013851-11-1	1.09
20	14.97	Nerolidol 1	1,000,285-43-5	1.09
21	15.14	Neral	000106-26-3	1.67
22	15.38	Geraniol	000106-24-1	1.41
23	15.58	Geranial	000141-27-5	2.13
24	16.20	δ-Terpinyl acetate	093836-50-1	1.07
25	16.32	Myrtenyl acetate	001079-01-2	1.19
26	16.53	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	057709-95-2	1.15
27	16.64	α-Terpinyl acetate	000080-26-2	1.76
28	16.83	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	000141-12-8	1.12
29	17.06	Dauca-5,8-diene	142,928-08-3	0.98
30	17.25	β-Elemene	000515-13-9	1.26
31	17.66	(E)-Caryophyllene	000087-44-5	1.95
32	17.86	α-Guaiene	003691-12-1	2.32
33	18.10	α-Humulene	006753-98-6	1.26
34	18.44	Germacrene D	023986-74-5	1.14
35	18.52	β-Selinene	017066-67-0	1.70
36	18.64	Aciphyllene	087745-31-1	2.42
37	18.74	α-Bulnesene	003691-11-0	2.98
38	18.92	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	000483-76-1	1.18
39	19.61	γ-Muurolene	030021-74-0	1.01
40	19.73	Caryophyllene oxide	001139-30-6	1.11
41	20.05	γ- Gurjunene	022567-17-5	1.18
42	20.55	α-Guaiene	003691-12-1	1.03
43	20.88	Guaia-1(10),11-diene	1,000,374-19-7	1.00
44	21.19	Isolongifolene, 9,10-dehydro-	1,000,151-67-1	1.01

conspecific plants. The high repellency of the blends of *O. suave* and *O. americanum* essential oils against *An. gambiae* compared to those of the individual essential oils could be due to additive or synergistic effects of individual constituents in the two essential oils. The GC-MSD results revealed that the major compounds in *E. citriodora* and *O. americanum* leaves were 1,8-Cineole,

Table 3 GC-MSD results of chemical components from the essential oils of E. citriodora

No	RT	Compound name	CAS	Relative %
1	9.28	Tricyclene	000488-97-1	1.04
2	9.50	Thujene	002867-05-2	1.01
3	9.62	α-Pinene	000080-56-8	2.49
4	9.94	Camphene	000079–92-5	1.08
5	10.08	3-Thujen-2-ol, stereoisomer	003310-03-0	1.10
6	10.55	β-Pinene	000127-91-3	1.41
7	10.89	Myrcene	000123-35-3	1.11
8	11.12	α-Phellandrene	000099-83-2	1.10
9	11.36	p-Mentha-2,4(8)-diene	000586-63-0	1.02
10	11.52	o-Cymene	000527-84-4	6.69
11	11.66	1,8-Cineole	000470-82-6	24.55
12	12.14	γ-Terpinene	000099–85-4	4.43
13	12.71	δ–2-Carene	000554-61-0	1.20
14	12.97	3-methyl-3-methylbutylbutanoate	000659–70-1	1.29
15	13.11	endo-Fenchol	014575–74-7	1.12
16	13.25	cis-p-Mentha-2,8-dien-1-ol	003886-78-0	1.07
17	13.34	α-Campholenal	004501-58-0	1.03
18	13.54	(Z)-Pinocarveol	019889-99-7	1.31
19	13.69	Camphenilanol	000465–31-6	1.06
20	13.97	Isoborneol	000124-76-5	1.40
21	14.14	Terpinen-4-ol	000562–74-3	3.65
22	14.36	α-Terpineol	000098-55-5	3.67
23	14.74	Sabinol	000471-16-9	1.34
24	14.99	(E)-p-mentha-1(7),8-dien-2-ol	1,000,374-16-7	1.36
25	15.44	2-isopropyl-5-methyl-3-cyclohexen-1-one	1,000,155-47-0	1.27
27	15.82	Thymol	000089-83-8	1.35
28	16.09	3-Methyl-4-isopropylphenol	003228-02-2	1.77
29	16.56	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	057709-95-2	1.19
30	16.88	Fumaric acid, cyclohex-3-enylmethyl isohexyl ester	1,000,345-14-2	1.04
31	17.02	Epizonarene	041702-63-0	1.00
32	17.53	10 s,11 s-Himachala-3(12),4-diene	060909-28-6	1.00
33	17.67	(E)-Caryophyllene	000087-44-5	1.00
34	17.84	Zonarene	041929-05-9	1.01
35	17.92	Aromadendrene	000489-39-4	1.19
36	18.20	allo-Aromadendrene	025246-27-9	1.05
37	18.36	1,2,3,4-tetrahydro-6,7-dimethylnaphthalene	001076–61-5	0.99
38	18.54	β-Selinene	017066-67-0	1.01
39	18.72	Dihydro-β-agarofuran	005956-09-2	1.00
40	18.86	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	150,320-52-8	0.99
41	18.95	1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-Naphthalene	000483-77-2	1.00
42	19.35	Globulol	051371-47-2	0.99
43	19.43	Viridiflorol	000552–02–3	1.16
44	19.53	(E)-Cadina-1(6),4-diene	020085-11-4	1.13
45	19.72	γ-Gurjunene	022567-17-5	2.21
46	19.82	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	022567-17-5	1.31
47	19.94	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1E,3a.alpha.,7a.beta.)-	056324-68-6	1.17
48	20.08	Caryophyllene oxide	001139-30-6	1.04
49	20.16	α-Bulnesene	1,000,374-19-9	1.12
50	20.24	β-Gurjunene	017334-55-3	1.35

Table 3 (continued)

No	RT	Compound name	CAS	Relative %
51	20.35	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]-	022469-52-9	1.14
52	20.51	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, (1Z,3a.alpha.,7a.beta.)-	056324-69-7	3.01

Table 4 Repellency of different essential oils, blends and Ballet (positive control) against *An. gambiae*

Sample	1.0 mg/mL	0.75 mg/mL	0.5 mg/mL	0.25 mg/mL
Eucalyptus citriodora	89.33 ± 4.40[a]	67.33 ± 3.36[b]	54.00 ± 2.45[c]	28.33 ± 2.04[d]
Ocimum americanum	96.67 ± 3.33[a]	92.67 ± 4.52[a]	89.81 ± 4.26[a]	80.33 ± 1.53[b]
Ocimum suave	88.67 ± 4.67[a]	85.33 ± 3.74[a]	75.33 ± 2.44[b]	58.67 ± 3.74[c]
Blend 1	100.00 ± 0.00[a]	96.00 ± 4.00[a]	96.00 ± 4.00[a]	96.00 ± 4.00[a]
Blend 2	90.23 ± 3.40[a]	86.67 ± 2.95[a]	85.33 ± 3.54[a]	83.62 ± 4.22[a]
Blend 3	89.32 ± 4.2[a]	85.43 ± 3.5[a]	86.22 ± 3.2[a]	81.78 ± 3.90[a]
Ballet	100.00 ± 0.00[a]	96.67 ± 3.33[a]	96.67 ± 3.33[a]	96.67 ± 3.33[a]

Values expressed as mean ± standard error of the mean (n = 5). Values with similar lower-case letters along the column are not significantly different using one way ANOVA and Tukey's post hoc (p > 0.05). Blend 1: 1:1 ratio of O. suave & *O. americanum*. Blend 2- E. citriodora & O. Americanum Blend 3- *E. citriodora* & *O. suave*, Ballet: Mosquito Repellent

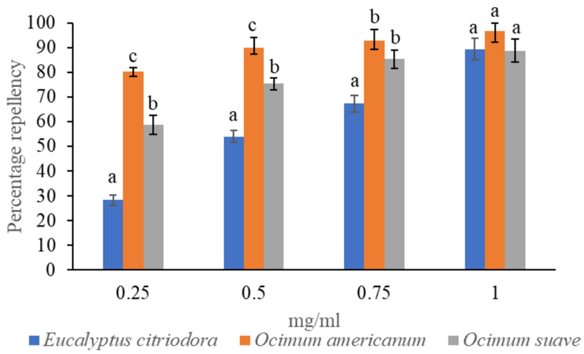

Fig. 5 Mean (± SE) percentage repellency of *E.citriodora*, *O.suave*, & *O.americanum* against *An. gambiae*

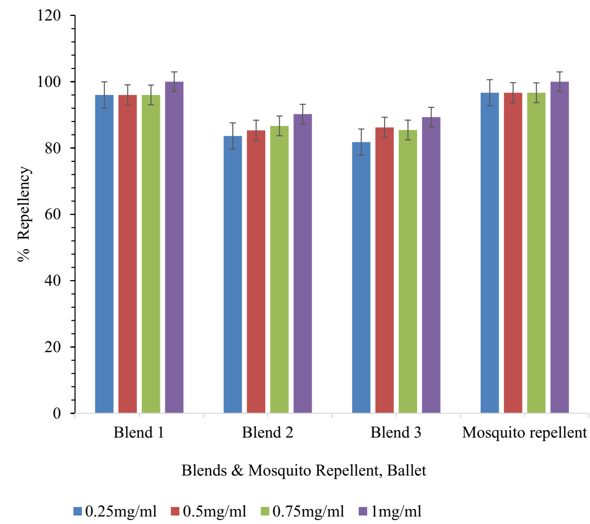

Fig. 7 Mean (± SE) percentage repellency of the blends & Ballet (Mosquito repellent) at various concentrations. Blend 1: 1:1 ratio of *O. suave* & *O. americanum*. Blend 2- *E. citriodora* & *O. americanum* Blend 3- E. citriodora & *O. suave*, Ballet: Mosquito Repellent against An. gambiae

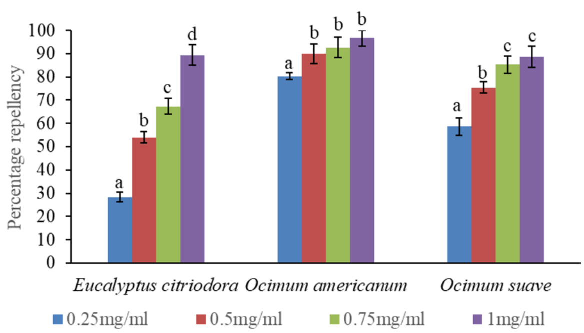

Fig. 6 Mean (± SE) percentage repellency of E. citriodora, *O. suave* & O. Americanum at various concentrations against *An. gambiae*

while *β*-Bisabolene was the major component/compounds in the *Ocimum suave* plant leaves.

Discussion

Blood-feeding and disease vector invertebrates are of health, economic, and scientific concern [11]. The most commonly known include mosquitoes, jiggers, blackflies, tsetse flies, fleas, chewing fleas, ticks, lice, mites, and bedbugs [12]. The major vector-borne diseases transmitted to humans by mosquitoes are malaria, dengue fever, lymphatic filariasis, and Zika virus disease [13]. Currently, the WHO does not recommend insecticide space-spraying due to a lack of evidence about its impact on malaria and the short life of the used chemicals [14]. Bioinsecticides are derived from natural products, such as bioactive compounds of plants, pheromones, and from microorganisms, such as bacteria, fungi, viruses, or protozoan form a better alternative [15]. There are four major classes of bioinsecticides based on their nature of origin: phytochemicals, microbial pesticides, plant-incorporated protectants (PIPs), and pheromones [16]. Plant-based repellents have been used for generations as personal protection against mosquitoes [17]. Ethnobotanical studies provide valuable knowledge for developing natural products. Commercial repellents with plant-based ingredients are popular [18]. The repellents from plant extracts are green, environmentally friendly, biodegradable, and non-toxic [19]. Many researchers have conducted studies on repellence to determine the efficacy of ethnobotanical plants for space fumigation against human-biting arthropods [20, 21]. The repellent effect of the emitted volatiles is attributed to the higher percentage of terpenoids in *O. suave* and *O. americanum*, respectively. This shows that the active compounds gain synergism between themselves, hence resulting to increased repellency [22].

The repellent effect of the emitted volatiles is attributed to the higher percentage of phellandrene and tricyclene *O. suave* and *O. americanum*, respectively. This shows that active compounds gain synergism between themselves, resulting in an increase in repellency [23]. Thus, subtractive assays provide additional insight into the relative contributions of these compounds to the repellency of the two-component blend [24]. Phytochemicals show multiple modes of action and exert their effects on multiple target sites in insects; their efficacy can be enhanced when used as a blend (e.g. mixture of oils) against mosquitoes [25]. The current study shows that multiple deployments of formulations of *O. suave,* and *O. americanum* essential oils can provide space protection against *An. gambiae* up to a certain level, after which no further enhancement in repellency occurs [26].

Conclusion

Phytochemicals have gained relevance and use to control and manage mosquito problems because they are natural, environmentally safe, less toxic, inexpensive, and, more importantly, less prone to mosquito resistance. This study revealed that the plant-based essential oils under study possess repellence properties against mosquitoes. Additionally, the study revealed that a 1:1 blend of *O. suave* and *O. americanum* essential oils is a potent repellent against *An. gambiae* and can be used to offer protection against *An. gambiae* bites thus reducing the spread of malaria.

Suggestions for further research

The repellence of the emitted volatiles was evaluated in a choice set-up in two screen houses. Full field trials need to be undertaken to rule out any possible differences in the repellence due to the overlap of repellences range of the treatment with that of the control and the behaviour of *An. gambiae* when they are constrained. The Gas Chromatography linked Electroantennography (GC-EAD) analysis of the essential oil should be conducted so as to identify all compounds perceived by the *An. gambiae* antennae, which can then be assayed as a full blend to determine its repellence and in subtractive modes to determine the relative contribution of each component.

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Author contributions

A.M, A.C, F.N and J.K.M did the experiment, analysed data, wrote and reviewed the manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate

The National Commission for Science Technology and Innovation (NACOSTI/P/23/3959) and the ethical committee of Chuka University (CU/ERC/NACOSTICOSTI/1423).

Institutional review board statement

Not applicable.

Competing interests

The authors declare no competing interests.

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