Repellency and Composition of Essential Oils of Selected Ethnobotanical Plants Used in Western Kenya against Bites of *Anopheles gambiae* Sensu Stricto

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Abstract: The essential oils of *Ocimum gratissimum* Linn, *Hyptis suaveolens* (L) Poit and *Vitex keniensis*, which are used traditionally in Western Kenya for personal and space protection against mosquito bites, were screened for repellence against *Anopheles gambiae* Sensu Stricto. Essential oils were extracted from their leaves by hydrodistillation, characterised by gas chromatography linked with mass spectrophotometer and electroantennogram detectors. The repellency of the oils and their selected blends was studied by the reduction in probing and feeding on the human arm. The oils showed promising repellency for *Anopheles gambiae*, *O. gratissimum* (RD_{50} = 2.77 × 10^{-5} mg cm^{-2}, 95 % CI), *Vitex keniensis* (RD_{50} = 5.68 × 10^{-5} mg cm^{-2}) and *Hyptis suaveolens* (6.27× 10^{-5} mg cm^{-2}) as compared to that of DEET (control) RD_{50} = 1.25×10^{-5} mg cm^{-2}). The bioactive constituents of each oil were identified by Gas chromatography-linked with Mass spectrometry and Electroantennography. Some compounds were confirmed by co-injections of the oil with available authentic standards. The results provide a scientific rationale for the traditional use of these plants in repelling disease vectors and other biting insects, and lay down some useful groundwork for downstream development of more effective products for personal and space protection.

Key words: *Anopheles gambiae*; ethnobotanicals; *Ocimum gratissimum*; *Hyptis suaveolens; Vitex keniensis*; repellency; essential oils; bioactive constituents.

Introduction

Malaria remains one of the most important parasitic diseases of the developing world. Despite ongoing efforts to control the disease, it still represents a serious public health problem in about 90 countries worldwide. In 2015, there were roughly 212 million malaria cases globally and an estimated 429,000 malaria deaths [1]. The burden is heaviest in Africa, where an estimated 90 % of all malaria deaths occur, particularly in children aged under 5 years [1].

To date, no method of malaria control has proven effective enough to significantly reduce the high transmission levels found in sub- Saharan Africa [2]. Even the most efficacious of these, such as pyrethroid-treated bed nets, have been difficult to

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implement on a sustainable basis for several reasons, including cost, availability, acceptability by communities [3,4] as well as resistance development and behavioural shifts in mosquitoes [5]. However, with increasing problems of toxicity to non-target organisms and resistance of mosquitoes to synthetic insecticides [6], there has been growing interest in ethnobotanicals used by different communities to control mosquitoes [7]. In this study, we compared the repellencies of essential oils of three plants, *Ocimum gratissimum, Vitex keniensis* and *Hyptis suaveolens*, used in Western Kenya for space protection against mosquitoes by hanging branches of the plants in households and/or by application of mashed plant parts on exposed parts of the body for personal protection [8]. Besides, the composition profiles of the oils were characterised by Gas Chromato-graphy linked Mass Spectrometry (GC-MS) and candidate active constituents were identified by Gas Chromatography-linked Electroantenno-graphy (GC-EAD).

O. gratissimum L. is a perennial plant indigenous to Africa, and has now spread widely in South America and Asia. The essential oil of the plant has been reported to be active against several pathogenic microorganisms, such as *Staphylo-coccus aureus, S. typhimurium, Escherichia coli* and *Salmonella typhi* [9]. It has also been shown to repel *Simulium damnosum*, the causative agent of *Onchocerca volvulus* in Nigeria [10,11]. *Hyptis suaveolens* (L.) Poit. grows in different parts of Tropical Africa and has been used for some ethnobotanical applications in rural communities [12,13]. No previous study has been reported on *Vitex keniensis.* Plants for the study were selected based on ethnobotanical information and chemo-taxonomic consideration [14] , supple-mented by observation of plants growing naturally in the wild that emitted a specific odour that showed signs of avoidance by insects.

Materials and methods

Plant materials

The aerial parts of *O. gratissimum, V. keniensis* and *H. suaveolens* (10.0 kg each) at their different stages of growth were collected from Mukhweso village in Mumias sub-county, Kakamega County (0° 17’3.19" N 34° 45’8.24" E), in the western region of Kenya, between May and July 2014. The plants were identified by a taxonomist namely Mr Lucas Karimi Kaime of Department of Pharmacy and Complementary/Alternative Medicine Research and the voucher specimens were deposited at the Herbarium at the Department of Botany of Kenyatta University, Nairobi, Kenya. The voucher specimen numbers were DY/01/08/2014, DY/02/08/2014 and DY/03/08/2014 for *O. gratissimum, V. keniensis* and *H. suaveolens* respectively. The samples (leaves, flowers or whole aerial parts) were air-dried under a shade for seven days before extraction.

Extraction of essential oils

The essential oils from the plants samples (leaves, flowers or aerial parts) were extracted by hydrodistillation using modified Clevenger apparatus. About 500 g of each of the plant material were put into a 2-litre round-bottom flask and 500 ml of water added. The flask was then fitted with the Clevenger apparatus and a double pocket condenser. The plant materials were hydrodistilled for 4 h. The essential oil was collected on the water layer in the Clevenger apparatus. The procedure was repeated three times for each plant sample. The essential oil was separated, dried with anhydrous sodium sulphate and stored in amber-coloured vials at 0_{o}C until use.

Mosquito repellency assay

The oil of each plant was tested for repellence on female *An. gambiae* s.s. (ex-Ifakara, Tanzania strain) that were reared at International Centre for Insect Physiology and Ecology (ICIPE) Duduville mosquito insectary. All assays were carried out using 5-7 days old female mosquitoes that had been starved for 18 h following access to 6% glucose solution. The use of human volunteers in mosquito repellency bioassay followed guidelines of the Declaration of Helsinki and Tokyo for humans and the research was conducted following Kenya Medical Research Institute (KEMRI) ethical rules on scientific research and development (reference KEMRI/ RES/7/3/1). The subjects provided written

informed consent before participating. Six human volunteers, who demonstrated no allergic reaction to mosquito bites or candidate essential oils were selected. Assays were carried out with 50 mosquitoes in aluminium-frame cages (50×50×50 cm) in a room at a temperature of 27-35°C and relative humidity of 65 -80 %. Test solutions (0.5 ml) were dispensed on one of the forearms from the wrist to the elbow. The rest of the hand was covered with a glove. Acetone (0.5 ml, HPLC grade) was dispensed on the other forearm to serve as a control. The different doses of each oil were then screened sequentially in 6 replicates according to WHO (1996) protocols [15] on laboratory and field evaluation of insecticides and repellency, starting from the lowest dose to the highest dose on forearms of the volunteers [16]. The control arm was the first to be introduced into the cage and was left for 3 minutes. The number of mosquitoes that landed on that arm during that duration was recorded. The treated arm was then introduced into the cage for the same period and the number of mosquitoes landing on the arm were recorded. For comparison, DEET was similarly tested in the same dose-range. Average protec-tive efficacy (PE) of each dose of the essential oils and DEET from six replicates were deter-mined [17].

Analyses of essential oils

Gas chromatographic separation was performed on a 6890N gas chromatograph (Agilent Technologies) equipped with split-splitless injector (230°C) and flame ionization detector (FID). The eluants leaving the GC column were mixed with hydrogen and the eluting compounds were burned by a flame surrounded by air and an oxygen-rich environment. The GC was equipped with an HP-1 capillary column (10 m × 0.53 mm i.d., 2.65 μm film thickness). The oven temperature was initially set at 30°C for 0.5 min, followed by gradient increase to 150°C (at 5°C/min for 0.1 min), and finally increased to 250°C (10°C/min) for 45 min. The components of essential oils were initially obtained with an enhance integrator (HP Chemstation).

GC -MS analyses were performed using a fused silica capillary column (50 m × 0.32 mm i.d., film thickness 0.52 μm, DB-1, J & W Scientific) attached to an on -column injector, which was directly coupled to HP 5972 MSD. Ionization was by electron impact (70 eV, source temperature 250°C). Helium was used as the carrier gas. The oven temperature was maintained at 30°C for 5 min, and programmed at 5°C/min to 250°C which was different from that of GC-MS since the column used in the GC was not the same (HP-1 capillary column, 10 m x 0.5 3mm i.d, 2.65 μm film thickness). The calculation of retention indexes was made through co-injection with an n-alkenes' series. Identification of the oil consti-tuents were based on their retention indices [18] and comparison of mass spectra with databases [19]. The quantification was done by an external standard method using calibration curves generated by running GC analysis of representa-tive authentic compounds.

Identification of electrophysiologically-active constituents

Electroantennography was carried out using Gas chromatography linked to electroantennogram detector (GC-EAG) with mosquitoes obtained from the London School of Hygiene and Tropical Medicine. Electroantennogram (EAG) recordings from 5 to 7-day old female *An. gambiae* mosquitoes were made using Ag-AgCl glass electrodes filled with ringer solution [20]. Each insect was anaesthetized by chilling, and it's head excised and inserted into the tip of an electrode. The tips of the antennae were inserted into the recording electrode. The signals were passed through a high impedance amplifier (UN- 06, Syntech, The Netherlands) and analysed by using a customised software package (Syntech, The Netherlands). Identification of EAG-active com-ponents was confirmed by peak enhancements associated with GC co-injection of the essential oils with pure authentic standards (α-pinene, β-pinene, hexyl acetate, (E, E)-decadienal, eugenol, p-cymene, E-caryophyllene, p-cuminol) [21] sourced from Sigma-Aldrich, Taufkirchen, Germany. The purity of the standards was confirmed by a single sharp peak from the GC spectroscopy ana-lyses [21].

Assessment of repellency of blends of EAG-active compounds

Available EAG-active compounds identified in

the essential oils were blended in the ratio found in GC-MS analyses. These included 5 of 9 EAG-active compounds of *O. gratissimum* oil: α-pinene, β-pinene, hexyl acetate, (*E, E*)-decadienal and eugenol (Blend A); 3 of 7 EAG-active compounds of *H. suaveolens* oil: β-pinene, p-cumenol and (*E*)-caryophyllene (Blend B); and 3 of 7 EAG-active compounds of *V. keniesis* oil: α-pinene, p-cymene and *E*-caryophyllene (Blend C). Each blend was tested in the same dose range as the parent essential oil.

Data analyses

Protective efficacy (PE) of each dose was calculated using the formula, PE = (% control mean – test mean)/ % control mean [17,22-24]. Mean P.E values of different doses of each essential oil were ranked transformed and subjected to Analysis of variance (ANOVA) followed by Student-Newman-Kuels (SNK) posthoc tests [25]. Doses capable of repelling half of mosquito population (RD_{50}) for the test samples were obtained by Probit analysis [26,27] using the PE values obtained from the replicated experiments [16].

Results and discussion

Yields and repellencies of essential oils

Yields of the essential oils from the dried aerial parts of *O. gratissimum, V. keniensis* and *H. suaveolens* were 0.60 g (0.12 %), 0.35 g (0.07 %) and 0.40 g (0.08 %) respectively. Highest amount of oil was found in *O. gratissimum* and minimum in *V. keniensis* The data from the repellent assays of the essential oils of the three plants and DEET against *An. gambiae* s.s. is summarised in Table 1. All the three essential oils showed significant ($p<0.05$) repellence against the mosquito as compared to the untreated arm. That of *O. gratissimum* was most repellent (RD_{50} = 2.77×10^{-5}) and close to that of the positive control DEET (1.25×10^{-5}). *V. keniensis* and *H. suaveolens* essential oils showed comparable repellencies with RD_{50} of 5.68×10^{-5} and 6.27×10^{-5}, respectively. In Nigeria, Oparaocha *et al*.[28] evaluated the fumigant toxicity of methanol extract of *O. gratissimum* and found it to be toxic against different species of mosquitoes. In a thermal fumigation experiment, *H. suaveolens* essential oil was found to be an effective source of repellent blend against *An. gambiae* [8]. In another study, essential oil of *H. suaveolens* was found to have significant repellent activity against the Asian tiger mosquito [29]. No previous reports on the mosquito repellence of *V. keniensis* essential oil have been reported.

Composition of the essential oils and EAG active compounds

A total of 57 compounds were identified in the essential oils of the three plants by GC-MS. Each essential oil showed different composition and proportion of chemical constituents. Some of these constituents have been reported to have repellent properties to *An. gambiae* mosquitoes [16,30]. Their

Table 1. RD_{50} (95 % CI) values of the three essential oils DEET and against *An. gambiae*

Dose	*O. gratissimum* % PE±SE	*V. keniensis* % PE±SE	*H. suaveolens* % PE±SE	DEET % PE±SE
10^{-5}	47.66±10.84$_a$	36.53±14.83$_a$	42.59±5.52$_a$	51.11±13.32$_a$
10^{-4}	64.94±10.01$_a$	57.78±9.35$_a$	58.06±12.06$_a$	86.22±4.51$_b$
10^{-3}	85.47±6.48$_b$	58.38±8.58$_a$	67.39±6.23$_a$	94.29±3.69$_b$
10^{-2}	100.00±0.00$_c$	73.34±5.92$_b$	68.79±11.23$_a$	100.00±0.00$_c$
10^{-1}	100.00±0.00$_c$	91.67±5.69$_c$	94.38±2.69$_b$	100.00±0.00$_c$
RD_{50} ($\times10^{-5}$ mg cm^{-2})	2.77(1.22-3.25)$_A$	5.68(4.12-6.72)$_B$	6.27(4.48-7.28)$_B$	1.25(0.82-2.13)$_A$

Mean percent repellencies of *O. gratissimum, V. keniensis, H. suaveolens* oils and DEET and their respective RD_{50} (95 % CI) values against *An. gambiae* (mean values followed by same small letters within the same column are not significantly different $p< 0.05$, while RD_{50} (95 % CI) values in the same row followed by the same capital letter(s) are not significantly different $p< 0.001$)

percentage composition and order of their elution from GC (HP-1) are given in Table 2a, 2b and 2c. Out of 24 constituents identified in the essential oil of *O. gratissimum* in this study,10 have been previously reported to have repellent effects against *An. gambiae* [16,30]. The oil was dominated by monoterpenes (45.8 %) and sesquiterpenes (29.2 %). The major compounds of *O. gratissimum* essential oil were (*Z*)-ocimene (29.73 %), eugenol (21.76 %), germacrene D (9.65 %), β-caryophyllene (5.86 %), β- linalool (4.13 %), and β–pinene (3.66 %) among others as indicated in Table 2a. Of the 24 detected compounds in *O. gratissimum* essential oil, only nine elicited electrophysiological responses with *An. gambiae* antennae.

Table 2b gives the 31 compounds identified in *V. keniensis* essential oil, five of which have been

Table 2a. Chemical composition of the essential oil of *O. gratissimum* aerial parts obtained by hydrodistillation and analysed by GC MS

No.	Compound	RT	Concentration (%)	RI	RI*	EAG activity
1	α-Thujene	17.69	0.24	933	926	
2	α-Pinene*	17.98	0.51	939	934	✓
3	1-Octen-3-ol*	19.20	0.46	966	967	
4	Sabinene*	19.34	0.45	969	969	
5	β-Pinene*	19.53	3.66	972	975	✓
6	Hexyl acetate	19.95	1.21	981	984	✓
7	α-Terpinene*	20.94	0.68	1002	1010	
8	β-Cymene	21.05	0.66	1006	1015	
9	(Z)-Ocimene	21.53	29.73	1024	1028	✓
10	(E)-Ocimene	21.95	2.44	1040	1041	
11	β-Linalool*	23.58	4.13	1098	1086	
12	(Z)-4,8-Dimethyl-1,3,7-nonatriene	24.14	0.76	1117	1115	✓
13	1-Terpinen-4-ol	26.25	1.27	1182	1175	
14	(E,E)-Decadienal	26.91	0.86	1201	1223	✓
15	Eugenol*	31.31	21.76	1350	1338	✓
16	α-Copaene	32.03	1.93	1375	1376	✓
17	β-Cubebene	32.86	1.80	1403	1383	
18	β-Caryophyllene*	34.09	5.86	1450	1432	
19	α-Humulene*	34.30	0.23	1458	1449	
20	Germacrene D	35.71	9.65	1510	1485	✓
21	Elemecin	36.41	1.94	1538	1554	
22	δ-Cadinene	36.59	0.14	1545	1556	
23	β-Caryophyllene oxide*	38.17	3.14	1607	1570	
24	Asarone	38.86	1.49	1652	1679	

*Compounds reported in the literature to be repellent against *An. gambiae*
RT: retention time
RI: values of calculated retention indices
RI*: values of retention indices found in literature and database
✓: EAG active
Hydrocarbon monoterpenes (1,2,4,5,7,8,9&10)
Oxygenated monoterpenes (11,13,14)
Hydrocarbon sesquiterpenes (16,17,18,19,20&22)
Oxygenated sesquiterpene (23)
Alcohol (3); Phenylpropenes (15,21&24); Ester (6), Olefin (12)

Table 2b. Chemical composition of the essential oil of *V. keniensis* aerial parts obtained by hydrodistillation and analysed by GC MS

No.	Compound	RT	Concentration (%)	RI	RI*	EAG activity
1	α-Pinene*	17.98	0.49	939	934	✓
2	1-Octen-3-ol*	19.21	0.51	966	967	
3	β-Thujene	19.30	0.40	969	971	
4	β-myrecene	19.93	0.53	981	984	
5	α-Phellandrene*	20.46	1.57	991	1000	
6	p-Cymene	21.07	1.40	1007	1010	✓
7	E-Ocimene	21.95	0.96	1040	1041	
8	β-Linalool*	23.58	1.25	1098	1095	
9	Cetronellol acetate	31.42	0.32	1354	1354	
10	α-Cubebene	32.03	0.30	1375	1356	
11	β-Cubebene	32.22	10.88	1381	1389	
12	α-Copaene	32.86	1.03	1403	1383	✓
13	β-Elemene	33.02	1.01	1413	1375	
14	α-Gurjunene*	33.13	2.03	1414	1410	✓
15	Trans-α-Bergamotene	33.77	0.21	1438	1431	
16	E-Caryophellene	34.09	2.71	1450	1432	✓
17	γ-Elemene	34.35	1.83	1459	1449	
18	(E)-β-Farnesene	34.65	1.00	1470	1450	
19	α-Humulene	34..97	1.88	1482	1465	
20	(Z,Z)-α-Farnesene	35.18	0.32	1490	1492	
21	α-Curcumene	35.31	1.14	1494	1483	
22	α-Selinene	35.42	1.15	1485	1489	
23	α-Muurolene	35.82	3.75	1498	1500	
24	δ-Cadinene	36.65	12.67	1547	1540	
25	Germacrene D-4-ol	36.89	2.39	1557	1568	
26	Spathulenol	37.02	0.90	1562	1580	✓
27	Patchulane	38.46	4.60	1619	1610	
28	Tau Murrolol	38.91	9.79	1648	1642	
29	δ-Eudesmol	39.39	0.64	1642	1650	
30	α-Cadinol	39.87	16.01	1658	1655	✓
31	α-Bisabalol	40.09	0.95	1678	1687	

*Compounds reported in the literature to be repellent against *An. gambiae*
EA: EAG-active
RT: retention time
RI: values of calculated retention indices
RI*: values of retention indices found in literature and database
✓: EAG active. Hydrocarbon monoterpenes (1,3,4,5,6&7)
Oxygenated monoterpene (8)
Hydrocarbon sesquiterpenes (10,11,12,13,14,15,16,17,18,19,20,21,22,23,24&27)
Oxygenated sesquiterpenes (25,26,28,29,30&31)
Alcohol (2)
Ester (9)

reported to have repellent effects against *An. gambiae* in literature [16,30]. Sesquiterpenes, (71.0 %) and monoterpenes, (22.6 %) dominated the oil. The major compounds of *V. keniensis* essential oil were α-cadinol (16.01 %), δ-cadinene (12.67 %), β-cubebene (10.88 %), *tau*-muurolol (9.79 %), and α-muurolene (3.75 %) among others. It is worth mentioning that this is the first report of chemical constituents of the essential oil of *V. keniensis*. Seven compounds from *V. keniensis* *essential* oil were consistently detected by the antennae of *An. gambiae*.

In the essential oil of *H. suaveolens*, 21 compounds were identified. The oil was dominated by sesquiterpene (66.7 %) and monoterpene (23.8 %) as shown in Table 2c. The major constituents of essential oil of *H. suaveolens* were (*E*)-caryophyllene (21.27 %), γ-elemene (9.75 %), *trans*-α-bergamotene (5.07 %), (*Z*)-α-*cis* bisabolene epoxide (4.54 %), and spathulenol (4.35 %)

Table 2c. Chemical composition of the essential oil of *H. suaveolens* aerial parts obtained by hydrodistillation and analysed by GC MS

No.	Compound	RT	Concentration (%)	RI	RI*	EAG activity
1	1-Octen-3-ol*	19.84	0.83	966	967	
2	Sabinene*	19.34	4.13	969	969	
3	β-Pinene*	19.50	0.63	972	975	✓
4	Limonene	21.39	1.02	1019	1024	
5	γ-Terpinene*	20.91	0.56	1000	1020	
6	α-Terpinolene*	22.38	1.60	1056	1090	
7	p-Cumenol	23.58	1.80	1287	1290	✓
8	α-Copaene	24.14	0.45	1403	1377	✓
9	α-Gurjunene*	24.40	3.72	1406	1409	✓
10	(E)-Caryophellene	25.84	21.27	1450	1432	✓
11	γ-Elemene	26.14	9.75	1459	1449	
12	*trans*-α-Bergamotene	26.24	5.07	1452	1450	
13	Bicyclogermacrene	32.86	2.19	1482	1490	✓
14	α-Selinene	33.12	2.01	1636	1494	
15	Z-α-*trans*-bisabolene epoxide	33.68	1.29	1662	1539	
16	Spathulenol	34.08	4.35	1586	1577	✓
17	β-Caryophellene oxide	34.35	3.70	1592	1581	
18	Ledol	34.96	1.16		1609	
19	Z-α-*cis*-Bisabolene epoxide	35.31	4.54	1662	1635	
20	Globulol	35.44	0.88	1623	1680	
21	Z-α-*trans*-Bergamotol	35.55	1.80	1693	1690	

*Compounds reported in the literature to be repellent against *An. gambiae*
RT: retention time
RI: values of calculated retention indices
RI*: values of retention indices found in literature and database
✓: EAG active
Hydrocarbon monoterpenes (2,3,4,5&6)
Hydrocarbon sesquiterpene (8,9,10,11,12,13&14)
Oxygenated sesquiterpenes (15,16,17,18,19,20&21)
Alcohol (1,)
Phenol (7)

among other compounds. Of the 21 constituents identified, six constituents have been reported to have repellent effects against *An. gambiae* [16,30]. Seven compounds in *H. suaveolens* essential oil were found to be EAG -active. The compounds from the essential oils of the three plants that elicited electrophysiological responses with *An. gambiae* are from different chemical classes, including monoterpenes, sesquiterpenes, an ester, and an olefin. This is in good agreement with a review by Nyasembe and Torto [31] who reported 29 plant volatiles from various chemical classes that have been detected by mosquitoes. The EAG-active compounds in the three essential oils can either elicit positive behavioural response (attractiveness) or negative behavioural response (repellency) [32].

Repellencies of *O. gratissimum, V. keniensis* and *H. suaveolens* essential oils and blends of available EAG-active compounds

The repellency data of essential oils of *O. gratissimum, V. keniensis* and *H. suaveolens* and their blends (A, C and B respectively) are provided in Table 3. Blend A and C exhibited significantly lower repellencies (RD_{50} 19.3×10^{-5} mg cm^{-2} and RD_{50} 66.6×10^{-5} mg cm^{-2}, respectively) compared with their parent oils (RD_{50} 2.77×10^{-5} mg cm^{-2} and RD_{50} 5.68×10^{-5} mg cm^{-2}, respectively). This indicated that other EAG-active components not included in the blend, may also be contributing to the repellent activity of the oil of these plants. On the other hand, Blend B exhibited higher repellency (RD_{50} 3.95×10^{-5} mg cm^{-2}, 95% CI) than the parent oil (RD_{50} 6.27×10^{-5} mg cm^{-2}, 95% CI). This implied that other components of the essential oil may inhibit the repellence of active compounds in the essential oil.

Conclusions

The findings of this study; (1) validate the traditional use of aerial parts of *O. gratissimum*, *V. keniensis* and *H. suaveolens* for personal and space protection against mosquito bites. (2) The essential oils of *O. gratissimum*, *V. keniensis* and *H. suaveolens* show a complex composition of hydrocarbon compounds. (3) The high repellency of essential oil of *O. gratissimum* compared to those of *V. keniensis* and *H. suaveolens* can be attributed to the presence of more compounds in the oil that have been reported to have a repellent effect against *An. gambiae*. (4) Our data suggest that the essential oil of *O. gratissimum* could be exploited to manage malaria vector. (5) The activity of an essential oil cannot be assigned to a particular compound but to a chemical finger-print which brings synergism in mosquito repellency.

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Table 3. RD_{50} of essential oils and synthetic blends of available EAG-active compounds from *O. gratissimum, V. keniesis* and *H. suaveolens*

Essential oil and blends	RD_{50} (×10-5 mg cm^{-2})
O. gratissimum oil	2.77_a
Blend A	19.30_b
V. keniensis oil	$5.68_{a'}$
Blend C	66.60_b
H. suaveolens oil	6.27_b
Blend B	3.95_a

Values in the same column with different letters are significantly different ($p<0.001$)
Blend A: α-pinene, β-pinene, hexyl acetate, (E,E)-decadienel and eugenol (from active compounds of *O. gratissimum* oil)
Blend B: β-pinene, para-cumenol and (*E*)-caryophyllene (from active compounds of *H. suaveolens* oil)
Blend C: α-pinene, p-cymene and *E*-caryophyllene (from active componds of *V. keniensis* oil)

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