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Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*

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Abstract

Essential oils of six plants growing in Kenya were screened for repellent activities against *Anopheles gambiae* sensu stricto. The oils of *Conyza newii* (Compositeae) and *Plectranthus marrubioides* (Labiateae) were the most repellent ($RD_{50} = 8.9 \times 10^{-5}$ mg cm^{-2}, 95% CI) followed by *Lippia javanica* (Verbenaceae), *Lippia ukambensis* (Verbenaceae), *Tetradenia riparia*, (*Iboza multiflora*) (Labiateae) and *Tarchonanthus camphoratus* (Compositeae). Eight constituents of the different oils (perillyl alcohol, *cis*-verbenol, *cis*-carveol, geraniol, citronellal, perillaldehyde, caryophyllene oxide and a sesquiterpene alcohol) exhibited relatively high repellency. Four synthetic blends of the major components (present in $\geqslant$1.5%) of the essential oils were found to exhibit comparable repellent activity to the parent oils.

Keywords: *Anopheles gambiae*; Repellent plants; Essential oils; Terpenoids; Alcohols; Epoxides

1. Introduction

In many parts of the world, plant-derived products have been used to repel or kill mosquitoes and other domestic insect pests (White, 1973; Chogo and Crank, 1981; Hwang et al., 1985; Curtis et al., 1991; Seyoum et al., 2002a,b). Solvent extracts and essential oils of many plants show varying levels of insect-repellent properties (Chogo and Crank, 1981; Curtis, 1990; Curtis et al., 1991; Trigg and Hill, 1996; Thorsell et al., 1998). Indeed, until the advent of synthetic compounds, essential oils and/or their mixtures formed the basis of most commercial repellent formulations (Curtis, 1990). However, due to their relatively high volatilities, they have been abandoned in favour of synthetic repellents, principally DEET (*N*,*N*-diethyltoluamide), which provides relatively long protection against blood-feeding insects (Fradin, 1998; Goodyer and Behrens, 1998). On the other hand, rapid skin penetration and bio-distribution of DEET in both humans and animals have raised concerns on its toxic side effects (Miller, 1982; Roland et al., 1985). Recently, a related repellent, 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester (KBR 3023 or Picaridin, or Bayrepel®) was developed by Bayer. On the basis of available evidence, KBR 3023 represents a promising alternative to DEET (Badolo et al.,

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Table 1
Mean percent repellencies of essential oils of six African plants

Concentration (g ml^{-1})	% Mean protective efficacy (PE) ± SE[a]		
	10^{-1}	10^{-3}	10^{-5}
T. camphoratus	98.5 ± 2.5^{a}	34.2 ± 2.8^{jk}	22.3 ± 1.5^{m}
L. javanica	90.3 ± 1.8^{bc}	57.9 ± 1.7^{e}	48.7 ± 2.4^{g}
P. marrubioides	100^{a}	58.3 ± 0.4^{e}	33.2 ± 0.9^{jk}
T. riparia	79.6 ± 3.2^{cd}	42.7 ± 2.5^{h}	37.7 ± 1.9^{j}
L ukambensis	83.9 ± 2.5^{bc}	52.2 ± 1.6^{f}	32.4 ± 1.9^{jk}
C. newii	100^{a}	45.5 ± 3.5^{g}	27.9 ± 2.6^{l}

Means with the same letters are not significantly different at $P = 0.0001$.
[a] Student–Newman–Kuels (SNK) test, SAS® Institute, 2000.

2004; Debboun et al., 2000). In the search for effective alternatives, there has been renewed interest in botanicals (Hwang et al., 1985; Curtis et al., 1991; Sakumar et al., 1991; Schreck and Leonard, 1991; Watanabe et al., 1993; Trigg, 1996; Govere et al., 2000; Grayson, 2000; Barasa et al., 2002).

The use of repellent plants and derived products for protection against mosquitoes is widespread in Africa (Srou et al., 1987; Curtis et al., 1991; Pålsson and Jaenson, 1999a,b). In our bioprospecting initiative for useful repellent and insecticidal plants, we have evaluated candidate plants in two principal ways: as sources of fumigants from intact plants from burning or thermal expulsion of plant materials, as used traditionally or improvements thereof (Seyoum et al., 2002a,b, 2003); and as essential oils obtained by hydrodistillation (Omolo et al., 2005). In both cases, we have sought to elucidate the chemical bases of the activities by identifying the individual plant constituents and their blends that are primarily responsible for the observed activities. In this communication, we report the repellent properties of the essential oils of 6 African plants, those of their constituents and synthetic blends of selected constituents against *An. gambiae* s.s., one of the principal Afro-tropical malaria vectors.

2. Results

2.1. Repellency assays of crude oils

Table 1 summarises the results of initial screening of the essential oils of six species of plants selected for detailed study. Probit analysis of repellency data (at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} g ml^{-1}) gave the following RD_{50} values: 8.9×10^{-5} (95% Confidence limits (CI): 1, 11×10^{-5}) (*Conyza newii* Oliv. & Hiern), 8.9×10^{-5} (1, 16×10^{-5}) (*Plectranthus marrubioides* Benth.), 2.6×10^{-4} (1.5, 3.1×10^{-4}) (*Lippia javanica* Spreng), 4.3×10^{-4} (1.8, 5.5×10^{-4}) (*Lippia ukambensis* Vatke), 5.0×10^{-4} (2.5, 7.5×10^{-4}) (*Tetradenia riparia* Hochst.) and 2.4×10^{-3} (1.6, 3.2×10^{-3}) mg cm^{-2} (*Tarchonanthus camphoratus* minor Less) (see also Table 4). DEET gave RD_{50} value of 3.3×10^{-4} (2.6, 4.2×10^{-4}) mg cm^{-2} under the same conditions.

Table 2
The chemical compositions of essential oils of the repellent plants

Compound	% Peak area of the plant oils					
	Cn	Lj	Pm	Lu	Tr	Tc
α-Pinene	0.35	t	0.17	1.13	t	16.62
Camphene	0.13	0.31	1.58	8.63	t	16.82
β-Pinene	0.18	0.08	0.81	1.00	0.78	0.78
Limonene	10.06	2.58	t	0.29	2.02	0.69
1,8-Cineole	6.84	t	9.00	2.42	1.50	6.51
Limonene oxide		38.99				
α-Terpinene	t	t	2.58	t	t	0.27
Linalool	0.11	2.69	t	0.40		0.88
p-Cymene	t		3.08	0.67	0.55	2.89
Citronellal		t				
γ-Terpinene	t		0.96	1.42	0.96	0.56
Geraniol	1.17					
Phenylethyl alcohol[a]	t					
α-Terpinolene	t		0.27	0.86		0.38
cis-Carveol			0.20			0.34
Camphor	0.17	0.75	48.80	39.84	0.13	0.38
α-Terpineol	t	2.04	0.38	t	t	3.78
Borneol	t	t	0.36	1.14	t	t
Myrtenol	t		t			t
Neral	t					
Carvone	t	0.4	t			
Perillaldehyde	29.28					
Perillyl alcohol	4.27					
Geranyl acetate	0.69					
Terpinen-4-ol		t	1.08		t	3.28
α-Fenchyl alcohol	0.21				0.73	14.76
Verbenone		6.06		0.78		
cis-Verbenol		11.33			t	0.30
Fenchone			1.75	0.17	64.82	0.43
Thujone					t	
Isocaryophyllene	0.58	1.38	1.67	0.43	0.10	1.36
Eugenol		0.46	t			
Aromadendrene		0.31				
Sesquiterpene alcohol[b]		t				
Caryophyllene oxide		0.20	1.13	t		1.06
Myrtenal[a]						0.38
4-Isopropyl benzaldehyde	0.78		t			

[a] Identified through GC-MS but not confirmed by GC co-injection standards; 't': present in trace amount (⩽0.1%) Cn, Lj, Pm, Lu, Tr, and Tc and refer to *C. newii*, *L. javanica*, *P. marruboides*, *L. ukambensis*, *T. riparia*, and *T. camphoratus*, respectively.
[b] Precise structure not worked out.

2.2. Essential oil compositions

A total of 36 compounds were identified in the essential oils of the six plants by GC-MS and GC co-injections with authentic samples. These and their relative proportions in the essential oils are given in Table 2.

Table 3
The RD_{50} (95% CI) values of the essential oil standards

Compound	$RD_{50} \times 10^{-5}$	Limits $\times 10^{-5}$
Camphene	221	5, 75
Limonene	180	26, 3
β-Pinene	156	4, 7897
p-Cymene	1	32052, 0
αTerpinene	240	20, 81
γ-Terpinene	274	2, 3166
α-Terpinolene	255	26, 123
α-Pinene	594	178, 5830
Isocaryophyllene	[a]	
Sesquiterpene alcohol	3	1, 5
Perillyl alcohol	6.3	0, 111
cis-Verbenol	7.5	0, 111
cis-Carveol	10	0, 73
Geraniol	11	1, 35
α-Terpineol	128	1, 443
Eugenol	132	2, 292
Terpen-4-ol	148	1, 1833
Linalool	153	22, 145
Citronellal	22	5, 85
Perillaldehyde	32	0, 490
Camphor	140	4, 23
Verbenone	156	21, 88
Fenchone	189	38, 258
Carvone	126	0, 1684
Caryophyllene oxide	120	0, 2016
Limonene oxide	147	15, 162
1,8-Cineole	124	8, 49
Citral	131	15, 63
α-Fenchyl alcohol	135	24, 1948
Borneol	165	8, 37
Myrtenol	154	45, 894
Geranyl acetate	326	178, 3051
Thujone	145	95, 2341
Myrtenal	165	96, 5975
Aromadendrene	498	237, 2915
4-Isopropylbenzaldehyde	163	85, 3598
DEET	33	26, 42

[a] RD_{50} not computable from the negative repellency data.

2.3. Repellency assays of individual constituents

Table 3 summarises the results of the repellency assay of 36 compounds identified in the essential oils of the six plants. Apart from isocaryophyllene (**1**) and *p*-cymene (**2**), all showed some repellent activities. Probit analyses of the repellency data (in the concentration range 10^{-5}–10^{-2} g ml^{-1}) for seven most potent repellent compounds, perillyl alcohol (**3**), *cis*-verbenol (**4**), *cis*-carveol (**5**), geraniol (**6**), citronellal (**7**), perillaldehyde (**8**) and caryophyllene oxide (**9**), gave the following RD_{50} values, respectively: 6.3×10^{-5} (95% confidence limits (CI): 0, 111×10^{-5}), 7.5×10^{-5} (1, 111×10^{-5}), 1.0×10^{-4} (0, 7.3×10^{-4}), 1.1×10^{-4} (0.1, 3.5×10^{-4}), 2.2×10^{-4} (0.1, 8.5×10^{-4}), 3.2×10^{-4} (0, 49×10^{-4}) and 1.2×10^{-3} (0, 20.16×10^{-3}) mg cm^{-2}.

2.4. Repellency assays of synthetic blends of major constituents of the essential oils

RD_{50} values obtained from repellency data of blends of the main (≥1.5%) constituents of oils of the six plants are given in Table 4. RD_{50} values of the parent oils show that repellencies of *C. newii*, *L. javanica*, *T. raparia* and *T. camphoratus* were comparable to the respective blends of the constituents. On the other hand, RD_{50} values of the experimental blends of *P. marrubioides* and *L. ukambensis* were much less than those of the corresponding essential oils.

3. Discussion

The present study constitutes part of our bioprospecting project to screen African plants with mosquito repellent constituents. Our overall aim is to identify (a) a pool of candidate plants with potential for use in traditional methods of reducing human-vector contacts, such as fumigation of households by direct burning, thermal expulsion or use of intact potted plants (Seyoum et al., 2002a,b, 2003); (b) key constituents of repellent plants that contribute to the repellent properties of the volatile blends; and (c) potential lead compounds with promising level of protection.

Dose–response studies of the oils indicate that the oils of *C. newii* and *P. marrubioides* are more potent

Table 4
RD_{50} (95% CI) values of (a) the crude essential oils, and (b) synthetic blends of the main constituents (present in ≥1.5%) of these oils

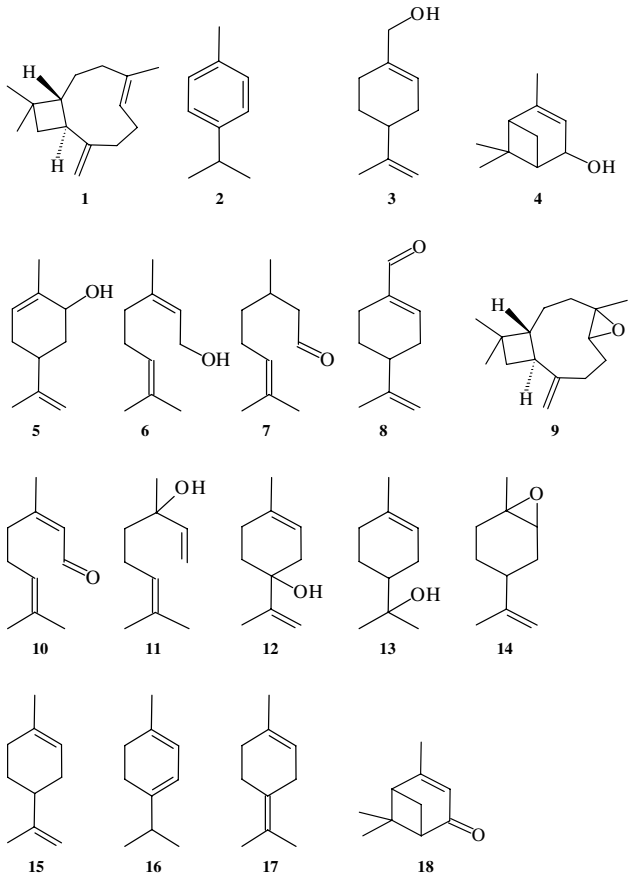
Plant	Essential oil $RD_{50} \times 10^{-5}$ (mg cm^{-2})	Limits $\times 10^{-5}$	Synthetic blend[a] $RD_{50} \times 10^{-5}$ (mg cm^{-2})	Limits $\times 10^{-5}$
C. newii	8.9	1, 11	12	1, 18
P. marrubiodes	8.9	1, 16	90	1, 95
L. javanica	26	15, 31	30	12, 45
L. ukambensis	43	18, 55	34	16, 39
T. riparia	50	25, 75	76	23, 88
T. camphoratus	240	155, 324	110	105, 385

[a] See Section 4.5.

than DEET. Those of *L. javanica*, *L. ukambensis*, and *T. riparia* are comparable to the synthetic repellent. The essential oil of *T. camphoratus* is less potent than DEET. The repellencies of the oils of *C. newii*, *L. javanica*, *T. riparia* and *T. camphoratus* are largely accounted for by blends of respective constituents present in $\geqslant$1.5% (Table 4). On the other hand, lower activities of synthetic blends of *P. marrubioides* and *L. ukambensis* relative to the corresponding parent oils indicate that minor constituents present in <1.5% also contribute to their repellencies and reflect the importance of compositional complexity in conferring bioactivity to natural terpenoid mixtures (Cates, 1996). Efforts to identify these behaviourally important minor components are in progress.

Of the seven more repellent constituents against *An. gambiae*, geraniol (**6**) and (*R*)-(+)-citronellal (**7**) were previously reported as mosquito repellents (Curtis et al., 1991; USDA, 1965; Dethier, 1956). The rest [(*S*)-(−)-perillyl alcohol (**3**), (*S*)-(−)-*cis*-verbenol (**4**), (*S*)-(−)-*cis*-carveol (**5**), (*S*)-(−)-perillaldehyde (**8**) and (±)-caryophyllene oxide (**9**)] are being reported for the first time as repellents of *An. gambiae*. Interestingly, different structural types of sesquiterpenoid and acyclic, monocyclic and bicyclic monoterpenes are represented by these compounds, although all are oxygenated and, with one exception (caryophyllene oxide), all are either alcohols or aldehydes. Given the structural diversity, no generalizations can be made at this juncture on structural requirements for potency in repellent activity against *An. gambiae*. However, within some groups of terpenoids, clear trends are discernible. Thus, within the acyclic monoterpenoid group, the relatively higher activity of geraniol (**6**) and citral (**10**) than (*R*)-(+)-citronellal (**7**) and (±)-linalool (**11**) suggests that the presence of 2,3-olefinic function and the position of hydroxyl group may be important for repellency. Likewise, within the cyclic monoterpenoids, (*S*)-(−)-perillyl alcohol (**3**), (*S*)-(−)-*cis*-carveol (**5**) and (*S*)-(−)-perillaldehyde (**8**) are relatively more potent than (±)-terpene-4-ol (**12**), (±)-α-terpineol (**13**), (±)-limonene oxide (**14**) and the non-oxygenated hydrocarbons such as (*R*)-(+)-limonene (**15**), α-terpinene (**16**) and γ-terpinene (**17**). Other noteworthy differences relate to (*S*)-(−)-*cis*-verbenol (**4**) and (*S*)-(−)-verbenone (**18**) (the alcohol being more active), and (±)-isocaryophyllene (**1**) and (±)-caryophyllene oxide (**9**) (the latter being active).

As a follow up, we have selected *C. newii* and *P. marrubioides* for evaluation as sources of fumigants (thermal expulsion and potted plants) in reducing man-vector contact in screenhouse experiments and in rural homesteads. The more potent individual terpenoids are being assessed in different formulations for personal protection.



4. Experimental

4.1. Plant materials

The leaves of the plants used were collected from different parts of Nyanza, Western, Rift Valley and Central provinces of Kenya in September 1999, February and June 2000. The collected plants were identified at the University of Nairobi (UoN), Botany Department. Voucher specimens were deposited at the UoN Herbarium: *C. newii* CNE/0376/2000; *L. ukambensis* LUK/0361/2000; *L. javanica* LJA/0373/2000; *P. marrubioides* PMA/0362/2000; *T. riparia* TRI/0385/2000; and *T. camphoratus* TCA/0364/2000. The leaves, flowers or whole aerial parts were dried under shade for one week before hydro-distillation.

4.2. Isolation

The essential oils were isolated by steam-distillation using Clavenger apparatus. The isolated oil was dried over anhydrous sodium sulphate, and stored in amber-coloured vials at 0 °C until required.

4.3. Analyses of essential oils

Analyses of the oils and identification of the components were carried out by GC, GC-MS, and GC co-injection of the essential oils with authentic samples. Analyses were performed on a capillary gas chromatograph, Hewlett Packard (HP) 5890 Series II, equipped with a split-less capillary injector system, 50 m × 0.2 mm (i.d.) crossed-linked methylsilicone (0.33 μm film thickness) capillary column, and FID coupled to HP 3393A Series II integrator. The carrier gas was N_2 at 0.7 ml min^{-1}. The temperature programme comprised of an initial temperature of 50 °C (5 min) to 280 °C at 5 °C/min and a hold at this temperature for 10 min. GC-MS analyses were carried out on a HP 8060 Series II Gas Chromatograph coupled to a VG Platform II Mass Spectrometer. The MS was operated in the EI mode at 70 eV and an emission current of 200 μA. The temperature of the source was held at 180 °C and the multiplier voltage at 300 V. The pressure of the ion source and MS detector were held at 9.4×10^{-6} and 1.4×10^{-5} mbar, respectively. The MS had a scan cycle of 1.5 s (scan duration of 1 s and inter-scan delay of 0.5 s). The mass and scan ranges were set at *m/z* 1–1400 and 38–650, respectively. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$, (Apollo Scientific Ltd., UK). The column used for GC-MS was the same as the one described for GC analysis except for the film thickness (0.5 μm). The temperature programme involved an initial temperature of 50 °C (5 min), to 90 °C at 5 °C min^{-1}, to 200 °C at 2 °C min^{-1}, to 280 °C at 20 °C min^{-1} and a hold at this temperature for 20 min.

4.4. Mosquito repellency assays of essential oils

The essential oils were assayed for their repellent activities against *A. gambiae* s.s. mosquitoes (ex-Ifakara, Tanzania, strain) that were reared under standard conditions at ICIPE, Duduville, mosquito insectary. Repellency assays were performed with 5–7 days old female *An. gambiae* that had been starved for 18 h, but previously fed on 6% glucose solution. Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or candidate oils. They had no contact with lotions, perfumes, oils or perfumed soaps on the day of the experiment. Initial screening for the bioactivity of the essential oils was carried out at 10^{-5}, $10^{-3}$and 10^{-1} g ml^{-1} according to WHO (1996) protocol. A total of 18 cages each measuring 50 × 50 × 50 cm were used, with 25 starved female *An. gambiae* in each cage. Test solutions (0.5 ml) were dispensed on one of the forearms of a volunteer 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 control. The control and treated arms were interchanged regularly to eliminate bias. The control arm was first introduced into the cage immediately after releasing the 25 experimental insects and kept there for 3 min. The number of insects that landed on that arm during the test duration was recorded. The treated arm was then introduced into the cage for the same period of time and the number of landing insects recorded. The different concentrations of each test sample were tested sequentially starting with the lowest dose. Repellency data from six replicates, expressed as protective efficacy (PE) at each dose, were calculated using the formula, PE = (% control mean − % test mean)/% control mean (Mehr et al., 1985). The data were transformed and subjected to analysis of variance (ANOVA) (SAS® Institute, 2000). Means were ranked using the Student–Newman–Keuls (SNK) test (SAS® Institute, 2000).

To obtain RD_{50} values, the essential oils were retested at 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} g ml^{-1} with 100 female *An. gambiae* per cage, following the same procedure as above. PE values from six replicates were obtained and the RD_{50} values for the oils calculated by probit analyses (Finney, 1971; Busvine, 1971).

4.5. Repellency tests of individual constituents and selected blends

Authentic samples of 36 compounds identified in the six more potent essential oils were tested in the concentration range 10^{-5}–10^{-2} g ml^{-1} as detailed in Section 4.4. Probit analyses data for eight most potent repellents were used to calculate their RD_{50}values. Synthetic blends of constituents present in ⩾1.5% (1.5–64%) in approximate relative amounts in the oils of six plants were prepared as follows:

1. *C. newii* – perillaldehyde, perillyl alcohol, 1,8-cineole, limonene (29:4:10:7).
2. *T. riparia* – fenchone, limonene, 1,8-cineole (64:2:1.5).
3. *P. marrubioides* – camphor, 1,8-cineole, *p* -cymene, α-terpenene, fenchone, isocaryophyllene (49:9:3:3:2:2).
4. *L. ukambensis* – camphor, camphene, 1,8-cineole (40:9:2.5).
5. *T. camphoratus* – camphene, α-pinene, α-fenchyl alcohol, 1,8-cineole, α-terpeneol, *p* -cymene, (17:17:15:7:4:3).
6. *L. javanica* – limonene oxide, *cis* -verbenol, verbenone, linanool, limonene, α-terpeneol (39:11:6:3:2.5:2).

These were tested in the 10^{-5}–10^{-2} g ml^{-1} concentration range and the repellency data were subjected to probit analysis.

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