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Constituents of Essential Oils from Three Plant Species Used in Traditional Medicine and Insect Control in Tanzania

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The essential oils of three aromatic plant species, Lantana viburnoides *sp.* viburnoides *var.* kisi *(A. Rich.) Verdc.,* Clausena anisata *(Willd.) Benth. and* Uvariodendron gorgonis *Verdc., were analysed for their chemical compositions and repellency activity against* Anopheles gambiae s.s. *The major chemical constituents were piperitenone (25.25%) and artemisia ketone (13.96%) in* L. viburnoides *oil; Estragol (88.38%) in* C. anisata*; and eugenol (89.82%) in* U. gorgonis. *The essential oils exhibited varying repellency properties against* An. gambiae *confirming the ethnobotanical usage as insect repellent. Further prospect exist of improving repellency of* U. gorgonis *and* L. viburnoides *essential oils by incorporating some inactive carriers.*

KEYWORDS Lantana viburnoides ssp *viburnoides* var *kisi,* Clausena anisata*,* Uvariodendron gorgonis*, essential oils, mosquito repellent*

INTRODUCTION

Ethnobotany has played a very important role in herbal medicine and traditional methods of protection against disease vectors, domestic insect pests, and stored cereal grain pests (2,3,7,27,28). Three highly aromatic plant species—*Lantana viburnoides* sp. *viburnoides* var. *kisi* (Verbenaceae);

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Clausena anisata (Rutaceae); and *Uvariodendron gorgonis* (Annonaceae)—found in central Tanzania and used in ethno-medicine and/or protection against insects in other parts of Africa were selected for the present study. The aerial parts of *L. viburnoides* are used in Iringa region in Tanzania for repelling mosquitoes (14), but no scientific confirmation of biological activity and associated chemical components has been reported. On the other hand, a related species, *L. camara*, and its extracts have been found to provide varying levels of protection against *Anopheles gambiae* (27), *Aedes* mosquitoes (8), and *Sitophilus zeamais* (22). *Clausina anisata* is widely used in traditional medicine for repelling ticks and insects (5) and in post-harvest protection (30). Significant qualitative differences in the repellency and composition of essential oils from *C. anisata* collected from different parts of Africa appear to occur (9,12,23,28), with some essential oils collections containing estragol, which is a genotoxic carcinogen and, therefore, unsuitable for human use (10,16). The oil from *C. anisata* growing in central Tanzania was therefore analyzed to assess the risks or otherwise associated with its traditional uses. No previous scientific study of the essential oil of *U. gorgonis* has been reported. The plant leaves are put in stored cereals and have a strong odor that resembles that of clove oil, the major component of which is a repellent compound known as eugenol (13,29). The essential oil of the plant was included in the present study to establish whether this phenolic mono-terpenoid formed a significant component.

MATERIALS AND METHODS

Plant Materials and Extraction

Lantana viburnoides sp. *viburnoides* var. *kisi* and *C. anisata* were collected from the Iringa region and *U. gorgonis* from Pugu Forest Reserve in Tanzania. The plant materials were authenticated at the Herbarium of the Department of Botany, University of Dar-es-Salaam, where voucher specimens are deposited. The leaves were dried in shade for 4 to 6 d and then hydro-distilled using a Clevenger-type apparatus for 8 h. The resulting oils were separated from the aqueous layers, dried over anhydrous Na_2SO_4, and stored at 4°C.

Gas Chromatography and Gas Chromatography–Mass Spectrometry

Analysis of the essential oil was carried out on a Hewlett Packard 5890A gas chromatograph (GC) equipped with a flame ionization detector (FID) and a Hewlett Packard 3396 series II integrator. A cross-linked methyl silicon capillary column (50 m × 0.2 mm ID × 0.33-μm film thickness, PerkinElmer LAS [UK] Ltd) was used for separation of the essential oil components. Nitrogen

was used as the carrier gas at a flow rate of 0.84 ml/min. The injector and detector temperatures were maintained at 250°C and 270°C, respectively. The temperature program comprised an initial temperature of 50°C, which was held for 5 min and then raised to 280°C at a rate of 5°C/min, where it was maintained for 20 min. Identification of the essential oil components was carried out on a Hewlett Packard 5790A series GC coupled to the VG Masslab 12-250 mass spectrometer (MS manufactured by Micromass, UK, formerly VG Biotech) with mass range m/z 1–1,400. This MS is equipped with a computerized data system running on MASSLYNX software with Wiley Version 6 and NIST Version 1.0 MS libraries. The MS was operated in the EI mode at 70 eV with the temperature of the source held at 180°C; multiplier voltage at 1,350 V; scan cycle of 1.5 s (scan duration of 1 s and inter-scan delay of 0.5 s); and scan range was m/z 38–650. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$, (Apollo Scientific Ltd., UK). The column and temperature program used for GC-MS was the same as for GC analysis except for the carrier gas, which was helium in this case. Where possible (depending on availability), identities of the essential oil components were confirmed by GC co-injections with authentic compounds obtained from Sigma Aldrich Chemical Co. (UK) or Fluka (Germany). Identification of the other compounds that were not commercially available was based on detailed comparison of their mass spectra with those in the libraries.

Mosquitoes

Mated female adult *An. gambiae s.s.* mosquitoes used in the study were obtained from a colony reared at ICIPE insectary. The larvae were reared in a room where the temperature was maintained at 32° to 36°C, and fed on TetraMin food (Tetra GmbH, Germany). Rearing temperatures and relative humidity in the adult insectary were 26° to 28°C and 70% to 80%, respectively. The adult mosquitoes were maintained on a 6% glucose solution, and females were fed on human blood thrice a week. Female mosquitoes used in the experiments were 5 to 7 days old, initially maintained on human blood but changed to glucose (6% solution) a day before the bioassays, and then starved for 18 h before being used in the experiment.

Ethical Clearance and Volunteer Safety

As the experiment required sources of human blood for mosquitoes and human landing catches, local volunteers were recruited with informed consent. A research protocol was submitted to the International Centre of Insect Physiology and Ecology based at Duduvile-Nairobi and to the Kenya National Ethical Review Committees based at the Kenya Medical Research Institute. Ethical clearance was obtained from Kenya National Ethics Board. The

discomfort and potential risks of mosquito bites were explained to the volunteers. The individuals had previously participated in similar studies, had good knowledge of malaria transmission, and showed mild or no allergic reaction to mosquito bites or the essential oil. Five adult volunteers (three male and two female) were involved in the experiments, and they do not object to being identified for publication. A parasite-free environment was ensured through regular screening of the volunteers' peripheral blood for *Plasmodium*. Sulphadine-pyriproxyfen prophylaxis was provided to each individual.

Repellency Assays

The repellency assay was performed in a dark room with red light as the only source of illumination (34). The room temperature and humidity were controlled at 28° ± 2°C and 75% ± 5%%, respectively, to mimic the feeding conditions for female *An. gambiae* s.s. mosquitoes. Cages (50 × 50 × 50 cm) made of aluminum sheet at the bottom, Pyrex window screen on sides and top, and a cotton stockinet sleeve for access on the front were used in the dose response assays. Different concentrations (10–10^{-2} % w/v) of the essential oil and selected constituents were prepared by dissolving 1 g of each sample in 10 ml of analytical grade acetone (99.5%, Technopharmchem/INDIA), followed by successive tenfold dilutions with acetone to obtain the other concentrations. Compounds whose repellency against *Anopheles gambiae* had previously been reported (15,21,24) were not evaluated. Acetone acted as a blank in all experiments and DEET (97%, Rieldel-De Haen/UK) as a positive control. Fifty test mosquitoes were used in each of five replicates involving five different adult volunteers for each concentration of a sample. The volunteers had no contact with any lotion, perfume, oil, or perfumed soap on the day of the bioassay. The forearm (average area of 696.6 cm^2) of each volunteer from the elbow to the hand was washed with water and left to dry. The test sample was spread once 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 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 for 3 min immediately after introduction of the mosquitoes. The number of mosquitoes that landed on the arm was recorded, and the insects were shaken off before they imbibed any blood. This was followed by exposure of a volunteer's arm first to the lowest concentration (10% w/v) of the test sample followed by sequential exposures to progressively higher concentrations (10–10^{-2} % w/v) of the sample, each time to fresh mosquitoes in a clean cage. The test arm of the volunteer was washed using a non-perfumed soap and tap water and allowed to dry naturally for at least 20 min before

dispensing the subsequent concentration. Only one compound/sample was tested per day.

Data Analysis

Percentage protective efficacy (PE) was calculated using the formula PE = (C-T/C) × 100%, where C and T are the mean numbers of mosquitoes that landed on the control and test arm, respectively. Means were subjected to analysis of variance and compared by Student-Newman-Keuls test (26). Probit to compute repellency concentration, which causes 50% response of the test mosquitoes (RC_{50}), was done by using the Lackfit inversel of the SAS programme (26).

RESULTS

Good yields of essential oils were obtained from the three plant species: *L. viburnoides* ssp *viburnoides* var *kisi* (0.36%); *C. anisata* (0.32%); and *U. gorgonis* (0.39%). The repellency activity against *Anopheles gambiae s.s.* of the crude oils was in the order of *U. gorgonis* ($RC_{50} = 0.38 \times 10^{-5}$ g/cm^2) > *L. viburnoides* ssp *viburnoides* var *kisi* ($RC_{50} = 0.56 \times 10^{-5}$ g/cm^2) > *C. anisata* ($RC_{50} = 1.1 \times 10^{-5}$ g/cm^2; Table 1). There was no significant difference ($p > 0.05$) in repellency between the crude essential oil from *U. gorgonis* and *L. viburnoides* ssp *viburnoides* var *kisi* up to a concentration of 1% w/v but significant difference with crude essential oil from *C. anisata* and DEET (see Table 1).

The composition of the essential oils from the three plant species based on GC-MS detectable peaks are shown in Figure 1 and Table 2. About 91.08% of the essential oil components from *L. viburnoides* ssp. *viburnoides* var. *kisi* were identified based on detectable peaks, with the least abundant component being 0.01% of the most abundant one in the individual essential oil (1,33). Sesquiterpenoids accounted for 11.72% while the rest were monoterpenoids. Piperitenone (25.25%), artemisia ketone (13.96%), limonene (7.80%), linalool (4.15%), *trans*-caryophyllene (4.47%), 1,6,9-tetradecatriene (6.64%), and ± verbenone (7.99%) were the major compounds in the essential oil (see Table 2). About 99.78% of the essential oil constituents from *Uvariodendron gorgonis* were identified, with the major compounds being eugenol (89.82%) and limonene (9.03%). Similarly 97.53% of the components of essential oil from *Clausena anisata* were identified with estragol (88.38%) and γ-terpinene (4.79%) as major components (see Table 2; Figure 1). Most of the constituents of the essential oils from leaves of *U. gorgonis* and *C. anisata* were monoterpenoids, which constituted 99.63% and 95.41%, respectively, of all the constituents of the essential oils.

TABLE 1 Protective efficacy against *An. gambiae s.s.* of essential oils, blends and compounds from three plant species

	Concentration (w/v)				RC_{50} (CI) (g/cm^2)
Treatment	10^{-2}	10^{-1}	10^{-0}	10	10^{-5}
U. gorgonis essential oil	29.68 ± 13.91^{aB}	48.90 ± 7.24^{bA}	57.88 ± 10.67^{Ab}	64.15 ± 2.37^{bA}	0.38 (0.21, 0.56)
C. anisata essential oil	13.45 ± 11.55^{bB}	21.91 ± 4.25^{cB}	42.93 ± 14.74^{Ab}	56.93 ± 13.68^{bA}	1.1 (0.96, 1.27)
L. vibunoides essential oil	26.47 ± 20.00^{aB}	46.05 ± 8.66^{bAB}	54.58 ± 21.37^{bAB}	62.66 ± 9.59^{bA}	0.56 (0.41, 0.74)
Estragol	10.47 ± 3.88^{cB}	17.79 ± 4.87^{cAB}	30.23 ± 8.30^{cAB}	37.98 ± 7.95^{Ac}	2.1 (1.76, 2.57)
α-Humulene	6.37 ± 2.97^{cB}	9.71 ± 1.39^{cdB}	27.34 ± 3.47^{Ac}	33.68 ± 5.93^{Ac}	2.2 (1.88, 2.64)
Endo-borneol	8.63 ± 1.66^{cB}	13.81 ± 2.76^{cB}	30.85 ± 4.60^{Ac}	30.28 ± 5.22^{Ac}	2.86 (2.29, 3.89)
DEET	13.10 ± 2.64^{bC}	82.68 ± 1.87^{aB}	100 ± 0^{aA}	100 ± 0^{aA}	0.085 (0.08, 0.09)

Mean values with the same small letters within the same concentration level and mean values with the same capital letters for a particular treatment are not significantly different at $p > 0.05$ by SNK; Value in parentheses represent lower and upper confidence limit at $p > 0.05$ by Lackfit inversel.

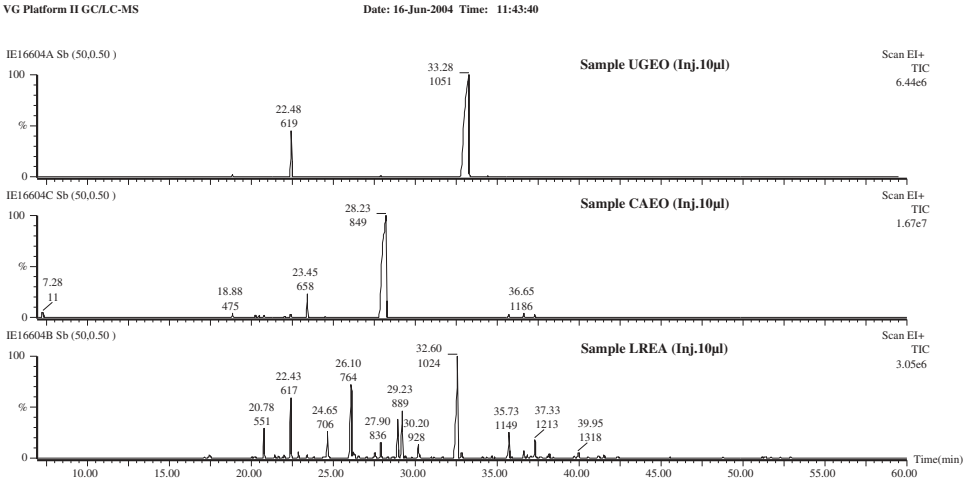

FIGURE 1 Total ion current chromatograms of *Uvariodendron gorgonis* (Sample UGEO), *Clausina anisata* (Sample CAEO) and *Lantana Viburnoides* Subsp *Viburnoides* Var *Kisi* (A. Rich) Verd (Sample LREA) essential oil constituents.

DISCUSSION

About 80% of the constituents of *L. viburnoides* were monoterpenoids, unlike those reported from one study on the essential oil of *L. camara*, which were mainly (~85%) sesquiterpenoids (17). These compounds may have been attributed to repellency activity of *L. viburnoides* oil against *An. gambiae s.s.* as it was found to be moderately active ($RC_{50} = 0.56 \times 10^{-5}$ g/cm^2). This bioactivity supports its traditional use in parts of Tanzania for repelling mosquitoes. Repellent property of some chemical constituents in *L. viburnoides* oil against *An. gambiae s.s* has been reported (15,21,24). However, piperitenone and 1,6,9-tetradecatriene, whose authentic samples were not available at time of experiment, need to be tested because in one study piperitenone oxide was found to be highly toxic but also having repellency effect against adult *An. stephensi* mosquitoes (31).

The major constituent of the essential oil of *C. anisata* collected from Tanzania was estragol (>88%), which was similar to oils obtained from plant materials collected in Nigeria (5,23) and unlike those collected close by in western Kenya and Zimbabwe (9,12,28). Despite the strong scent produced by fresh leaves from the plant species collected from Tanzania, repellency effects against *An. gambiae s.s.* of the essential oil was low (1.1×10^{-5} g/cm^2) unlike those of western Kenyan plant species (28). This suggests the existence of at least two major chemo-types of *C. anisata*. Likewise, toxicological data of estragol show that this compound is a naturally occurring genotoxic carcinogen, and hence it is unsuitable for human use (10). In view of risks posed by high estragol-containing plants to human health (10,16), an

TABLE 2 Percentage composition of compounds detected in the essential oil from the three plant species

		% Peak area		
*Peak no.	Name of the compound identified from the oil	*L. vibunoides* essential oil	*C. anisata* essential oil	*U. gorgonis* essential oil
1	α-Pinene	–	0.63	0.43
2	Camphene	0.04	–	0.03
3	Sabinene	0.17	0.42	–
4	β-Pinene	–	0.30	0.03
5	β-Myrcene	3.66	0.48	0.03
6	α-Phellandrene	–	0.02	–
7	β-Phellandrene	–	0.04	–
8	*p*-Cymene	0.43	0.20	–
9	Limonene	7.80	0.74	9.03
10	β-Ocimene	0.85	0.08	–
11	γ-Terpinene	0.46	4.79	–
12	Linalool	4.15	0.02	0.02
13	Artemisia ketone	13.96	–	–
14	Camphor	1.05	–	–
15	*Endo*-bornel	–	–	0.02
16	Terpinene-4-ol	0.83	–	–
18	α-Terpineol	2.50	–	0.22
19	Estragol	–	88.38	–
20	1,6,9-Tetradecatriene[a]	6.64	–	–
21	(+)-Verbenone	7.99	–	–
22	Isopiperitenone[a]	2.47	–	–
23	(+)-Carvone	0.21	–	–
24	Piperitenone[a]	25.25	–	–
25	Eugenol	0.9	–	89.82
26	α-Copaene	0.11	–	0.10
27	α-Bourbonene	0.31	–	–
28	*trans*-Caryophyllene	4.47	0.57	0.03
29	α-Humulene	1.02	0.85	–
30	Isocaryophylene	0.50	–	–
31	Germacrene-D	2.92	0.67	–
32	δ-Cadinene	0.53	0.03	0.02
33	β-Caryophyllene	0.83	–	–
34	β-Copaen-4α-ol[a]	0.30	–	–
35	β-Atlantone[a]	0.16	–	–

*Components are presented in order of increasing retention times; [a]except this all constituents confirmed by co-injection with authentic samples.

extensive survey of the volatile chemistry of the plant growing in different geographical locations is therefore warranted.

Eugenol (89.82%) and limonene (9%) were the major constituents in the essential oil of *U. gorgonis* and were attributable to its repellency property. Eugenol is an effective repellent and insecticide for insects in the order Hymenoptera (32), Isoptera (25), Diptera (4), and Coleoptera, including post-harvest pests (13,20). Therefore, the great abundance of this compound in the leaves of *U. gorgonis* indicates that it can be exploited for protection

against malaria transmitting mosquitoes as well as many other nuisance insects.

Previous reports on repellency properties of some plant essential oils indicated lower protection efficacy that DEET (6,11), but was improved significantly upon blending or formulation (18,19). The effectiveness of *U. gorgonis* and *L. viburnoides* essential oils as *An. gambiae* s.s. mosquito repellent can be improved further by incorporating some inactive carriers.

CONCLUSION

Crude essential oils and individual chemical constituents from the investigated plant species demonstrated repellency levels slightly lower than that of DEET (see Table 1). Nonetheless, there is still a possibility of using the oils from *U. gorgonis* and *Lantana viburnoides* sp. *viburnoides* var. *kisi* for controlling mosquitoes in small-scale programs as they exhibited moderate repellency activity.

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