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Draw inspiration from research on the insecticidal, irritant and mosquito-repellent properties of plants used by chimpanzees to build their nests

Eurydice Peti-Jean[1,2,3], Camille Lacroux[1,2], Harold Rugonge[2], Xavier Fernandez[4], Djallel Mansouri[4], Fabrice Chandre[5], Marie Rossignol[5], Sophie Durand[6], Kevin Calabro[3] and Sabrina Krief[1,2*]

Abstract

Background Vector-borne diseases are still responsible for the deaths of one million people worldwide every year, particularly in African countries. Plans to combat this worldwide burden, including strategies to control vectors, are still being investigated. Among them, the behavior of chimpanzees, our closest relatives living in African forests, has been studied. In Kibale National Park in Uganda, chimpanzees ingest plants that are biologically active against *Plasmodium falciparum* responsible for malaria but also select tree species to build their nests. The essential oils extracted from their leaves have repellent effects on *Anopheles gambiae*, which are vectors of *Plasmodium falciparum*.

Methods To investigate the chemodiversity of trees used by chimpanzees, essential oils (EOs) from the leaves of *Vepris nobilis, Lepisanthes senegalensis, Turraeanthus africanus*, and volatile extracts from the leaves of *Celtis africana, which are* not used for nesting by chimpanzees, were studied via gas chromatography–mass spectrometry. The repellent, irritant and toxic activities of the compounds selected on the basis of their abundance, availability and previously studied properties were subsequently tested under laboratory conditions alone and in mixtures on female *An. gambiae*.

Results Volatile compounds abundant at concentrations greater than 0.1% in the four plants were identified. We demonstrate different chemical profiles between the three EOs and the volatile extract, with molecules present in the essential oils such as β-elemene, δ-elemene, caryophyllene, α-humulene, or germacrene D. Chemical families specific to *Celtis africana* include aldehydes, ketones, carboxylic acids, furans, and vinylphenols. Only linalool was present in all four extracts. The mix we prepared and tested on mosquitoes, which contained α-humulene, caryophyllene, linalool and citral, is toxic and irritant to *An. gambiae*.

Conclusions This study describes volatile compounds present at more than 0.1% in the leaves of four species of Ugandan trees. Certain molecules present only in species used by chimpanzees in their nests can be combined to prepare solutions with anti-mosquito properties. The outcome of this work could lead to the formulation of a repellent spray inspired by chimpanzee behavior and the environment against *An. gambiae* to add a means of malaria prevention.

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Keywords *Pan troglodytes*, *Anopheles*, Essential oils, Zoopharmacognosy, Uganda, Vector-borne diseases, Malaria

Background

Vector-borne diseases are human illnesses caused by parasites, viruses and bacteria that are transmitted by vectors, which are essentially blood-sucking insects [1]. These diseases cause more than 700,000 deaths each year worldwide [1]. Among those vectors, female mosquitoes transmit many pathogens, including *Plasmodium* sp., which are responsible for malaria. In 2023, this disease affected 263 million people in 83 malaria-endemic countries, causing 597,000 deaths, and in Africa, 246 million people were affected, representing almost 94% of reported cases worldwide World Malaria Report (2024) [2].

Malaria has affected human survival since at least the Neolithic period [3] and possibly even since the Paleolithic period [4]. The first documented cases of fevers likely caused by malaria parasites appeared in ancient records from China (2700 BC) and Greece (850 BC) [5], with the discovery of the malaria parasite in blood [6]. The bark extract of the cinchona tree, native to the tropical Andes, was the first efficient treatment discovered in the seventeenth century. Quinine was isolated from this strain and purified in France in 1820 [7, 8]. Quinine remained the only treatment for malaria until the 1940 s; however, this drug presented a wide range of adverse effects, and as early as the 1910 s, resistance to this molecule by the *Plasmodium* parasite began to develop. In the 1930 s, research intensified, leading to the development of various synthetic molecules with other modes of action, such as mepacrine, resochin or sontochin [8, 9]. In the 1940 s, resochin was renamed chloroquine and became the main antimalarial drug because of its efficacy and low cost [10]. At the same time, the World Health Organisation (WHO) was launching a global campaign to eradicate malaria through insecticides by spraying dichlorodiphenyltrichloroethane (DDT). The toxicity of DDT to wildlife—especially birds—soon became evident, contributing to its agricultural ban in 1970. Moreover, resistance among mosquito populations has begun to emerge [11]. Despite all efforts, the number of deaths from malaria has risen again because of parasite resistance to treatments and mosquito resistance to insecticides. Some new compounds, such as mefloquine and artemisinin, were discovered in the 1970 s [8, 12]. In the 2000 s, artemisinin-based combination therapies were the only drugs recommended by the WHO to treat and control the disease [13]. However, the development of new resistance to drug treatments and the consequences of global changes could favor the emergence of new host/parasite relationships, such as the transfer of *P. vivax* from humans to various monkey species observed in America [14]. In addition, the redistribution of vectors and the spread of vector-borne diseases worldwide [15] point to the essential need to rethink therapies. Vaccine research is also underway to target the different stages of the parasite's life cycle, and to date, only two vaccines belonging to subunit vaccines preventing the sporozoite invasion in hepatocytes, RTS, S/AS01 and R21/Matrix-M, have been approved by the WHO [16]. Some countries with high levels of parasite transmission, such as Ghana, Kenya and Malawi, are now using them for children [17]. However, this treatment is lengthy, specific to a restricted species of parasite and not very effective in the long term [8]. Thus, the World Health Organization advises direct vector control, targeting *Anopheles* as the definitive host of *Plasmodium falciparum* [18]. Some of these control strategies include efforts to improve habitats and clean up water, distributing insecticide-impregnated mosquito nets (pyrethroids) to the population and spraying residual insecticides inside homes [1].

Terrestrial and marine natural organisms, such as plants, animals, and microbes, are inexhaustible sources of new, diverse, and biologically active compounds, and have always been a source of inspiration for mankind [19, 20]. Today, with the development of new analytical chemistry tools, the isolation of natural products (NPs) has been facilitated, enabling the development of other applications ranging from the cosmetics and perfumery sectors to agriculture with the development of biocontrol agents [21]. Among ecosystems, tropical forests are promising sources of new compounds, as plants present a greater number of defense metabolites than do plants in temperate forests. Some authors have proposed that such differences could be explained by the fact that tropical plants are more susceptible to attack by pathogens, herbivorous mammals and especially insects than are plants in temperate forests [22]. Greek, Roman and Indian scholars have also reported ancestral practices of using burnt plants to ward off mosquitoes [23]. Currently, ethnobotanical studies indicate that smoking remains the most common method of repelling mosquitoes in the tropics [24]. This efficiency could be due to the release of repellent volatile compounds such as β-ocimene [25]. However, burning plants in houses is harmful to health [24]. The mosquito-repellent properties of volatile compounds from tropical plants have not been studied

extensively, except for *Eucalyptus* sp., from which certain bioactive compounds, such as citronellal, eucalyptol, α-citral and α-pinene, have been isolated [20, 23, 26]. While tropical forests stand out as the richest biodiverse ecosystems, harboring 50–90% of all terrestrial species, they are also the most threatened biome [27]. Among the strategies that could be used to discover the biological properties of forest plants, observing how our closest relatives—nonhuman primates—self-medicate or prevent disease may be a successful option [28]. A study carried out in 2010 in Uganda and the Democratic Republic of the Congo revealed that nonhuman primates of the *Pan* genus (chimpanzees and bonobos) can carry different strains of *Plasmodium* [29–32]. The strong similarities between human beings and chimpanzees, whether genetic (98.8%), physiological, behavioral, cognitive or cultural, for example, with the use of tools they make [33] or the medicinal plants they use [34–37], encourage us to draw inspiration from the knowledge and behavior of chimpanzees. Chimpanzees practice self-medication by consuming plants that have low nutritional value but antimalarial properties [28]. It has also been reported that chimpanzees in Kibale National Park (Uganda) enhance the antiplasmodial properties of the bitter leaves of *Trichilia rubescens* ingested by consuming soil a few minutes later [38]. Several other antiparasitic compounds, particularly some active against *Plasmodium falciparum*, have been isolated from the bark or leaves of plants consumed by chimpanzees in Kibale National Park [37, 39, 40]. Furthermore, blood sample analysis of some of these wild chimpanzees revealed multispecies infections but low parasitemia [29]. We assume that chimpanzees, in addition to ingesting plants with antimalarial activities, may also use repellent plants to protect themselves from mosquitoes. For example, every evening, great apes build a nest by interweaving branches, stems and leaves to form a circular structure in a tree, in which individuals spend the night safely [41]. The locations where chimpanzees build their nests are thought to protect them from predators, parasites and pathogens [42]. The daily construction of nests reduces the risk of exposure, and nests represent physical barriers against both parasites and the vectors of these parasites [42]. In a previous study conducted by Krief et al. in 2012, anopheles species were trapped to survey their diversity and abundance in Kibale National Park according to environmental patterns, revealing that the height of the nest on the tree and the altitude chosen for nesting sites are locations where the risk of being exposed to the vectors is low [43]. To build their nests, chimpanzees bend and intertwine branches chosen according to some specific biomechanical and morphological characteristics, then lie down on the thick mattress [44]. In addition to providing stable and comfortable support, the nest creates an aromatic environment due to the crushed and crumpled leaves. In a study published in 2022, Lacroux et al. [45] tested the hypothesis that volatile extracts of leaves prevent mosquito bites, volatile extracts of leaves were tested against *Anopheles gambiae*. The essential oils of 1% of the preferred species were significantly more repellent and irritant than the essential oils of species that were abundant and not used by chimpanzees for nesting, suggesting the selection of tree species on the basis of their aromatic properties [45].

In this work, we aimed to follow-up and study the composition of four repellent/irritant essential oils of tree leaves from the Sebitoli chimpanzee habitat that are used in traditional medicine. Three of them are selected by chimpanzees for the construction of their nests (*Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus*), and one is abundant in the environment consumed but not used by chimpanzees for nesting (*Celtis africana*). The plant species used in traditional medicines. For example, the leaves and bark of species of the *Vepris* genus (Rutaceae) are commonly used in traditional African pharmacopoeia as a treatment for chronic illnesses and bacterial or parasitic infections [46]. *Lepisanthes senegalensis* (Sapindaceae) is used in traditional Senegalese medicine as an analgesic, anti-inflammatory, antibacterial and antifungal [47]. *Turraeanthus africanus* (Meliaceae) is used in traditional medicine to treat malaria in southern Cameroon [48]. Finally, *Celtis africana* (Ulmaceae) is used in African ethnomedicine for the treatment of various pathologies [49]. Despite these many properties, the compositions of the essential oils of *Lepisanthes senegalensis*, *Turraeanthus africanus* and *Celtis africana* have not yet been studied. The composition of the essential oils of *V. nobilis* is slightly better known, with the main volatile compounds found belonging to the monoterpene and sesquiterpene families [50, 51].

Once the composition was determined, we used the literature to determine whether repellent activities were identified previously against *Anopheles gambiae* for the most abundant compounds in the studied essential oils. Depending on commercial availability, we tested the toxic, irritant (i.e., repellent by tarsal contact) and repellent properties of the selected compounds, pure and in a mixture, on female *Anopheles gambiae* mosquitoes. The final goal of this study is to propose a solution inspired by chimpanzee nesting behavior and selection that may be used by humans without harvesting the tree leaves from their environment, which deserves careful preservation.

Methods

Mosquitoes

Behavioral assays were performed on female *An. gambiae* aged 2–7 days, originating from the insecticide susceptible reference strain Kisumu. This strain, originally collected in Kenya in 1953, has been reared at Institut de Recherche pour le Développement, Montpellier, France, where the assays were conducted. The insecticide susceptibility of the Kisumu strain was confirmed by World Health Organization (WHO) toxicity bioassay with WHO diagnostic doses (i.e. 4% DDT, 0.75% permethrin) and its genotypes for *kdr* and *ace.1R* mutations are controlled by PCR every 4 months as recommended by the iso 9001 norm. The colony has been maintained in a climatic room at 27 ± 2 °C, 80 ± 10% RH and with a photoperiod cycle of 12 h Light: 12 h Dark. Mosquito larvae were fed with fish food, then emerged adults were mechanically aspirated and transferred into 25 × 25 × 25 cm cages and provided access to 10% honey-water solution every morning [52].

Leaf sample collection

Leaves were collected from the Sebitoli forest located in the northern part of the Kibale National Park (KNP), southwestern Uganda, Africa. The climate of this equatorial area is composed of two rainy (from March to May, and September to November) and two dry seasons in between. Species were identified at the herbarium of the Laboratoire de Phanérogamie at the Muséum National d'Histoire Naturelle (Paris, France).

The leaves were collected by Camille Lacroux. *Celtis africana* leaves were harvested in 2018 from a previous survey [45], and the leaves of *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus* were collected in 2021 [45]. For *Celtis africana*, leaves were harvested from three different trees during the wet season. *Vepris nobilis* leaves were collected from five different trees during the dry season and the rainy season, while *Lepisanthes senegalensis* and *Turraeanthus africanus* leaves were each collected from a single tree during the dry season and the wet season, respectively.

The leaves remaining after the essential oil extraction were air-dried and stored in clean tea bags.

Extraction of volatile compounds

When the yield was sufficient, essential oils (EOs) were produced from the harvested leaves. For *Celtis africana*, the essential oil extraction yield was too low (0.0275% according to [45]), so a volatile extract was prepared from the remaining dry leaves for this study.

Essential oils

The essential oils of *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus* were weighed and extracted from fresh leaves via a Clevenger apparatus in Uganda. Fresh leaves were cut into small pieces of 1–2 cm^2. The cut leaves were placed in the distillation chamber with 20 L of water and boiled for 2 hours. The essential oil above the hydrolat, was collected in a separating funnel and stored in clean vials (Table 1).

Volatile extract

The leaves of *Celtis africana* were dried in the shade and stored at room temperature. A total of 150 g of dry leaves was boiled in water for 4 h. At the start of condensation, 3 mL of distilled hexane (Sigma Aldrich™) was added to the top of the Clevenger recovery burette in order to trap volatile compounds. The volatile extract condensed above the hydrolat was collected in a separately funnel. A total of 1.13 g of volatile extract of *Celtis africana* was obtained.

Volatile compound analysis by GC–MS and GC–FID

The essential oils were filtered and diluted 1:10 (v/v) in distilled dichloromethane (Sigma Aldrich™) before being deposited in a gas chromatograph (GC) (Agilent Technologies©). The volatile extract was directly injected into the GC.

GC–MS and GC–FID analyses

To identify and quantify the compounds, analysis of the essential oils and volatile extracts was performed via GC–MS and GC–FID via an Agilent 6890N gas chromatograph (Palo Alto, CA) equipped with an Agilent MSD5973N mass selective detector, a flame ionization detector (FID), an electronic pressure control (EPC) injector, and a multifunction automatic sampler (Combi–Pal, CTC Analytics, Zwingen, Swiss). Separations were achieved on an apolar HP-1 capillary column (100% polydimethylpolysiloxane; 50 m × 200 µm, 0.33 µm film thickness; Agilent Technologies). One microliter of sample (80 mg/mL) was injected in split mode (1/10), and helium (carrier gas) was used at a flow rate of 0.8 mL/min. The injector temperature was set to 250 °C, and

Table 1 Mass of fresh leaves and essential oils extracted from *Vepris nobilis*, *Lepisanthes senegalensis* and *Turraeanthus africanus*

Species of plant	Mass of fresh leaves (kg)	Mass of essential oils (g)	Essential oil yield (%)
Vepris nobilis	14.6	5.93	0.041%
Lepisanthes senegalensis	16.0	18.0	0.113%
Turraeanthus africanus	27.0	7.69	0.028%

the oven temperature was programmed from 40 °C to 270 °C at 2 °C/min. For GC–MS, a solvent delay of 5 min was selected. Mass spectra were recorded in electronic ionization (EI) mode at 70 eV, scanning the m/z 35–500 range (3.15 scan/s). For GC-FID, samples were injected in triplicate for quantification. The average of these three values and the standard deviation were determined for each identified compound.

Identification of complete and residual compounds

Data treatment was performed via MSD ChemStation (E02.02) software (Agilent Technologies). The identification of compounds involved the comparison of mass spectra with those recorded by internal librairies and commercial mass-spectral libraries (NIST and Wiley), as well as the comparison of linear retention index (LRIs) with those in the literature (NIST, ESO) and articles for the missing data in the previous databases. The retention index (RIs) were calculated via a formula according to van Den Dool and Kratz and according to the retention times of standard n-alkane C6–C27 homemade mixtures. Alkane mixtures diluted to 0.1% in diethyl ether were analyzed via GC–MS and GC–FID according to the methods described above.

Selection of compounds and preparation of solutions for bioassays

We proposed an identification for compounds with an abundance greater than 0.1% in the three essential oils whose repellent activity against *Anopheles gambiae* was demonstrated by Lacroux et al. 2022 [45] (i.e., EOs of *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus*). This allowed us to calculate the average percentage of each compound of interest, which we scaled down to 100 to determine the concentration of the compounds in a "synthetic" essential oil.

Compounds whose calculated abundance in this "synthetic" essential oil is greater than 2% are selected. Out of these six compounds, a more drastic choice was made according to their commercial availability and cost in order to propose an economically affordable mix. On the basis of these criteria, caryophyllene and α-humulene were selected. In addition, bibliographic studies enabled us to select compounds that were also present in the essential oils in this study and whose repellent activity had been demonstrated. Some of these compounds, such as linalool and citral, which are mixtures of the neral and geranial isomers, were present in the laboratory, so we also used them in our tests.

Thus, three solutions (Mixes 1, 2 and 3) of these compounds, as detailed in Table 2, were prepared in ratios corresponding to their abundance in the "synthetic" essential oils (Table 5). Before mixing them, the pure molecules caryophyllene (product reference 22,075, Sigma Aldrich™ purity ≥ 98.0%) and α-humulene (product reference CRM40921, Sigma Aldrich™ C = 2000 µg/mL) were tested individually at 1%, and α-humulene was also tested at 1.18%, the concentration at which it is in Mix 1.

Table 3 Summary of the chromatographic profiles obtained after analysis of the volatile compounds of the four plants

Species	Number of peaks in GC–MS	Number of compounds > 0.1% identified	Identified (% of EO)
Vepris nobilis	191	67	93.75
Lepisanthes senegalensis	71	24	96.06
Turraeanthus africanus	121	45	91.58
Celtis africana	86	46	77.54

Bioassays against *An. gambiae* mosquitoes

For each compound or solution of compounds, three tests were carried out at the Institute de la Recherche pour le Développement by the MIVEGEC team (Montpellier, France) to test their toxicity, irritability and repellency toward female mosquitoes of the genus Anopheles (species *Anopheles gambiae*, sensitive Kisumu strain), aged between 2 and 7 days. For each of the tests, three replicates were carried out along with their negative controls. The temperature and hygrometry data were recorded to ensure the validity of the test (Table S2). The tests performed in this study are conducted in tubes, identical to those performed and described in the study by Lacroux et al. 2022 and are referred to as 'Spatial

Table 2 Composition of the three solutions prepared for bioassays against mosquitoes

Compound	Caryophyllene	α-Humulene	Linalool	Citral
Product reference	22,075, Sigma Aldrich™	CRM40921, Sigma Aldrich™	Sevessence™	Sevessence™
Mix 1	1.64%	1.18%	0%	0%
Mix 2	0%	0%	0.25%	0.059%
Mix 3	1.64%	1.18%	0.25%	0.059%

repellent assays', 'Contact irritancy assays' and 'Toxicity assays' following the procedure described in [52]. The control solution consisted of an ethanol/silicone mixture (V/V 63%/37%). Two pure molecules (caryophyllene and α-humulene) and three solutions, Mix 1, Mix 2 and Mix 3 (Table 3), were tested.

Toxicity assays

The toxicity assay is used to determine the lethal property of a substance 24 h after exposure, which is a tarsal contact for one hour. The test was considered valid when the percentage of dead mosquitoes in the control experiment was less than 20%, and the solution was considered toxic when 20% of the mosquitoes died 24 h after exposure [53]. The confidence interval used is the Wald confidence interval, i.e., $1.96 \times (\sqrt{}((\%\ \text{mortality treated} \times (1 - \%\ \text{mortality treated})/\text{N treated}))$. If the mortality of mosquitoes in the control experiments was between 5 and 20%, we calculated the corrected mortality via Abott's formula [54], which allows natural mortality to be considered. It is calculated according to the following formula: (Σ % treated mortality − Σ % control mortality)/(1 − Σ % control mortality). A 95% corrected mortality confidence interval is calculated as the Wald mortality confidence interval/(1 − % control mortality).

Contact irritability assays

An irritability assay was carried out to determine the irritant property of a substance via tarsal contact for 10 min by recording the number of mosquitoes that escaped from the treated tube (the WHO test kit was used). The percentage of escaped mosquitoes was calculated as the sum of the number of escaped mosquitoes for all replicates over the total number of mosquitoes. The confidence interval used is the Wald confidence interval, i.e., $1.96 \times (\sqrt{}((\%\ \text{mortality treated} \times (1 - \%\ \text{mortality treated})/\text{N treated}))$. The test was considered valid when the percentage of escaped mosquitoes in the control experiment was less than 50%, and the solution was considered irritant when more than 50% of the mosquitoes escaped from the treated tube at the end of the experiment.

Spatial repellency assays

In the repellent assay, in contrast to the irritant assay, mosquitoes were not in direct contact with the products to be tested. We used the same device with a grid between mosquitoes and the treated sheet, and the percentage of escaped mosquitoes, calculated as in the irritation assay, enabled us to estimate the repellent properties of the product tested.

Data analysis

The proportions of escaped or dead mosquitoes in control and treated assays were compared using Binomial test by pooling the replicates. The proportions of escaped or dead mosquitoes were corrected by the control assay values using Abbot's formula [54]. The graphs were created using RStudio® [55].

Results

Identification of volatile compounds

We identified volatile compounds with an abundance of more than 0.1% in *Vepris nobilis, Lepisanthes senegalensis, Turraeanthus africanus* and *Celtis africana* leaves. The three essential oils of *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus* have similar chemical profiles, with a majority of sesquiterpenes (51.5%, 94.6% and 88.5%, respectively), whereas the volatile extract of *Celtis africana* contains a majority of sesquiterpenoids (33.5%). Only linalool is found in the volatile composition of the four plant species studied, and thirteen compounds are common to all three plant species used by chimpanzees to build their nests (Table S1).

Vepris nobilis

The chromatograms obtained after analysis of the essential oil of *V. nobilis* contained 191 peaks, two of which were predominant: germacrene D (32.88%) and (E)-β-ocimene (18.03%) (Table 4). A total of 63 compounds

Table 4 Most abundant volatile compounds present in the leaves of *V. nobilis, L. senegalensis, T. africanus* and *C. africana*

Compounds	CAS number	Th. RI	Exp. RI (FID)	*Vepris nobilis* (%)	*Lepisanthes senegalensis* (%)	*Turraeanthus africanus* (%)	*Celtis africana* (%)
(E)-β-Ocimene	3779-61-1	1038	1046	18.0			
δ-Elemene	20,307-84-0	1337	1337	0.27	69.7	3.04	
Germacrene D	37,839-63-7	1477	1489	32.9	14.91	0.96	
Ledene	21,747-46-6	1492	1492			33.8	0.39
Spathulenol	6750-60-3	1577	1576			1.92	12.3

Th. RI: Theoretical retention index; Exp. RI: Experimental retention index

with abundances greater than 0.1% were identified, representing 93.75% of the essential oil (Table 3). These include compounds belonging to 13 families, with a majority of linear or polycyclic sesquiterpenes (51.19%), linear or cyclic monoterpenes (20.13%), sesquiterpenoids (13.58%), phenylpropanoids (3.33%), alkanes (1.21%), monoterpenoids (1.65%), alkenes (1.21%) and benzyl esters (0.72%) (Table S1).

Lepisanthes senegalensis

A total of 71 peaks were detected from the chromatogram of the essential oil of *Lepisanthes senegalensis*. The two main compounds identified are δ-elemene (69.74%) and germacrene D (14.91%) (Table 4). The 24 compounds with abundances greater than 0.1% represented 96.1% of the essential oil (Table 4). These include compounds belonging to six families, with a majority of cyclic sesquiterpenes (94.64%), sesquiterpenoids (1.13%), monoterpenoids (0.17%) and benzyl esters (0.72%) (Table S1).

Turraeanthus africanus

The chromatogram of the essential oil of *Turraeanthus africanus* shows 121 peaks, with ledene (33.8%) identified as the main volatile compound (Table 4). A total of 43 compounds with an abundance greater than 0.1% were detected. They represent 94.1% of the essential oil (Table 3). These include compounds belonging to 10 families, with a majority of cyclic sesquiterpenes (88.5%), sesquiterpenoids (3.16%), esters (0.77%), diterpenes (0.54%), diterpenoids (0.52%), monoterpenoids (0.17%) and alkenes (0.10%) (Table S1).

Celtis africana

The chromatogram of the volatile extract of *Celtis africana* contains 86 peaks. Spathulenol was the main compound identified (12.3%) in the volatile extract of *Turraeanthus africanus* (Table 4). A total of 45 other compounds were present at concentrations greater than 0.1% and accounted for 78.3% of the volatile extract (Table 3). The compounds annotated belong to 15 families, with a majority of sesquiterpenoids (33.5%), aldehydes (13.1%), cyclic sesquiterpenes (1.98%), linear and cyclic monoterpenes (9.45%), ketones (9.19%), diterpenoids (4.46%), esters (1.93%), furans (1.35%), carboxylic acids (1.26%), monoterpenoids (0.81%), phthalates (0.74%) and vinylphenols (0.43%) (Table S1).

Tests on *Anopheles gambiae* mosquitoes

Drastic choice for a "synthetic" essential oil

We aimed to prepare a "synthetic" essential oil by pooling the active compounds from the extracts of the species with repellent properties against Anopheles according to Lacroux et al. (2022) [45]. To achieve this goal, the abundances of the compounds in the three repellent EOs (*Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus*) were combined, and their respective proportions were reported in the "synthetic" essential oil. Compounds whose calculated abundance in this "synthetic" essential oil was greater than 2% were selected. These included δ-elemene (25.95%), germacrene D (17.32%), ledene (12.01%), (E)-β-ocimene (6.40%), caryophyllene (3.75%) and α-humulene (2.62%) (Table 5). However, δ-elemene, germacrene D, ledene, and (E)-β-ocimene were excluded due to commercial unavailability.

Furthermore, linalool and citral were selected because of their known repellent activities against mosquitoes [56–62]. The test concentration chosen for these compounds is proportional to their concentration in the synthetic essential oil.

Pure molecules of commercial solutions diluted to 1%

The caryophyllene in the commercial solution used, diluted to 1%, is not toxic (1.5%), irritant (10%) or repellent (0.0%). Similarly, the α-humulene in the commercial solution used, diluted to 1%, was not toxic (14%), irritant (7.3%) or repellent (4.0%). A more concentrated solution of α-humulene at 1.18% did not increase any toxic (3.1%) or repellent (2%) activity but had greater irritant activity

Table 5 Most abundant compounds (> 2%) in *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus* EOs pooled

Compounds	CAS number	Th. RI	Exp. RI (FID)	*Vepris nobilis* (%)	*Lepisanthes senegalensis* (%)	*Turraeanthus africanus* (%)	*Celtis africana* (%)	Synthetic EO %
δ-Elemene	20,307-84-0	1337	1337	0.27	69.74	3.04		25.95
Germacrene D	37,839-63-7	1477	1489	32.9	14.91	0.96		17.32
Ledene	21,747-46-6	1492	1492			33.8	0.39	12.01
(E)-β-Ocimene	3779-61-1	1038	1046	18.0				6.40
Caryophyllene	150,320-52-8	1419	1422	0.67	0.92	8.97		3.75
α-Humulene	6753-98-6	1451	1455	1.38	0.25	5.75		2.62

Th. RI: Theoretical retention index; Exp. RI: Experimental retention index; EO: Essential oil

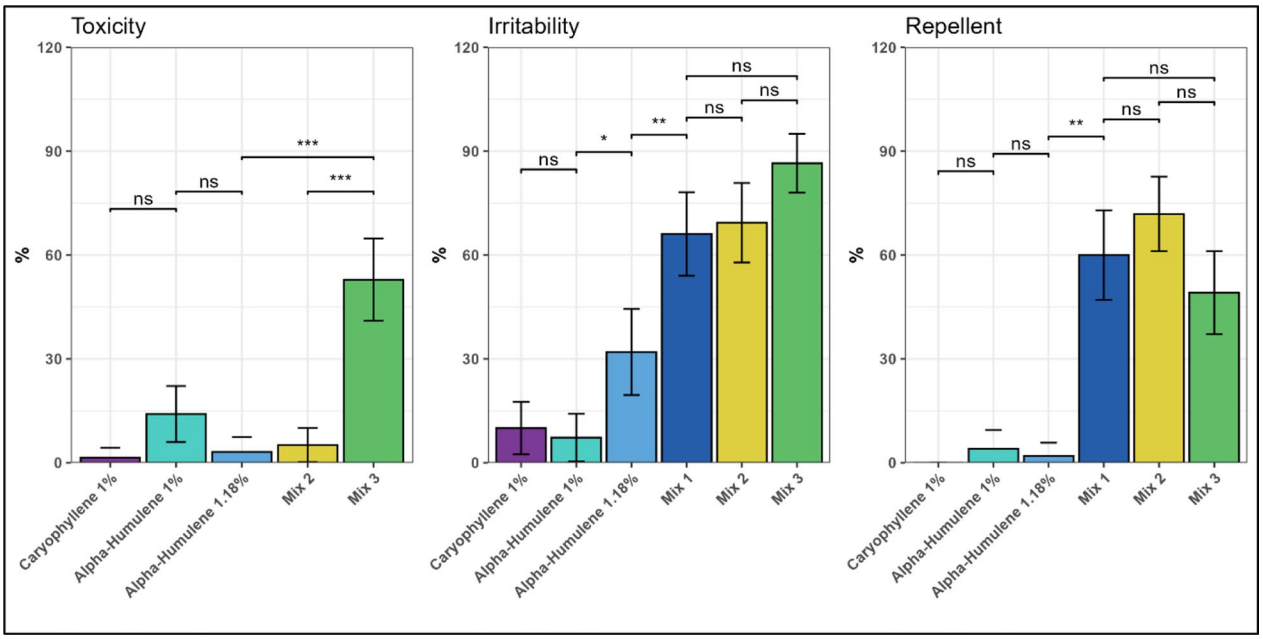

Fig. 1 Toxic, irritant and repellent properties of caryophyllene purity ≥ 98.0% tested at 1%, alpha-humulene. C = 2000 µg/mL tested at 1%, alpha-humulene at 1.18% pure molecule and mixtures 1, 2 and 3. n = 3. Composition of Mix 1: caryophyllene at 1.64% pure molecule and alpha-humulene at 1.18% pure molecule; composition of Mix 2: linalool at 0.25% and citral at 0.059%; composition of Mix 3: caryophyllene at 1.64% pure molecule, alpha-humulene at 1.18% pure molecule, linalool at 0.25% and citral at 0.059%. Squares: toxicity as a percentage of dead female mosquitoes, stripes: irritability as a percentage of escaped female mosquitoes, dots: repellency as a percentage of escaped female mosquitoes

than the same compound at a lower concentration (1%) (Fig. 1). Owing to their limited quantity, linalool and citral have not been tested separately.

Mixtures 1, 2 and 3 were diluted according to the ratios corresponding to the essential oils studied.

A-humulene at 1.18% and caryophyllene at 1.64% when pulled together (Mix 1) presented noticeable irritant (66%) and repellent (60%) activities. The toxicity of this solution could not be measured because of the high mortality rate of control mosquitoes (> 20%). Mix 2, containing a mixture of 0.25% linalool and 0.059% citral, was not toxic (5.1%) but was irritant (69%) and repellent (72%) toward the *Anopheles gambiae* adult female mosquitoes. Finally, highly toxic (53%), irritant (87%) and repellent (49%) activities were observed for Mix 3, which combined mixtures 1 and 2 (Fig. 1).

Discussion

In a previous study by Lacroux et al. published in 2022, essential oils extracted from the leaves of tree species used by chimpanzees to nest, as well as abundant plants in their forested habitat in Uganda, were shown to be repellent and irritant to *Anopheles gambiae* at a concentration of 1% [42]. This follow-up study proposes the identification of 183 volatile compounds in four of these plant species, selected because of their bioactivities against *Anopheles gambiae* and attempts to attribute anti-mosquito properties to some of these compounds. We established a chemical profile and tentatively identified the most abundant (> 0.1%) volatile compounds in the leaves of three plant species used by chimpanzees to build nests, namely, *Vepris nobilis, Lepisanthes senegalensis* and *Turraeanthus africanus*, and one ingested species and abundant species in their habitat, *Celtis africana*. The mass spectrometry analysis method coupled with gas chromatography revealed a majority of sesquiterpenes, with germacrene D, δ-lemene and ledene as the main compounds. On the other hand, the sesquiterpenoid spathulenol was the main compound detected in the leaves of *Celtis africana.* We carried out toxicity, contact irritability and repellency tests on female *An. gambiae* mosquitoes using the same protocols and strains as Lacroux et al. [42]; four compounds were selected based on their abundance, cost and commercial availability in the goal of proposing an affordable solution inspired by chimpanzee behavior of selective choice of nesting materials. Both mixtures of (1) linalool and citral and (2) caryophyllene and α-humulene had significant repellent effects. Solution (3), composed of solutions (1) and (2), had a slightly lower repellent effect but had synergistic effects on the irritant effects (87%).

From the leaves of *Lepisanthes senegalensis*, we proposed the identification of 24 compounds, i.e., 96.1% of the essential oil extracted, and from the leaves of *Turraeanthus africanus,* 43 compounds, representing 91.6% of the essential oil extracted. The volatile compounds present in the leaves of *L. senegalensis* and *T. africanus* have never been studied before. The compounds abundant in more than 0.1% of the essential oil of *V. nobilis* leaves represented 29.9% of the essential oil and 53 compounds. Our findings are consistent with those of previous studies that proposed a majority of monoterpenes and sesquiterpenes with germacrene D as the major compounds [50]. Finally, for *Celtis africana*, the volatile extract made from the leaves contains 45 compounds for which we propose identification, i.e., 77.5% of the extract. The volatile compounds in the leaves of *C. africana* differ from those in the other three, with a majority of sesquiterpenoids and only nine compounds also found in at least one essential oil analyzed. Species of the *Celtis* genus are used in South Africa to treat epilepsy or as antiseptic or antibacterial agents, for example [49]. Studies have also shown that compounds such as a sphingolipid glucoside isolated from aerial parts are cytotoxic and could be promising for the treatment of cancers [63]. However, no studies on the volatile compounds of *C. africana* plant have been published. Furthermore, this plant does not appear to have been used as an insect repellent in ancestral practices or today. Additionally, following the hydrodistillation of *Celtis africana* carried out in this study, we can deduce that it would be interesting to test the repellent properties of the volatile compounds in the leaves of this plant again. Indeed, with respect to the composition of the volatile extract, compounds such as eucalyptol or globulol are present in the essential oil of *Eucalyptus globulus,* which, at a concentration of 20%, repels *Aedes aegypti* mosquitoes for up to 90 min [64]. Other compounds present, such as geranyl acetone, nerolidol and caryophyllene oxide, are known for their repellent effects on *Anopheles aegypti* and *Anopheles quadrimaculatus* [65].

According to the assay results for *Anopheles*, α-humulene is more irritant but less toxic and repellent at higher concentrations (1.18%). It is also more irritating than caryophyllene at a concentration of 1%. Interestingly, a solution composed of these two sesquiterpenes at the average concentrations found in the EO studied (Mix 1) becomes irritant and repellent. Caryophyllene and α-humulene are isomers frequently found in essential oils and are known for their antitumour, anti-inflammatory and antimicrobial effects [66]. Furthermore, these two compounds are among the most abundant in hop EO, exhibiting contact toxicity in the adult *Sitophilus granarius* (LD50 of 41.87 µg/adult for α-humulene and LD50 of 138.51 µg/adult for caryophyllene) [67]. They have also been shown to have a synergistic larvicidal effect on *Aedes aegypti* and *An. gambiae* [68, 69]. On the other hand, the solution composed of linalool and citral (Mix 2) is also irritant and repellent. These two compounds have been identified in several EOs with proven mosquito repellent activity [56, 57, 59, 61, 62]. Gas chromatography coupled with electroantennographic detection measurements revealed that the monoterpenoid citral induced strong antennal responses in *Aedes aegypti* receptors [60]. Linalool, a hydroxyl monoterpene widely found in terrestrial plants, is highly prized for its repellent activity, which is highly dependent on the linalool concentration [58]. Following these results, we aimed to produce active solutions inspired by natural essential oils using commercially available compounds: three solutions were formulated and tested. The solutions composed of caryophyllene, α-humulene, linalool and citral (Mix 3) act in synergy at the concentrations tested for toxic and irritant effects. However, this solution seems to be less repellent than the other two tested solutions. The effects on mosquitoes depend on the concentration of each molecule, which could be explained by the antagonistic action of the compounds used. Combining different chemical families that may have different modes of action could increase effectiveness and/or overcome the problem of resistance [69].

To pursue this study, it would be interesting to test the properties of the Mix 3 formulation on mosquitoes, as well as a mixture of the three essential oils. Such a solution could be reproduced where the tree species are not present by mixing commercial EO. If tree crops could be produced locally, the extraction of essential oils would be less expensive and easier in African countries, which suffer from malaria and other vector-borne diseases.

Conclusion

Finally, this study demonstrated the chemodiversity of living organisms through the composition of the volatile molecules of four Ugandan plant species belonging to four different botanical families. We identified the main volatile compounds in the leaves of *Lepisanthes senegalensis, Vepris nobilis, Turraeanthus africanus* and *Celtis africana*. We also demonstrated the anti-mosquito properties of some of these compounds at specific concentrations present in the leaves of trees used by chimpanzees to build their nests. We have also illustrated the putative synergistic effects that certain molecules can have. This study needs to be continued if we want to be able to provide additional defense to Ugandan populations and, more generally, to African populations in the face of the risks posed by malaria. Finally, at a more global level, this work highlights the importance and richness of tropical

forests and the urgency of protecting both flora and fauna, including our closest relatives, chimpanzees. This survey clearly illustrates the 'One Health' concept by unifying human, animal and environmental health.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-026-05805-3.

Supplementary Material 1. Table S1: Volatile compounds and chemical families with their percentages present in the leaves of *V. nobilis*, *L. senegalensis*, *T. africanus*, *Celtis africana* and in the "synthetic" essential oils. Th. RI: Theorical retention index; Exp. RI: Experimental retention index; in bold: most abundant compounds.

Supplementary Material 2. Table S2: Temperatureand hygrometryconditions during toxicity, irritability and repellent assays

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Author contribution
Conceptualization: SK; CL; Data curation SK, EP, CL; Formal analysis EP, KC; Funding acquisition SK; Methodology SK, XF, DM, FC, MR, SD, KC; Project administration SK, HR; Writing—original draft EPJ, SK, KC; Writing—review and editing: EPJ, SK, KC, CL, HR, XF, DM, FC, MR, SD, KC.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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