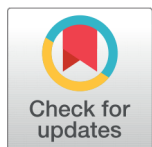


ORIGINAL ARTICLE



OPEN ACCESS

Received: 13/11/2025

Accepted: 20/11/2025

Published: 23/11/2025

Citation: Jerome SZ, Lancine T, Benson BB, Janat Akhanovna MB, Yves-Alain B (2025) GC(FID) and GC-MS Composition and Mosquito-Repellent Activity of the Leaf Essential Oil of *Citrus limon* L. (Rutaceae) from Côte d'Ivoire. Indian Journal of Science and Technology 18(42): 3383-3395. <https://doi.org/10.17485/IJST/v18i42.1696>

* **Corresponding authors.**

sahijeromez@gmail.com

lancine.traore@univ-man.edu.ci

Funding: None

Competing Interests: None

Copyright: © 2025 Jerome et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Published By Indian Society for Education and Environment (iSee)

ISSN

Print: 0974-6846

Electronic: 0974-5645

GC(FID) and GC-MS Composition and Mosquito-Repellent Activity of the Leaf Essential Oil of *Citrus limon* L. (Rutaceae) from Côte d'Ivoire

Sahi Zlankeuhon Jerome^{1*}, Traore Lancine^{1,2*}, Boua Boua Benson¹, Mamyrbekova-Bekro Janat Akhanovna¹, Bekro Yves-Alain¹

¹ Laboratoire de Chimie Bio-Organique et de Substances Naturelles, Université NANGUI ABROGOUA, 02 BP 801 Abidjan 02, Côte d'Ivoire

² UFR Sciences et Technologies, Université de Man, BP 20 Man, Côte d'Ivoire

Abstract

Objectives: This study aimed to characterize the chemical composition of the essential oil (EO) from the leaves of *Citrus limon* L. and to evaluate its repellent activity against *Anopheles gambiae*. **Methods:** The EO was obtained by hydrodistillation with a yield of 0.70% and analyzed by gas chromatography with flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Repellency tests, conducted according to the WHO standard protocol, involved the application of ointments at 5% and 10% on guinea pigs exposed to adult mosquitoes. **Findings:** The EO was obtained with a yield of 0.70%. A total of 22 constituents were identified, representing 93.45% of the oil, dominated by limonene (45.36%), monoterpenes (87.23%), and a smaller fraction of sesquiterpenes (3.67%). At 10%, it offers a Complete Protection Time (CPT = 100%) of 4 hours compared to 3 hours 30 minutes for the natural reference substance (*C. citratus*) against adult female *Anopheles* mosquitoes (14 days). **Novelty:** Considering its extractive value and long-lasting repellent properties (100% for 4 hours and 98.12% for 7 hours), *Citrus limon* L. should be recommended for the development of natural, biodegradable, long-lasting alternative repellents for skin application.

Keywords: *Citrus limon* L.; Essential oil; GC-MS; Limonene; Repellent activity; *Anopheles gambiae*

1 Introduction

Mosquitoes, major vectors of numerous parasitic and viral diseases such as malaria, dengue, and yellow fever, represent a serious public health problem in tropical regions. Côte d'Ivoire is among the 10 countries with the highest number of malaria cases and deaths. In 2023, the country reported 2.8% of malaria cases and deaths⁽¹⁾. Children under five are most vulnerable to malaria, according to preliminary results of the 2021 Demographic and Health Survey (DHS), an increase from 18% in 2011 to 26% in 2021 of malarial infection among children was observed⁽²⁾. In Côte d'Ivoire, mosquito

control programs rely heavily on the regular use of chemical insecticides, which lead to the spread of vector resistance, as well as other problems such as environmental pollution, biomagnification, and damage to public and animal health (Sengottayan, 2020)⁽³⁾. The risk of diseases transmitted by *Anopheles* mosquitoes, the main vectors of malaria, as well as their consequences on morbidity and mortality, underscore the need for effective alternative arsenals. It is therefore urgent to develop new pesticides, particularly repellents made from new natural substances with biodegradable, non-toxic repellent properties at prescribed doses. That's why we've been looking into developing long-lasting, effective plant-based repellents.

In this context, essential oils (EOs), complex mixtures of volatile compounds mostly belonging to the terpene family, represent promising alternatives. These secondary metabolites, derived from the isoprenoid pathway, exhibit a wide chemical diversity and are well known for their bioactivity (Yamina, 2025)⁽⁴⁾. Their analysis is mainly based on gas chromatography (GC), combined with retention indices and mass spectrometry (Adams, 2007)⁽⁵⁾, which are reference tools for the identification of volatile constituents. Several studies have demonstrated the repellent efficacy of certain EOs rich in monoterpenes. For example, *Cymbopogon citratus*, rich in terpenic aldehydes (geranial, neral), is widely used as a natural repellent (Sonja *et al.*, 2021)⁽⁶⁾. The present study therefore aims to determine the chemical composition of the leaf essential oil of *C. limon* L. collected in Côte d'Ivoire and to evaluate its mosquito-repellent activity against *Anopheles gambiae*, in comparison with *C. citratus* used as a natural reference.

2 Methodology

2.1 Plant and Biological Material

The plant material consisted of leaves of *Citrus limon* L., collected in the Adzopé department (06°06'25" N; 03°51'43" W), and leaves of *Cymbopogon citratus* (reference plant), collected in Bingerville (05°21'20.9" N; 03°53'07.3" W), Côte d'Ivoire. Botanical identification of the specimens was performed at the Centre National de Floristique, Université Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire).

The biological material consisted of 14-day-old adult females of *Anopheles gambiae* sensu lato, Kisumu strain (insecticide-susceptible), reared at the Medical Entomology Laboratory of the Swiss Centre for Scientific Research in Côte d'Ivoire.

2.2 Essential Oil Extraction

Essential oils were obtained by continuous hydrodistillation for three hours using a Clevenger-type apparatus connected to a 10 L flask. The distillates were collected and stored in amber vials, tightly sealed, wrapped in aluminum foil to protect from light, and kept at 4 °C until further use.

2.3 GC (RI) and GC-MS Analysis of Essential Oils

Gas chromatographic analyses were performed using a Perkin-Elmer Clarus 500 system equipped with a split injector and two capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm): one non-polar (BP-1, polymethylsiloxane) and one polar (BP-20, polyethylene glycol), coupled to two flame ionization detectors (FID). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min, followed by an isothermal hold at 220 °C for 20 min. The injector and detector temperatures were set at 250 °C. Samples were prepared in chloroform (50 μL EO/500 μL CHCl₃) and injected in split mode (1:60), with hydrogen as carrier gas (1 mL/min). Relative composition was calculated from the peak areas.

GC-MS analyses were carried out using a Perkin Elmer AutoSystem XL gas chromatograph equipped with an automatic injector and a non-polar Rtx-1 column (60 m × 0.22 mm i.d., film thickness 0.25 μm), coupled to a TurboMass mass detector. Helium was used as the carrier gas (0.8 mL/min; inlet pressure 25 psi). Injection conditions were: temperature 250 °C, split mode (1:50), injection volume 0.2 μL. The oven program ranged from 60 °C to 230 °C at 2 °C/min, with a final isothermal hold of 45 min at 230 °C. The detector temperature was set at 280 °C. Ionization was achieved by electron impact (70 eV), and mass spectra were acquired in the range of 35–350 m/z.

Constituents were identified by comparison of calculated retention indices (RI), obtained using a homologous series of n-alkanes (C7–C28) and linear interpolation, with literature data via the *Target Compounds* software (Perkin-Elmer), as well as by comparing mass spectra with commercial and in-house libraries Adams, 2007)⁽⁵⁾.

2.4 Biological Assay

Essential oils were formulated into repellent ointments at two concentrations: 5% and 10%. Ointments based on *Citrus limon* EO were coded P5 and P10, whereas those prepared with *Cymbopogon citratus* EO (reference plant) were coded PC5 and PC10.

2.5 Rearing and Source of *Anopheles gambiae* s.l.

Mosquito rearing was conducted in accordance with WHO (2014) recommendations described in the malaria entomology and vector control training manual⁽⁷⁾. To avoid contamination by wild populations potentially exposed to synthetic repellents or insecticides, the laboratory strain *Anopheles gambiae* s.l. Kisumu (insecticide-susceptible) was used. Larvae were fed with finely ground cat food and reared under controlled conditions: 27 ± 3 °C, $80 \pm 10\%$ relative humidity, and a 12:12 h (light:dark) photoperiod. Adults were kept in entomological cages and continuously supplied with a 10% sucrose solution (Figure 1A). To obtain unfed females for bioassays, the sucrose solution was removed from cages 24 h before the experiments (Figure 1B).

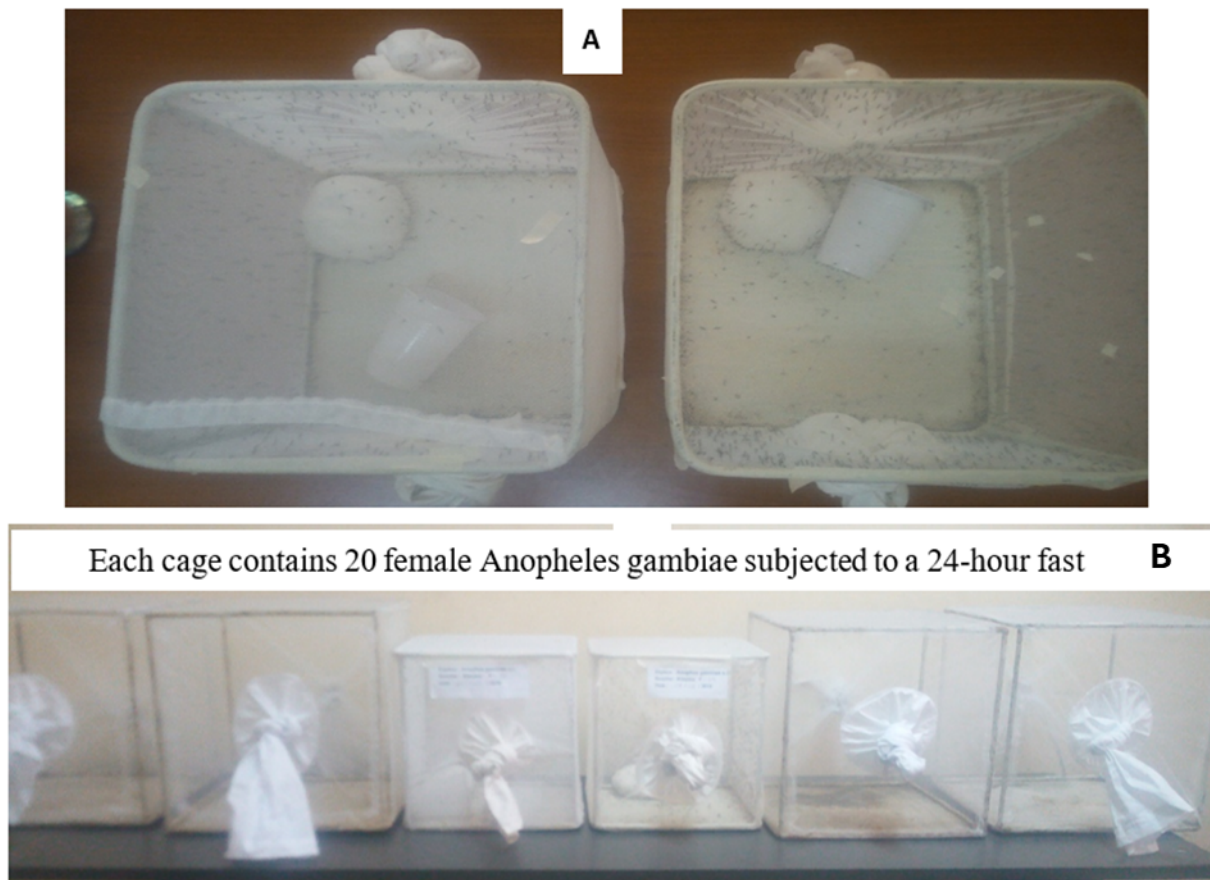


Fig 1. Holding cage used for maintaining adult *Anopheles gambiae* adults

2.6 Repellency Tests

Repellency assays were carried out in accordance with WHO (1996) guidelines⁽⁸⁾, with specific adaptations inspired by the Kaplan-Meier method (2009). For each experiment, twenty unfed adult female *Anopheles gambiae* were introduced into separate entomological cages. Tests were performed on laboratory-reared male guinea pigs (400–500 g). A dorsal surface area of 5×8 cm² was shaved and cleaned with ethanol to standardize the application zone. Ointments containing essential oils of *Citrus limon* (P5 : 5%, P10 : 10%) and *Cymbopogon citratus* (PC5: 5%, PC10: 10%) were applied to different animals, while the control received plain petroleum jelly.

Each treated animal was positioned on top of the cage, with the exposed area directly in contact with mosquitoes for a duration of seven hours. A test was considered valid when at least five mosquitoes landed on the control animal. Bites were assessed by abdominal examination of mosquitoes, allowing identification of blood-fed females. Observations were recorded every minute during the first 15 minutes, then every 15 minutes for up to 7 h. Each experiment was repeated twice using new mosquitoes and new guinea pigs.

The effectiveness of the formulations was expressed using three parameters: (i) the tested dose, (ii) the bite reduction rate (protection P), and (iii) the complete protection time (CPT). These variables were calculated according to standardized equations and definitions:

- Protection (bite reduction rate) provided by the ointment (P) at each dose was calculated as follows:

$$P = 1 - (T / C) = (C - T) / C$$
P: protection rate (%)
T: number of mosquitoes on the treated animal
C: number of mosquitoes on the control animal
- The complete protection time (CPT) was calculated as the number of minutes elapsed between the application of the repellent and the first mosquito landing and/or detection.

For the calculation of these variables, a data processing software was used, namely the Kaplan online survival function calculator. The entomological efficacy parameters are defined by the WHO and state that:

- If a product shows a protection rate (P) < 90%, it is considered ineffective.
- If a product shows a protection rate (P) ≥ 90%, it is considered effective.
- When several products achieve P ≥ 90%, the most effective one is the formulation with the longest complete protection time (CPT).

3 Results and Discussion

3.1 Extraction Yield

The essential oil obtained from the leaves of *Citrus limon* was pale yellow in color, aromatic and liquid at room temperature. The extraction yield was 0.7%. According to AFNOR standards, *C. limon* leaves are considered rich in essential oil⁽⁹⁾. This yield is lower than those reported in Algeria by Himed et al (2020)⁽¹⁰⁾ and Mehda and Makhoulfi (2021)⁽¹¹⁾ using the same method (hydrodistillation) but on other organs. Indeed, they obtained respectively a yield of 2.20±0.773% on the zests and a yield of 3% on the flowers of *C. limon*. These differences clearly show the variation in essential oil content from one organ to another for the same plant. This variability in yield could be justified according to several factors, namely their chemical composition and their physiological role⁽¹²⁾.

3.2 Chemical Composition of Essential Oils

3.2.1. Chemical composition of *Citrus limon* L.

Figure 2 shows the gas chromatographic profile of the essential oil (EO) obtained from the leaves of *Citrus limon*. Each numbered peak corresponds to an identified constituent.

Like the other peaks, peak 7, corresponding to the major compound, was identified based on its mass spectrum (Figure 3). This identification was further confirmed by the close agreement between the experimental retention indices (RIa/RIp: 1021/1199) and those reported in the reference database (RIa/RIp: 1020/1199). Ionization was performed by electron impact (EI, 70 eV).

The positive ion mass spectrum of the major compound exhibited a molecular ion peak at m/z 136 [M⁺], consistent with a molecular weight of 136 and corresponding to the molecular formula of limonene (C₁₀H₁₆). The main fragmentation patterns observed in this spectrum are illustrated in Figure 4.

Based on these analyses, 22 compounds were identified, accounting for 93.45% of the essential oil constituents (Table 1). Limonene (45.36%) was the predominant compound. Other major constituents included β-pinene (14.56%), citronellal (10.97%), and sabinene (5.43%). The oil was characterized by a high proportion of monoterpenes (87.23%), with a predominance of hydrocarbon monoterpenes (71.07%). In contrast, hydrocarbon sesquiterpenes were present in only small amounts (3.67%).

Our results regarding the chemical profile of this oil are consistent with those reported by Himed (2020)⁽¹⁰⁾ and colleagues in Algeria. They showed that lemon essential oils are characterized by high concentrations of limonene (61.26%), α-pinene (9.65%), α-citral (4.22%), and γ-terpinene (3.84%). They also showed that lemon oils are generally dominated by monoterpene compounds (≈78.98%), while other constituents such as alcohols, aldehydes, and esters are present only in minor amounts (1.8%). Similar results were also reported by Kacaniova et al (2024) in Slovakia⁽¹³⁾. Their study revealed that limonene (60.7%), β-pinene (12.6%) and γ-terpinene (10.3%) are the major constituents of the oil. However, slight variations were observed

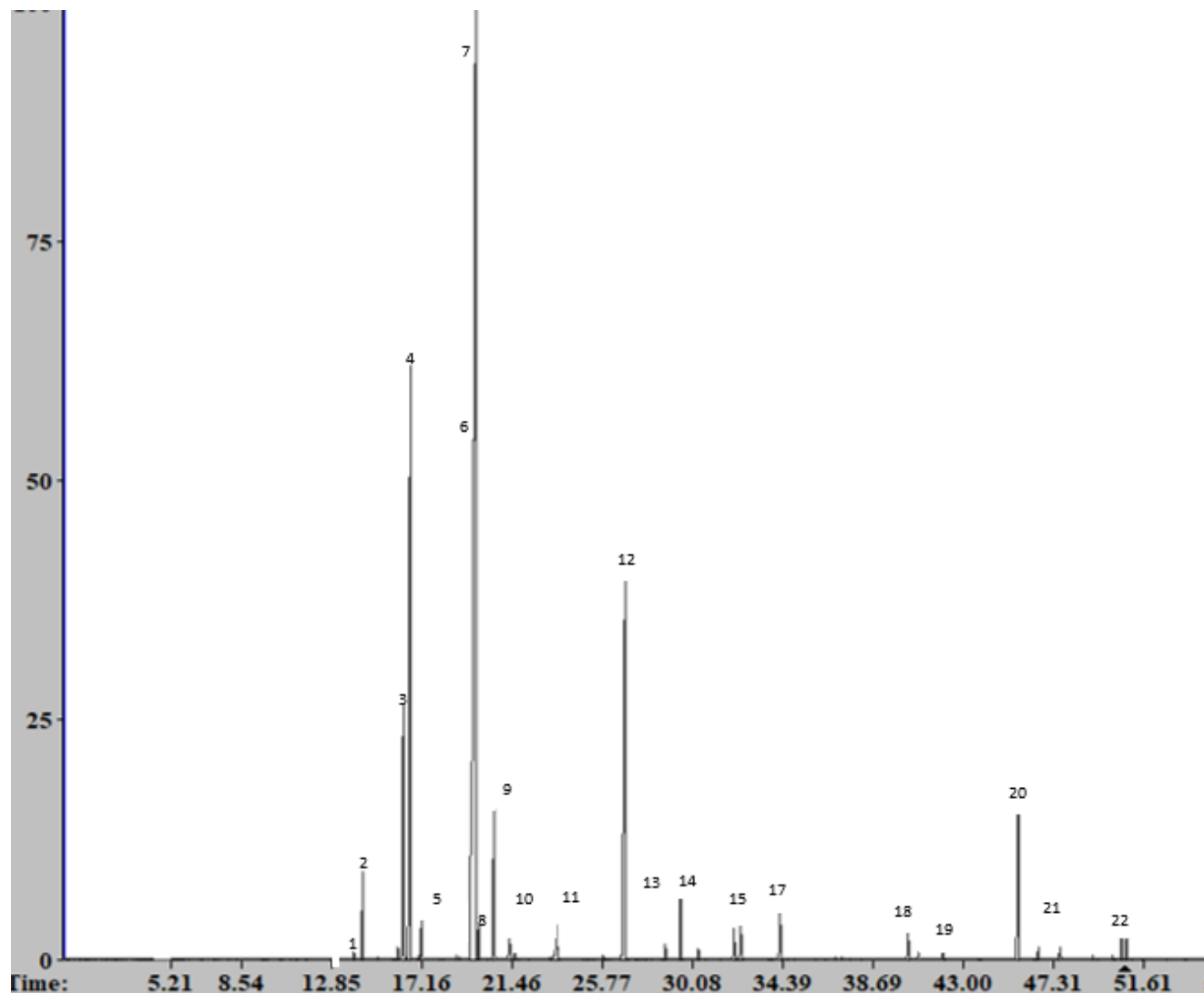


Fig 2. Gas chromatographic profile of *Citrus limon* essential oil obtained on a non-polar column

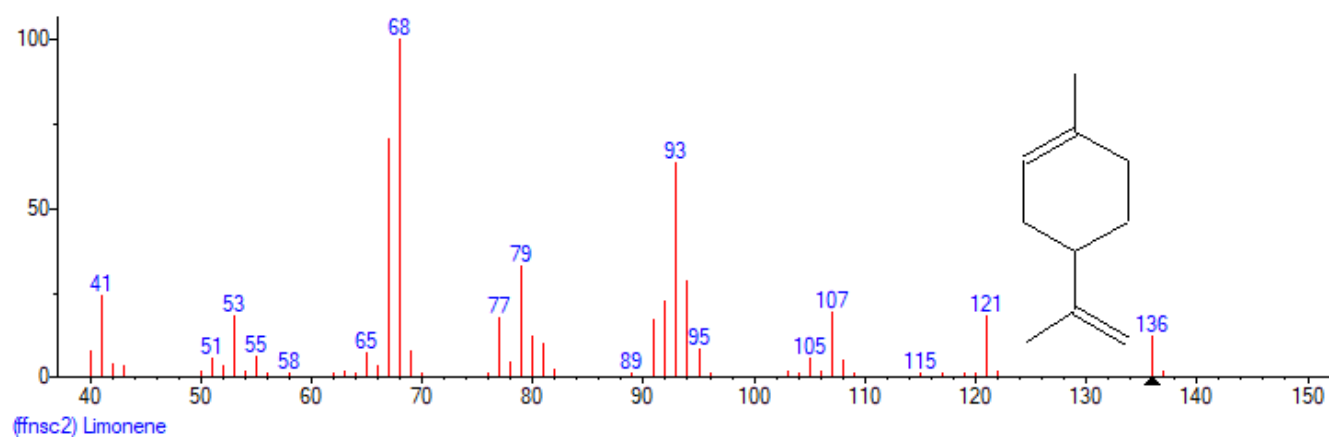


Fig 3. Positive ion mass spectrum of limonene, showing the molecular ion peak at m/z 136 $[M^+]$ and the main fragmentation pattern

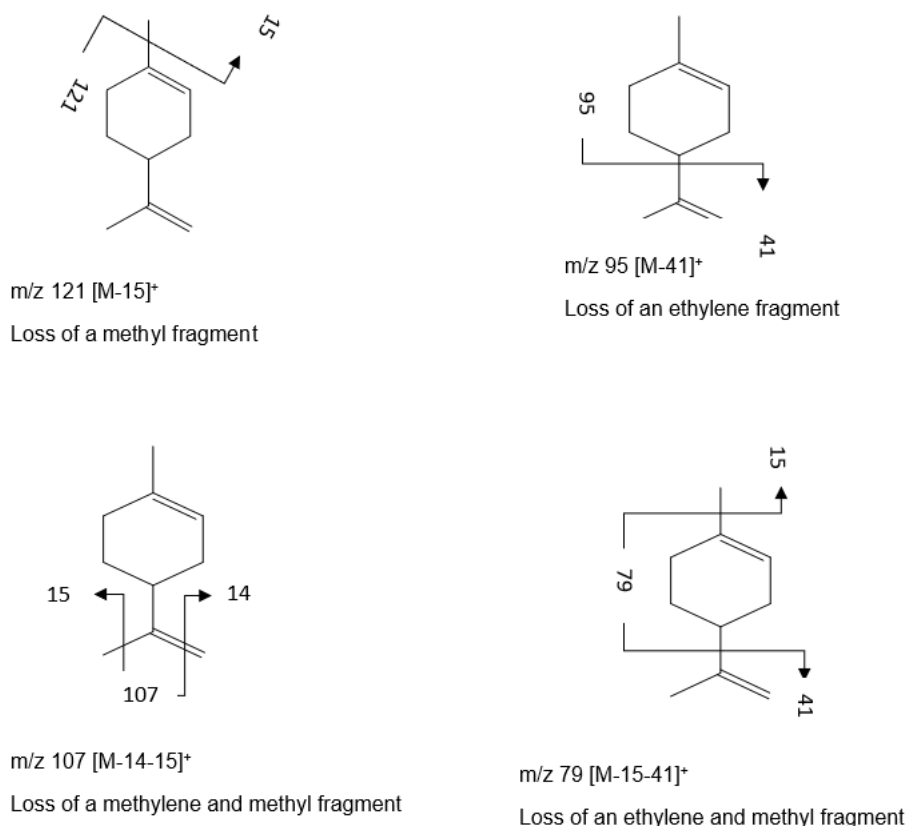


Fig 4. Fragmentation pattern of limonene obtained from electron impact (EI, 70 eV) mass spectrometry

compared to the work of Posadino *et al.* (2024) in Italy⁽¹⁴⁾. The latter reported very high levels of the major compound limonene (82%). However, slight qualitative and quantitative variations are observed in the chemical composition of lemon oil from one study to another. This variability can result from several factors. These include genetic factors, the age of the plant, etc.⁽¹²⁾.

3.2.2. Chemical Composition of *Cymbopogon citratus* Essential Oil

Figure 5 shows the gas chromatographic profile of the essential oil of *Cymbopogon citratus*. Each numbered peak corresponds to an identified compound listed in Table 2.

Peak 11, which exhibited the highest intensity, corresponded to the major compound (geranial). Its identification was achieved based on its mass spectrum (Figure 6) and confirmed by the close agreement between the experimental retention indices (RIa/RIp: 1021/1199) and those reported in the reference database (RIa/RIp: 1243/1730). Ionization was carried out by electron impact (EI, 70 eV). This same procedure was systematically applied to the identification of all detected compounds.

The positive ion mass spectrum of the major compound displayed a molecular ion peak at m/z 152 [M⁺], consistent with a molecular weight of 152 and corresponding to the molecular formula of geranial (C₁₀H₁₆O). The main fragmentation patterns observed in this spectrum are illustrated in Figure 7.

A total of thirteen (13) compounds, representing 92.05% of the essential oil constituents, were identified (Table 2). *Cymbopogon citratus* essential oil was predominantly monoterpene (91.37%), with a strong predominance of oxygenated compounds (76.91%). Four major constituents were identified: geranial (35.92%), neral (28.24%), myrcene (13.90%), and geraniol (6.14%). Our results are consistent with several studies conducted in other countries. Among others, we can recall the results of the work of Lulekal and collaborator in Ethiopia (2019)⁽¹⁵⁾. They reported that the essential oil of *Cymbopogon citratus* contains mainly citral (71.297%), myrcene (19.034%), 4,5-epoxycarene (2.780%), linalool (1.713%), (S)-cis-verbenol (1.110%), and undecan-2-one (1.001%). But also, those carried out by Nebie *et al.* in Burkina Faso (2023)⁽¹⁶⁾. They identified 15 compounds representing 96.16% of the total chemical composition of the oil. It mainly contains geranial (41.49%), neral (32.83%) or 74.32% of citral and β -myrcene (13.66%). These studies have demonstrated that the essential oil of *Cymbopogon*

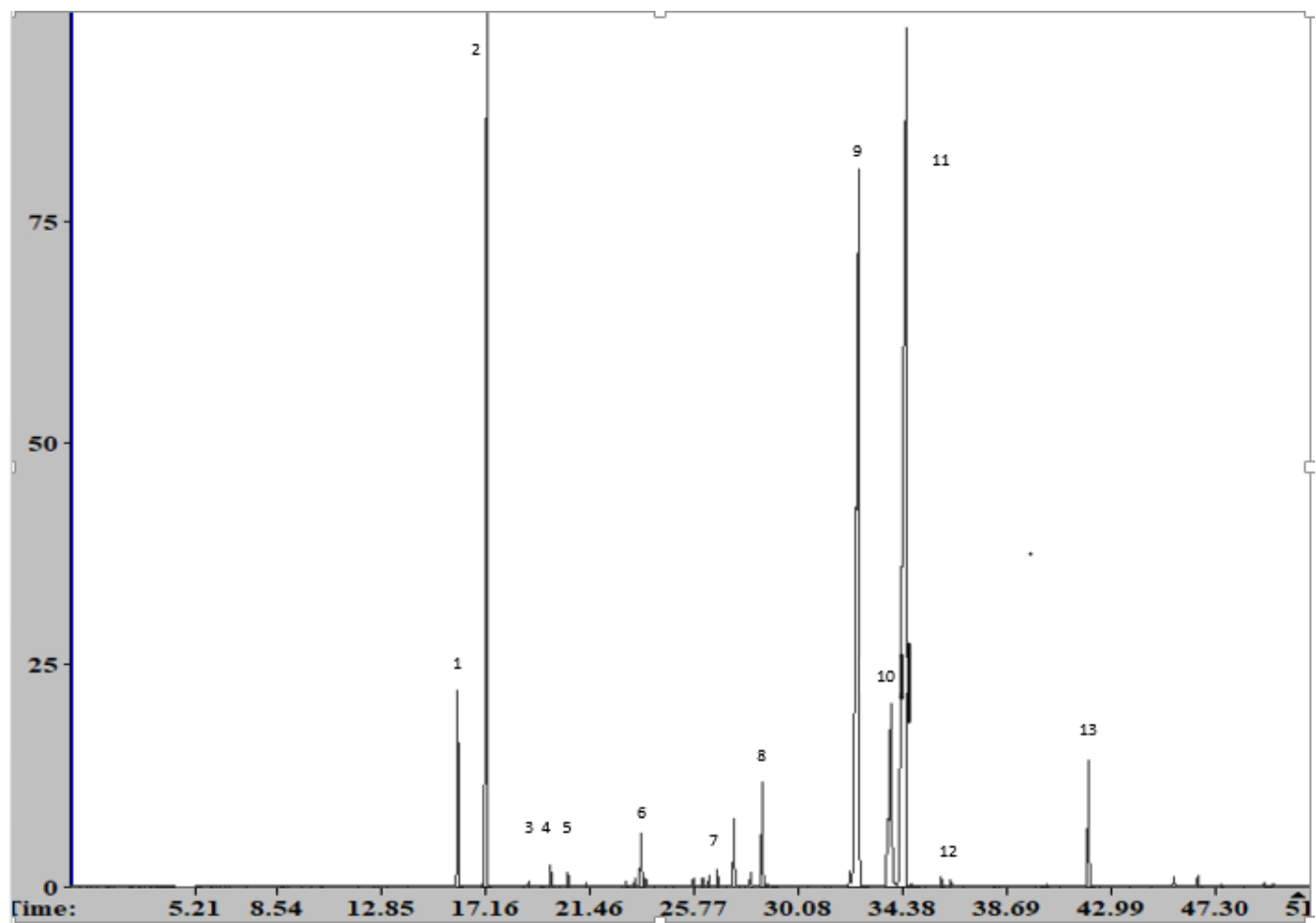


Fig 5. Gas chromatographic profile of Cymbopogon citratus essential oil obtained on a non-polar column

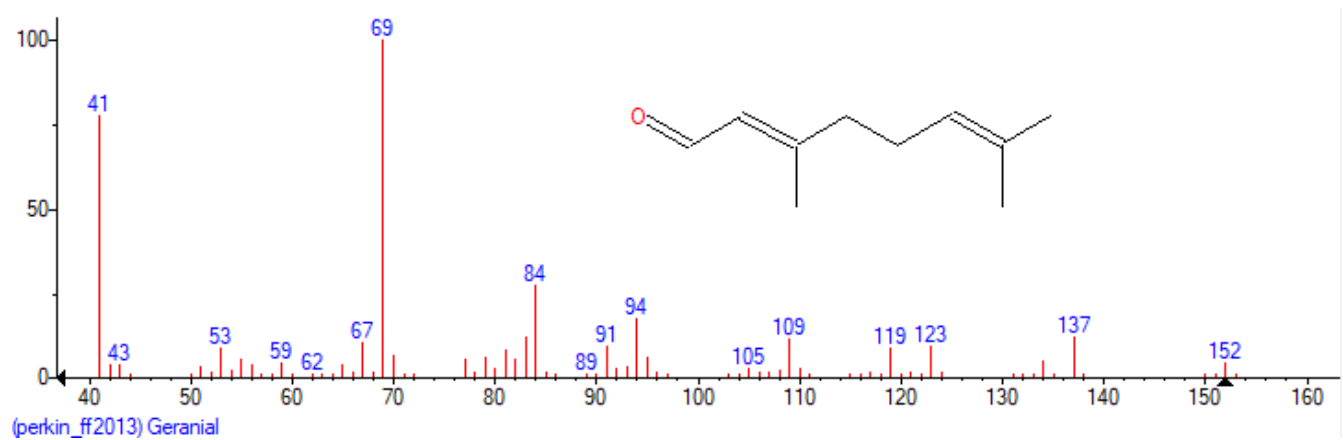


Fig 6. Positive ion mass spectrum of geranial, showing the molecular ion peak at m/z 152 [M^+] and the main fragmentation pattern

Table 1. Chemical composition of the essential oil from *Citrus limon* leaves as determined by GC (RI) and GC–MS

Compounds	Retention indices		Compound content (%)	Identification
	Irap Exp/ Bd	Irp Exp/ Bd		
1 α -thujene	922/ 922	1015/ 1023	0,13	Ir, SM
2 α -pinene	930/ 931	1013/ 1022	1,42	Ir, SM
3 sabinene	964/ 964	1119/ 1120	5,43	Ir, SM
4 β -pinene	970/ 970	1118/ 1110	14,56	Ir, SM
5 myrcene	980/ 979	1156/ 1159	1,33	Ir, SM
6 <i>p</i> -cymene	1011/ 1011	1267/ 1268	0,62	Ir, SM
7 limonene	1021/ 1020	1199/ 1199	45,36	Ir, SM
8 (<i>Z</i>)- β -ocimene	1024/ 1024	1228/ 1230	0,45	Ir, SM
9 (<i>E</i>)- β -ocimene	1035/ 1034	1246/ 1247	1,96	Ir, SM
10 γ -terpinene	1047/ 1047	1241/ 1243	0,28	Ir, SM
11 linalol	1082/ 1081	1542/ 1544	0,96	Ir, SM
12 citronellal	1130/ 1131	1476/1479	10,97	Ir, SM
13 terpinen-4-ol	1159/ 1159	1596/ 1597	0,37	Ir, SM
14 α -terpineol	1170/ 1171	1691/ 1691	1,46	Ir, SM
15 citronellol	1207/ 1208	1760/ 1761	0,92	Ir, SM
16 neral	1213/ 1214	1677/ 1679	0,92	Ir, SM
17 geranial	1240/ 1245	1728/ 1721	1,22	Ir, SM
18 acetate de citronellyle	1331/ 1331	1656/ 1657	0,59	Ir, SM
19 acetate <i>de</i> neryle	1339/ 1340	1721/ 1725	0,26	Ir, SM
20 (<i>E</i>)- β -caryophyllene	1413/ 1424	1588/ 1591	3,08	Ir, SM
21 trans α -bergamotene	1428/ 1432	1576/ 1580	0,32	Ir, SM
22 α -famesene	1496/ 1479	1741/ 1721	0,59	Ir, SM
Hydrocarbons monoterpenes			71,07	
Oxygenated monoterpenes			16,16	
Total monoterpenes			87,23	
Hydrocarbons sesquiterpenes			3,67	
Oxygenated sesquiterpenes			00	
Total sesquiterpenes			3,67	
Total Hydrocarbons			74,74	
Total oxygenates			16,16	
TOTAL			90,90	

IrapExp/ Bd: retention index of experimental Kovats on non-polar column calculated using C7-C28 n-alkanes / literature databases (Kondjoyanet Berdague, 1996). **IrpExp/ Bd:** retention index of experimental Kovats on polar column calculated using C7-C28 n-alkanes / literature databases (Kondjoyanet Berdague, 1996).

citratius is mainly composed of monoterpenes, the majority of which is made up of oxygenated compounds, and that these same constituents dominate the chemical profile. These molecules are recognized for their repellent activity, which justifies the use of *C. citratius* essential oil as a natural reference substance for anti-mosquito formulations⁽¹⁷⁾.

3.3 Biological Activity

3.3.1. Repellent activity of *Citrus limon* L. essential oil

The results of the mosquito repellent bioassays performed with ointments containing *Citrus limon* essential oil at 5% (P5) and 10% (P10) are presented in Table 3.

Table 2. Chemical composition of the essential oil from *Cymbopogon citratus* leaves as determined by GC (RI) and GC–MS.

Compounds	Retention indices		Compound content (%)	Identification
	Irap Exp/ Bd	Irp Exp/ Bd		
1 6-methylhept-5-en-2-one	960/ 963	1336/ 1337	3,02	Ir, SM
2 myrcene	980/ 979	1157/ 1159	13,90	Ir, SM
3 <i>p</i> -cymene	1011/ 1011	1267/ 1268	0,16	Ir, SM
4 (<i>Z</i>)- β -ocimene	1024/ 1024	1228/ 1230	0,24	Ir, SM
5 (<i>E</i>)- β -ocimene	1035/ 1034	1246/ 1247	0,16	Ir, SM
6 linalol	1082/ 1081	1543/ 1544	1,00	Ir, SM
7 citronellal	1129/ 1131	1479/1479	0,31	Ir, SM
8 terpinen-4-ol	1156/ 1159	1600/ 1597	1,14	Ir, SM
9 neral	1215/ 1214	1679/ 1679	28,24	Ir, SM
10 geraniol	1233/ 1232	1842/ 1837	6,14	Ir, SM
11 geranial	1243/ 1245	1730/ 1721	35,9	Ir, SM
12 thymol	1266/ 1266	2179/ 2189	0,16	Ir, SM
13 acetate de geranyle	1358/ 1358	1752/ 1748	2,12	Ir, SM
Hydrocarbons monoterpenes			14,46	
Oxygenated monoterpenes			78,05	
Total monoterpenes			92,51	
Hydrocarbons sesquiterpenes			00	
Oxygenated Sesquiterpenes			00	
Total Sesquiterpenes			00	
Total Hydrocarbons			14,46	
Total Oxygenates			78,05	
TOTAL		92,51		

IrapExp/ Bd: retention index of experimental Kovats on non-polar column calculated using C7-C28 n-alkanes / literature databases (Kondjoyanet Berdague, 1996). **IrpExp/ Bd:** retention index of experimental Kovats on polar column calculated using C7-C28 n-alkanes / literature databases (Kondjoyanet Berdague, 1996).

Table 3 illustrate the decreasing protection of P5 over time. Protection initially reached 55% within the first 15 minutes but dropped below 50% after 1 h, with an average protection of 31.25% and a complete protection time (CPT) of zero.

In contrast, P10 provided 100% protection for 4 h, corresponding to a CPT of 4 h. Protection then decreased slightly to 95% at 4.5 h and stabilized at 85% until the end of the assay, resulting in an average protection of 98.12%.

According to WHO criteria, any substance with an average protection $\geq 90\%$ ($P \geq 90\%$) is considered effective. On this basis, the 10% ointment (P10) demonstrated strong repellent activity against adult female *Anopheles gambiae*, with an average protection of 98.12%, compared with only 31.25% for the 5% ointment (P5) (Table 4).

3.3.2. Repellent activity of *Cymbopogon citratus* essential oil (reference substance)

The results of the mosquito repellent bioassays conducted with ointments containing *Cymbopogon citratus* essential oil at 5% (PC5) and 10% (PC10) are presented in Table 5.

Table 5 shows the rapid decline in protection provided by PC5 over time. Indeed, PC5 afforded 50% protection during the first 30 minutes, which decreased to 45% between 30 min and 1 h, then to 20% between 1 h and 5 h 30 min, before stabilizing at 10% between 6 h and 7 h. PC5 exhibited an average protection rate of 26.87%, with no complete protection time (CPT = 0) and an effective protection time limited to 30 min.

In contrast, PC10 provided full (100%) protection for 210 min (3 h 30 min), corresponding to its CPT. Protection then decreased slightly to 95% and was maintained at this level until the end of the experiment. The average protection rate of PC10 was 97.5% (Table 6).

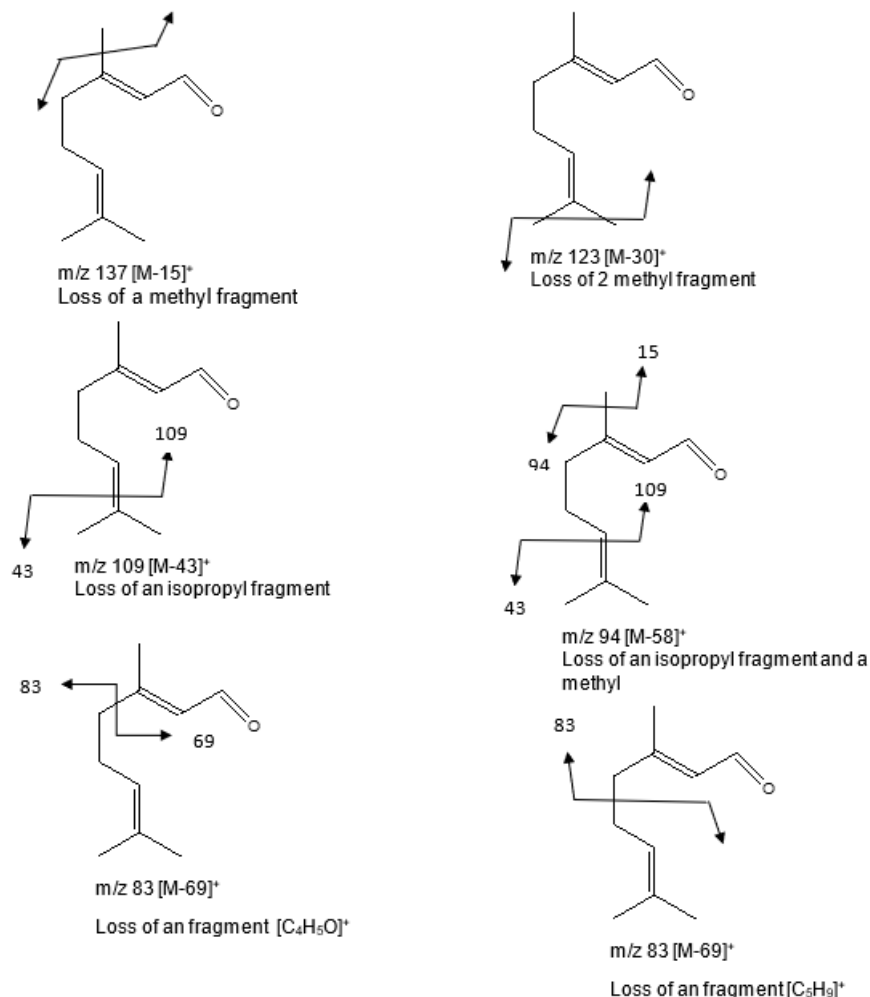


Fig 7. Fragmentation pattern of geranial obtained from electron impact (EI, 70 eV) mass spectrometry

Overall, PC10 not only achieved a significantly higher mean protection rate (97.5%) compared with PC5 (26.87%) but also provided a longer CPT of 210 min (3 h 30 min) versus 0 min for PC5 against adult *Anopheles gambiae* (Table 6). On this basis, PC10 can be considered more effective than PC5, as it consistently maintained a higher level of repellency.

3.4 Comparative Study of the Persistence of Repellent Effects of P10 and PC10

Repellency tests showed that P10 exhibited a remarkable repellent effect comparable to that of the reference substance PC10 against adult *Anopheles gambiae*. However, P10 was more persistent, providing 100% protection (CPT) for 4 h, while PC10 maintained full protection for only 3 h 30 min (Figure 8). According to WHO standards, the persistence of repellent efficacy is directly related to CPT, with higher values indicating greater effectiveness.

The strong repellent activity of P10 is attributable to the chemical composition of its essential oil, particularly the presence of monoterpenes such as limonene (45.36%), β -pinene (14.56%), citronellal (10.97%), and the sesquiterpene (E)- β -caryophyllene (3.08%), all of which are recognized for their repellent properties (Lima et al. (2024))⁽¹⁸⁾. These molecules may also act synergistically. This is supported by Nebie et al. (2023)⁽¹⁶⁾, who demonstrated that co-distillation of 80% *Cymbopogon citratus* leaves and 20% *Mentha piperitha* improved the antioxidant efficacy of essential oils.

These aromatic compounds are thought to neutralize host-derived odors such as lactic acid, carbon dioxide, ammonia, carboxylic acids, oct-1-en-3-ol, and other sweat constituents used by female mosquitoes to locate their hosts. Such volatile

Table 3. Repellent activity of *Citrus limon* essential oil ointments at 5% (P5) and 10% (P10) against *Anopheles gambiae*

Observation times	Cumulative number of bites on the control ©	Cumulative number of bites on treated animal (T)		Cumulative number of dead mosquitoes	P as a percentage of P (5%) and P (10%)	
		P ₁ (5%)	P ₁ (10%)		P ₁ (5%)	P ₁ (10%)
15 min	20	9	0	0	55	100
30 min	20	10	0	0	50	100
45 min	20	10	0	0	50	100
1h	20	10	0	0	50	100
1h30	20	12	0	0	40	100
2h	20	14	0	0	30	100
2h30	20	14	0	0	30	100
3h	20	14	0	0	30	100
3h30	20	14	0	0	30	100
4h	20	16	0	0	20	100
4h30	20	16	1	0	20	95
5h	20	16	3	0	20	95
5h30	20	16	3	0	20	95
6h	20	16	3	0	20	95
6h30	20	16	3	0	20	95
7h	20	17	3	0	15	95
P. Average (%)					31,25	98,12

Table 4. Repellency parameters (mean protection rate and complete protection time) of *Citrus limon* essential oil ointments (P5 and P10)

	Dose	TPC (minute)	Average percentage (%)
P _{S5}	5%	0	31,25
P _{S10}	10%	240	98,12

molecules may interfere with olfactory receptors, disrupting host-seeking behavior or even rendering the mosquitoes insensitive to normally attractive odors, as demonstrated by the Vosshall team and reported by Sahi et al⁽¹⁷⁾.

The greater persistence of the repellent effect of P10 (*C. limon* EO) compared with PC10 (*C. citratus* EO) can be explained by differences in chemical classes, particularly the balance between efficacy and volatility. The synergistic action of both volatile monoterpenes and less volatile sesquiterpenes in *C. limon* oil likely accounts for its prolonged activity, whereas *C. citratus* oil contains only oxygenated monoterpenes, which are more volatile and less persistent⁽¹⁷⁾.

4 Conclusion

The essential oil from the stem bark of *Citrus limon* L. was extracted by continuous hydrodistillation for 3 h, yielding 0.12%. The oil was pale yellow, aromatic, and liquid at room temperature. A total of 22 compounds were identified, representing 93.45% of the oil, with limonene (40.36%) as the major constituent. The chemical profile was dominated by monoterpenes (87.23%), particularly hydrocarbon monoterpenes (71.07%), while hydrocarbon sesquiterpenes were present at low levels (3.67%).

At 10% concentration, *C. limon* essential oil exhibited significant repellent activity, providing a mean protection rate of 95.29% against adult female *Anopheles gambiae*. Moreover, it demonstrated greater persistence than *Cymbopogon citratus*, a well-known natural repellent, with a complete protection time (CPT) of 4 h. This strong repellency can be attributed to the presence of active compounds such as limonene (45.36%), β -pinene (14.56%), citronellal (10.97%), and (E)- β -caryophyllene (3.08%).

Table 5. Repellent activity of *Cymbopogon citratus* essential oil ointments at 5% (PC5) and 10% (PC10) against *Anopheles gambiae*

Observation times	Cumulative number of bites on the control ©	Cumulative of bites on animal (T)		Cumulative number of dead mosquitoes	P as % of PC	
		PC(5%)	PC(10%)		PC(5%)	PC(10%)
15 min	20	10	0	0	50	100
30 min	20	10	0	0	50	100
45 min	20	11	0	0	45	100
1h	20	12	0	0	40	100
1h30min	20	12	0	0	40	100
2h	20	14	0	0	30	100
2h30min	20	15	0	0	25	100
3h	20	16	0	0	20	100
3h30min	20	16	0	0	20	100
4h	20	16	1	0	20	95
4h30min	20	16	1	0	20	95
5h	20	16	1	0	20	95
5h30min	20	16	1	0	20	95
6h	20	18	1	0	10	95
6h30min	20	18	1	0	10	95
7h	20	18	1	0	10	95
P. Average (%)					26,87	97,5

Table 6. Repellency parameters (mean protection rate and complete protection time) of *Cymbopogon citratus* essential oil ointments (PC5 and PC10)

	Dose	TPC (minute)	Average percentage (%)
PC (5%)	5%	0	26,87
PC (10%)	10%	210	97,5

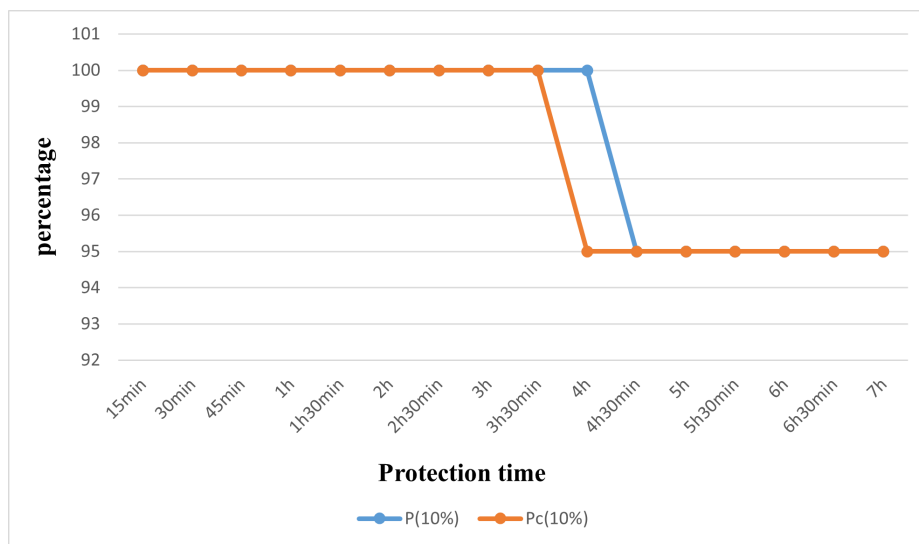


Fig 8. Comparative persistence of repellent activity between *Citrus limon* (P10) and *Cymbopogon citratus* (PC10) essential oil ointments against adult female *Anopheles gambiae*

This work provides the first scientific contribution on the chemical composition and repellent activity of *C. limon* leaf essential oil against adult *A. gambiae*. Given its satisfactory extraction yield and proven repellent efficacy, *C. limon* essential oil should be strongly considered for the development of natural skin-applied mosquito repellents.

5 Acknowledgements

The APC, partially deferred by Indian Society for Education and Environment, is gratefully acknowledged.

References

- 1) Organization WH. World Malaria Report. 2024;p. 12–14. Available from: <https://www.google.com/url?sa=t&source=web&rc=t=j&opi=89978449&url=https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024&ved=2ahUKEwjD15CX2LQCAxUDNPsDHWnnEQgQFnoECBgQAQ&usg=AOvVaw0lEkrYSxc31nPED04TNfuP>.
- 2) D'Ivoire USPMIC. Malaria Operational Plan FY. 2023;p. 10–12. Available from: <https://www.google.com/url?sa=t&source=web&rc=t=j&opi=89978449&url=https://reliefweb.int/report/cote-divoire/presidents-malaria-initiative-cote-divoire-malaria-operational-plan-fy2023&ved=2ahUKEwjPrMfi2LQCAxW9UaQEHRkEs4QFnoECBgQAQ&usg=AOvVaw1w86hcKidWgfl48liLpiO9>.
- 3) S NS. A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. *Frontiers in physiology*. 2020;25(10):1591. <https://doi.org/10.3389/fphys.2019.01591>.
- 4) Yamina BN. Essential Oils: Chemical Composition and Diverse Biological Activities : A Comprehensive Review. *Journal Name*. 2025;20(1):1–29. <https://doi.org/10.1177/1934578X241311790>.
- 5) Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th ed. Carol Strea. Allured. 2007.
- 6) G S, K B, A M, SJ J, C M, B V, et al. Repellent activity of cymbopogon citratus essential oil against four major stored product pests: plodia interpunctella, sitophilus oryzae, acanthoscelides obtectus and tribolium castaneum. *Journal Name*. 2021;70(3-4):140–148. <https://doi.org/10.2478/contagri-2021-0020>.
- 7) Organization WH. Malaria Entomology and Vector Control: A Learner's Guide. 2014. Available from: <https://www.google.com/url?sa=t&source=web&rc=t=j&opi=89978449&url=https://www.paho.org/sites/default/files/2012-cha-manuel-entomologie-paludisme.pdf&ved=2ahUKEwjR1Jex2rCQAxWqUaQEHSgGdFkQFnoECBgQAQ&usg=AOvVaw1wLUZUE3HZE2ZhzH6YGovT>.
- 8) Organization WH. Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. World Health Organization. 1996. Available from: <https://www.who.int/publications/i/item/ctd-whopes-ic-96.1>.
- 9) AFNOR. Essential oils, sampling and method of analysis. *Paragraph edition*. 2000;(1):471. Available from: https://www.google.com/url?sa=t&source=web&rc=t=j&opi=89978449&url=https://www.doc-developpement-durable.org/file/Fabrications-Objets-Outils-Produits/Huiles-essentielles/FICHES_PLANTES%26HUILES/Normes_AFNOR_HE.pdf&ved=2ahUKEwiD17Dd2rCQAxVGRKQEHSONI0UQFnoECBYQAQ&usg=AOvVaw3SbvyIMdLtsXm428Moz1H.
- 10) Himed L, Merniz S, Benbraham M, Boudjouada E, Barkat M. Preservation of tomato paste with an antifungal agent (lemon essential oil). *African Journal of Food Agriculture Nutrition and Development*. 2020;20(2):15608–15618. <https://doi.org/10.18697/ajfand.90.18870>.
- 11) Mehda A, Makhloufi N. Application of Citrus limon essential oil for the preservation of certain food products. *Thesis*. 2021.
- 12) Kone Y, Boua BB, Ouattara Z, Mamyrbekova-bekro JA, Bekro YA. Influence of flowering on the chemical composition and anti-sinusitis activity of the essential oil of the leaves of *Diphasia klaineana* Pierre (Rutaceae) from Ivory Coast. *Journal of Applied Biosciences*. 2019;135:13742–13749. <https://doi.org/10.4314/jab.v134i1.10>.
- 13) Kačániová M. <https://doi.org/10.3390/plants13040524>.
- 14) Posadino AM, Maccioccu P, Eid AH, Giordo R, Pintus G, Fenu G. Citrus limon var. pompia Camarda var. nova: A Comprehensive Review of Its Botanical Characteristics, Traditional Uses, Phytochemical Profile, and Potential Health Benefits. *MDPI Journal*. 2024;16(16):2619. <https://doi.org/10.3390/nu16162619>.
- 15) Lulekal E, Tesfaye S, Gebrechristos S. Phytochemical analysis and evaluation of skin irritation, acute and sub-acute toxicity of *Cymbopogon citratus* essential oil in mice and rabbits. *Toxicol Rep*. 2019;6:1289–1294. <https://doi.org/10.1016/j.toxrep.2019.11.002>.
- 16) Nebie B, Dabire CM, Bationo RK, Sosso S, Nebie RCH, pale E, et al. Chemical composition and antioxidant potential of the essential oil obtained by co-distillation of *Mentha piperita* L. and *Cymbopogon citratus* (DC.) Stapf from Burkina Faso. *International Journal Biological and Chemical Sciences*. 2023;17(2):689–700. <https://doi.org/10.4314/ijbcs.v17i2.32>.
- 17) Sahi Z, Boua BB, Ouattara AF, Mamyrbekova-Bekro JA, Bekro Y. Analysis by GC (Ir), GC/MS and mosquito repellent effect of essential oils against *Anopheles gambiae*: Case of stem bark of *Sterculia tragacantha* Lindl (Sterculiaceae) from Côte d'Ivoire. *Journal of Medicinal Plants Research*. 2021;15(7):309–320. <https://doi.org/10.5897/JMPR2021.7132>.
- 18) Lima EAS, Campos DR, Soares EFMS, Chaves DSA, Cid YP, Coumendouros K. Insecticidal and repellent activity of essential oils from *Copaifera reticulata*, *Citrus paradisi*, *Lavandula hybrida* and *Salvia sclarea* against immature and adult stages of *Ctenocephalides felis*. *Research Square*. 2024. <https://doi.org/10.21203/rs.3.rs-4139452/v1>.