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Investigation on chemical composition and insecticidal activity against *Anopheles gambiae* of essential oil obtained by co-distillation of *Cymbopogon citratus* and *Hyptis suaveolens* from Western Burkina Faso

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Abstract

Background Essential oils of *Cymbopogon citratus* and *Hyptis suaveolens* are known for their insecticidal properties, but remain ineffective against mosquitoes resistant to synthetic insecticides. In order to improve insecticidal properties of these plants, this study aimed to investigate the chemical composition and insecticidal activity against *Anopheles gambiae* mosquitoes of essential oil obtained by co-distillation of dry leaves of *C. citratus* and *H. suaveolens*.

Methods Essential oils were extracted by hydrodistillation from dry leaves of *C. citratus* and *H. suaveolens*, separately, then from the mixture of the dry leaves of the two plants in mass ratio 50/50. Each pure essential oil and the mixture obtained either by co-distillation or by combining pure essential oils in volume ratio 50/50 were then analysed by GC/MS. All essential oils and Deltamethrin 0.05% (positive control) were tested on two species of mosquitoes of the genus *Anopheles gambiae* according to the World Health Organization standard methods.

Results Essential oil obtained by co-distillation mainly contained piperitone (40.80%), 1,8-cineole (24.64%), p-menth-4(8)-ene (13.20%), limonene (6.09%) and α-pinene (4.73%). However, the mixture of pure essential oils of these two plants mostly contained geranial (20.74%), neral (16.42%), 1,8-cineole (19.79%), sabinene (6.03%) and β-pinene (3.87%). The essential oil of *C. citratus* mainly contained geranial (41.49%), neral (32.83%), β-myrcene (13.66%) and geraniol (3.49%) while the major constituents of essential oil of *H. suaveolens* were 1,8-cineole (39.58%), sabinene (12.06%), β-pinene (7.73%), α-terpinolene (6.72%) and (E)-caryophyllene (7.49%). At the dose of 1%, all essential oils, except that of *H. suaveolens,* induced about 100% of mortality on the sensitive species of *An. gambiae*. However, on the resistant species at the same dose, the essential oil obtained by co-distillation induced the highest mortality (53.44%). The essential oils of *C. citratus, H. suaveolens* and the mixture of the two pure essential oils caused respectively 2.47, 15.28 and 18.33% of mortality. The synthetic insecticide caused 100 and 14.84% of mortality respectively on the sensitive and resistant species of *An. gambiae*.

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Conclusion Essential oil obtained by co-distillation showed good insecticidal efficacy against a resistant species of *An. gambiae* and might constitute a new solution to fight against mosquitoes resistant to synthetic insecticides.

Keywords *Cymbopogon citratus*, *Hyptis suaveolens*, Co-distillation, Essential oil, Insecticidal activity, Resistant *Anopheles gambiae*

Background

Malaria remains one of the most worrying endemic disease in sub-Saharan Africa. The infection is caused by a parasite of the genus *Plasmodium*, transmitted to humans by female mosquitoes of the genus *Anopheles*. According to the World Malaria Report in 2022, the African region had 95% of the 241 million malaria cases and 96% of the 627,000 deaths from the disease globally [1]. In Burkina Faso, it remains the first cause of consultation and mortality. According to the Ministry of Health, in 2021 the number of registered malaria cases was 12.2 million, including 4355 deaths [2]. To fight this disease, many methods have been developed. The strategy of controlling vectors is integral to the success of the fight against malaria. This strategy uses insecticide-impregnated mosquito nets and indoor spraying of insecticides from the family of pyrethroids, organochlorines, organophosphates and synthetic carbamates [3]. The use of insecticides has interesting, sometimes spectacular results, such as the reduction of malarial morbidity, which is associated with a reduction in the aggressiveness and the rate of entomological inoculation of the vectors [4]. Unfortunately, the use of synthetic chemicals has many disadvantages, such as environmental pollution and ecological disorders [5]. In addition, people are more and more aware of the upsurge in the resistance of *Anopheles* mosquitoes to most synthetic insecticides [6, 7]. In Burkina Faso, previous reports showed that mosquitoes of the genus *An. gambiae* have developed resistance to all four classes of insecticides (organochlorines, organophosphates, carbamates, pyrethroids) currently available for public health use [8]. Faced with this resistance, and the toxic effects of synthetic insecticides, the use of natural substances extracted from plants with proven insecticidal properties, is now encouraged. Indeed many studies showed that essential oils of aromatic plants could constitute an alternative to the use of synthetic insecticides, because they can kill all insects at all stages of development [9]. Nevertheless, the practical application of essential oils as an insecticide generally requires the use of high doses and/or long treatment times [10]. This would limit their use because of their fairly high cost due to their low extraction yield and the high energy costs of extraction [11]. Consequently, a major challenge for the practical application of essential oils is to develop optimised combinations, which will allow the use of low doses but sufficiently effective [12, 13]. To this end, recent studies have reported that essential oils obtained by co-distillation (simultaneous distillation) of aromatic plants have improved antioxidant and antifungal properties compared to the essential oils of each plant and the pure essential oil mixtures of co-distilled plants [14, 15].

Cymbopogon citratus and *Hyptis suaveolens* are two aromatic plants used as pesticidal plants by the local population [16]. Essential oils of these two plants have insecticidal properties against *Anopheles gambiae* mosquitoes [17]. However, in our akwnoledgement, no study has been conducted on the combinations of essential oils of these two plants. The present work aims to determine the chemical composition and insecticidal activity of the essential oil obtained by co-distillation of *C. citratus* and *H. suaveolens* against *An. gambiae* sensu lato (*s.l.*) mosquitoes, the major vector of malaria in Burkina Faso.

Methods

Plant material

The plant material consisted of the dry leaves of *C. citratus* and *H. suaveolens*. The samples were collected in August 2019 on the site of the Nazi Boni University, Burkina Faso (11°12'N; 4°24'W). The samples were dried in the shade for seven days at room temperature before essential oils extraction. The plants were identified as numbers 961 and 964 respectively for *C. citratus* and *H. suaveolens* and samples were deposited at the herbarium of the Nazi Boni University.

Biological material

The biological material consisted of two species of *An. gambiae s.l.*: the sensitive species (Kisumu) and the resistant species (VK-Labo). Mosquitoes were provided by the insectarium of the Research Institute in Health Sciences Western Regional Office, Burkina Faso.

Extraction of essential oils

Essential oils were obtained by hydrodistillation using a Clevenger type apparatus from the dry leaves of *C. citratus* and *H. suaveolens*, separately, then from their mixture in the mass proportion 50/50. The extraction yield of essential oils (R) was determined according to the formula:$R = \frac{Mh}{Mv} \times 100$, where Mh is the mass of essential oil obtained and Mv, the mass of dry plant material used [18].

Chemical analysis of essential oils

The essential oils extracted and a mixture of the pure essential oils in a volume ratio 50/50 were chemically analysed using gas chromatography/mass spectrometry techniques (GC/MS). The Agilent 8860 brand chromatograph was used. It was coupled to an Agilent 5977B type mass spectrometer equipped with a quadripolar analyser. The separation of the various constituents was carried out using a DBWAX capillary column of the polyethylene glycol (PEG) type (60 mx 0.25 mm), (thickness of the film 0.25 cm) under the following experimental conditions: gas vector (helium: 1 mL min^{-1}), ionization energy (70 eV), injector temperature (250 °C), detector temperature (250 °C). The oven is programmed from 60 to 250 °C for 10 min with a gradient of 2 °C min^{-1}. The injection was in split mode 1–10. The identification of the different constituents of essential oils was carried out both, by comparing their mass spectra with those of NIST 2008 database [19] and by comparing their retention index (RI) with those of the literature [20].

Evaluation of the insecticidal activity of essential oils

All essential oils and a synthetic insecticide (Deltamethrin 0.05%) used as a positive control, were tested according to the World Health Organization's tube mosquito sensitivity testing protocol as described by Wangrawa [21].

Impregnation of N°1 Whatman papers with essential oils

Essential oils were diluted in acetone to afford solutions at doses of 0.01; 0.1 and 1%. Two (2) mL of each solution were poured homogeneously using a Pasteur pipette on 15 cm × 12 cm N°1 Whatman paper. The impregnated papers were kept, for drying, at the the laboratory for 10 min, then were wrapped in aluminum foil and kept in the refrigerator at 4 °C until use.

Test procedure

For each essential oil solution, 100 *An. gambiae* females aged 3 to 5 days are exposed for 1 h in the test tubes (marked red) lined with impregnated papers at the rate of 25 mosquitoes per tube. After this exposure period, the mosquitoes are transferred to the observation tubes (marked green) lined with non-impregnated paper. Cotton soaked with 10% of glucose was placed above each observation tube. A group of 25 mosquitoes exposed only to acetone constituted the negative control. The mortality rate is determined after 24 h of observation using Abbott's formula [22]:

$$\text{Mortality rate} = \frac{\text{Mortality in essential oil} - \text{Mortality in control}}{100 - \text{Mortality in control}} \times 100.$$

All the tests were done in triplicate.

Statistical analysis

Bioassay data were analysed using the Microsoft Excel spreadsheet and reported as mean ± standard deviation. The one-way ANOVA analysis of variance was performed using IBM SPSS Statistics 22 software. The comparison of the means was made at the 5% level by the Student–Newman–Keuls test (S–N-K test).

Results

Extraction yield of essential oils

Extraction yields of the essential oils are given in Table 1. The essential oil of *H. suaveolens* has the lowest extraction yield (0.75%) while that obtained by co-distillation (*C. citratus/ H. suaveolens*) has the yield of highest extraction (1.22%).

Chemical composition of essential oils

The results of the chromatographic analyses of the essential oils are presented in Table 2. Figure 1 presents the structures of main constituents of the essential oils.

In the essential oil of *C. citratus*, 14 compounds representing 96.16% of the total mixture were identified. Geranial (41.49%), neral (32.83%), β-myrcene (13.66%) and geraniol (3.49%) are the major constituents of this essential oil.

Twenty-eight (28) compounds representing 93.27% of the total mixture of *H. suaveolens* essential oil were identified. This essential oil mainly contained 1,8-cinelole (39.58%), sabinene (12.06%), β-pinene (7.73%), (E)-caryophyllene (7.49%), α-terpinolene (6.72%) and α-pinene (3.48).

The essential oil obtained by co-distillation contained 23 compounds, representing 95.59% of the total mixture. It mainly contained piperitone (40.80%), 1,8-cineole (24.64%), p-menth-4(8)-ene (13.20%), limonene (6.09%) and α-pinene (4.73%).

In the mixture of pure essential oils of the two plants, it was identified 38 compounds representing 93.27%. This mixture mostly contained geranial (20.75%), neral (16.42%), 1,8-cineole (19.79%), β-pinene (7.37%) and sabinene (6.03%).

Table 1 Extraction yields of essential oils

Essential oil	Yield (%)
C. citratus	1.05 ± 0.08
H. suaveolens	0.75 ± 0.03
C. citratus / H. suaveolens	1.22 ± 0.12

Table 2 Chemical composition of essential oils

RI_{Lit}[20]	RI_{Exp}	Compounds	Content (%)			
			C. citratus	*H. suaveolens*	CC/HS*	CC/HS
921.5	921	Butanal, 3-methyl-	–	–	–	0.96
1025.4	1025	*α*-Pinene	–	**3.48**	1.74	**4.73**
1026.6	1027	*α*-Thujene	–	0.32	0.16	–
1110.0	1117	*β*-Pinene	–	**7.73**	**3.87**	–
1122.0	1128	Sabinene	**-**	**12.06**	**6.03**	–
1282.4	1138	p-Menth-4(8)-ene	–	–	–	**13.20**
1146.8	1153	3-Carene	–	0.30	0.15	–
1160.9	1164	*β*-Myrcene	**13.66**	1.07	**7.37**	0.13
1167.7	1170	*α*-Phellandrene	–	0.26	0.13	0.26
1177.8	1185	*α*-Terpinene	–	0.77	0.39	0.12
1198.2	1206	**Limonene**	–	1.99	1.00	**6.09**
1211.1	1219	**1,8-Cineole**	–	**39.58**	**19.79**	**24.64**
1234.5	1236	β-Ocimene, cis-	0.36	–	0.18	–
1245.0	1249	*γ*-Terpinene	–	1.09	0.55	0.17
1250.4	1253	β-Ocimene, trans-	0.25	–	0.13	–
1270.1	1274	*p*-Cymene	–	–	–	0.44
1282.4	1288	**α-Terpinolene**	–	**6.72**	**3.36**	0.13
1336.9	1340	5-Hepten-2-one, 6-methyl-	1.14	–	0.57	–
1391.9	1398	3-Octanol	–	0.15	–	–
1444.2	1454	1-Octen-3-ol	–	0.86	0.43	–
1460.2	1470	Cis-sabinene hydrate	–	0.37	0.19	–
1475.3	1481	Citronellal	0.17	–	–	–
1523.2	1523	β-Bourbonene	–	0.38	0.19	–
1543.3	1551	Linalool	1.14	–	0.57	–
1548.9	1555	Sabinene hydrate, trans-	–	0.19	0.10	–
1584.2	1569	p-Menth-2-en-1-ol, Trans-	–	–	–	0.36
–	1574	Isoneral	1.46	–	0.73	–
1559.1	1589	α-Bergamotene, trans-	–	1.03	0.52	–
1590.9	1593	*β*-elemene	–	0.20	0.10	0.24
1598.5	1600	**Caryophyllene, (E)-**	–	**7.49**	**3.75**	0.45
1598.1	1601	2-Undecanone	0.39	–	0.20	–
1601.2	1607	Terpinen-4-ol	–	1.65	0.83	0.23
1614.1	1634	p-Menth-2-en-1-ol, cis-	–	–	–	0.25
1661.2	1661	Pinocarveol, trans-	–	–	–	0.17
1673.2	1673	*β*-Humulene	–	0.49	0.25	–
1678.5	1688	**Neral**	**32.83**	–	**16.42**	–
1694.0	1703	***α*-Terpineol**	–	–	–	**1.52**
1708.2	1712	Germacrene D	–	1.43	0.72	–
1716.9	1722	*β*-Selinene	–	0.28	0.14	–
1729.9	1736	**Piperitone**	–	–	–	**40.80**
1734.5	1737	Bicyclogermacrene	–	2.63	1.32	–
1725.0	1738	**Geranial**	**41.49**	–	**20.74**	–
1751.1	1760	Geranyl acetate	0.49	–	0.25	–
1755.7	1761	*δ*-Cadinene	–	–	–	0.13
1763.9	1771	Citronellol	0.30	–	0.16	–
1803.1	1803	p-Mentha-1(7),8-dien-2-ol, trans-	–	–	–	0.14
1794.6	1805	Nerol	0.21	–	0.11	–
1808.1	1812	2-Tridecanone	0.23	–	0.12	–

Table 2 (continued)

RI_{Lit}[20]	RI_{Exp}	Compounds	Content (%)			
			C. citratus	*H. suaveolens*	CC/HS*	CC/HS
1848.3	1852	*p*-Cymene-8-ol	–	0.16	–	–
1839.3	1852	**Geraniol**	**3.49**	–	1.75	0.24
1894.9	1893	p-Mentha-1(7),8-dien-2-ol, cis-	–	–	–	0.11
1986.2	1988	Caryophyllene oxide	–	0.34	0.17	0.11
Total			**96.16%**	**93.27%**	**94.60%**	**95.59%**
Hydrocarbon monoterpenes			**14.27%**	**35.79%**	**25.05%**	**25.27%**
Oxygenated monoterpenes			**80.78%**	**41.95%**	**61.64%**	**68.46%**
Hydrocarbon sesquiterpenes			–	**14.15%**	**6.99%**	**0.79%**
Oxygenated sesquiterpenes			–	**0.34%**	**0.17%**	**0.11%**
Other compounds			**1.37%**	**1.01%**	**0.75%**	**0.96%**

The bold values are correspond to those of the major constituents of essential oils

RI_{Lit}: Literature Retention Index; RI_{Exp}: Experimental Retention Index; CC/HS*: mixture of pure essential oils of *C. citratus* and *H. suaveolens*; CC/HS: essential oil obtained by co-distillation of *C. citratus* and *H. suaveolens*

Insecticidal activity of essential oils

Insecticide test results revealed that all essential oils are effective against the two species of *An. gambiae* (Fig. 2). ANOVA analysis of variance showed a highly significant difference between mosquito mortality rates from one essential oil to another and depending of tested dose (Table 3). The essential oils were more effective on the sensitive species than on the resistant species. All EOs caused mortality rates of approximately 100% on the sensitive species at the dose of 1% except that of *H. suaveolens* which caused 59.55% of mortality. On the resistant species, at the dose of 1%, mortality rates were 2.47, 12.65, 19.17 and 53.48% respectively for the essential oils of *C. citratus, H. suaveolens*, the mixture of pure essential oils and the essential oil obtained by co-distillation. The synthetic insecticide caused 100 and 14.84% of mortality respectively on the sensitive and the resistant species of *An. gambiae*.

Discussion

Extraction yields of essential oils from *C. citratus* and *H. suaveolens* are different from those reported by many authors. Indeed, the extraction yield of the essential oil of *C. citratus* in this study (1.05%) is higher than that of the species from Benin (0.71%) and Ivory Coast (0.70%) [23, 24]. However, the yield obtained by Djibo [25] of the species from the center of Burkina Faso (1.4%) was higher than that of the present study. The extraction yield of the essential oil of *H. suaveolens* is generally less than 1% whatever the geographical origin. The extraction yield obtained in this study (0.75%) is much higher than those of the species harvested in different regions of Ivory Coast, which were between 0.04 and 0.12% [26]. The species from central Burkina Faso had a yield comparable to that of the present study. Indeed, the essential oil yield of this species was between 0.23 and 0.88% depending on the period and the harvest site [25]. Genetic factors, collection area, stage of plant development, season and/or plant environment and reactions over time within the plant would justify differences in yield for the same plant species [27]. Moreover, the higher yield of essential oil obtained by co-distillation compared to those of pure essential oils, could be due to the appearance of new compounds and/or to the increase in the content of certain molecules of pure essential oils.

The composition of the essential oil of *C. citratus*, dominated by citral (74%) is comparable to that described in several countries, in particular in Benin [27], in Algeria [28] and in Burkina Faso [25]. However, the essential oil of *H. suaveolens* presents a very significant quantitative and qualitative difference compared to those described in the literature. Indeed, six (6) majority compounds (caryophyllene, sabinene, germacrem, pinene, terpinolene and humulene) were most often reported in the different chemotypes studied in the West African region and in South Asia, particularly in Burkina Faso [25], in Senegal [29], in Nigeria [30], in Togo [31], in Ivory Coast [26], in Thailand [32] and in India [33]. In contrast, the essential oil of the species from the Dutch island of Aruba had a fairly high content of 1,8-cineole (35.9%). However, it differs from that of the present study by the low presence of caryophyllene (0.3%) [34].

The mixture of pure essential oils of the two plants contained all the major constituents identified in the pure essential oils of each plant but at lower contents. The low contents would be related to a dilution effect caused by the mixture of essential oils. However, in the essential oil obtained by co-distillation, the major

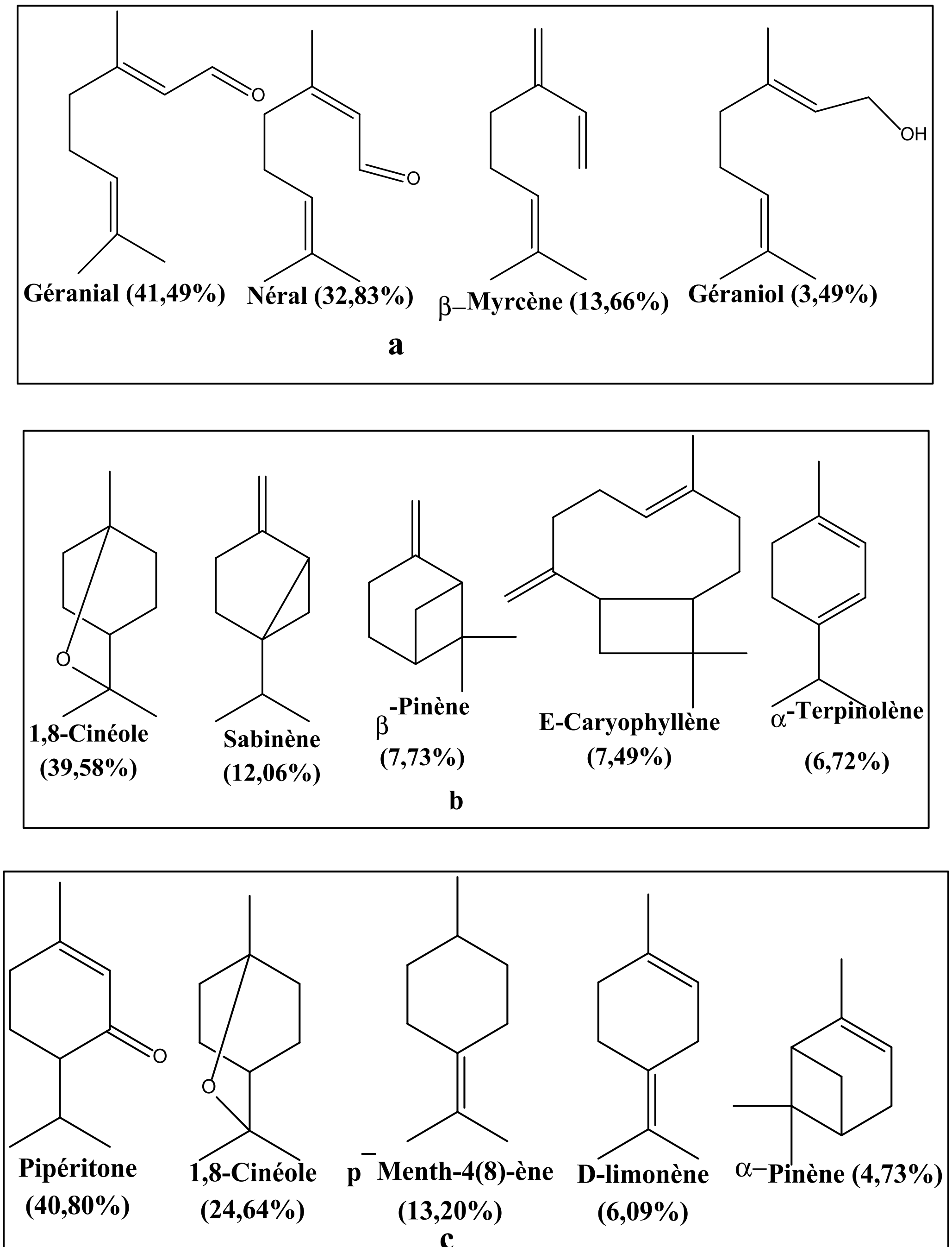

Fig. 1 Structures of main constituents of essential oil of *C. citratus* (**a**), *H. suaveolens* (**b**) essential oils and that obtained by co-distillation of the two plants (**c**)

constituents were piperitone (40.80%) and p-menth-4(8)-ene (13.20%). Yet, these compounds were not present in the two pure essential oils. On the other hand, β-pinene (7.73%) and sabinene (12.06%), major constituents of the essence of *H. suaveolens* were absent in that obtained by co-distillation. Similarly, neral (32.83%) and geranial (41.49%), major constituents of the essence of *C. citratus* were not identified in the essence obtained by co-distillation.

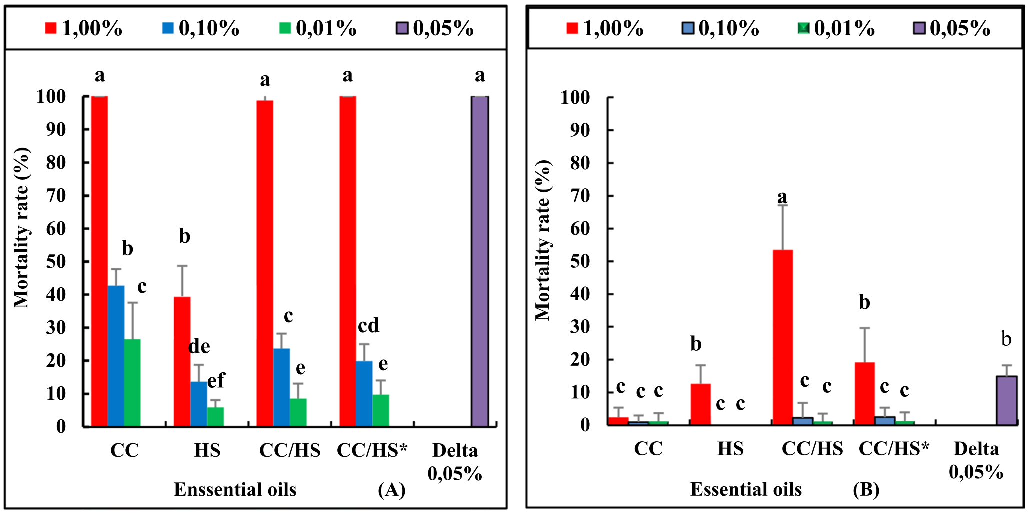

Fig. 2 Mortality rate caused by essential oils on sensitive (**A**) and resistant (**B**) species. *CC* essential oil of *C. citratus*, *HS* essential oil of *H. suaveolens*, *CC/HS* essential oil obtained by co-distillation of the two plants, *CC/HS** mixture obtained by combining pure essential oil, *Delta 0.05%* Deltamethrin 0.05%. The bands assigned the same letter are not significantly different according to the Student-Newman and Keuls test at the 5% threshold

Table 3 ANOVA analysis of variance of different treatments on the two mosquito strains

Treatment	sum of squares	ddl	F-test	P-value
Sensitive species	73,965,528	12	227,477	0.000
Resistant species	10,654.060	12	28.819	0.000

Furthermore, limonene (1.99%) and α-pinene (3.48%) present only in the essence of *H. suaveolens* were identified in that obtained by co-distillation at higher levels (6.09% and 4.73% respectively) unlike α-terpinolene (6.72%), (E)-caryophyllene (7.49%) and β-myrcene (13.66%) which were identified with sufficiently low levels (0.41; 0.45 and 0.13% respectively). A similar study also reported the appearance of new compounds (limonene and carvacrol) in the essential oil obtained by co-distillation of *Rosmarinus officinalis*, *Thymus vulgaris* and *Camaldula officinalis* and not having been identified in the pure essence of each plant extracted separately [35]. These results show that chemical reactions would have occurred during the co-distillation of *C. citratus* and *H. suaveolens* under the influence of certain parameters (acid pH, heat and catalysts). Indeed, according to many authors, the heat, the acid pH and the presence of certain molecules in the distillation medium can promote reactions of cyclization, elimination and dehydration of certain constituents of essential oils during the process of distillation [36]. Fisher et al. [37] showed that sabinene would degrade upon hydrodistillation to give terpen-4-ol, α-terpinene and γ-terpinene. The study conducted by Chiou et al. [38] on the thermal stability of major constituents of Japanese mint essential oil showed the conversion of menthol acetate to menthol, menthol to menthone, menthone to piperitone and piperitone to thymol. With regard to structures of the new molecules formed and those disappeared, piperitone would come from the cyclization of neral and geranial (Fig. 3). This analysis corroborates that of Mikkola et al. [39] who reported that the hydrogenation of citral led to the formation of many products including piperitone. As for p-menth-(4)8-ene, it could come from a rearrangement of sabinene (Fig. 4). Myrcene and β-pinene would transform into limonene, thus increasing the content of this molecule in the mixture unlike other molecules (Fig. 5). However, more in-depth studies need to be carried out to elucidate these different transformations.

The results of insecticidal tests showed that the insecticidal activity varies from one essential oil to another and from one species of mosquito to another. The variation in the insecticidal effectiveness of essential oils for the same species of mosquito would be linked to the chemical composition of each essential oil. On the other hand, the difference in genetic heritage of the two species of *An. gambiae* tested could justify the difference in mortality recorded for the same essential oil from one species to another [38]. Indeed, the essential oil of *C. citratus,* which was very effective on the sensitive strain with a mortality rate of 100% at the dose of 1% was found to be less effective on the resistant species with a mortality rate of 2.47% at the same dose. This result agrees with that obtained by Nonvhio et al. [39] who reported that the essential oil of *C. citratus* which caused 100% mortality on the sensitive species (Kisumu) of *An. gambiae* at a concentration of

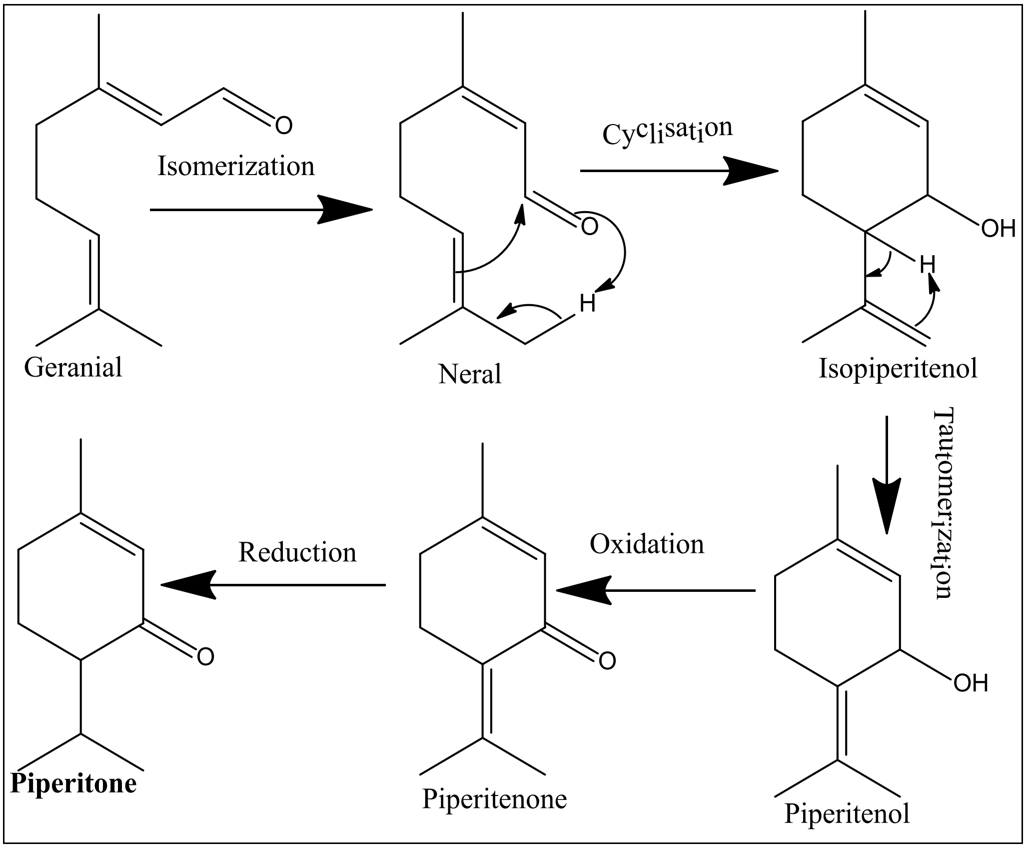

Fig. 3 Probable mechanism of piperitone formation from neral and geranial

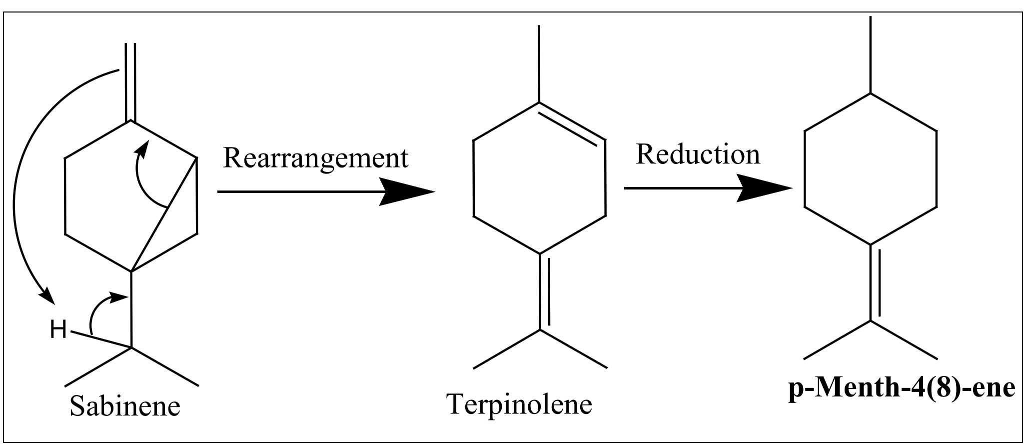

Fig. 4 Probable mechanism of p-menth-(4)8-ene formation from sabinene

22.6 µg/cm^2 had induced only 4% mortality on the resistant strain at the same concentration. Similarly, Wangrawa et al. [40] obtained with the essential oil of *H. suaveolens* from central Burkina Faso lethal concentrations LC_{50} and LC_{90} respectively of 0.85 and 1.38% on the sensitive species (Kisumu) while on the resistant species the LC_{50} and LC_{90} were 1.86 and 2.82%, respectively.

The essential oil obtained by co-distillation of *C. citratus* and *H. suaveolens* was 21.6, 3.5, 3 and 3.6 times more effective respectively than essential oils of *C. citratus*, *H. suaveolens*, the mixture of the two pure essential oils

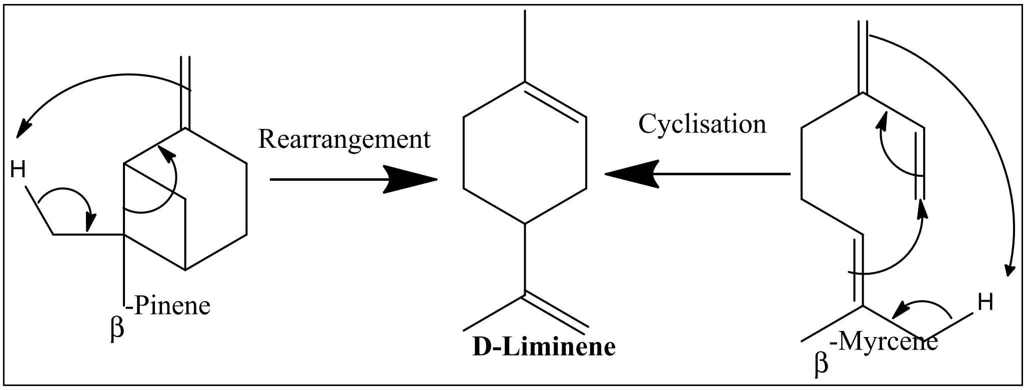

Fig. 5 Probable mechanism of D-limonene formation from myrcene and β-pinene

and the synthetic insecticide on the resistant species of *An. gambiae*. This result shows that the co-distillation allows improving significantly the insecticidal efficacy of essential oils of these two plants. This effectiveness would be due to the synergistic actions of certain molecules, thus increasing the biological action of the essential oil. Thus, piperitone (ketone) and 1,8-cineole (ether); piperitone and terpineol (alcohol); piperitone and limonene (hydrocarbon); 1,8-cineole and limonene; piperitone and p-menth-4(8)-ene (hydrocarbon) may act synergistically. This analysis is in agreement with that carried out by Aissaoui et al. [41] who reported that combinations of camphor (ketone) and 1,8-cineole (ether), camphor and linalool (alcohol), α-pinene (hydrocarbon) and linalool (alcohol) produced synergistic insecticidal activity against the mite *Tetranychus urticae* [42]. Van Vuuren and Viljoen [43] also reported that the combination of limonene and 1,8-cineole produced a synergistic effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Nébie et al. [11] also reported the synergistic insecticidal effect on *Callosobruchus maculatus* of the mixture of pure essential oils of *Cymbopogon schoenanthus* and *Ocimum basilicum*, which would be due to a synergistic action of piperitone and eugenol (alcohol).

Conclusion

The co-distillation of *C. citratus* and *H. suaveolens* made it possible to obtain an essential oil which contains new majority molecules such as piperitone and p-menth-4(8)-ene. This essential oil exhibited insecticidal activity 21.6, 3.5, 3 and 3.6 times higher, respectively, than essential oils of *C. citratus, H. suaveolens*, the mixture of the pure essential oils and the synthetic insecticide on a resistant species of *An. gambiae*. These results show that the co-distillation of *C. citratus* and *H. suaveolens* makes it possible to improve the insecticidal efficacy of essential oils of these two plants on mosquitoes resistant to synthetic insecticides. Therefore, they pave the way for the development of a new natural insecticide that is more effective and safer against mosquito vectors of malaria. In addition, other studies are finally necessary to know the mass proportions of the two plants to be co-distilled in order to obtain an optimal insecticidal activity.

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Author contributions
This work was carried out in collaboration among all authors. The authors DDS, MN and RKD allowed the evaluation of insecticidal activity. The author PD allowed the analysis by CPG/MS of essential oils. The author CMD contributed to the writing of this manuscript. All authors have read and approved the final manuscript.

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