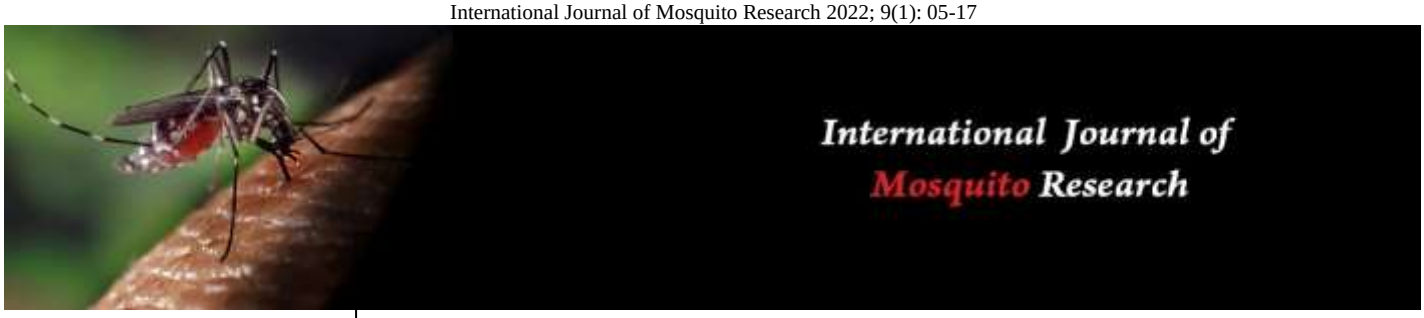

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(1): 05-17

www.dipterajournal.com
Received: 18-10-2021
Accepted: 05-12-2021

PN Akono
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

GT Magne
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

JA Mbida
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

C Tonga
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

EF Fongnzossie
Laboratory of Forest Resources and Wood Valorization, University of Douala, PO Box 1872, Douala, Cameroon

JN Mekoulou
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

MPD Jazet
Laboratory of Biochemistry, Faculty of Science, University of Douala. PO Box. 24 157 Douala, Cameroon

C Menut
IBMM, Montpellier-UMR 5247, Faculty of Pharmacy, 15 Avenue Charles Flahault, 34093, Montpellier, France

Corresponding Author:
GT Magne
Laboratory of Biology and Animal Physiology, Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

In vitro insecticidal activity on *Anopheles gambiae giles*, 1902 and antiplasmodial activity on *Plasmodium falciparum* welch, 1897 of essential oils of some plants of the Cameroonian flora

PN Akono, GT Magne, JA Mbida, C Tonga, EF Fongnzossie, JN Mekoulou, MPD Jazet and C Menut

DOI: https://doi.org/10.22271/23487941.2022.v9.i1a.575

Abstract
For decades, pest control has been confronted with the double resistance of vectors to usual insecticides and of parasites to conventional molecules. The search for new natural molecules is therefore a necessity. The present study aimed to evaluate the insecticidal and anti-plasmodial potential of essential oils from the leaves of 10 species of plants belonging to the Myrtaceae family (*Syzygium aromaticum*, *Psidium littorale*, *Eucalyptus globulus*, *Eugenia uniflora* and *Callistemon rigidus*), Verbenaceae (*Lippia adoensis* and *Lantana camara*) and Cupressaceae (*Cupressus sempervirens*, *Cupressus macrocarpa and Cupressus lusitanica*). The essential oils were extracted by steam distillation using a Clevenger-type apparatus. Their chemical composition was determined by GC-MS. Insecticidal tests were performed according to the WHO protocol and the *in vitro* anti-plasmodial activity of these essential oils was evaluated by the radioisotope method. Except for *S. aromaticum* and *E. uniflora*, the essential oils of the plants studied yielded higher content in monoterpenes (49.01% to 100%) than in sesquiterpenes (0% to 49.82%). At 50 ppm, laboratory adults of *A. gambiae* (tkd_{95} = 10.32mn) and those of *A. gambiae s.l.* (tkd_{95}=7.9 min) were more sensitive to the essential oils of *S. aromaticum* and *L. adoensis* respectively. Mature larvae of *A. gambiae S.l.* (LC_{95}=35.4 ppm) and laboratory larvae of *A. gambiae* (LC_{95}=35.4ppm) were more sensitive to *E. uniflora* oil. In addition, the essential oils studied had shown *in vitro* antiplasmodial activity against *Plasmodium falciparum*. *E. globulus* (IC_{50} = 12.13 ppm) and *L. camara* (IC_{50} = 12.34 ppm) appeared to be the most active. These oils can be used as a basis for the development of new biocides and natural antimalarials.

Keywords: Cupressaceae, Myrtaceae, Verbenaceae, essential oils, biocides, natural antimalarials

Introduction
Parasitic infections remain one of the major public health problems for many countries at the beginning of this 21st century. This is the case of malaria, whose pathogen, Plasmodium, is responsible for 300 to 500 million cases and kills 2 to 3 million people per year, mainly children under 5 years of age, in tropical and subtropical regions of the world [1]. In Cameroon, the disease is one of the leading causes of death [2]. Globally, African countries supported by development partners are implementing a diversity of solutions to deal with this disease. The actions carried out range mainly from preventive measures to the rapid management of patients based on early diagnosis and the accessibility and administration of drugs. The implementation of these measures has led to more or less encouraging results. Current data indicate a relative decline in malaria prevalence over the past decade, though with some disparities across regions [3]. This situation is believed to be inherent to, among other things, a poor compliance to existing preventive and curative measures, and especially to the emergence of dual resistance of plasmodial strains and their vectors to antimalarials and conventional insecticides respectively [4]. Resistant strains of Plasmodium and their vectors are on the rise in sub-Saharan Africa [5, 6, 7]. Moreover, the conventional molecules generally used have many limitations: environmental pollution, significant side effects, intoxication of non-target animal populations [8, 9, 10]. In this context, the search for natural molecules with effective biological properties is a necessity.

Plants from the Cameroonian flora have been an extensive source of new molecules for thousands of years, which simply need to be explored. To date, many studies highlighting the biological activity of plant species from Cameroonian flora have been carried out [11, 12, 13, 14, 15]. From the results obtained, it appears that the biological activities of plants are boosted when they are used in the form of essential oils [16, 17]. Volatile essences are endowed with several functional groups and have the ability to diffuse easily through cell membranes [18]. They are therefore recognised as having a broad spectrum of antibacterial [19], antifungal [20, 21], antiparasitic [22, 23, 14] and insecticidal activities [24, 25, 14].

Callistemon rigidus, Eugenia uniflora, Psidium littorale, Syzydium aromaticum (Myrtaceae), *Lantana camara, Lippia adoensis* (Verbenaceae), *Cupressus macrocarpa, Cupressus sempervirens Cupressus lusitanica* (Cupressaceae) are plants of the Cameroonian flora traditionally used by the populations as insect repellent and in the treatment of many diseases such as amoebic dysentery, malaria, cancer and bacterial infections [26, 27, 28]. The traditional use of these plants probably makes them potential reservoirs of active molecules. This hypothesis has never been verified in the existing scientific literature. With this in mind, the present study proposes to evaluate the insecticidal and antiplasmodial potentials of the essential oils of the above listed plants.

Materials and Methods

Plant material

Ten (10) plant species belonging to 3 botanical families were selected because of their traditional use as insect repellents and in the treatment of malaria in most localities of the Littoral and West Regions of Cameroon. The basic information on the plants used, quantities, harvest period and their essential oils yield are presented in Table I.

Essential oils extraction

The fresh leaves of each plant species were washed with spring water, cut into small units and then subjected to hydrodistillation for 5 hours using a Clevenger-type apparatus. The essential oil collected by decantation at the end of the distillation was filtered on an anhydrous sodium sulphate column to eliminate any residual water and then introduced into dark glass bottles that were hermetically sealed. The whole was kept in a refrigerator at a temperature of 4 °C.

Chemical characterisation of the essential oils

Chemical analysis of the essential oils was performed using a Varian CP-3380 chromatograph equipped with a flame ionisation detector and a capillary column (length 30 m, internal diameter 0.25 mm) with an apolar methylsilicone stationary phase (DB-1, film thickness 0.25 µ). Nitrogen was used as carrier gas with a flow rate of 0.8 ml.min-1. The injector temperature is 220 °C; the detector was set at 250 °C. The oven is programmed from 50 °C to 200 °C with a temperature gradient of 5 °C.min-1. The retention indices of the different constituents were calculated in relation to the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration considering that their response factors were all equal to 1 [25].

The gas chromatography-mass spectrometry coupling was carried out using a Hewlett Packard HP 5970 A apparatus, equipped with an apolar capillary column (30 m x 0.25 mm) made of fused silica of the HP-1 type (film thickness 0.25 µ) and a quadrupole detector (ionisation energy 70 eV). The injector temperature was 220 °C and the interface zone temperature was 210 °C. Injection in split mode (1/100) of 1 µl of a 10% solution of essential oil in dichloromethane. The oven temperature was programmed from 70 °C to 200 °C with a gradient of 10 °C.min-1. The carrier gas was helium with a flow rate of 0.6 ml.min-1. The acquisition was carried out in scan mode [35-300 amu] at 2.96 scan.sec-1 [25].

The identification of the constituents of the essential oils was done on the basis of their retention indices and mass spectra by comparison with data from the literature [29, 25].

Collection and rearing of *Anopheles gambiae s.l.* larvae

Larval populations of Anopheles were collected in natural sites (pits, cesspools and truck tyre tracks, puddles) following the Dipping method [30], from May to July 2019 at a rate of five consecutive days per month in the Youpwe district (Douala). The larvae were reared in the insectarium of the entomology unit of the University of Douala. Thus, they were introduced into basins (30 x 20 x 10cm). The larval density used was 100 larvae per 100 cl of spring water. These larvae were fed with a protein and mineral rich powder, Tetrababy fish food [31], diluted in a small quantity of water and poured into the rearing tanks. The rearing water was renewed every three days to avoid death by asphyxia of the larvae due to decomposition of the food. The pupae from this rearing were introduced into the cages wrapped with mosquito net until they emerged. The adults obtained were identified morphologically using the Anopheles identification keys [32, 33]. The morphological identification carried out on individuals of the *Anopheles gambiae* complex allowed the breeding to be continued only with individuals of the species *Anopheles gambiae s.l.* The blood meal required for the maturation of the oocytes laid by the females was taken from a rabbit held immobile and placed prone on a board one meter long and 25 cm width with a hole in the centre. After one or more eggs had been laid, they were transferred to the closed petri dishes and kept there for 2 days to complete their maturation. After hatching, the larvae obtained were fed with tetrababy fish food according to Desfontaines *et al.* (1991) [31] until the adult stage. The adults obtained were subjected to biological tests. The Kisumu strain (*Anopheles gambiae*) reared under similar conditions served as a control.

Plasmodium falciparum culture

The strain used was the chloroquine resistant FcB1/Colombia strain. This strain was maintained on human red blood cells in RPMI 1640 medium, containing 25 mM HEPES, pH 7.3, 2 g/L sodium bicarbonate, 2 g/L glucose, penicillin and streptomycin [34]. The medium was supplemented with 10% heat-activated human serum. The red blood cells and serum used were from the “Etablissement Français du Sang”. The culture was performed with a haematocrit of 2% and a parasitaemia of 0.5 to 1%. The culture medium was changed once a day. A Giemsa-stained blood smear was taken daily to monitor parasitaemia.

Assessment of biological activities of essential oils

Larvicidal activities against *Anopheles gambiae s.l.*

The related tests consisted in evaluating the mortality of immature (L1 and L2) and mature (L3 and L4) stages of Anopheles in the presence of diluted solutions of essential oils

following a methodology inspired by the World Health Organization protocol [35]. Indeed, 20 larvae were collected using a Pasteur pipette and placed in 8 cm diameter bowls each containing 99 ml of well water to which 1 ml of diluted test solution was added. Preliminary experiments were used to select a range of concentrations for the actual tests. For this purpose, stock solutions of essential oils of each sample were prepared in 70° ethanol. From these, dilutions were made to obtain final experimental concentrations of 50, 100 and 150 ppm. Three replicates were made for each dilution. Two control bowls were also set up under the same conditions as the test bowls. The negative control contained only ethanol (in the same proportions as for the tests, i.e. 1%) without any trace of essential oil. Larvae were counted every 5 minutes for 1 hour, then every hour for 10 hours and finally after 24 hours of exposure to the volatile extracts solubilised in water.

Adulticidal activities against *Anopheles gambiae s.l.*

The methodology used was inspired by the WHO protocol for sensitivity testing in cylindrical tubes [36]. It consists of exposing mosquitoes to discriminatory doses of insecticides on impregnated paper; assessing the time required to knock down 50% and 95% of the mosquitoes (tkd50 from the prepared essential oil solutions), and then determining the mortality of the mosquitoes after 24 hours. Preliminary experiments had allowed the selection of a range of essential oil concentrations for the actual tests by diluting the raw essential oils in acetone (organic solvent). The following experimental concentrations were obtained: 150 ppm, 100 ppm, 50 ppm. 2500 microlitres of each essential oil solution was prepared in a test tube and then transferred to a glass petri dish. A Wattman paper was inserted for impregnation, removed and spread on a bench for drying for 03 to 05 minutes. The dry Wattman paper was then threaded into an exposure tube.

Thus, 20 to 25 previously identified non-gorging adult females of *A. gambiae s.l.* of 2-5 days of age were introduced into an observation tube lined with paper not impregnated with essential oil. After one hour of observation, the mosquitoes were transferred to an exposure tube lined with Wattman No. 1 paper (12x15 cm^2) impregnated with diluted essential oil. Knocked-down mosquitoes were counted at regular 5-minute intervals for one hour. At the end of the exposure period (60 minutes), the mosquitoes were transferred back to the observation tube. A swab soaked in a 10% sugar solution was placed over the observation tube and mortality was determined after 24 hours of observation. For each defined concentration of essential oil, 3 sets of 20-25 mosquitoes were prepared. A set of 20-25 mosquitoes exposed only to acetone was the control. Mortality rates were interpreted according to the criteria proposed by the WHO [36].

***In vitro* anti-plasmodial activity**

The *in vitro* anti-plasmodial activity of essential oils was assessed by the radioisotopic method [37]. This method determines the inhibition of parasite growth in culture in the presence of various concentrations of molecules by measuring the incorporation of [3H] tritiated hypoxanthine into the nucleic acids of the parasites. The tests were performed in 96-well plates [38].

Each essential oil sample was prepared in culture medium and then added to asynchronous parasite cultures (1% parasitaemia, 1% final haematocrit, 200 µL final volume per well). The contact time between the parasite culture and the essential oil sample was 24 hours. Subsequently, 0.5 µCi of [3H] hypoxanthine (1-5 Ci/mmol; Amersham, Les Ulis, France) was added per well. The plates were then incubated at 37°C under a humid atmosphere containing 5% CO2 and then frozen at (-80°C). After thawing the plates, the contents of the wells were collected on glass fibre filters (Wallac®, USA) using a cell collector (Filter Harvester, USA). After the addition of scintillation fluid (Perkin Elmer®, USA), the radioactivity (counts per minute) was measured with a spectrophotometer (450-Microbeta Trilux, USA). The inhibition of parasite growth induced by each concentration of essential oil was determined by comparing the radioactivity incorporated in the treated culture with that of the control culture (without oil). The sensitivity of the chloroquine-resistant strain FcB1/Colombia to the different essential oils was assessed according to their IC50 values (g/Ml) as follows: IC50< 10 (strong); 10 ≤ IC50 ≤ 50 (Medium, moderate); >50 ≤ IC50 ≤ 100 (weak); IC50 >100 (inactive) [39].

Data analysis for bioassay

Analyses were performed by using the statistical software SPSS version 19.0. Chi-square statistics was used to compare the mortality rate of larvae. Differences were considered statistically significant at $P < 0.05$. The Henry simplified table that transforms the percentages of larval mortality into probit was used to determine the lethal concentration required to kill 50% (LC95) of larvae. The WINDL software, version 2.0 was used to calculate Kdt 50 and Kdt 95.

Results and Discussion

Chemical composition of essential oils

The chemical compositions and yields vary from one essential oil to another (Tables 1, 2 and 3). The yields vary from 0.07% for the species *Lantana camara* to 2.01% for *Syzygium aromaticum.* This variation is thought to be related to the plant organ from which the oil is extracted, the nature of the soil, the time of collection, and the time required for extraction [40, 25]. Probst (2012) [41] and Tchoumbougnang *et al.* (2005) [12] have also shown that several other factors can influence plant performance, such as the developmental phase of the plant, the pollination cycle, seasonal variations and the pathophysiological condition of the plant.

Syzygium aromaticum

The analysis of the chemical composition of the essential oil of *Syzygium aromaticum* shows that eugenol is the major constituent (90.25%). The essential oils of *Syzygium aromaticum* samples from Indonesia (75.04%) and Madagascar (83.58%) also showed a high proportion of eugenol [42, 43]. There is a similar evidence from the study conducted by Bhuiyan *et al.* (2010) [44] who reported eugenol (74.3%) as the majority compound. From the above, it should be suggested that eugenol is the characteristic molecule of the essential oil of *Syzygium aromaticum.*

Lippia adoensis

The chemical composition of the *Lippia adoensis* essential oil sample corroborates that obtained in Côte d'Ivoire by Soro *et al.* (2015) [45] with respect to the main compound. Indeed, during their work, the essential oil sample of *Lippia adoensis* from the forest area of Côte d'Ivoire showed a high content of geranial (29.1%) and neral (21.9%). However, contradictory

results have been recorded [46, 47, 48]. The latter showed that the essential oil of *Lippia adoensis* originating from certain areas of Côte d'Ivoire and Nigeria had a high content of oxygenated monoterpenic compounds dominated by 1,8-cineole and eucalyptol respectively.

Cupressus macrocarpa

The essential oil of *Cupressus macrocarpa* was dominated by α-pinene (20.78%). Similar results were obtained with samples from Argentina and Greece [49, 50, 51]. Furthermore, it should be noted that α-pinene has been reported as a majority compound in essential oils of other species of the genus *Cupressus*; namely *Cupressus arizonica* from Tunisia, *Cupressus atlantica* from Morocco and *Cupressus sempervirens* from Algeria [52, 53]. From the above, it should be suggested that α-pinene is the characteristic molecule of Cupressus essential oils, although conflicting results showed that some samples of *Cupressus macrocarpa* from Egypt had neral (35%) and α-terpineol (19.01%) as majority compounds [54, 55].

Lantana camara

β-caryophyllene (20.37%) was the main compound in the essential oil of *Lantana camara*. Some works have shown some variability in the chemical composition of this plant species depending on the collection site with β-caryophyllene (9.8%) as the main compound for the sample from Egypt [56]; (E)-nerolidol (16.6%) for the sample from Cuba [57]; davanone (44.4%) for the sample from Nepal [58], sabinene (16.9%) for the sample from Yemen [58].

Psidium littorale

Psidium littorale is predominantly composed of 1,8-cineole (eucalyptol) (39.55%). Although this result corroborates those recorded by Scur *et al.* (2016) [59] and Marques *et al.* (2008) [60], it contrasts with the result recorded by Soliman *et al.* (2016) [61] and Adam *et al.* (2011) [62]. The latter showed that β-caryophyllene was the majority compound in samples of *Psidium littorale* from Egypt and French Polynesia.

Cupressus lusitanica

The essential oil of *Cupressus lusitanica* leaves obtained in our results contains Sabinene (20.86%) as the main compound, followed by terpinen-4-ol (16.87%), and α -pinene (13.27%). Sabinene and α -pinene were also found among the main compounds in the essential oil of the leaves of samples of the same plant species from Portugal [63]. However, our results are in contrast to those obtained with some Cameroonian samples of *C. lusitanica* by Kuiaté *et al.* (2006) [64] and Teke *et al.* (2013) [65]. In these samples, molecules such as umbellulone, α-pinene, germacrene D, epi-zonarene were predominant.

Cupressus sempervirens

The essential oil sample of *Cupressus sempervirens* is dominated by α-pinene (12.28%). This result corroborates those obtained by [51, 66, 67] Athanassios *et al.* (2013), Jahani *et al.* (2019), Nacira and Yousra (2017) respectively with the samples originating from Greece, Iran and Algeria.

Eucalyptus globulus

The essential oil of *Eucalyptus globulus* is monoterpene-dominant with α -Pinene (35.84%) being the main compound, followed by eucalyptol. These results are contradictory to previous work conducted on samples from Brazil, Argentina and Ethiopia [68, 69, 70]. The latter found Eucalyptol as the main compound of *Eucalyptus globulus* essential oil in their respective countries. However, although Eucalyptol is positioned as the second main compound in our sample, it should be suggested that this molecule is characteristic of all *Eucalyptus globulus* samples.

Callistemon rigidus

The essential oil of *Callistemon rigidus* is predominantly composed of oxygenated monoterpenic compounds (85.03%), of which eucalyptol (77.47%) is the majority compound. Eucalyptol was also found in the majority of the essential oil in the leaves of samples of the same plant species from Cameroon and China [71, 72].

Eugenia uniflora

The essential oil of *Eugenia uniflora* leaves obtained in our results contains Eugenol (37.65%) as the main compound, followed by Germazone (21.66%). However, this chemical composition contrasts with those determined from Brazilian samples by Gallucci *et al.* (2011); Klinger *et al.* (2013); Sviech *et al.* (2018) [73, 74, 75] with respect to the majority compounds found and reflects a high variability in the chemical composition of the essential oil of the leaves of this plant species within the same country.

Table 1: Data on essential oils from the leaves of the collected plants

	Plant			collection			Essential oil	
Family	**Species**	**Organ**	**Mass(g)**	**Location**	**Date**	**Colour**	**Mass (g)**	Yield **(%)**
Myrtaceae	*Eugenia uniflora*	leaves	2,400	Douala	17.11.2018	Jaune	6.6	0.28
Myrtaceae	*Psidium littorale*	leaves	4,100	Douala	26.09.2018	Yellow	9.5	0.118
Myrtaceae	*Callistemom rigidus*	leaves	2,200	Douala	12.08.2018	Yellow	20.95	0.952
Myrtaceae	*Syzygium aromaticum*	leaves	3,400	Penja	19.01.2019	Milky white	68.34	2.01
Myrtaceae	*Eucalyptus globulus*	leaves	6,800	Douala	01.10.2018	Yellow	20.35	0.299
Verbenaceae	*Lippia adoensis*	leaves	3,500	Douala	15.08.2018	Yellow	17.05	0.48
Verbenaceae	*Lantana camara*	leaves	3,900	Douala	24.09.2018	Yellow	1.65	0.076
Cupressaceae	*Cupressus lusitanica*	leaves	2,500	Douala	01.10.2018	Yellow	16.9	0.676
Cupressaceae	*Cupressus sempervirens*	leaves	3,800	Douala	18.09.2018	Yellow	9.65	0.254
Cupressaceae	*Cupressus macrocarpa*	leaves	2,200	Douala	18.09.2018	Yellow	14.8	0.673

Table 2: Chemical composition of essential oils of *Psidium littorale*, *Eugenia uniflora*, *Eucalyptus globulus*, *Syzygium aromaticum* and *Callistemon rigidus*

Family	**Myrtaceae %**				
Compounds	**Essential oils composition**				
	P. littorale	***E. uniflora***	***E. globulus***	***S. aromaticum***	***C. rigidus***
Monoterpenes	50.27	4.02	90.72		100
Hydrocarbon monoterpenes	7.27	3.45	65.24	-	14.97
α-thujène	4.36	-	-	-	-
Sabinene	1.37	-	-	-	
α -Pinène	-	0.63	35.84	-	14.11
Camphene	-	-	1.75	-	-
β-pinène	0.32	0.86	0.36	-	0.86
Myrcène	0.41	-	-	-	-
P-Cymene	-	-	19	-	-
Limonene	-	0.24	3.99	-	-
α-phellandrène	0.81	-	-	-	-
α –Ocimene	-	1.45	-	-	-
γ—Terpinene	-	0.27	3.67	-	
Terpinolene	-	-	0.63	-	-
Oxygenated monoterpenes	43.00	0.57	25.48	-	85.03
1,8-Cineole (eucalyptol)	39.55	-	7.6	-	77.47
Linalol	-	0.21	-	-	0.59
Carvacrol	-	0.36	-	-	-
Fenchyl-Alcool	-	-	1.25	-	-
Camphor	-	-	0.85	-	-
Pinocarveol	-	-	0.71	-	-
Isopinocarveol	-	-	-	-	0.55
Borneol	-	-	3.02	-	-
Terpineol-4	-	-	1.3	-	-
α –Terpineol	-	-	5.76	-	6.42
cis-Menth-2-en-1-ol	0.68	-	-	-	-
Terpineol <5->	1.17	-	-	-	-
Cinnamaldehyde <(E)'>	0.15	-	-	-	-
3-Cyclohexene-1-methanol, 5-hydroxy-2,3,4-trimethyl	-	-	0.7	-	-
Methyl geranate	0.48	-	-	-	-
6-Isobutyryl-2,2,4,4-tetramethylcyclohexane-1,3,5-trione	-	-	1.67	-	-
2,2,4,4-Tetramethyl-6-(2-methylbutanoyl)cyclohexane-1,3,5-trione	-	-	2.62	-	-
Neryl acetate	0.97	-	-	-	-
Sesquiterpenes	47.66	54.86	2.95	9.75	
Hydrocarbon sesquiterpenes	20.46	24.38	0.48	7.92	-
α –copaène	5.62	-	-	-	-
m-Cymene	-	0.45	-	-	
α –Caryophyllene	0.72	3.95	-	-	-
β-Caryophyllene	0.69	0.32	0.48	-	-
ß-Copaene	2.63	-	-	-	-
(+)-Aromandrene	0.28	-	-	-	-
α-humulène	0.28	-	-	7.09	
Δ-Elemene	-	0.42	-	-	-
β-Elemene	-	2.56	-	-	-
Epizonarene	-	0.36	-	-	-
α-selinène	1.39	-	-	-	-
Trans-β-guaiene	0.7	-		-	-
β-bisabolène	1.17		-	-	-
γ-Cadinène	2.35	-	-	-	
Nookatène	1.06	-	-	-	-
δ-Cadinène	0.46	-	-	-	-
9-Epi-β-Caryophyllene	-	0.58	-	-	-
D-germacrene	0.68	1.83	-	0.83	-
α –Selinene	-	0.51	-	-	-
β-Selinene	-	0.85	-	-	-
α-calacorene	0.21	-	-	-	-
γ-Elemene	-	6.06	-	-	-
Δ-Cadinene	-	0.56	-	-	-
α –Gurgujene	-	0.57	-	-	-
Selina-3,7(11)-diene	0.73	-	-	-	-

Germacrene B	-	5.36	-	-	-
trans-Cadina-1,4-diene	1.49	-	-	-	-
Oxygenated sesquiterpenes	27.20	30.48	2.47	1.83	-
Cubebol	2.94	0.56	-	-	-
Isoaromadendrene epoxide	-	-	-	1.49	
(E)-Nerolidol	1.96	-	-	-	-
Spathulenol	-	1.56	0.95	-	-
Oxyde de caryophyllène	0.31	-	-	-	-
(-)-Globulol	-	1.97	0.82	-	-
Cubenol-1-epi	0.46	-	-	-	-
α-Farnesol	-	-		0.34	-
Viridiflorol	-	1.28	0.7	-	-
Cubenol	1.52	-	-	-	-
α-Muurolol	0.88	-	-	-	-
Muurolol	-	0.38	-	-	-
Neointermedeol	-	0.67	-	-	-
α-Cadinol	0.81	-	-	-	-
Germacrone	-	1.18	-	-	-
(E,E)-Farnesol	0.38	-	-	-	-
Rosifoliol	-	0.29	-	-	-
epi-β-Bisabolol	2.87	-	-	-	-
Germazone	-	21.66	-	-	-
α-Bisabolol	8.56	-	-	-	-
Shyobunol	2.43	-	-	-	-
(E)-Nerolidol acetate	1.18	-	-	-	-
(2Z,6E)-Farnesol	2.21	-	-	-	-
Perhydrocyclopropa[E]Azulene-4,5,6-Triol, 1,1,4,6-Tetramethyl	-	0.93	-	-	-
(E,E)-Farnesyl acetate	0.69	-	-	-	-
Aromatic coumpound	-	37.65	-	90.25	-
Eugenol	-	37.65	-	90.25	-
Others	-	2.16	6.32		-
Isobutyric acid, isobutyl ester			0.73		
Cyclobutaneacetonitrile, 1-Methyl-2-(1-Methylethenyl)-	-	0.59	-	-	-
Methyl 10,13-octadecadiynoate					
3,6-Dimethyl-4H-Furo[3,2-C]Pyran-4-One	-	0.3	-	-	-
Phthalic acid, 3,5-dimethylphenyl 2-pentyl este	-	0.2	-	-	-
Isoamyl butyrate	-	-	0.53	-	-
Cyclopropa[C,D]Pentalene-1,3-Dione, Hexahydro-4-(2-Methyl-2-Propenyl)-2,2,4-Trimethyl	-	0.85	-	-	-
Ingol 12-Acetate	-	0.42	-	-	-
Dihydroxanthin	-	-	0.93	-	-
1,5-Cyclooctadiene, 3,4,7,8-tetrakis(1-methylethylidene)-	-	-	0.51	-	-
Cyclohexanecarboxaldehyde, 6-methyl-3-(1-methylethyl)-2-oxo-1-(3-oxobutyl)-	-	-	3.03	-	-
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	-	-	0.59	-	-

Table 3: Chemical composition of essential oils of *Cupressus sempervirens, Cupressus lusitanica, Cupressus macrocarpa, Lippia adoensis* and *Lantana camara*

Family	**Cupressaceae %**			**Verbenaceae %**	
Compounds	***C. sempervirens***	***C. lusitanica***	***C. macrocarpa***	***Lippia adoensis***	***Lantana camara***
Monoterpenes	70.83	94.87	83.59	85.83	49.01
Hydrocarbon monoterpenes	47.17	66.62	59.2	4.15	30.27
α –Thujene	1.43	3.59	1.51	-	0.52
α –Pinene	12.28	13.27	20.78	-	3.9
α-Sabinene	6.77	20.86	-	-	-
Camphene	-	-	0.45	-	1.46
β-Pinene	0.86	4.2	8.81	3.14	17.99
Myrcene	2.56	-	3.64	1.00	1.93
δ-3-carène	4.14	-	4.97	-	1.50
α-Terpinene	1.95	5.1	-	-	0.28
δ-terpinène	-	-	2.95	-	-
P-cymène	-	-	0.95	-	-
cis-β-ocimène	-	-	-	-	0.89
trans-β-ocimène	-	-	-	-	0.56
Terpinolène	-	-	3.38	-	0.48

α-phellandrene	-	-	-	-	0.18
o-Cymene	2.39	4.07	-	-	-
Limonene	9.24	4.98	9.96	-	-
γ -Terpinene	2.39	7.52	-	-	0.58
(E)-β-Terpinolene	3.16	3.03	-	-	
Oxygenated monoterpenes	23.66	28.25	24.39	81.68	18.74
Isocineole	-	0.33	-	-	-
Linalool	3.55	0.86	4	0.38	14.68
trans-thujanol	-	0.47	-	-	-
Thujone	-	0.28	-	-	-
trans-Menth-2-en-1-ol	-	0.93	-	-	-
cis-p-menth-2-en-1-ol	-	0.71	2.56	-	-
Cis-β-terpineol	-	-	0.27	-	-
Borneol	-	-	-	0.47	-
Trans-β-terpineol	-	-	0.44	-	-
3-cyclohexen-1-ol	-	-	-	1.12	-
Citronelol	-	-	-	2.77	-
myrtenyl methyl ether	-	0.36	-	-	-
Neral	-	-	-	24.74	-
Camphor	0.42	2.42	-	-	-
Geraniol	-	-	-	19.05	
Geranial	-	-	-	33.15	-
Bornyl acetate	0.42	0.52	0.13	-	-
Umbellunone	8.24	-	-	-	-
Terpinen-4-ol	6.42	16.87	9.21	-	0.76
2-cis-dihydro acétate de tepinyl	-	-	0.2	-	-
α-Terpineol	0.97	1.37	6.01	-	-
Déca-(2E,4E)-dien-1-ol	-	-	0.18	-	-
Linalyl Isovalerate	0.68	-	-	-	-
Piperitone	0.26	-	0.56	-	-
p-Cymen-8-ol	-	0.34	-	-	-
cis-Piperitol	-	0.31	0.83		-
trans-Piperitol	-	0.49	-	-	
cis-Linalool oxide	0.27	-	-	-	-
neo-dihydro carveol	-	-	-	-	1.35
Lilac aldehyde D	-	-	-	-	1.06
Methyl salicylate	-	-	-	-	0.89
Piperityl acetate	-	1.09	-	-	-
α -Terpinyl acetate	2.43	0.6	-	-	-
Bornyl formate	-	0.3	-	-	-
Sesquiterpenes	22.60	0.0	16.25	14.17	49.82
Hydrocarbon sesquiterpenes	15.74	0.0	8.93	14.17	40.11
α –Copaene	0.31	-	-	-	-
α –Cedrene	0.31	-	-	-	-
α-Cubebene	-	-	2.18	-	-
Cyclosativene	-	-	0.13	-	1.32
β-Bourbonene	-	-	-	1.13	0.57
cis-thujopsene	-	-	-	3.59	-
α –Caryophylene	0.59	-	-	-	-
Cadina-3,5-diene	0.39	-	-	-	-
cis-Muurola-4(15),5-diene	2.31	-	-	-	-
Elemène	-	-	-	-	0.85
β-Caryophyllene	-	-	-	-	20.37
ß-Copaene	-	-	0.46	-	-
D-germacrene	-	-	-	-	8.79
α-humulene	-	-	0.57	6.38	-
α-Selinene	-	-	-	3.07	-
Bicyclogermacrene	-	-	-	-	2.22
cis-Muurola-4(15),5-diene	1.33	-	-	-	-
G-muurolène	-	-	1.59	-	-
D-germacrène	-	-	0.51	-	-
α –germacrene	-	-	1.61	-	4.70
α-Curcumene	2.13	-	-	-	-
Zonarene	2.17	-	-	-	-
α-calacorene	-	-	-	-	0.21
ç-Muurolene	1.94	-	-	-	-
δ-Cadinène	4.01	-	1.72	-	0.28

7-Hydroxyfarnesene	-	-	-	-	0.80
α-Cadinène	-	-	0.16	-	-
α –Amorphene	0.25	-	-	-	-
Oxygenated sesquiterpenes	6.86	-	7.32	-	9.71
Piperityl acetate	0.38	-	-	-	-
Caryophyllene Oxide	0.43	-	-	-	-
Cedrol	0.73	-	-	-	-
Tau,-Cadinol	1.9	-	-	-	-
Muurolol	1.62	-	-	-	-
α-Bisabolol	1.01	-	-	-	-
p-cymenol	0.79	-	-	-	-
10-epi-Cubebol	-	-	.,7		-
Longipinanol	-	-	-	-	3.65
Viridiflorol	-	-	-	-	0.23
Fokienol	-	-	-	-	0.28
β-atlantol	-	-	-	-	0.59
1,10-di-epi-Cubenol	-		0.48	-	-
Eremoligenol	-	-	-	-	0.67
Humulène époxyde I	-	-	1.1	-	-
α-Cadinol	-	-	1.25	-	2.47
γ-Eudesmol	-	-	1.11	-	-
14-Hydroxy-9-epi-(E)-caryophyllene	-	-	-	-	1.61
α-Eudesmol	-	-	0.68	-	-
(E)-Nerolidol acetate	-	-	-	-	0.21
Aromatic compounds	-	1.61	-	-	-
1-(2-pyridyl)piperazine	-	1.24	-	-	-
benzyl ether	-	0.37	-	-	-
Non-terpenic oxygenated compound	-	-	-	-	0.29
2-Methylbutyl 2-methylbutyrate	-	-	-	-	0.29
Others	6.61	3.5	-	-	-
1H-Imidazole, 4-(2-propenyl)-	0.45	-	-	-	-
Carbonic acid, heptyl vinyl ester	2.47				
9-Cyclohexylbicyclo (3.3.1)nonan-9-ol	0.44	-	-	-	-
5-Methyl-2-hexanol, 2-methylpropionate	0.61	-	-	-	-
Butanoic acid, 1-methylhexyl ester	0.33				
Propanoic acid, 2-octyl ester, (R or S)	0.28				
1-Pentene, 5-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene	0.64		-	-	-
Benzenehexanenitrile, á,á-dimethyl-î-oxo	0.47	-	-	-	-
Spiro[(tricyclo[6.2.2.0(2,7)]dodeca-5,9-diene)-4,1'-cyclobutane]-11,2'-dione, 1,3,3,5,12,12-hexamethyl	0.31	-	-	-	-
2-Propionyloxytridecane	0.31	-	-	-	-
Benzene, (3-octylundecyl)-	0.3	-	-	-	-
Dehydroabietan	-	0.57	-	-	-
Abietadiene	-	2.93	-	-	-

Anti-plasmodial activities of essential oils

Various *in vitro* antiplasmodial activities of the essential oils studied against *Plasmodium falciparum* were noted. *Eucalyptus globulus* (IC50 = 12.13 ppm) and *Lantana camara* (IC50 = 12.34 ppm) appeared to be the most active (Table 4). The high antiplasmodial activity of theessential oil of *Lantana camara* would be due to its high content of linalool (14.68%) and β-Caryophyllene (20.37%). Indeed, some studies have shown that terpenes such as farnesol, nerolidol, limonene, and linalool inhibit dolichol biosynthesis in the trophozoite and schizont cycle of *P. falciparum in vitro*. These terpenes also have the ability to inhibit the biosynthesis of the isoprenic side chain of the benzoquinone of the schizogonic cycle [76, 77, 78]. Furthermore, (E)-Nerolidol (1.96%) although present in *Psidium littorale* essential oil did not result in an interesting IC50. The same observation is made with the essential oil of *Cupressus macrocarpa* containing linalool (4%) and limonene (9.96%). From the above, the high toxicity of *Lantana camara* oil cannot be attributed to the isolated effect of Linalool. β-Caryophyllene (20.37%), one of the major compounds in *Lantana camara* essential oil is also known to have antimalarial properties against *P. falciparum* and anticancer properties [79]. The hypothesis of interaction between linalool and β-Caryophyllene in order to boost the toxicity of *Lantana camara* essential oil against *P. falciparum* is to be suggested. This hypothesis remains to be verified in our future work. Moreover, the high antiplasmodial activity of *Eucalyptus globulus* would be due to its high α -Pinene content (35.84%). According to Salehi *et al* (2019) [80], this molecule has excellent antimalarial properties.

Table 4: Anti-plasmodial activity of essential oils on *Plasmodium falciparum*

Essential oils	IC50 (ppm)	Statut
Eugenia uniflora	96,87	low
Psidium littorale	115,45	Inactive
Callistemom rigidus	154,35	Inactive
Eucalyptus globulus	12,13	Medium
Lippia adoensis	141,26	Inactive
Lantana camara	12,34	Medium
Cupressus lusitanica	44,86	Medium
Cupressusse sempervirens	230,31	Inactive
Cupressus macrocarpa	147,29	Inactive

Insecticidal activities of essential oils

Insecticidal tests showed that the sensitivity of *A. gambiae* (laboratory strain) and *A. gambiae s.l.* to the doses of the essential oils is a function of the botanical origin of the essential oil and the place of collection of the anopheles strain.

Regarding the botanical origin of the essential oil, the LC_{95} values as well as the tkd_{50} and tkd_{95} values (at 50 ppm) show that the tested anopheline strains are more sensitive to essential oils of *Syzygium aromaticum* (laboratory strain of *A. gambiae* adult), *Lippia adoensis* (*A. gambiae s.l.* adult), and *Eugenia uniflora* (*A. gambiae s.l.* and laboratory strain of *A. gambiae* old larvae) than the other essential oils (Tables 5, 6, 7). According to [81] Pellecuer *et al.* (1976), the toxicity of an essential oil is strongly related to its chemical composition. The essential oil of *S. aromaticum* is characterised by its high Eugenol content (90.85%). This molecule has been shown to be active on certain crop pests such as *Ceratitis capitata* and *Rhopalosiphum padi* [82]. It is therefore suggested that the high insecticidal activity on *A. gambiae* adults (laboratory strain) is due to Eugenol, which acts either alone or in synergy with other molecules. Some oxygenated monoterpenes such as carvacrol and linalool are also known for their insecticidal activities [83, 84, 85]. These molecules are thought to be responsible for the larvicidal toxicity of *Eugenia uniflora* on *A. gambiae s.l.*. In addition, the high content of Neral (24.74%) and geranial (35.15%) in the essential oil of *Lippia adoensis* is thought to be the cause of the toxicity of this oil towards *A. gambiae s.l.* adults. The biological activity of these compounds was previously mentioned in the *in vivo* evaluation of lemongrass essential oil against *Plasmodium berghei* [12] and in the evaluation of *in vitro* antifungal activity of essential oils of citrus on the mycelial growth of *Phaeoramularia angolensis* [21].

The high sensitivity of the laboratory strain of *A. gambiae* compared to other strains in our study corroborates the work of Fekadu *et al.* (2009) and George and Vincent (2005) [86, 87]. Indeed, Fekadu *et al.* (2009) [86] showed that laboratory strains of *A. arabiensis* larvae were more sensitive to essential oils than field strains. George and Vincent (2005) [87] had similar results on *Culex quinquefasciatus* strains. These observations could be explained by the fact that the larvae collected in the field were on the one hand apparently better adapted to adjust to environmental variations and therefore required a higher concentration of active molecules to cause the required mortality. On the other hand, these field larvae were genetically more heterogeneous and systematically exposed to various insecticides, which gave them a higher general tolerance to toxic compounds [88]. This resistance can also be attributed to the highly polluted nature of the Youpwe district. Indeed, Youpwe is a densely populated area of the city of Douala where the main activities are fishing and trade. Intensive fishing is done with the use of chemicals. Moreover, household waste is not always evacuated by the hygiene services in charge of cleaning up the environment. In many places, there are blocked gutters that prevent the proper circulation of water during the rainy season, leading to the formation of stagnant water that emits foul odours. Recent studies in the city of Douala have shown a strong link between toxic waste and the resistance of malaria vectors to conventional insecticides [89, 90]. It is therefore quite possible that the resistance of Anopheles is associated with the polluted nature of this area.

Table 5: Knockdown times and mortality of adult female *Anopheles gambiae s.l.* exposed to diluted essential oils

Doses	50			
Essential Oils and control	**kdt_{50} (min)**	**kdt_{95} (min)**	**Mortality (%)**	**Susceptibility**
Eugenia uniflora	13.94	72.59	55,33	resistant
Psidium littorale	6.66	22.86	100	sensitive
Lippia adoensis	1.06	7.9	100	sensitive
Lantana camara	5.48	34.37	66.6	resistant
Eucalyptus globulus	5.79	17.33	100	sensitive
Cupressus macrocarpa	1.57	9.07	100	sensitive
Cupressus sempervirens	5.85	10.96	100	sensitive
Syzygium aromaticum	2.42	8.82	100	sensitive
Callistemom rigidus	5.18	14.61	100	sensitive
Negative control				

Table 6: Knockdown times and mortality of adult females of *Anopheles gambiae* (laboratory strain) exposed to diluted essential oils

Doses (ppm)	**50**			
Essential Oils and control	**kdt50 (min)**	**kdt95 (min)**	**Mortality (%)**	**Susceptibility**
Eugenia uniflora	5,6	18,7	100	sensitive
Psidium littorale	4,96	16,73	100	sensitive
Lippia adoensis	3.27	10.92	100	sensitive
Lantana camara	6.32	16.73	96.66	Probable resist
Eucalyptus globulus	5.3	19.1	100	sensitive
Cupressus macrocarpa	5.26	16.5	100	sensitive
Cupressus sempervirens	5.22	16.18	100	sensitive
Syzygium aromaticum	2.34	10.32	100	sensitive
Callistemom rigidus	6.84	23.25	96.66	Probable resist
Negative control	0	0	0	-

Table 7: Lethal concentration 95 (LC95) and mortality (%) of *Anopheles gambiae s.l.* larvae exposed to diluted essential oils

			Larval mortality (%)							
	LC95 (ppm)		***A. gambiae s.l.***				***A. gambiae***			
Essential Oils and control	**Old larvae of *A. gambiae s.l***	**Old larvae of *A. gambiae***	**50 ppm**	**100 ppm**	**150 ppm**	**p**	**50 ppm**	**100 ppm**	**150 ppm**	**p**
Eugenia uniflora	35,4	35.4	98.3	100	100	0.74	98.3	100	100	0.74
Lippia adoensis	86,03	53.13	66	95	96.6	0.005	90	100	100	0.06
Psidium littorale	88,68	66.78	81.6	93.3	98.3	0.36	88.3	98.3	100	0.02
Syzygium aromaticum	119,25	55	1	26	28.3	0.06	88.3	100	100	0.06
Eucalyptus globulus	141,27	138.53	13	78	91.6	0.04	0.55	83.3	96.6	0.03
Cupressus macrocarpa	267,55	78.35	36	85	91.6	0.06	76.6	95	100	0.04
Lantana camara	270,03	136.75	83	90	95	0.005	53.3	86.6	96.6	0.02
Cupressus sempervirens	315,17	81.03	75	80	95	0.03	78.3	90	100	0.02
Cupressus lusitanica	502,05	90.52	76	85	91.6	0.04	65	80	100	0.02
Callistemom rigidus	779,15	990.94	0	1	10	0.07	43.3	48.3	71.6	0.05
Negative control	-	-	0	0	0	-		0	0	-

Conclusion

The aim of this research was to characterise and determine the insecticidal and anti-plasmodial properties of the essential oils of ten plants from the Cameroonian pharmacopoeia traditionally used for their repellent effects against mosquitoes and their effectiveness in the treatment of malaria. The results showed that these oils are made up of a mixture of molecules whose number, nature and proportions depend on the plant species. Anopheles adults were found to be more sensitive to the essential oils of *Syzygium aromaticum* and *Lippia adoensis*, while their larvae were more sensitive to the essential oils of *Eugenia uniflora*. Furthermore, the essential oils of *Eucalyptus globulus* and *Lantana camara* appear to be the most active against *P. falciparum in vitro*. Their richness in bioactive molecules and their obvious accessibility in the local environment make these five plants potential raw materials for the development of new biocides and natural antimalarials.

Acknowledgement

The authors are grateful to the communities of Youpwe (Douala) for their collaboration.

References

1. WHO. Rapport mondial sur le paludisme, Genève, Suisse, 2010, 24.
2. WHO. Rapport mondial sur le paludisme, Genève, Suisse. 2012, 24.
3. WHO. Points essentiels: Rapport sur le paludisme dans le monde. 2017.
4. Bouyahyaoui A. Contribution à la valorisation des substances naturelles: Etude des huiles essentielles des cupressacées de la région de l'Atlas algérien. Thèse université de Mostaganem, Algérie. 2017, 115.
5. Etang J, Chandre F, Guillet P, Manga L. Reduced bio-efficacy of permethrin EC impregnated bednets against an Anopheles gambiae strain with oxidase-based pyrethroid tolerance. Malaria Journal. 2004;3:46-52.
6. Overgaard HJ, Reddy VP, Abaga S, Matias A, Reddy MR, Kulkarni V, *et al*. Malaria transmission after five years of vector control on Bioko Island, Equatorial Guinea. Parasites & Vectors. 2012;5:253-266.
7. Nwane P, Etang J, Chouaibou M, Toto J, Koffi A, Mimpfoundi R, *et al*. Multiple insecticide resistance mechanisms in *Anopheles gambiae* s.l. populations from Cameroon, Central Africa. Parasit Vectors. 2013;6:41–54.
8. Yang YC, Lee SG, Lee HK, Kim MK, Lee HS. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. Journal of Agricultural and Food Chemistry. 2002;50(13):3765-3767.
9. Kiran R, Bhavani K, Devi ST, Rajeswara RBR, Janardhan RK, Show M. Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi*. Bioresource Technology. 2006;97(18):2481-2484.
10. Senthikumar A, Kannathasan, Venkatesalu. Chemical constituents and larvicidal property of essential oil of *Blumea mollis* (D.Don) Merr. Against *Culex quinquefasciatus*. Parasitolology Research 2008;103(4):959-962.
11. Amvam ZPH, Bivity L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet P. Aromatic plants of tropical central Africa, Part XXXII: chemical composition and antifungal

activity of thirteen essential oils from aromatic plant of Cameroon. Flavour and Fragrance Journal. 1998;13:107-114.
12. Tchoumbougnang F, Amvam ZPH, Dagne E, Mekonnen Y. *In vivo* antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* on mice infected with *Plasmodium berghei*. Planta medica. 2005;71:20-23.
13. Bigoga JD, Saahkem PA, Ndindeng SA, Ngondi JL, Nyegue M, Oben JE *et al*. Larvicidal and Repellent Potential of *Chenopodium ambrosioides* Linn Essential Oil against *Anopheles gambiae Giles* (Diptera: Culicidae). The Open Entomology Journal. 2013;7:16-22.
14. Akono NP, Baldovini N, Mouray E, Mambu L, Belong P, Grellier P. Activity of *Ocimum basilicum*, *Ocimum canum*, and *Cymbopogon citratus* essential oils against *Plasmodium falciparum* and mature-stage larvae of Anopheles funestus S.S. Parasite. 2014;21:33. Doi: 10.1051/parasite/2014033.
15. Akono NP, Jazet DPM, Tonga C, Kouotou S, Kekeunou S, Magne GT, *et al*. Larvicidal activity of essential oils from pericarps of ripe Citrus fruits cultivated in Cameroon on pyrethroids sensitive and resistant strains of *Anopheles gambiae Giles*, 1902. Journal of Entomology and Zoology Studies. 2015;3(4):334-339.
16. Kassir JT, Mohsen ZH, Mehdi NS. Toxic effect of limonene against *Culex quinquefasciatus* Say larvae and its interference with oviposition. Anzeiger fur Schadlingskunde Pflanze-Nschutz Umweltschutz. 1989;62:19-21.
17. Mansour SA., El-Sharkawy AZ, Ali AR. Mosquitocidal activity of Citrus peel oils with respect to their limonene content. Journal of Natural Toxins. 2004;1:111-134.
18. Boyom F, Ngouana V, Amvam ZPH, Menut C, Bessiere JM, Gut J, *et al*. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry. 2003;64:1269-1275.
19. Derwich E, Benziane Z, Boukir A. Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. International Journal of Agriculture and Biology. 2010;12:199-204.
20. Dambolena JS, Zunino MP, López AG, Rubinstein HR, Zygadlo JA, Mwangi JW *et al*. Essential oils composition of *Ocimum basilicum* L & *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*. Innovative Food Science and Emerging Technologies. 2010;11:410-414.
21. Jazet DPM, Kuate J, Boyom FF, Ducelier D. Composition chimique et activité antifongique *in vitro* des huiles essentielles de Citrus sur la croissance mycélienne de *Phaeoramularia angolensis* Cambridge University Press. 2002. https://doi.org/10.1051 /fruits:200200
22. Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. International Journal of Food Microbiology. 2004;94:223-253.
23. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological Effects of Essential Oils--a Review. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association. 2008;46(2):446-475.
24. Foko G, Tamesse J, Messi J. Insecticidal effects of *Capsicum annuum* on *Anopheles gambiae* Giles under laboratory conditions. Journal of Entomology. 2007;4(4):299-307.
25. Tchoumbougnang F, Dongmo PMJ, Sameza ML, Mbanjo EGN, Fotso GBT, Zollo PHA. Larvicidal activity against *Anopheles gambiae* Giles and chemical composition of essential oils from four plants cultivated in Cameroon. Biotechnology, Agronomy, Society and Environment. 2009;13(1):77-84.
26. Parh IA, Forbuzo BC, Matheney EL, Ayafor JF. Plants used for control of insects pests on stored grains in parts of North west Highland Savannah Zone of Cameroon. Sciences Agronomique et Développement. 1998, 54-60.
27. Tapondjou LA, Bouda H, Fontem DA, Zapfack L, Lontsi D, Sondengam BL. Local plants used for traditional stored product protection in the Menoua division of the western highland of Cameroon. Integrated Protection of Stored Products, IOBC Bulletin, 2000;23(10):73-77.
28. Mpondo EM, Dibong SD, Pouha M. Etude Ethnobotanique des plantes médicinales utilisées dans le département du Haut-Nkam (Sud Cameroun) International Journal of Innovation and Applied Studies. 2017;21:574-595.
29. Adams RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy (essential oils). Illinois USA: Allured Publishing Corporation. 2001;3:9-456.
30. Service MW. Mosquito ecolology: fields sampling methods. Applied science publishers, London, 1976, 583.
31. Desfontaines M, Tchikangwa I, Le Goff G, Robert V, Carnevale P. Influence de l'alimentation des larves d'*Anopheles gambiae* (Diptera, Culicidae) sur le développement préimaginal en insectarium. Bulletin de liaison et de documentation de l'O.C.E.A.C. 1991;98:12–14.
32. Gillies MT, De Meillon B. The Anophelinae of Africa, south of the Sahara (Ethiopian Zoogeographical Region). Publications of the South African Institute for Medical Research. 1968;54:1-343.
33. Gillies MT, Coetzee MA. Supplement to the Anophelinae of Africa south of the Sahara.The South African Institute for Medical Research, Johannesburg. 1987, 143.
34. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193:673-675.
35. WHO. Bioassay method for the titration of *Bacillus sphaericus*: consultation on the developpement of *Bacillus sphaericus* as a microbial larvicide. World Health Organisation. 1985;3:85-95.
36. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organisation. 2013;18:40.
37. Desjardins RE, Graig JC, David H, Jeffrey DC. Quantitative assessment of antimalarial activity *in vitro* by a semi-automated micro-dilution technique. Antimicrobial Agents and Chemotherapy. 1979;16:710-718.
38. Guillon J, Moreau S, Mouray E, Sinou V, Forfar I, Belisle-Fabre S *et al*. New ferrocenic pyrrolo[1,2-a] quinoxaline derivatives: synthesis, and *in vitro* antimalarial activity. Bioorganic & Medicinal Chemistry. 2008;16:9133-9144.
39. Ogunjinmi OE, Olawore NO, Aliyu AA. Comparative studies of Chemical Constituents and Antimalarial

Activity of Essential oils Extracted from the Stem, Root and Fruit peel of *Citrus paradisi* Grown in Nigeria. IOSR Journal of Applied Chemistry. 2017;10:01-11.
40. Vieira RF, Simon JE. Chemical Characterization of Basil (*Ocimum* spp.) Found in the Markets and Used in Traditional Medicine in Brazil Economic Botany. Published By: Springer. 2000, 207-216.
41. Probst IS. Atividade Antibacteriana de óleos essenciais e avaliação de potencial sinérgico. Dissertação de Mestrado – Programa de Pós-Graduação em Biologia Gerale Aplicada, Universidade Estadual Paulista, 2012, 112.
42. Razafimamonjison G, Jahiel M, Duclos, Ramanoelina P, Fawbush F, Danthu P. Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. International Journal of Basic and Applied Sciences. 2014;3(3):224-233.
43. Hamad A, Mahardika MGP, Yuliani I, Hartanti D. Chemical constituents and antimicrobial activities of essential oils of *Syzygium polyanthum* and *Syzygium aromaticum*. Rasayan Journal of Chemistry. 2017;10(2):564-569.
44. Bhuiyan MNI, Begum J, Nandi NC, Akter F. Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston). African Journal of Plant Science. 2010;4(11):451-454.
45. Soro LC, Grosmaire L, Ocho-Anin AAL, Munier S, Menut C, Pelissier Y. Variabilité de la composition chimique de l'huile essentielle des feuilles de *Lippia multiflora* cultivées en Côte d'Ivoire. Journal of Applied Biosciences. 2015;88:8180-8193.
46. Kanko C, Sawaliho BE-H, Kone S, Koukoua G, N'Guessan YT. Étude des propriétés physico-chimiques des huiles essentielles *de Lippia multiflora*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon giganteus*. Comptes Rendus Chimie. 2004;7:1039-1042.
47. Konan JLK, Turquin L, Attah H, Yao-Kouame A, Allou K, Ake S. *Lippia multiflora* (verbenaceae) en Côte d'Ivoire : point des premiers resultats de recherche et enjeu cultural. Agronomie Africaine. 2010;22(2):121-130.
48. Babarinde SA, Oladoye SO, Adesina GO, Adebayo MA. Chemical Composition and Bioactivity of *Lippia adoensis* Hochst ex. Walp (Verneneaceae) Leaf essential oil against *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae). Journal of Northeast Agricultural University (English Edition). 2016;23(4):8-14.
49. Malizia RA, Cardell DA, Molli JS, González S, Guerra PE, Grau RJ. Volatile constituents of leaf oils from the Cupressaceae family: part I. *Cupressus macrocarpa* Hartw. *C. arizonica* Greene and *C. torulosa* Don species growing in Argentina. Journal of Essential Oil Research. 2000;12:59-63.
50. Adams RP. Geographic variation in the leaf essential oils of *Hesperocyparis* (cupressus) *abramsiana, H. goveniana* and *H. macrocarpa*: Systematic implications. Phytologia. 2009;91(1):226-243.
51. Athanassios G, Danae P, Fotini P, Dimitrios PP, George KN, Olga E *et al*. Essential oil composition, adult repellency and larvicidal activity of eight Cupressaceae species from Greece against *Aedes albopictus* (Diptera: Culicidae). Parasitology Research, 2013;112:1113-1123.
52. Chéraif I, Ben JH, Hammami M. Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica* Greene. Biochemical Systematics and Ecology. 2007;35(12):813-820.
53. Boukssaim H, Ghanmi M, Satrani B, Aafi A, Aberchane M, Khia AMSB *et al*. Caractérisation chimique et microbiologique des huiles essentielles des rameaux, des cônes et du bois de *Cupressus atlantica*, arbre forestier endémique du Maroc. Phytothérapie. 2013;11:294-300.
54. El-Ghorab AH, El-Massry KF, Shaaban HA. Effect of Drying on the Chemical Composition of the Egyptian *Cupressus macrocarpa* (Hartw.ex Gordon) Essential Oils and their Biological Characteristics. Journal of essential oil bearing plants. 2007;10(5):399-411.
55. Saad AM, Mohammed MMD, Mosad A, Ghareeb WS, Ahmed MAF. Chemical Composition and Antimicrobial Activity of the Essential Oil of the Leaves of *Cupressus macrocarpa* Hartweg. ex Gordon. Journal of Applied Pharmaceutical, 2017;7(9):207-212.
56. Abdel-Hady NM, Abdei-Halim AS, Al-Ghadban AMJ. Chemical composition and insecticidal activity of the volatile oils of leaves and flowers of *Lantana camara* L. cultivated in Egypt. Journal of the Egyptian Society of Parasitology. 2005;35(2):687-98.
57. Satyal P, Crouch RA, Monzote LA, Cos P, Awadh ANA, Mehdi AAHX *et al*. The chemical diversity of *Lantana camara*: analyses of essential oil samples from Cuba, Nepal, and Yemen. Chemistry & biodiversity. 2016;13(3):336-342.
58. Sousa EO, Colares AV, Rodrigues FFG, Campos AR, Lima SG, Costa JGM. Effect of Collection Time on Essential Oil Composition of *Lantana camara* Linn (Verbenaceae) Growing in Brazil Northeastern. Records of Natural Products. 2010;4(1):31-37.
59. Scur MC, Pintoa FGS, Pandinia JA, Costab WF, Leiteb CW, Temponi LG. Antimicrobial and antioxidant activity of essential oil and different plant extracts of *Psidium cattleianum* Sabine. Brazilian Journal of Biology. 2016;76(1):101-108.
60. Marques FA, Wendler EP, Sales MBHLN, Coffani-Nunes JV, Campana J, Guerrero JPG. Volatile oil of sabine from the Brazilian Atlantic Fores. *Psidium cattleianum*. Journal of Essential Oil Research. 2008;20(6):519-520.
61. Soliman FM, Fathy MM, Salama MM, Saber FR. Comparative study of the volatile oil content and antimicrobial activity of *Psidium guajava* L & *Psidium cattleianum* Sabine leaves. Bulletin of Faculty of Pharmacy, Cairo University. 2016;54:219-225.
62. Adam F, Vahirua-Lechat I, Deslandes E, Menut C. Aromatic Plants of French Polynesia. V. Chemical Composition of Essential Oils of Leaves of *Psidium guajava* L. and *Psidium cattleyanum* Sabine. Journal of Essential Oil Research. 2011;23(1):98-101.
63. Adams RP, Zanoni TA, Lara A, Barrero AF, Cool LG. Comparison among *Cupressus arizonica* Greene, *Cupressus benthamii* Endl., *Cupressus lindleyi* Klotz. Ex Endl. and *Cupressus lusitanica* Mill. using leaf essential oils and DNA finger printing. Journal of Essential Oil Research. 1997;9(3):303-309.
64. Kuiaté JR, Bessière JM, Vilarem G, Amvam ZPH. Chemical composition and antidermatophytic properties of the essential oils from leaves, flowers and fruits of *Cupressus lusitanica* Mill. from Cameroon. Flavour and

Fragrance Journal. 2006;21:693-697.
65. Teke GN, Kemadjou NE, Kuiate JR. Chemical composition, antimicrobial properties and toxicity evaluation of the essential oil of *Cupressus lusitanica* Mill. leaves from Cameroon. BMC complementary and alternative medicine. 2013;13:130.
66. Jahani M, Akaberi M, Khayyat MH, Emami SA Chemical Composition and Antioxidant Activity of Essential Oils from *Cupressus sempervirens*. var. *sempervirens*, *C. sempervirens*. cv. Cereiformis and *C. sempervirens* var. horizentalis. Journal of Essential Oil Bearing Plants. 2019;22(4):917-931.
67. Nacira A, Yousra B. Activité Antimicrobienne de l'Huile Essentielle du Cyprès Vert (*Cupressus Sempervirens* L.). Algerian Journal of Natural Products. 2017;5(2):455-462.
68. Maciel MV, Morais SM, Bevilaqua CML, Silva RA, Barros RS, Sousa RN *et al*. Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. Veterinary Parasitology. 2010;167:1-7.
69. Barbosa LCA, Filomeno CA, Teixeira RR. Chemical Variability and Biological Activities of Eucalyptus spp. Essential Oils Molecules. 2016;21(12):1671. Doi: 10.3390/molecules21121671.
70. Kassahun A, Feleke G. Chemical Composition and Physico-Chemical Analysis of *Eucalyptus Globulus*. Leave and Oil Science Journal of Chemistry. 2019;7(2):36-38.
71. Xiao-duo J, Quan-long P, Garraffo HM. The Essential Oil of the Leaves of *Callistemon rigidus* R. Br. Journal of Essential Oil Research. 1991;3:465-466.
72. Jazet PMD, Tatsadjieu LN, Ndongson BD, Kuate J, Zollo APH, Menut C. Correlation between chemical composition and antifungal properties of essential oils of *Callistemon rigidus* and Callistemon citrinus of Cameroon against *Phaeoramularia angolensis*. Journal of Medicinal Plants Research. 2009;3(1):9-15.
73. Gallucci S, Neto AP, Porto C, Barbizan D, Costa I, Marques K *et al*. Essential Oil of *Eugenia uniflora* L.: an Industrial Perfumery Approach. Journal of Essential Oil Research. 2010;22:176-179.
74. Klinger A da FR, Layane VA, Jamylla MGde O, Clarice ND, Denise FCM, Eloisa Hde AA *et al*. *Eugenia uniflora* L. Essential Oil as a Potential *Anti-Leishmania* Agent: Effects on *Leishmania amazonensis* and Possible Mechanisms of Action. Evidence-Based Complementary and Alternative Medicine, 2013, 279726.
75. Sviech F, Pianoski KE, Kruger RL, Valescakotovicz BEM, Sakata GSB, Bombardelli MCM. Biological activity of essential oil of pitanga (*Eugenia uniflora* L.) leaves. Boletim Centro de Pesquisa de Processamento de Alimentos, 2018; https://revistas.ufpr.br/alimentos/article/view/58308. Consulté le 12 février 2020.
76. Menezes AMS, Almeida FRC, Rao VSN, Matos MEO. Anti-inflammatory activity of the essential oil of *Vanillosmopsis arborea*. Fitoterapia. 1990;61:252-254.
77. Christensen SB, Kharazmi A. Bioactive compounds from natural sources: isolation, characterization and biological properties. London: Taylor & Francis. 2001, 379-432.
78. Zyl RLV, Seatlholo ST, Viljoen AM. Pharmacological interactions of essential oil constituents on the *in vitro* growth of *Plasmodium falciparum*. South African Journal of Botany. 2010;76:662-667.
79. Chinnaperumal K, Govindasamy B, Chinnadurai S, Manickam R. Nanoparticules d'Ag synthétisées à l'aide de beta caryophyllène isolé de Murraya koenigii : antipaludéen (*Plasmodium falciparum* 3d7) et activité anticancéreuse (lignées cellulaires A549 et HeLa). Journal of Cluster Science. 2017;28:1667-1684.
80. Salehi B, Upadhyay S, Orhan IE, Jugran AK, Jayaweera SLD, Dias DA *et al*. Therapeutic Potential of alpha and beta Pinene: A miracleGift of Nature. Biomolecules. 2019;9(11):738.
81. Pellecuer J, Allegrini J, de Buochberg SM. Huiles essentielles bactéricides et fongicides. Revue de l'Institut Pasteur de Lyon. 1976;9:135-159.
82. Hamraoui A, Regnault-Roger. Comparaison des activités insecticides des monoterpènes sur deux espèces d'insectes ravageurs des cultures: *Ceratitis capitata* et *Rhopalosiphum padi*. Acta botanica. 1997;144(4):413-417.
83. Kim DH, Ahn YJ. Constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. Pest Management Science. 2001;57:301-306.
84. Park BS, Lee SE, Choi WS, Jeong CY, Song C, Cho KY. Insecticidal and acaricidal activity of piperonaline and piperoctadecalidine derived from dried fruits of *Piper longum* L. Crop Protection. 2002;21:249-251.
85. Papachristos DP, Karamanoli K, Stamopoulos DC, Menkissoglu-Spiroudi U. The relationship between the chemical composition of three essential oils and their Insecticidal activity against *Acanthoscelides obtectus* (Say). Pest Management Science. 2004;60(5):514-520.
86. Fekadu M, Mekuria T, Tesfaye B, Meshesha B, Teshome G-M. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. African Journal of Biotechnology. 2009;8(17):4183-4188.
87. George S, Vincent S. Comparative efficacy of *Annona squamosal* Linn & *Pongamia glabra* Vent to Azadirachta indica A. Juss against mosquitoes. Journal of Vector Borne Disease. 2005;42:159-163.
88. Kabir KE, Khan AR, Mosaddik MA. Goniothalamin- a potent mosquito larvicide from *Bryonopsis laciniosa* L. Journal of Applied Entomology. 2003;127:112-115.
89. Antonio-Nkondjio C, Tene FB, Ndo C, Menze DB, Zebaze TS, Awono AP *et al*. *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution. Malaria Journal. 2011;10:154.
90. Antonio-Nkondjio C, Defo-Talom B, Tagne-Fotso R, Tene-Fossog B, Ndo C, Lehman LG *et al*. High mosquito burden and malaria transmission in a district of the city of Douala, Cameroon. BMC Infectious Diseases. 2012;12:275.