

Full Length Research Paper

Analysis by GC (I_r), 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

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The objectives of this study were to determine the chemical composition and to evaluate the mosquito repellent property of essential oils extracted from the bark of the stem of *Sterculia tragacantha* Lindl on *Anopheles gambiae*, vector of malaria. The essential oil of the stem bark of *S. tragacantha* Lindl is extracted by hydrodistillation and their chemical compositions were identified by GC (I_r) and GC-MS. This oil has been tested on sensitive « kisumu » strains of *A. gambiae* adults, according to the World Health Organization (WHO) guidelines for laboratory testing of the repellency of chemicals. The extraction yield obtained from this oil is 0.12%. By means of GC (I_r) and GC-MS, 29 compounds representing 88.4% of the HE were identified. β-elemol (40.54%) is the major compound of this oil. It is rich in sesquiterpenes (79.47%) with a predominance of oxygenated sesquiterpenes (55.86%). As for the oxygenated monoterpenes, they represent 8.93%. The repellency test against adult female *Anopheles*, revealed that the EO of *S. tragacantha* (10%) has repellent properties against mosquitoes. Indeed, it induces an average reduction of 98.2% of the blood meal (TIRS) of mosquitoes on guinea pigs with a complete protection time (CPT) of 5 h compared to 3 h 30 min for the natural reference substance (*Cymbopogon citratus*). In view of its extractive value and prolonged repellent properties, *S. tragacantha* could be a good alternative in the vector borne diseases control if used as natural repellents for skin application.

Key words: *Anopheles gambiae*, *Sterculia tragacantha* Lindl, essential oil, chemical composition, mosquito repellent.

INTRODUCTION

In recent years, global health authorities have alerted us to the worrying upsurge in vector-borne diseases.

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These pathologies, transmitted from one individual to another via a vector, are at the origin of many historical pandemics. Today, malaria is the most important of these diseases in such a way that 3.2 billion people are at risk, representing half of the world's population (WHO, 2015). It thus constitutes a real public health problem for the underprivileged tropical areas of Africa, Asia and Latin America. Children under 5 and pregnant women are the most vulnerable because, for example, a child dies from malaria every 30 s in Africa (WHO, 2019). In Côte d'Ivoire, malaria accounts for approximately 32.53% of all causes of mortality (Kouadio et al., 2006) and in 2020 remains the leading cause of consultations in health centers (MSHP, 2020). Malaria is an infectious parasitosis caused by a protozoan *Plasmodium* transmitted to humans by an infected female mosquito of the genus *Anopheles* (Pasteur Institute, 2021).

The current national strategy to eradicate this disease in Côte d'Ivoire is based on vector control which consists of the distribution and use of long-acting insecticide-treated mosquito nets and spraying intra-domiciliary pesticides in highly endemic areas to supplement the use of impregnated mosquito nets (MSHP). Unfortunately, these synthetic chemicals have harmful effects on humans and their environment because of their persistence in the environment (Corbel et al., 2009; Fradin and Day, 2002; Paluch et al., 2010). But overall, they may be faced with limited action following resistance developed by vectors (Chandre et al., 1999; Lundwall et al., 2005; Katz et al., 2008); hence, the interest in finding other arsenals of natural active ingredients. Our choice fell on *Sterculia tragacantha*, a plant traditionally used in the south-east of Côte d'Ivoire in the lagoon region as an anti-mosquito repellent in order to extract its essential oil, to study its chemical composition and to compare its repellent activity to that of *Cymbopogon citratus* taken as a natural reference substance because it is recognized for its insect repellent properties (Moore et al., 2007; Pushpanathan et al., 2006).

METHODOLOGY

Plant and biological material

The plant material is composed of essential oils extracted from the bark of the stem of *S. tragacantha* collected on April 27, 2019 in the Department of Adzopé (latitude: 6.1069400 and longitude - 3.8619400) and leaves of *C. citratus* (reference substance) collected in Bingerville (50 21'20.9 " North, 30 53'7.3 ") in the district of Abidjan on April 29, 2019. These plants were identified at the National Floristic Center of the University Félix Houphouët Boigny of Côte d'Ivoire.

The repellency was evaluated using a bio test on unfed females of *Anopheles gambiae* sensu lato aged 5 to 7 days after emergence (Kisumu strains sensible to insecticides) from the medical entomology laboratory of Swiss Center of Scientific Research in Ivory Coast (SCSR).

Extraction of essential oils

The leaves or husks dried for 3 to 4 days under air conditioning were used for extraction. Thus a quantity (0.5 kg) of the plant organ (leaves or shell) was placed on a grid, kept about 5 cm from the bottom of the casserole dish, above the water level in a pressure cooker with a capacity of 10 L containing 1 L of water connected to the Clevenger type device. Using a hot plate, the pressure cooker was heated to a boil. The duration of the extraction for each sample is 3 h 30 min from the time of obtaining the first drop of EO for each of the two plants. During this time the water vapor destroys the structure of plant cells, releases the molecules contained and carries these volatile components to the cylindrical column and then to the refrigerant where the gas phase condenses. The water-essential oil mixture obtained is collected in a test tube through the gasoline. The essential oil separated from the water by decantation is removed using a Pasteur pipette in a tube and then dried over anhydrous sodium sulfate (NaSO₄). Finally, the essential oils of each plant organ are collected separately in glass bottles and then stored in the refrigerator at 4°C. Essential oils were coded S and C, respectively for *S. tragacantha* and lemongrass.

The essential oil yield is the ratio between the mass of oil extracted and the mass of the plant material used for hydrodistillation (AFNOR, 2000). The yield (R) expressed as a percentage of the two organs was calculated by the following formula:

$$R (\%) = (\text{Mass of essential oil extracted (g)} / \text{Mass of plant material (g)}) \times 100$$

Analysis by GC (I_r)

The analyses were carried out using a gas chromatograph of the Perkin-Elmer Clarus 500 type, equipped with a divider injector, two columns of nonpolar and polar silica 50 × 0.22 mm in internal diameter, film thickness 0.25 µm, filled with polydimethylsiloxane (C₂H₆O_{Si})_n and polyethylene glycol, respectively, and two flame ionization detectors.

The operating conditions are as follows: the carrier gas is hydrogen; the pressure at the top of the column is 20 psi; the temperature of the injector and the detectors is 250°C. The temperature program is 60°C up to 220°C at a rate of 2°C/min, with a step of 20 min at 220°C; the injection was done by divider mode with a 1/60 ratio. The amount of sample injected is 0.5 µL from a solution containing 50 µL of EO in 350 µL of CDCl₃.

The volatile substances were identified by comparing their retention indices (I_r) on polar and non-polar columns. These I_r were calculated from the retention times of the separated compounds and the linear n-alkanes (Browning, 1971).

Analysis by GC/MS

GC/MS analyses were performed using a Perkin Elmer autosystem XL chromatograph coupled to a Perkin Elmer Turbo Mass detector. The chromatograph is equipped with an automatic injector and an apolar column (Rtx-1) with an internal diameter of 60 m × 0.22 mm with a film thickness of 0.25 µm.

The carrier gas is hydrogen (1 mL) which exerts a column head pressure of 25 psi. The temperature of the injector is 250°C and that of the detector 280°C. The temperature is programmed at 60°C followed by an increase to 230°C at a rate of 2°C/min, with a plateau of 45 min at 230°C. The injection is done by split mode with a division ratio of 1/50. The amount of sample injected is 0.2 µL. Mass spectra are obtained using a quadrupole filter detector and

ionization is done by electron impact with a 70 eV electron beam.

Identification of constituents of EO

The identification of EO compounds was made by:

- (1) The comparison of their GPC retention indices, obtained on polar and non-polar columns (Ira and Irp), determined as a function of the retention times of a series of n-alkanes by linear interpolation with those of the authentic compounds or of the data literature;
- (2) The correspondence of their spectral data with those of commercial mass spectra libraries and the laboratory (König et al., 2004; Adams, 2007; McLafferty, 1994).

For the analysis of the spectroscopic data, the AMDIX32 processing software was used. The Automatic Mass Spectral Deconvolution and Identification System (AMDIS) allows you to automatically find any of a set of target compounds in a gas chromatography/mass spectrometry (GC/MS) data file. The program first deconvolutes the GC/MS data file to find all of the separate components. Each of these components is then compared against a library of target compounds. The match factor between the target spectrum and the deconvoluted component spectrum is then reported, if it is above a user set value, and the matched target spectrum is called a library hit.

Biological test

The main experiments were carried out according to the guidelines of the World Health Organisation (WHO) related to laboratory testing of the repellency of chemicals (WHO, 2009, 2013). The general objective of these laboratory studies is to estimate the effective dose of a repellent and the complete protection time provided by a repellent after application to the skin by determining the specific objectives which are:

- (1) The dose-response and effective dose of the repellent;
- (2) Protection (p);
- (3) Full protection time (CPT)

This test is preferably performed on human subjects (final users of the repellent) in order to obtain results that are relevant to the actual conditions of use (WHO, 2013). Laboratory animals were chosen in this study to perform the first tests for safety and then apply to human skin if the product is found to be effective.

Mosquito breeding

Mosquito rearing was carried out according to the standard procedure described in the book for participant guide entitled Malaria Entomology and Vector Control (WHO, 2014). Indeed, instead to use wild strains which were likely in contact with repellents or synthetic insecticides and might carry some resistance genes, sensible strains from the medical entomology laboratory of SCSR were used. Mosquito larvae were reared with ground kibbles for cats and then kept at 27°C plus or minus 3°C and at 80 plus or minus 10% relative humidity under a photoperiod at 12:12 (night: day). The adults were kept in cages, and fed with a 10% sucrose solution (Figure 1A), but 24 h before the experiments, the sucrose solution was removed from the female mosquito storage cages (Figure 1B).

Repellency test

Initially, the readiness of mosquitoes to land and/or probe was assessed by holding an untreated Guinea pig abdominal skin (that is, treated with simple petroleum jelly) kept still on top of a first cage, whose shaved face was exposed to mosquitoes for 30 s or until that 2 to 5 landings/soundings are counted. The procedure is repeated with another untreated animal in a second cage. If this landing and/or sounding level is not reached in either cage, the experiment must be abandoned (WHO, 2013).

Repulsion test

Each essential oil was used to make two ointments of 5 and 10% concentration which are tested to identify the most effective dose. Ointments formulated with the EO of *S. tragancatha* have been coded PS₅ and PS₁₀. Those designed with the essential oil of the reference substance *C. citratus* at concentrations of 5 and 10% noted PC₅ and PC₁₀. So for each test, 10 unfed female mosquitoes were placed in different cages. A 5x8 cm² area of abdomen hair was removed from laboratory-reared male Guinea pigs weighing an average of 400 to 500 g then washed, ethanol cleaned and dried. PC₅, PC₁₀, PS₅ and PS₁₀ ointments were applied to different animals. Each treated animal was kept on top of the cage in which the treated face is exposed to mosquitoes for 7 h. The number of bites was counted by examining the abdomen of all mosquitoes for blood fed. Thus, the number of bitches in the trials was recorded every minute for the first 15 min and then every 15 min for 7 h. Each test was repeated 2 times while using new mosquitoes and a new animal (WHO, 2009, 2013).

Data analysis

The parameters for evaluating the effectiveness of the products are determined as follows (WHO, 2009, 2013):

- (1) The dose-response lines and the effective doses (ED) of a repellent corresponding to 50% (ED₅₀) and 99.9% (ED_{99.9}) of protection against landing and/or detection of mosquitoes. They are estimated by calculating the protection P.
- (2) The protection P offered by the ointments for each dose was calculated according to the following formula:

$$P = (1 - (T / C)) \times 100 = ((C - T) / C) \times 100$$

where T: number of mosquitoes on the treated animal and C: number of mosquitoes on the control animal.

- (3) Full Protection Time (CPT) is calculated as the number of minutes from the time of repellent application to the first landing and/or probing of the mosquito.

For the calculation of these parameters, data processing software was used. This is Kaplan Meier's online survival function calculator.

Entomological standards of efficacy are defined by the WHO and state that:

- (1) If a product has protection P < 90% then it is ineffective.
- (2) If a product has P ≥ 90% protection then it is effective.
- (3) If several products have P ≥ 90% protection, the most effective at the longest CPT.

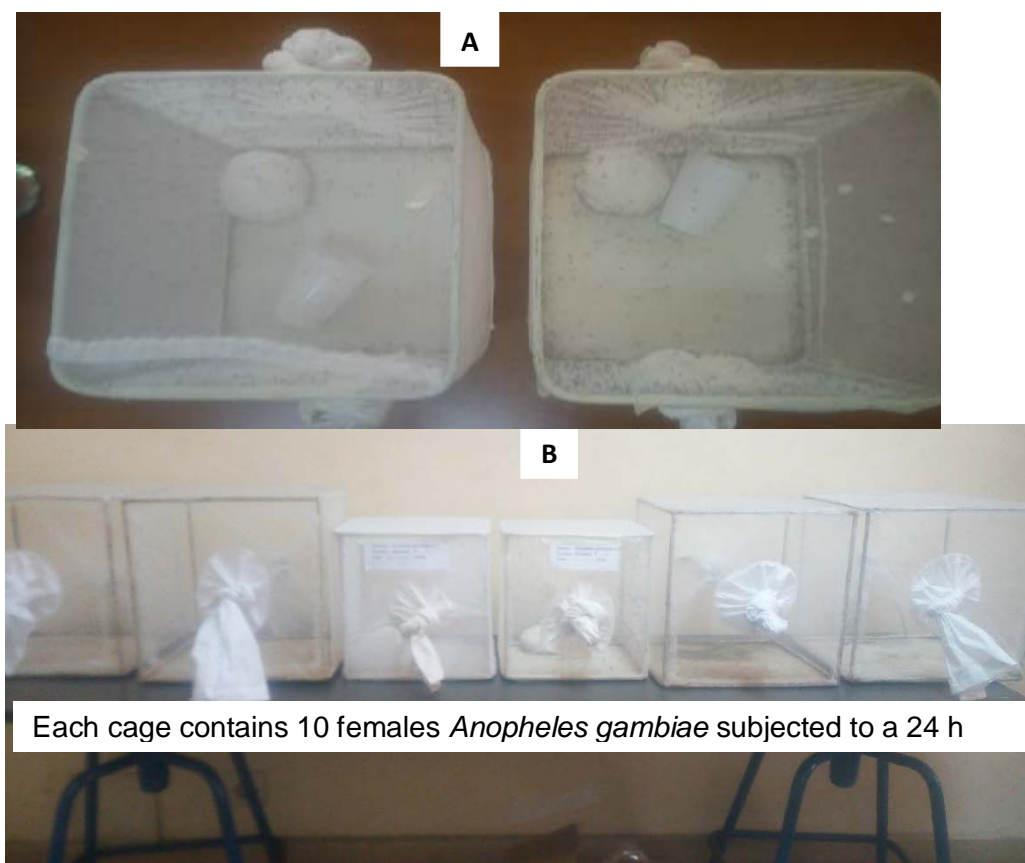


Figure 1. Conditioning cage of adults *Anopheles gambiae*.

RESULTS AND DISCUSSION

Extraction yield

The essential oil extracted from the stem bark of S-coded *S. tragacantha* is light yellow with an aromatic odor. This oil is liquid at room temperature. The oil is extracted with a yield of 0.12%. According to AFNOR standards (2008), the stem barks of *S. tragacantha* are rich in essential oil. The extraction yield obtained in our study is lower than that reported by Aboaba et al. (0.73%) working on the species from Nigeria.

Chemical composition of essential oils

Chemical composition of S

Constituents (29) were identified in S representing 88.4% of its chemical composition (Table 1). A major compound (β -elemol 40.54%) and 4 compounds of high content (E) - caryophyllée 8.14% and α -copaene 7.94%, α -eudesmol 4.74% and β -eudesmol 4.45% have been revealed.

These dominant compounds identified in the study are different from those reported by Aboaba et al. (2016). They are hexahydrofarnesylacetone (32.5%), viridiflorol (18.9%) and 1,8-cineole (14.79%). This difference can be linked to several factors such as climate, harvest period and area, etc.

The essential oil is rich in sesquiterpenes (79.47%) with a predominance of oxygenated sesquiterpenes (55.86%). As for the oxygenated monoterpenes, they represent 8.93% (Figure 2).

Chemical composition of reference substance C

Analysis of the chemical composition of C identified 13 compounds representing 92.05% of the compounds (Table 2). Four (4) major compounds emerge, namely geranial (35.92%), neral (28.24%), myrcene (13.90%) and geraniol (6.14%).

Essential oil C consists only of monoterpenes, of which 78.05% are oxygenated monoterpenes and 14.46% hydrocarbon monoterpenes (Figure 3). It has a composition identical to that of other countries. Indeed,

Table 1. Chemical composition of S.

Identified compound	Retention indices		Content of compounds (%)
	IrapExp/Bd	IrpExp/Bd	
α -thujene	922/ 922	1015/ 1023	0.20
α -pinene	930/ 931	1013/ 1022	0.62
camphene	943/ 943	1061/ 1066	0.32
β -pinene	970/ 970	1108/ 1110	0.40
myrcene	980/ 979	1157/ 1159	0.37
α -phellandrene	996/ 997	1161/ 1164	0.38
3-carene	1004/ 1005	1144/ 1147	0.41
<i>p</i> -cymene	1011/ 1011	1268/ 1268	2.90
limonene	1020/ 1020	1197/ 1199	2.32
(<i>Z</i>)- β -ocimene	1024/ 1024	1229/ 1230	0.31
cis linalol oxide	1057/ 1055	1464/ 1467	0.37
linalol	1080/ 1081	1543/ 1544	0.33
α -cubebene	1345/ 1350	1450/ 1252	0.23
cyclosativene	1364/ 1376	1472/ 1483	0.55
α -copaene	1373/ 1379	1485/ 1488	7.94
β -elemene	1384/ 1388	1582/ 1589	0.88
cyperene	1396/ 1406	1519/ 1525	1.00
(<i>E</i>)-caryophyllene	1414/ 1424	1589/ 1591	8.14
β -copaene	1422/ 1427	1597/ 1582	0.54
α -humulene	1446/ 1456	1660/ 1665	1.05
germacrene D	1471/ 1480	1700/ 1704	2.18
γ -cadinene	1510/ 1507	1748/ 1748	1.10
β -elemol	1533/ 1531	2076/ 2076	40.54
caryophyllene oxide	1565/ 1576	1973/ 1980	2.14
muurolo-4,10(14)-dièn-1 β -ol	1613/ 1611	2159/ 2159	2.38
γ -eudesmol	1620/ 1620	2161/ 2167	1.27
β -eudesmol	1630/ 1642	2211/ 2223	4.45
α -eudesmol	1635/ 1646	2220/ 2218	4.74
α -bisabolol oxide	1647/ 1646	1723/ 1973	0.34
Hydrocarbon monoterpenes			8.23
Oxygenated monoterpenes			0.70
Total monoterpenes			8.93
Hydrocarbon sesquiterpenes			23.61
Oxygenated sesquiterpenes			55.86
Total sesquiterpenes			79.47
Total			88.40

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

studies carried out on species from Burkina Faso (Djibo, 2000), Brazil (Blanco et al., 2009; Machado et al., 2012) and Benin (Bossou et al., 2013) have shown that EO consist mainly of monoterpenes with a predominance of oxygenates. The same majority compounds were identified by these authors. These are respectively geraniol (2.3, 6.14, 1.3, and 1%), geranial (44.6%, 35.92,

45.7, and 44.3%), neral (33, 28.24, 32.5, and 33.1%) and myrcene (10.7, 28.24, 11.5, and 12.4%). Likewise for the essential oil of lemongrass from Réunion which is rich in oxygenated monoterpenes (90.16% with geranial, neral and geraniol) associated with a hydrocarbon monoterpene (8.49%) myrcene (Miora, 2017). Also, the work of Tchoumboungang and Dongmo (2009) stated that the

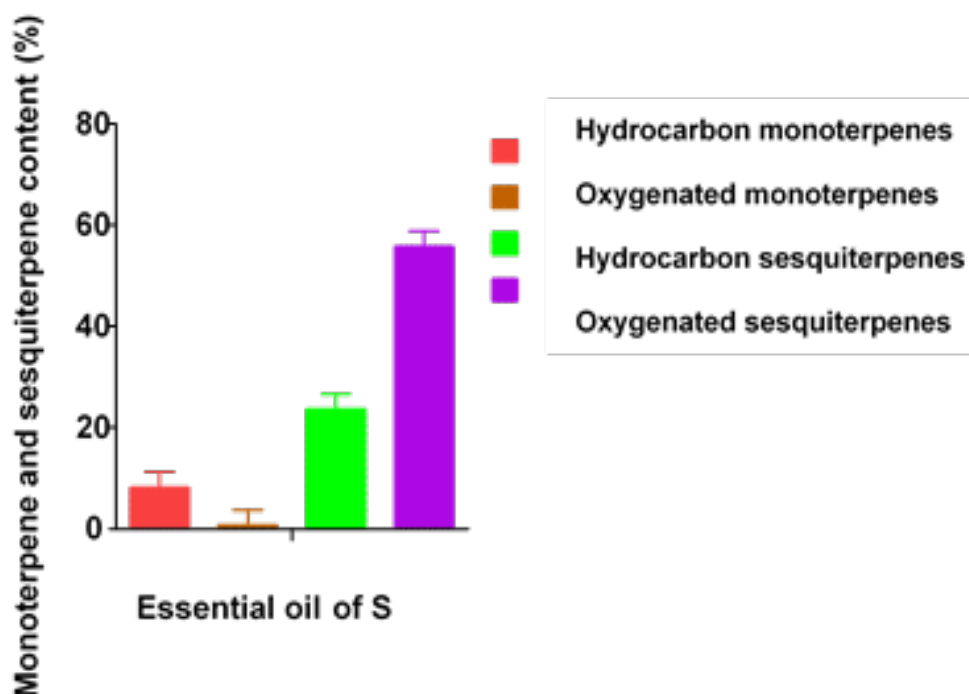


Figure 2. Monoterpenes and sesquiterpenes content of S.

essence of *C. citratus* is predominantly monoterpene (97.4%), with a preponderance of oxygenates (81.0%) namely geraniol (15.6%), geranial (39.3%) and neral (21.9%). All this work on the essential oil of *C. citratus* has shown that the quality of the gasoline hardly varies and this is explained by the existence of major molecules such as geraniol, geranial, neral and myrcene all recognized as compounds with repellent activity, thus justifying its use as a natural reference substance with repellent effect.

Biological activity

Repellent activity of the essential oil of *S. tragacatha* (S)

The results of the mosquito repellent activity tests carried out using the product P_{S5} and P_{S10} are shown in Table 3.

In view of Table 3 and Figure 4, the protection offered by P_{S5} is low, very rapidly falling over time. Indeed P_{S5} offers a protection of 60 to 50% which extends over 1 h 30 min and drops to 30% in 3 h before stabilizing at 20% from 3 h 30 min until the end of the experience. P_{S5} has a protection (P) of 32.5% and zero CPT (Table 4).

For P_{S10} , the 100% protection period lasts for 300 min or 5 h which corresponds to his CPT. During this time the unfed mosquitoes remained calm and away from the

animal. The second period corresponds to 95% protection. P_{S10} has a medium protection of 98.12% while 7 h (Table 4).

In view of the efficacy parameters P_{S10} is more effective than P_{S5} . Indeed according to WHO standards, the most effective product has a medium protection $\geq 90\%$ and the longest CPT.

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

The results of the mosquito repellent activity tests carried out using the P_{C5} and P_{C10} products are shown in Table 5.

Table 5 and Figure 5 show the rapid drop in protection offered by P_{C5} over time. Indeed, it offers 50% protection for the first 30 min and drops to 45% (from 30 min to 1 h) then to 20% (1 to 5 h 30 min) to stabilize at 10% (6 to 7 h). P_{C5} has an average protection percentage of 26.87%, a zero full protection time and an effective time of 30 min (Table 6).

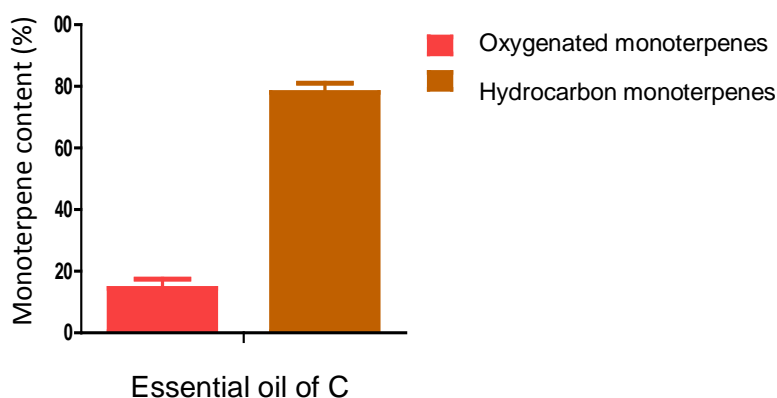
As for P_{C10} , its 100% protection extends over 210 min or 3 h 30 min which corresponds to its Full Protection Time (CPT = 210 min). This protection drops to 95% and is maintained until the end of the experience. The medium protection of P_{C10} is 97.5% (Table 6).

In short, P_{C10} is more effective than P_{C5} because it has

Table 2. Chemical composition of C.

Identified compound	Retention indices		Content of compounds (%)
	IrapExp/ Bd	IrpExp/ Bd	
6-methylhept-5-èn-2-one	960/ 963	1336/ 1337	3.02
myrcene	980/ 979	1157/ 1159	13.90
<i>p</i> -cymene	1011/ 1011	1267/ 1268	0.16
(<i>Z</i>)- β -ocimene	1024/ 1024	1228/ 1230	0.24
(<i>E</i>)- β -ocimene	1035/ 1034	1246/ 1247	0.16
linalol	1082/ 1081	1543/ 1544	1.00
citronellal	1129/ 1131	1479/1479	0.31
terpinen-4-ol	1156/ 1159	1600/ 1597	1.14
neral	1215/ 1214	1679/ 1679	28.24
geraniol	1233/ 1232	1842/ 1837	6.14
géranical	1243/ 1245	1730/ 1721	35.92
thymol	1266/ 1266	2179/ 2189	0.16
géranyl acetate	1358/ 1358	1752/ 1748	2.12
Hydrocarbon monoterpenes			14.46
Oxygenated monoterpenes			78.05
Total monoterpenes			92.51
Total			92.51

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

**Figure 3.** Monoterpene content of C.

a high protection (97.5%) compared to that of P_{C_5} (26.87%) and better protection with CPT. 210 min versus 0 min for P_{C_5} on adults of *A. gambiae* s.l.

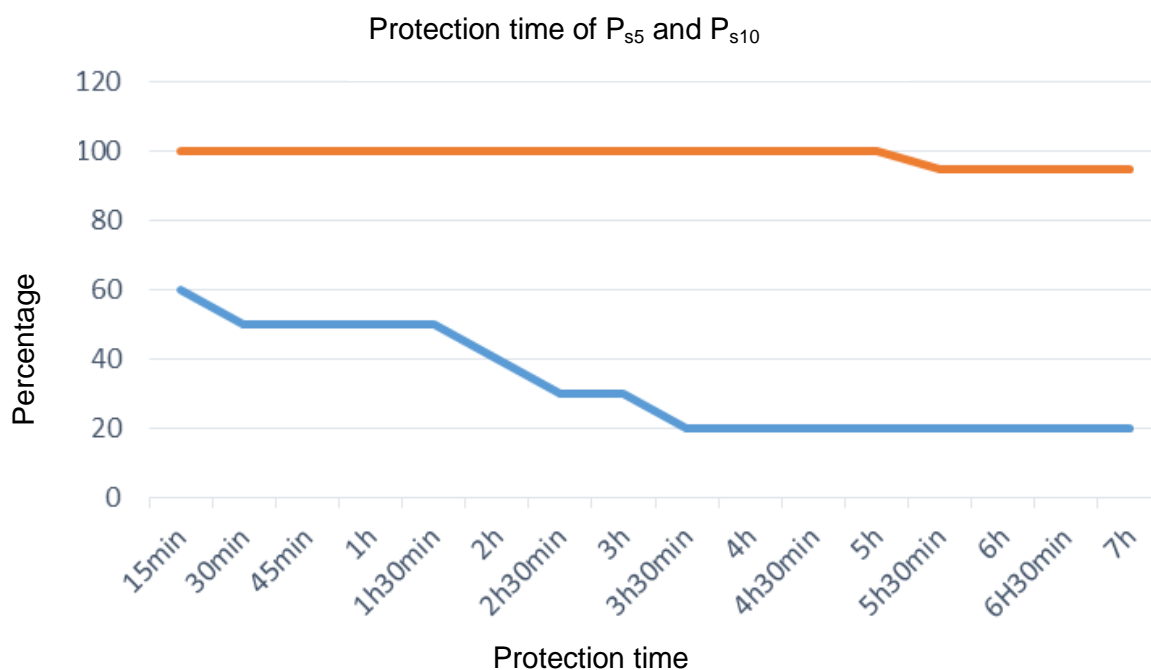
Comparative study of the persistence of the repellent effect of PC_{10} and PS_{10} ointments

Insect repellent tests have shown that PS_{10} has a

remarkable repellent effect like that of the reference substance (PC_{10}) on adults of *A. gambiae* s.l because their protection (P) is greater than 90%. However, PS_{10} is more persistent than PC_{10} because it has 100% complete protection (CPT) which lasts 5 h 30 min while PC_{10} provides the same protection for 3 h 30 min (Figure 6). However, according to WHO standards, the persistence of the effectiveness of a repellent is linked to its CPT; the higher it is, the more effective the repellent. This

Table 3. Repellency against *Anopheles gambiae* as a function of the time of S.

Observation time	Number of cumulated sips on the control (C)	Number of cumulated sips on treated animal (T)		Number of dead mosquitoes	TIRS in % de P _{S5} and P _{S10}	
		P _{S5}	P _{S10}		P _{S5}	P _{S10}
15 min	20	8	0	0	60	100
30 min	20	10	0	0	50	100
45 min	20	10	0	0	50	100
1 h	20	10	0	0	50	100
1 h 30	20	10	0	0	50	100
2 h	20	12	0	0	40	100
2 h 30	20	14	0	0	30	100
3 h	20	14	0	0	30	100
3 h 30	20	16	0	0	20	100
4 h	20	16	0	0	20	100
4 h 30	20	16	0	0	20	100
5 h	20	16	0	0	20	100
5 h 30	20	16	1	0	20	95
6 h	20	16	1	0	20	95
6 h 30	20	16	1	0	20	95
7 h	20	16	1	0	20	95

**Figure 4.** Evolution of the repellency of P_{S5} and P_{S10} as a function of time.

persistence of the repellent effect of P_{S10} (essential oil of *S. tragacatha*) against P_{C10} (essential oil of *C. citratus*) is linked to the chemical composition of its essential oil in particular to molecules such as myrcene (0.37%),

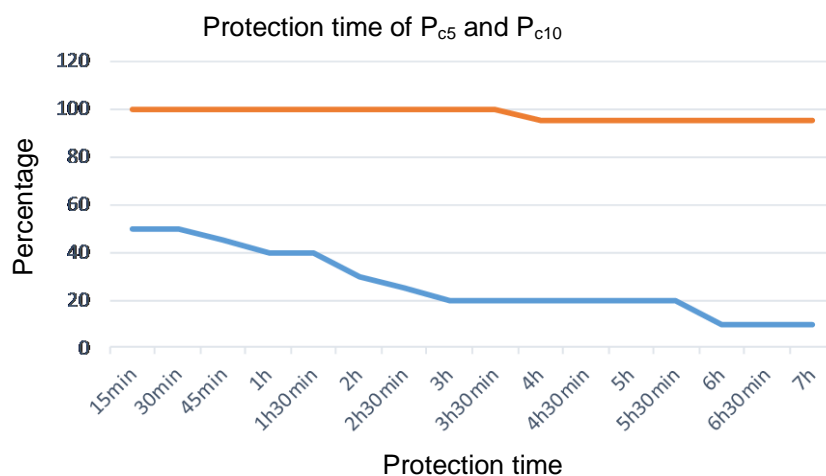
limonene (2.32%), α -copaene (7.94%), (E)-caryophyllene (8.14%) and β -elemol (40.54%) recognized for their repellent property (Miora, 2017). The synergistic action of these compounds could justify the activity of P_{S10}. This

Table 4. Efficiency parameters (ET₅₀, TIRS and CPT) of PS₅ and PS₁₀.

Parameter	Dose (%)	CPT (min)	Protection P (%)
P _{S5}	5	0	26.87
P _{S10}	10	350	98.12

Table 5. Repellency against *Anopheles gambiae* as a function of the time of C.

Observation time	Number of cumulated sips on the control (C)	Number of cumulated sips on treated animal (T)		Number of dead mosquitoes	TIRS in % de P _c	
		P _c (5%)	P _c (10%)		P _c (5%)	P _c (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
1 h	20	12	0	0	40	100
1 h 30	20	12	0	0	40	100
2 h	20	14	0	0	30	100
2 h 30	20	15	0	0	25	100
3 h	20	16	0	0	20	100
3 h 30	20	16	0	0	20	100
4 h	20	16	1	0	20	95
4 h 30	20	16	1	0	20	95
5 h	20	16	1	0	20	95
5 h 30	20	16	1	0	20	95
6 h	20	18	1	0	10	95
6 h 30	20	18	1	0	10	95
7 h	20	18	1	0	10	95

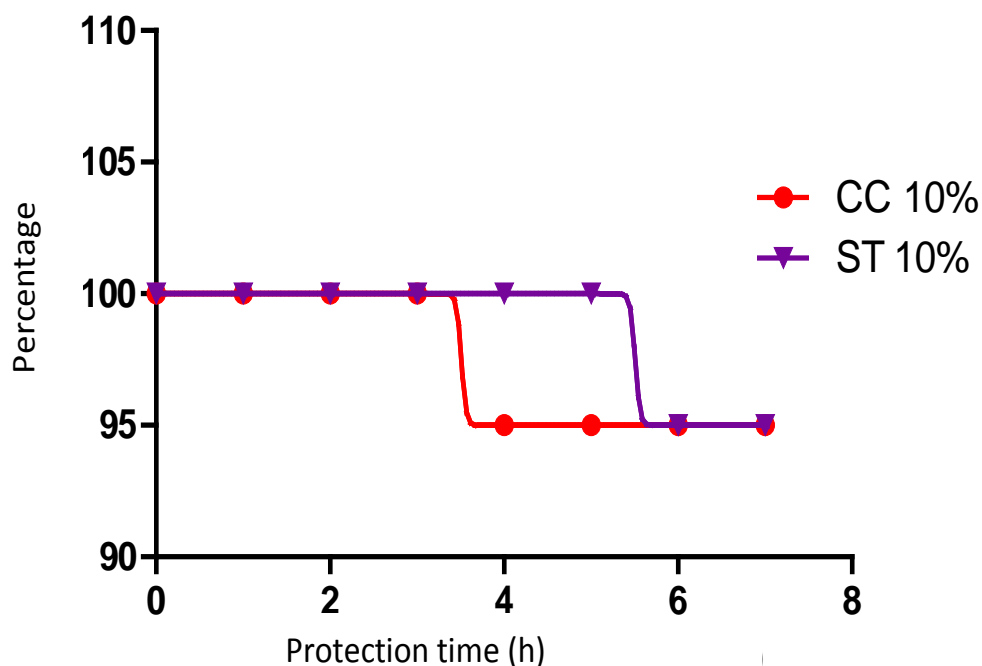
**Figure 5.** Repellency of PC₅ and PC₁₀ as a function of time.

is supported by Odalo et al. (2005) by showing that the individual repellent activity of compounds of some oils from Kenya on *A. gambiae* is below the repellent activity

of total essential oil. This PS₁₀ activity is also linked to oxygenated sesquiterpenes, in particular the β -elemol, the major compound, the repellent effect of which has

Table 6. P_{C5} and P_{C10} repellency parameters.

Parameter	Dose (%)	CPT (minute)	TIRS (%)
P _{C5}	5	0	26.87
P _{C10}	10	210	97.5

**Figure 6.** Comparison of the protection time of the HE of PC₁₀ and PS₁₀

already been proven by Nerio et al. (2010). Indeed, PS₁₀ is rich in less volatile oxygenated sesquiterpenes, which explains its prolonged action compared to the more volatile oxygenated monoterpenes contained in PC₁₀ (Kaloustian et al., 2012) (Figure 7).

The repellent property observed previously is explained by the fact that the compounds are for the most part endowed with an aromatic property that can neutralize body odors (lactic acid, carbon dioxide, ammonia, carboxylic acids, oct-1-en-3-ol and other sweat compounds) that female mosquitoes use to locate their prey (Guillaumot, 2009; Keswani and Bellare, 2006; McBride, 2016). These volatile substances confuse the female *Anopheles* by inactivating her receptors just enough to disturb her, or even render them insensitive to odors that she would find attractive, according to Vosshall's team (Pellegrino, 2011).

Conclusion

The essential oil of the stem bark of *S. tragacantha* (S)

was extracted by continuous hydrodistillation for 3 h with a yield of 0.12%. S is light yellow in color with an aromatic odor and liquid appearance at room temperature. Compounds (29) representing 88.4% of the EO have been identified, including a major compound β -elemol (40.54%). HE is rich in sesquiterpenes (79.47%) with a predominance of oxygenated sesquiterpenes (55.86%). As for the oxygenated monoterpenes, they represent 8.93%. The essential oil of *S. tragacantha* (S) is endowed with repellent property at 10%. At this dose, it offers a protection of 98.12% on adult females of *A. gambiae* s.l and TPC at 5 h versus 3 h 30 min for the natural reference substance. This activity is due to the presence of certain molecules such as myrcene (0.37%), limonene, α -copaene, (E)-caryophyllene and β -elemol. This search is a scientific evaluation of the repellent activity against adults of *A. gambiae* s.l of the essential oil of the stem bark of *S. tragacantha*. Based on the good extraction performance and proven repellent properties against *A. gambiae* s.l, *S. tragacantha* should be strongly recommended for the development of natural skin repellents after in deep further analysis.

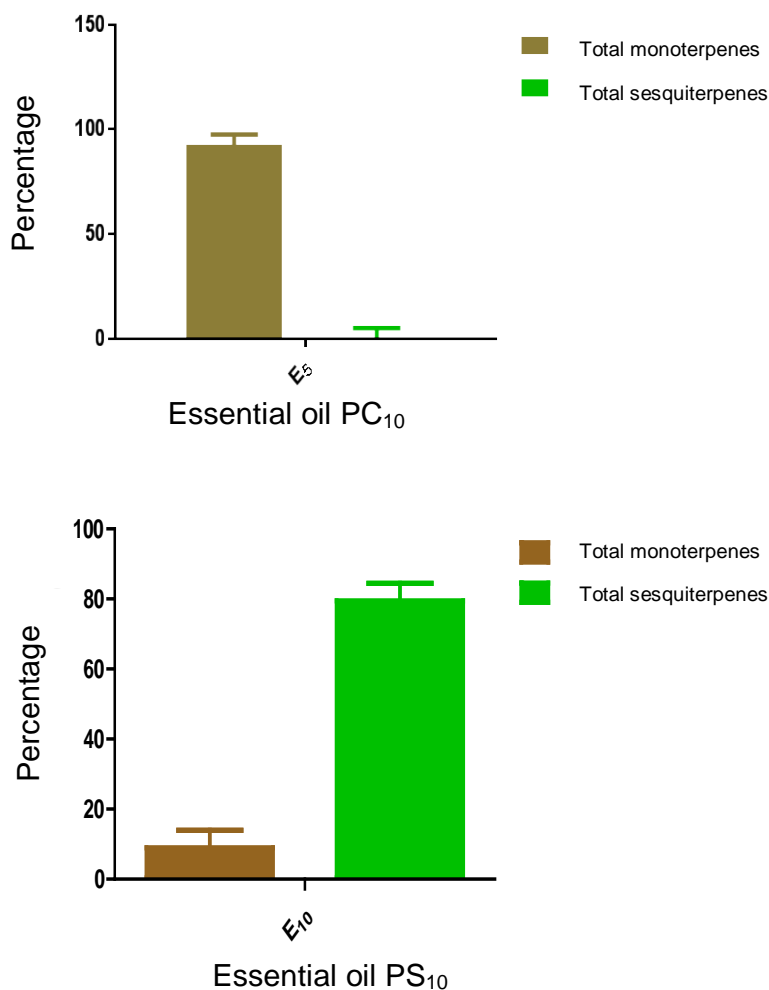


Figure 7. Monoterpenes and sesquiterpenes content of PC₁₀ and PS₁₀.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

REFERENCES

- Aboaba SA, Igumoye E, Flamini G (2016). Composition chimique des feuilles et de l'écorce de tige de *Sterculia tragacantha*, *Anthocleista vogelii* et des feuilles de *Bryophyllum pinnatum*. Journal of essential oil research 29(1):85-92, doi : 10.1080/10412905.2016.1178182.
- Adams RP (2007). Identification of essential oils components by gas chromatography / mass spectroscopy. 4 the edition. Allured Publishing Corporation: Carol Stream P 804.
- AFNOR (2000). Essential oils, sampling and method of analysis. Paragraph Edition (1):471.
- AFNOR (2008). Publication of essential oils, ISO standards, standards: [http://portailgroupe.afnor.fr/public_espacenormalisation/AFNORT75A/%20\(consulted%20on%2004/23/2016\)](http://portailgroupe.afnor.fr/public_espacenormalisation/AFNORT75A/%20(consulted%20on%2004/23/2016)).
- Blanco MM, Costa C, Freire AO, Santos JG, Costa M (2009). Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 16(2-3):265.
- Bossou AD, Manginckx S, Yedomonhan H, Boko PM, Akogbeto MC, De Kimpe N, Avlessi F, Sohounhloue DCK (2013). Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles). Parasite and Vectors 6(1):1-7.
- Browning DR (1971). Chromatographie, Masson & Cie, Paris, p 164.
- Chandre F, Darrier F, Manga L, Akogbeto M, Faye O, Mouchet J, Guillet P. (1999). Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. Bulletin of World Health Organization 77(3):230-234.
- Corbel V, Stankiewicz M, Pennetier C, Fournier D, Stojan J, Girard E, Dimitrov M, Molgó J, Hougard JM, Lapied B (2009). Evidence for inhibition of cholinesterases in insect and mammalian nervous systems by the insect repellent deet. BMC Biology 7(1):1-11.
- Djibo KA (2000). Analysis of essential oils from some plants of the flora of Burkina Faso belonging to the lamiaceae (*Hyptis spicigeralam.*, *Hyptis suaveolens* Poit., *Ocimum americanum* L.) and poaceae (*Cymbopogon schoenanthus* L., *Cymbopogon giganteus* chiovet *Cymbopogon citratus*Dc) families. PhD thesis University of Ouagadougou Burkina Faso pp. 129-131.
- Fradin LS, Day JF (2002). Comparative Efficacy of Insect Repellents against Mosquito Bites. New England Journal of Medicine 347(1):13-18.
- Guillaumot L (2009). Mosquitoes and dengue fever. Institut Pasteur of New Caledonia. [Online], <http://www.institutpasteur.nc/spip.php?article160#generalites,consult>

- ed on 03/08/2020.
- Kaloustian J, Hadji-Minaglou F (2012). Knowledge of essential oils. Quality and aromatherapy Spring 210 p. <https://dumas.ccsd.cnrs.fr/dumas-01515314/document> consulted the 06/08/2020
- Katz TM, Miller JH, Hebert AA (2008). Insect repellents: historical perspectives and new developments. *Journal of American Academy of Dermatology* 58(5):865-871.
- Keswani RK, Bellare JR (2006). A Review of Mosquito Attraction Studies: Important Parameters and Techniques. *Research Journal of Parasitology* 1(1):31-41.
- Kondjoyan N, Berdagre J L (1996). A compilation of relative retention indices for the analysis of aromatic compounds. Edition Flaveur Laboratory, Meat Research Station, INRA of Theix, St Genes Champanelle, France 234 p.
- König WA, Hochmuth DH, Joulain D (2004). Terpenoids and Related Constituents of Essential Oils. University of Hamburg, Institute of Organic Chemistry: Hamburg. Library of MassFinder, 2..
- Kouadio AS, Cissé G, Obrist B, Wyss K, Zingsstag J (2006). Fardeau économique du paludisme sur les ménages démunis des quartiers défavorisés d'Abidjan, Côte d'Ivoire. *Vertigo-la revue électronique en sciences de l'environnement, (Hors-série 3)*.
- Lundwall E, Pennetier C, Corbel V (2005). Paludisme : où en est la prophylaxie d'exposition ? *La Revue du Praticien* 55(8):3-11.
- Machado M, Pires P, Dinis AM, Santos-Rosa M, Alves V, Salgueiro L, Cavaleiro C, Sousa MC (2012). Monoterpenicaldehydes as potential anti-Leishmania agents: Activity of *Cymbopogon citratus* and citral on *L. infantum*, *L. tropica* and *L. major*. *Experimental Parasitology* 130(3):223-231.
- McBride CS (2016). Genes and Odors Underlying the Recent Evolution of Mosquito Preference for humans. *Journal Current Biology* 26(1):41-46.
- McLafferty FW, Stauffer DB (1994). The WileyRegistry of Mass Spectral Data; 6th ed. Spectrometry Library Search System Bench-Top / PBM, version 3:10.
- Ministry of Health and Public Hygiene (MSHP) (2020). Malaria and anemia parasite prevalence survey (eppa-ci). <https://dhsprogram.com/pubs/pdf/FR330/FR330.pdf>.
- Miora R (2017). Chemical and biological characterization of three repellent essential oils from regional biodiversity against Ross River alphavirus. Doctoral thesis, University of Reunion pp. 45-46.
- Moore SJ, Hill N, Ruiz C, Cameron MM (2007). Field Evaluation of Traditionally Used Plant-Based Insect Repellents and Fumigants against the Malaria Vector *Anopheles darlingi* in Riberalta, Bolivian Amazon. *Journal Medecinal Entomology* 44(4):624-630.
- Nerio LS, Olivero-Verbel J, Stashenko E (2010). Repellent activity of essential oils: A review. *Bioresource Technology Journal* 101(1):372-378.
- Odalo JO, Omolo MO, Malebo H, Angira J, Njeru PM, Ndiege IO, Hassanali A (2005). Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. *Acta Trop., Malaria Research in Africa - Multilateral Initiative on Malaria* 95(3):210-218.
- Paluch G, Bartholomay L, Coats J (2010). Mosquito repellents: a review of chemical structure diversity and olfaction. *Pest Management Science* 66(1):925-935.
- Pasteur Institute (2021). Malaria: information and treatment. <https://www.pasteur.fr/fr/centre-medical/%20/%20sickness%20cards/%20malaria.%20Accessed%205/02/2021%20at%208:23%20am%20GMT>.
- Pellegrino M, Steinbach N, Stensmyr MC, Hansson BS, Vosshall LB, (2011). A natural polymorphism alters odour and DEET sensitivity in an insect odorant receptor. *Nature* 478(7370):511-514.
- Pushpanathan T, Jebanesan A, Govindarajan M (2006). Larvicidal, ovicidal and repellent activities of the essential oil of *Cymbopogon citratus* Stapf (Graminae) against the filarial mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Tropical Biomedicine* 23(1):208-212.
- Tchoumboungang F, Dongmo JMP (2009). Larvicidal activity on *Anopheles gambiae* Giles and chemical composition of oils extracted from four plants cultivated in Cameroon, *Plantamedica. Biotechnology, Agronomy, Society and Environment* 13(1):77-84.
- World Health Organization (WHO) (2010) World Malaria Report 15 p.
- World Health Organization (WHO) (2014). Malaria entomology and vector control: a learner's guide, pp. 132-139.
- World Health Organization (WHO) (2013).). Guidelines for laboratory and field testing of long-lasting insecticidal nets http://apps.who.int/iris/bitstream/handle/10665/80270/9789241505277_eng.pdf?ua=1?sequence=1. Consulted the 05/07/2019
- World Health Organization (2009). Guidelines for efficacy testing of mosquito repellents for human skin. World Health Organization. <https://apps.who.int/iris/handle/10665/70072> consulted the 22/07/2019