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Adulticidal and repellent activity of the methanolic leaf extract and essential oils of *Hyptis suaveolens* and *Lippia adoensis* against *Anopheles gambiae* ssp Giles 1902 (Diptera: Culicidae)

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Abstract
Malaria disease is a serious public health problem in tropical regions and the use of botanical insecticides and repellent products can provide practical means of preventing this disease. Adulticidal and repellent effects of methanol extracts and essential oils of the leaves of *Hyptis suaveolens* and *Lippia adoensis* were evaluated on *Anopheles gambiae*. Adulticidal activity was tested using 1000 to 125 mg/bottle of methanol extracts and from 100 to 12.5 mg/bottle of essential oils following CDC method. Repellency test was performed with cream formulation containing 15% of both plants extracts or essential oils each and applied at 2, 4 and 8 mg/cm^2 for each extract and 1, 2 and 4 mg/cm^2 for each essential oil on dorsal hand of volunteers according to WHO (1996) method. In result, up to 93.33% mortality of *An. gambiae* was registered with methanol extracts and essential oils tested at 1000 and 100 mg/bottle, respectively. The LC_{50} values recorded after 24 h post exposure were 139.6 and 178.3 mg/bottle for extracts, 3.22 and 6.15 mg/bottle for essential oils of *H. suaveolens* and *L. adoensis*, respectively. Total protection for up to 120 min without receiving bites was recorded with creams formulation applied at dose of 8.0 mg/cm2. With essential oil cream formulation of both plant applied at 4.0 mg/cm^2, no bites were received for at least 150 min. In results, methanol extracts and essential oils extracted from the leaves of these plants have the potential to be used as a natural source insecticide and repellent plant to control *An. gambiae*.

Keywords: Adulticidal, repellency, *Hyptis suaveolens*, *Lippia adoensis*, *Anopheles gambiae*, Cameroon

1. Introduction
Mosquitoes transmit dreadful diseases such as malaria, which are widespread in the world [1]. In sub-Saharan Africa, many *Anopheles* species are among the most important vectors of malaria, with *Anopheles gambiae* being the most widely distributed and most efficient. It remains a major public health problem, particularly in sub-Saharan Africa where the highest prevalence rate of up to 90% of the world malaria burden was recorded [2]. In Cameroon, malaria is by far the leading cause of morbidity (15.6%) and mortality (13%) [3]. The diseas inflicts great economic loss and social disruption such as school absenteeism, loss of productivity, aggravation of poverty, high costs for health care etc. [4]. The application of insecticides remains one of the most important strategies in the prevention and control of malaria. In numerous malaria programmes, indoor residual spraying (IRS) of insecticides and long lasting insecticidal treated nets (LLINs) are the two important tools utilized [2]. Moreover, synthetic repellents DEET-based products made in lotions, creams, pastes, coils or vaporizers were widely used throughout the world [4]. However, repeated application of synthetic insecticides is increasing the selection pressure for resistance in malaria vectors [5]. IRS insecticides are harmful to human beings and animals, and some synthetic repellents may affect the dermis, cause skin irritation and unpleasant smell [6]. Environmental pollution and deleterious effects on non-target organisms, added to high operational cost are among others, the problems faced by using synthetic products for vector control [7]. With these problems in focus, the development of new strategies for selective mosquito control is required and plant-based products or botanicals are attractive alternatives. Botanicals are affordable, environmentally safe, and generally available in developing countries and are known to produce several biological effects in mosquitoes [8].

More than 1,200 plant species having potential insecticidal value were described by Roark [9]. The most promising botanicals were found in the families of Rutaceae, Labiatae, Meliaceae, Asteraceae, Annonaceae, and Malvaceae which provided numerous insecticide principles [10]. *Hyptis suaveolens* L. (Laminaceae) is an annual sub-shrub, distributed evenly in the tropic of West Africa. Several studies reported the medicinal uses of this plant [11]. *Lippia adoensis* Hochst (Verbanaceae) is an herbaceous plantdistributed throughout West Africa [12]. Extracts of the plant have been documented as a promising fumigant against a number of insect pests of cultivated crops. It was also reported for its pediculocidal and scabicidal activities against body lice, head lice and scabies mites [13]**.** Nukenine *et al.* [14] reported the efficacy of this plant against *Sitophilus zeamais* Motsch. The choice of the two plant species in the present study was based mainly on their insecticidal effects reported in the literature. Other reasons include their abundant availability in the northern part of Cameroon and their accessibility by local populations. The overall objective of this work is to investigate the adulticidal activities of methanolic extract and the repellency of essential oils of the leaf of *Hyptis suaveolens* and *Lippia adoensis* agains *Anopheles gambiae* ssp in order to contribute to malaria vector control.

2. Materials and Methods

2.1 Site of study

2.2 Collection and processing

The fresh young green leaves of *H. suaveolens* were collected at Dang, Vina Division, Adamawa region, Cameroon in April 2015. Similar leaves for *L. adoensis* were collected at Mbe of the Vina Division in June 2015. Dang (Plateau) and Mbe (plain) are situated 15 km and 70 Km north of the capital city of the region, Ngaoundere, respectively. The site where the leaves were harvested had the following coordinates: altitude (1090 and 616 masl), latitude (7° 41‟ and 7° 86‟ N), Longitude (13° 52‟ and 13° 60‟ E) for Dang and Mbe, respectively. The agro-ecology at Dang is Sudano-Guinean savanna and at Mbe Sudano-Sahelian savanna. The Vina Division is characterized by two seasons a dry season from November to March and a wet season spanning April to October. Mbe is located 70 km north of Ngaoundere in the Adamawa region. The identity of plant species was confirmed by the Botanist, Prof. Mapongmetsem Pierre-Marie of the Department of Biological Sciences, University of Ngaoundéré. The collected leaves were dried under shade during 7 days and pulversied into powder until the particles passed through a 0.4-mm mesh sieve. The powders were stored in a deep-freezer at -18 °C until needed for extraction.

2.3 Extraction of Methanol crude extract

From the collection of plant powder, 500 g for each plant was weighed with a balance (KERN
440 – 45 N made, Max 1000g and d = 0.1g specification, IKA®: RH b manufacturer, Balingen city Germany) and extracted for 72 h by cold maceration in 2.5 L of methanol (Sigma Aldrich), shaking twice a day (morning and afternoon) in the laboratory of Chemistry, University of Ngaoundéré. To obtain the methanol extract, 500 g of powder of each plant were macerated in 2500 ml of methanol for 3 days at room temperature and then the maceration was filtered using what man No.1 filter paper. The residue of maceration was rinsed and filtrated several times with fresh methanol until a clear phase was obtained. The filtrate was summited to Rotary Evaporator apparatus to obtain a residue called crude extract. The crude extract was stored in a refrigerator at 4 °C until needed for bioassay.

2.4 Extraction of essential oil

One thousand gram of leaf powder each plant species was used separately for essential oil extraction. The plant powder was subjected to hydrodistillation for 3 hours using a Dean Spark apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C in refrigerator until needed for bioassay. The yield of oil obtained from plant materials was calculated [15] following the method: Oil (% w/w) =× 100 31.

2.5 Mosquito strain (*Anopheles gambiae*)

Eggs of *Anopheles gambiae* from a culture at OCEAC Yaoundé were reared to adults according to the protocol [16] of WHO in the biology laboratory at the University of Ngaoundere. The larvae were fed on Tetra Min ® (Tetra GmbH, Germany). Nutritional powder made from cray fish and biscuit was also used as food for the larvae. Well Water was use for breeding of the aquatic stages of the mosquito in trays. The water in the tray was renewed every other day to avoid water pollution resulting from the presence of the nutritional powder [17]. Pupae were pippetted from the water in the tray and were transferred into transparent glasses containing well water, and then placed inside mosquito cage with volume 300 x 300 x 300 cm. A Petri dish was placed in each cage, containing 10% sucrose soak in cotton pads, to serve as food to the emerging adults. Adult mosquitoes destined for 33 mortality test were feed on chick blood twice a week. Those for repellency test were not feed with blood. The *Anopheles gambiae* was reared under ambient laboratory conditions.

2.6 Adulticidal test using CDC bottles

Adulticidal bioassay was performed following the method [18]. CDC [19] protocol was followed to prepare and coat bottles. The extracts and essential oils were dissolved in 98% ethanol. The choice of this chemical was about the ability of this solvent to evaporate very fast. To obtain the concentrations of 12.5, 25, 50 and 100 mg/bottle of essential oils; 125, 250, 500 and 1000 mg/bottle of the methanol extracts, products were dissolved in adequate quantity of acetone to make 10 ml of total solution. Each 1 ml of this solution would contain 12.5, 25, 50 and 100 mg of the essential oils and 125, 250, 500 and 1000 mg of methanol extracts. After cleaning and drying the bottles, 1 ml of the solution of each concentration of the prepared insecticide was added to the bottles. Only 1 ml of 98% ethanol was added to the control bottle. The content of each bottle was swirled and inverted by gently rotating so that the sides, all the way around are coated. After that, the caps were removed and continued rolling bottles on their side until all visible signs of the liquid were gone from inside and the bottles were completely dry. The bottles were left for 24 hours on their sides and covered with aluminum foil that will keep them protected from light.

2.7 Bioassay procedure

The bioassay was performed with the cleaned bottles of 250 ml in a lying position**.** Preliminary screening test was done in an increasing series of concentrations 1000, 500, 250 and 125

mg/ bottle to identify the lowest concentration that inhibits the mosquito adults. Die-Once® (Diclorvos) was used as positive test. Standard methodology according to WHO and CDC was followed in the determination of the lethal concentration doses adult‟ s mortality LC_{50} and LC_{90} CDC [20]. Mosquitoes Treated bottles untreated bottle using a mouth aspirator, 10–25 mosquitoes were introduced into each test bottle including the control bottle. At start time (Time 0), the bottles were examined to count the number of dead and live mosquitoes. The number of mosquitoes dead or alive was subsequently recorded every 15 minutes up to 24 h or in a shorter time if all the insects died. However, data were grouped such that mortality counts were reported for 1, 6, 12, 18 and 24 h post-treatment. The mortality in the control bottle was taken into consideration at 24 hours (end of the bioassay) when reporting the results of the bioassay. Abbott‟ s formula was used to correct results if the mortality in the control bottle was between 3% and 10%. The bioassay results were discarded, if mortality in the control bottle at the end of the test was >10%. Mosquitoes were considered dead if they can no longer stand.

2.8 Repellent test

[21] WHO method was performed to carry out the repellency test against of *An. gambiae* females. Cream formulation in percentage was prepared in three game concentrations for preliminary screening of the different plant products: 25%, 15% and 10% for methanolic extract and 15%, 10% and 5% for essential oils. Formulation 15% was retained for bioassay because of its mean effect. The formulation was done using petroleum (TOF BANDR) 100%. This petroleum was used as negative control (C-). The different mass for Mouth aspirator Mosquitoes in the cage formulation were weighed using a balance, max 2200 g- min 0.5g with capacity and e= 0,01g-0.01g of accuracy. Doses of 1.0, 2.0 and 4.0 mg/cm² of essential oils cream formulation and 2.0, 4.0 and 8.0 mg/cm2 extract cream repellents were applied for repellent test using human bait method. Three human volunteers were selected for the experiment from those who showed mild or no allergic reaction to mosquito bites or candidate oils. The volunteers were asked not to apply any lotions, perfumes, oils or perfumed soaps on the day of the assay [22]. An area of 7 by 7 cm (49 cm^2) was marked and cut open on plastic disposable hand gloves. The edges of the cut area were lined with masking tape**.** The control and test arms were interchanged regularly to eliminate any bias. By gently tapping the sides of the experimental cages, the mosquitoes were activated; the controls arms were introduced into the cage first and keep there for the experiment time. Pure petroleum jelly was used as negative control, while a commercial repellent cream (30% DEET) was used as positive control. The species *An. gambiae* was tested during night time from 18.00 h - 05.00 h GMT +1. Previously, the arms of three human volunteers were washed and cleaned thoroughly with distilled water before application of the repellent cream. Both arms were covered with rubber glove and a window area of 7 cm × 7 cm was opened on the dorsal part of the hand of the volunteers. The right hand was used for treatment and the left as control. The left hand, which acted as a control was treated only with pure petroleum jelly and was exposed for up to 30 sec in a mosquito cage (30 cm × 30 cm × 35 cm) containing 50 nulliparous female mosquitoes (5-7 days old). If at least two mosquitoes landed on or bit the control arm, the repellency test was then continued for 3 min after every 30 min until 210 min. Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. An attempt of the mosquito to insert its sty lets was considered as a bite. The mosquitoes that landed on the hand were recorded and then shook off before imbibing any blood. Subsequently, the test arm was introduced into the cage for the same duration and the number of landing *An. gambiae* recorded. The different sample concentrations were tested sequentially starting with the lowest one The experiments were repeated five times in separate cages and in each replicate different volunteer were used to nullify any effect of skin differences on repellency. The percentage protection was calculated by using the following formula [23]:

% protection = [(NC – NT)/NC] × 100 Where NC = number of mosquitoes that bit/landed on the control and NT = number of mosquito that bit/landed on the treated areas of the volunteer.

1.9 Data analysis

The adult's per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott‟ s formula [24]. SPSS 16.0 version package was used for determination of LC_{50} and LC_{90} values Finney [25]. Mortality data was subjected to ANOVA procedure and significantly different means were separated using Tukey‟ s test (P= 0.05) (SPSS version 16.0)

A B

Fig 1: (AB) Different products for the repellent test and Control and treated arms introduced into cage for record landing of mosquito

3 Results

3.1 Yield and Phytochemical screenings from *H. suaveolens* and *L. adoensis*

The yield and phytochemical are presented in table 1 and 2 respectively.

Table 1: Yields of products from *H. suaveolens* and *L. adoensis.*

Product	Plantes	Masse totale	Masse/ volume	Yield
extraits	*H. suaveolens*	500g	28,89g	5,778
	L. adoensis	500g	48,80g	9,760
huiles	*H. suaveolens*	1000g	1,2mL	0,12
	L. adoensis	1000g	2,6mL	0,56

3.2 Adulticidal activity

3.2.1 Methanol leaf extract

Until 12 h post-treatment, there was no mortality observed in the control group, while in positive control adult mortality significantly varied (F (4, 20) = 49.85, $P<0.001$) to 97.50% after 24 h post-exposure. After 1 h post-exposure, no mortality of mosquito was recorded with methanol leaf extract of *Hyptis suaveolens* at the lowest concentration (125 mg/bottle) but, 29.17% mortality was observed at the highest concentration (1000 mg/bottle). After 24 h post-treatment, a significant (F(4, 20)= 345.13, $P<0.001$) dose dependent mortality of *An. gambiae* adults was recorded with the methanol leaf extract of *H. suaveolens* ranging from 49.17% mortality at the concentration of 125 to 93.33% at 1000 mg/bottle. In general, the lethal concentration of methanol leaf extract of *H. suaveolens* decreased with time post-exposure (table 3). A good lethal concentrations of LC_{50}= 139.6 mg/bottle and LC_{90}= 705.5 mg/bottle of methanol leaf extract of *H. suaveolens* was obtained after 24 h post treatment. The lethal concentrations for this plant that killed 90% decreased also with increasing exposure time. For methanol extract of *L. adoensis*, the moderate mortality of adults *An. gambiae* was observed and significantly varied (F (4, 20) = 132.19, $P<0.001$) from 1.67% at the lowest concentration (125 mg/bottle) to 32.50% at the highest dose of 1000 (mg/bottle) after 1 h post treatment. After 24 h post-treatment, a significant (F (4, 20) = 330.07 $P<0.001$) dose dependent mortality of adults *An. gambiae* was recorded with the methanol leaf extract, ranging from 46.67% at the concentration of 125 (mg/bottle) to 97.50% mortality at 1000 mg/ bottle. As in the case of *H. suaveolens*, the lethal concentration of methanol leaf extracts of *L. adoensis* also declines with time post-exposure **(**table 3**)**. A low lethal concentration values of LC_{50}= 178.3 mg/bottle and LC_{90}= 1375mg/bottle were recorded after 24 h post-treatment against

Table 2: Qualitative phytochemical screening of methanolic leaf extract of *Hyptis. Suaveolens* and *Lippia. Adoensis.*

Plant species	Alkaloids	Flavonoids	polyphenol	Saponoids	Tannin	Terpenoids
H. suaveolens	++	++	++ +	++	+++	+++
L. adoensis	++	++	+ ++	+++	+++	+++

+++ High concentration of compound; ++ Moderate concentration.

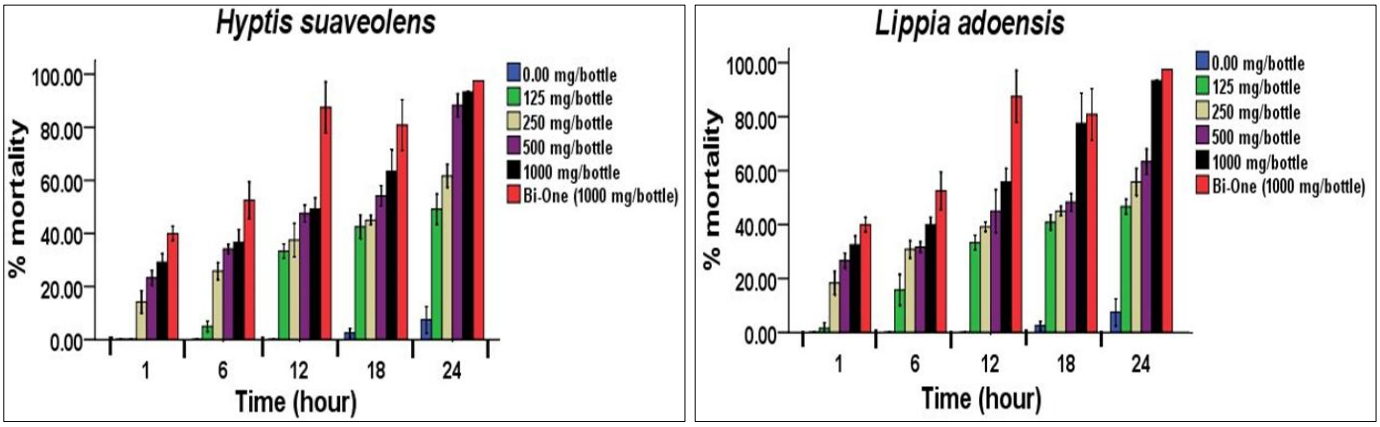

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Fig 2: Adulticidal activity of *Hyptis suaveolens* and *Lippia adoensis* methanol leaf extracts against adults of *Anopheles gambiae* under laboratory conditions

3.2.2 Essential oils

Indeed, the essential oils of *H. suaveolens* caused significant mortality to adults of *An. gambiae*, which increased with concentration and time post-exposure. Within 24 h of exposure, no significant difference was observed on adults mortality of *An. gambiae* tested with essential oil of *H. suaveolens* at doses 50 mg/bottle (93.33% mortality), 100 mg/bottle (93.33% mortality) as well as the positive control (97.50% mortality). A low mortality percent of 2.50% and 6.67% of adults *An. gambiae* was noticed after 18 and 24 h post-exposure, respectively. The commercial insecticide (Positive control) caused significant mortality (F (4, 20) = 49.85, $P<0.001$) of mosquito adults ranging from 40.00% to 97.50% after 1 and 24 h, respectively. The LC_{50} values of essential oils of *H. suaveolens* decreased with increasing exposure time **(**table 4**).** The low dose that killed 50% mosquito adults was obtained after 24 h post treatment. The LC_{50} values of *H. suaveolens* of adults of mosquito species tested were 103.04 and 3.22 mg/bottle respectively after 1 h and 24 h post-exposure. The LC_{90} values were 2667 and 49.50 mg/bottle respectively after 1, and 24 h post-treatment. Similarly, the essential oil of *L. adoensis* significant caused mortality of the adults of *An. gambiae* species, which increased with concentration and time post-exposure. The

mortality significantly varied from 30.83 to 50.52% (F= 141.83, *P*<0.001), 1 h post-exposure. After 24 h post-exposure, significant mortalities of 66.67, 76.67, 93.33 and 93.33% were recorded at doses of 12.5, 25, 50 and 100 mg/bottle, respectively. After 24 h post exposure, no significant difference was noticed in the mortality of adults mosquitoes caused by essential oils of *L. adoensis* at doses of 50 and 100 mg/bottle and the commercial insecticide used as the positive control. The positive control significant caused mortality ranging from 40.00 after 1 h to 97.50% after 24 h to mosquitoes species tested. The negative control caused 2.50 and 6.67% mortality of adults after 18 and 24 h, respectively. The LC_{50} value of essential oils of *L. adoensis* decreased with increasing exposure time. The lowest lethal concentration which killed 50% mosquitoes was observed after 24 h post treatment (6.15 mg/bottle). The LC_{90} values also decreased with increasing time post-exposure. These values were 8078, and 54.01 mg/bottle recorded respectively after 1and 24 h post-treatment.

Table 3: LC_{50} and LC_{90} values (mg/ bottle) of *Hyptis suaveolens* and *Lippia adoensis* methanol

Plants	Time(H	Slope±SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
H. suaveolens	1	1.50±0.13	0,75	1883 (1266.8-3941.6)	13410 (5695.1-72706.3)	43.99***
	6	1.17±0.11	0.60	1451 (973.7-3097.8)	18230 (6602.4-149884)	43.99***
	24	1.82±0.12	0.77	139.6 (113.9-163.7)	705.5 (586.6-902.2)	37.73*
L. adoensis	1	1.36±0.12	0.71	1737 (1174.4-3561.8)	15230 (6232.2-87297.9)	41.23***
	6	0.73±0.10	0.65	2044 (1364.3-4008.1)	1.2E5 (34418.5-1.04E6)	18.56ns
	24	1.45±0.11	0.96	178.3 (123.5-228.5)	1375(916.1-2836.4)	55.49***

^{Ns}P>0.05, *P<0.05 and ***P<0.001, FL= Fiducial Limit, LC= Lethal concentration.

3.3. Repellent activity

2.3.1 Plant extracts

The results of repellent activity of *Hyptis suaveolens* and *Lippia adoensis* methanol extracts creams tested against females of *An. gambiae* are presented in table 5. Overall, the cream formulated caused a significant concentration dependent repellent activity against adults of *An. gambiae*, which declined with time post exposure. The methanolic crude extracts cream formulation of *H. suaveolens* exhibited 75, 65% protection, without mosquito bites within 180min 8.0 mg/cm^2. Cream (30% DEET) used as positive control, its repellency effect was comparable to the *H. suaveolens* extracts cream tested at 8.0 mg/cm^2, since no skin mosquito bites was noticed after 180 min post exposure. Within 30 min of exposure to the methanolic crude extracts cream formulation of *Lippia adoensis*, 100% protection against mosquito bites was achieved for the concentration 2.0, 4.0 and 8.0 mg/cm^2 at 30min, 180 and 24h post-exposure, respectively.

3.3.2 Essential oils

Results of the repellent efficacy of *H. suaveolens* and *L. adoensis* leaf essential oils cream formulation against adult females of *An. gambiae* are presented in table 6**.** In general, the cream formulations of both plant assessed, exhibited a significant repellence activity against the females mosquitoes, which increased with concentration, but reduced with time post-exposure. The cream formulated with the essential oil of *H. suaveolens*, completely protected the skin from the bites of *An. gambiae* for 180 min and 24h when the concentration rate was respectively 2.0 and 4.0 mg/cm^2. Commercial cream formulation containing 30% DEET, used as positive control totally protected the skin from the bites of the malaria vector for 150 min less than *H. suaveolens* creams (24h) applied at the rate of 4.0 mg/cm^2. The negative control made of pure petroleum jelly repelled mosquitoes at the low rate ranging from 1.45 to 4.76% during entire period of evaluation. For the cream made with the *L. adoensis* essential oil, the 100% protection from the bites of *An. gambiae* that was recorded at 1.0 and 2.0 mg/cm^2 150 min and 24h respectively high than the total protection time of 180 min obtained with the first plant (*H. suaveolens*).

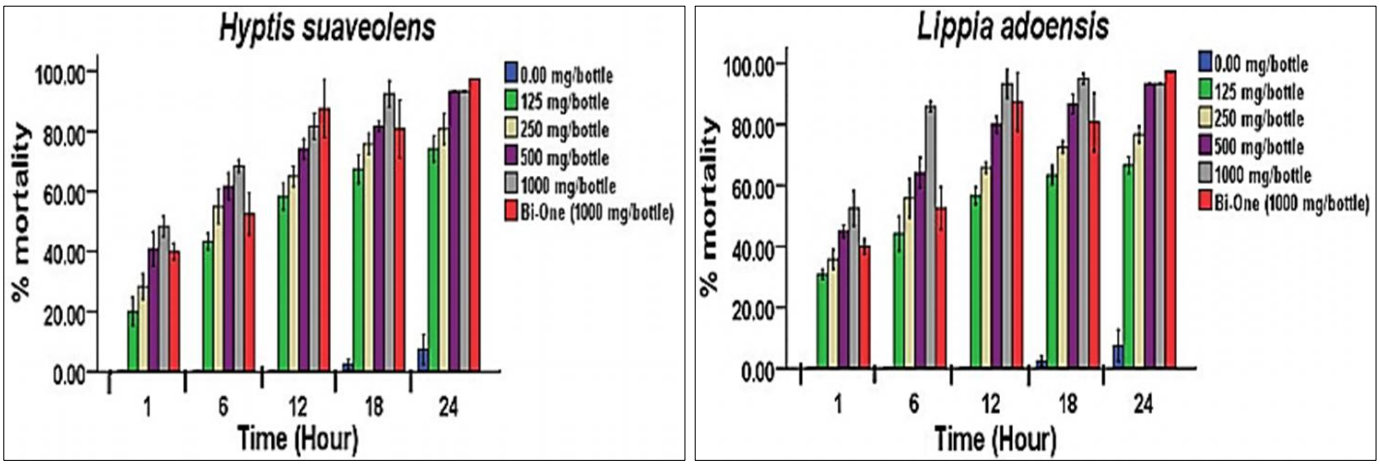

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Fig 3: (AB) Adulticidal activity of *Hyptis suaveolens* and *Lippia adoensis* essential oils against adults of *Anopheles gambiae* under Laboratory conditions

Table 4: LC_{50} and LC_{90} values (mg/bottle) of *Hyptis suaveolens* and *Lippia adoensis* leaf essential oils post-treatment against adults of

Anopheles gambiae under laboratory conditions.

Plants	Time(h)	Slope± SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
H. suaveolens	1	0.91±0.10	0.79	103.04 (81.75-142.92)	2667(1242.23-8616.21	13.27^{ns}
	6	0.70±0.09	0.73	19.36 (14.15-24.26	1302(594.29-4962.85	8.80 ^{ns}
	12	0.77±0.10	0.81	7.16 (4.07-10.20)	325.86 (197.93-738.12	9.49 ^{ns}
	18	1.00±0.11	0.85	4.79 (2.76-6.89)	92.21 (76.60133.22)	16.16 ^{ns}
	24	1.08±0.12	0.64	3.22 (1.67-4.93)	49.50 (41.05-63.42)	15.77 ^{ns}
L. adoensis	1	0.64±0.09	0.83	81.22 (61.84-123.80)	8078(2350.56-75533.9)	6.47 ^{ns}
	6	1.24±0.10	0.91	18.51 (14.19-22.57)	200.32 (136.31-366.09)	25.86*
	12	1.39±0.11	0.92	10.84 (8.68-12.87	90.36 (74.98-115.23)	18.89 ^{ns}
	18	1.41±0.12	0.87	7.96 (6.02-9.81)	64.59 (54.84-79.77	10.29 ^{ns}
	24	1.36±0.12	0.70	6.15 (3.34-7.92)	54.01 (46.09-66.11)	14.00 ^{ns}

^{Ns} P>0.05, *P<0.05, FL= Fiducial Limit, LC= Lethal concentration.

At the dose of 2.0 mg/cm^2, total protection without receiving bite from *An. gambiae* female was achieved for 24h. During the whole period of repellency test, an insignificant repellency effect of pure petroleum jelly (negative control) ranging from 1.45 to 4.76% was recorded.

4. Discussion

During laboratory experiments, methanolic leaves extracts and essential oils revealed to possess adulticidal and repellent activity against adults of *Anopheles gambiae* ssp. The mortality of these plant products increased with increasing concentration and time post-exposure. Percentage of repellency increased with increasing concentration but decrease with time post – exposure. Indeed, in the present study, methanol extraction yield varied from one plant to another. The methanolic extract yield of *H. suaveolens* was 5.78% less than 9.76% in yield obtained with *L. adoensis* methanolic extraction after 72h cold maceration process. Similar methanol yield extraction close to *L. adoensis* were reported on *Moringa oleifera* (10.30%), *Azadirachta indica* bark (11.10%) and *Terminalia Arjuna* bark (12.20%) [26]. We attribute those differences to the factors affecting efficiency of the extraction such as part, structure and plant species as well as chemical composition, polarity of solvents used for extraction, method and procedure of plant extraction.

Table 5: Repellent effect of *Hyptis suaveolens* and *Lippia adoensis* methanolic leaf extract against adults of *Anopheles gambiae* under laboratory conditions.

Plant	Time	% repellency - Concentration (mg/cm^2)					
		0	2	4	8	+DEET 30	F
H.suaveolens H.	30min	5.00±5.00C	26.67±6.67B	100.0±0.00A	100.0±0.00A	100.0±0.00A	157.21***
	180min	2.18±1.44E	21.74±4.14D	55.65±1.67C	75.65±2.46B	95.65±0.87A	254.34***
	24h	1.45±1.03E	15.94±4.27D	53.62±1.18C	67.39±3.21B	94.20±1.18A	221.02***
L.adoensis	30min	5.00±5.00B	100.0±0.00A	100.0±0.00A	100.0±0.00A	100.0±0.00A	361.00***
	180min	2.18±1.44E	46.09±1.00D	64.35±1.67C	80.87±3.33B	95.65±0.87A	369.48***
	24h	1.45±1.03E	36.23±3.74D	57.97±1.87C	74.63±1.39B	94.20±1.18A	292.92***

Means ± S.E. in the same row followed by the same letter do not differ significantly at P = 0.05 (Turkey's test). Each datum represents the mean of three replicates. *** P<0.00

Essential oils obtained by hydrodistillation process in this present study present a low extraction yield of 0.12% for *H. suaveolens* and 0.56% for *L. adoensis*, compared to high yield of up to 1.24% of *Piper nigrum* and 4.6% of *Eucalyptus citriodora* [27]. The yields comparable to *L. adoensis* yield, up to 0.60% and 0.80% of *Ocimum basilicum* and *O. Gratissimum* respectively were obtained by Anwar *et al.* [28]. Low yield of essential oils obtained might be ascribed to the long dryness duration of 7days in our case, causing the loss of the essential oils content by evaporation. Among other factors influencing essential oils yield, site of collect, plant species, and duration of distillation, chemical composition and extraction procedure. Both local plants methanol leaf extracts contained all organic classes compound targeted and supposed to have insecticidal effects. Alkaloids and flavonoids were moderately concentrated while tannins and terpenoids were highly concentrated in the both plant methanol extracts assessed. Saponoids were moderately concentrated in *H. suaveolens* extract and highly concentrated in *L. adoensis* methanol extract. Similarly, Amerasan *et al.* [29] reported the presence of alkaloids, steroids, saponins, flavonoids at moderate concentration in the methanol leaf extract of *Mimusops elengi*. Qualitative and quantitative variations in phytochemical components observed were earlier highlighted by previous studies, which reported numerous factors determinative the composition of the plant extract including seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant used and postharvest drying and storage [30]. Creams formulated with the methanol leaf extracts of *H. suaveolens* and *L. adoensis* have demonstrated significant dose-dependent repellent activity against adults of *An. gambiae*. Up to 24 hours without bites of *An. gambiae* females were observed with methanol creams extracts of *H. suaveolens* and *L. adoensis* applied at 8 mg/cm^2 on the volunteer's skin. Comparatively, complete protection (100% repellency) for 120 min without bites of *An. stephensi* was reported with methanol extracts of the leaves of *Cassia tora* [31]. The present results are in accordance with results obtained by Pushpin than *et al.* [32] using neem oils against mosquito bites of *Anopheles*. The cream formulations of both plant assessed, exhibited a significant repellence activity against the females mosquitoes, which increased with concentration, but reduced with time post- exposure. In this study, 100% protection for at

least 2.50 h from bites of *An. gambiae* females on dorsal hand skin treated with creams formulated with *H. suaveolens* and *L. adoensis* leaves essential oils was recorded. Similarly, essential oil of *Tagetes minuta*, providing a repellency of 90% protection for 2 h against *An. stephensi*, was reported by Tyagi *et al.* [33]. The essential oils of *Zingiber officinalis* showed repellent activity at 4.0 mg/cm^2, which provided 100% protection up to 120 min against *Cx. quinquefasciatus* [32]

Table 6: Repellent effect of *Hyptis suaveolens* and *Lippia adoensis* leaf essential oils against adults of *Anopheles gambiae* under laboratory conditions.

Plant	Time	% Repellency - Concentration (mg/cm^2)					
		0	1	2	4	+DEET 30	F
H. suaveolens	30min	0.00±0.00B	87.50±7.22A	100.0±0.00A	100.0±0.00A	100.0±0.00A	183.00**
	180min	2.18±1.44E	48.70±1.66D	86.09±0.00C	100.0±0.00A	94.78±1.00B	1443***
	24h	1.45±1.03D	45.65±1.82C	76.09±1.39B	97.83±1.39A	92.75±2.51A	547.30**
L. adoensis	30min	0.00±0.00B	93.75±6.25A	100.0±0.00A	100.0±0.00A	100.0±0.00A	249.00**
	180min	2.18±1.44C	81.74±6.25B	94.78±1.00AB	99.13±0.87A	95.65±0.87A	186.76**
	24h	1.45±1.03D	65.94±2.47C	84.06±1.87B	96.38±2.74A	92.75±2.50AB	311.74**

5. Conclusion

The results of the present investigation conducted in juin 2015 in Ngaoundéré clearly evidenced the adulticidal and repellent activities of *H. suaveolens* and *L. adoensis* methanol extracts and essential oils against the malaria vector, *An. gambiae*. In general, mosquitocidal activity of the both plant products tested increased with ascending concentration and time post-exposure. Regarding the repellent effect, total protection for 120 min without receiving bites from *An. gambiae* females was observed with methanol extract creams of both plants applied at the dose of 8 mg/cm^2. The essential oil cream formulations applied at 4 mg/cm^2, produced total protection from *An. gambiae* female bites for 180 min with *H. suaveolens* cream and 150 min with *L. adoensis* cream. From these results, methanol extracts and essential oils of the leaves of *H. suaveolens* and *L. adoensis* could be applied indoor or outdoor to reduce *An. gambiae* populations as well as to avoid bites from the adult females.

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