International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2016; 3(6): 11-19

Received: 03-09-2016
Accepted: 04-10-2016

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Larvicidal and oviposition-deterrence activities of four local plant extracts from Burkina Faso against *Anopheles gambiae* S. l. (Diptera: Culicidae)

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Abstract
Mosquitoes have developed resistance to various synthetic insecticides, making their control increasingly difficult. As part of the search for natural biocides, an alternative to synthetic insecticides, larvicidal and oviposition-deterrence activities of ethanol, acetone and hexane extracts of *Lantana camara* L., *Hyptis suaveolens* Poit., *Ocimum canum* Sims. and *Hyptis spicigera* Lam. on sensitive and local strains of *An. gambiae* s. l. were evaluated.
Larvicidal activity was assessed according to the WHO standard protocol. Oviposition deterrence activity was evaluated using gravid *Anopheles gambiae* s. l. Phytochemical screening of these plant extracts revealed the presence of terpenes, tannins, saponins, alkaloids, flavonoids, steroids and phenols. The LC_{50} and LC_{90} values determined for these extracts varied according to plants and solvents. The hexane extracts of *L. camara* were the most toxic to *An. gambiae* local strain larvae with LC_{50} value of 20.19 ppm (95% CL=14.35-26.67) and LC_{90} value of 49.29ppm (95% CL=37.13-65.40). Extracts also showed high oviposition- deterrence of *An. gambiae*. For *L. camara* acetone extract, The average number of eggs was 0 ± 0, 10 ± 1 and 128 ± 16 eggs in treated plastic cups while in control plastic cups it was 258 ± 36, 224 ± 55 and 256 ± 31 eggs at 100 ppm, 500 ppm, 1000 ppm respectively. Our results indicated that these extracts, mainly extracts *of Lantana camara*, could be used for *An. gambiae* control as ecofriendly natural products.

Keywords: Mosquito, bioassay, plant extract, larvicidal, oviposition-deterrence

1. Introduction
Anopheles gambiae complex mosquitoes transmit both lymphatic filariasis and malaria, the later being the most deadly endemic disease in sub-Saharan Africa [1]. There were an estimated 214 million of malaria cases with 438,000 deaths worldwide in 2015 [2]. Malaria contributes to the slowing down of economic growth in African countries, maintaining them in a vicious circle of poverty [3]. Burkina Faso is one of the most affected countries [5]. Indeed, malaria is responsible for 55.14% of patients treated in the public health centers, 65.56% of hospitalizations and 59.60% deaths in children aged less than 5 years [4].
Currently recommended methods by the World Health Organization (WHO) for malaria control include insecticide-treated mosquito nets and indoor sprays for the control of the mosquito vector [5]. These methods used in sub-Saharan Africa have yielded interesting results in term of mortality and morbidity reduction [2]. Efficient and good quality mosquito nets and synthetic insecticides are still expensive for populations at risk [6]. The accumulation of synthetic insecticides in ecosystems because of agricultural activities, leads to a pollution problem that is harmful for non-target organisms [7]. In addition, these insecticides are hazardous to handle, leave toxic residues in food products, and are not easily biodegradable [8]. Furthermore, the intensive usage of system insecticides in agricultural as well as domestic environments causes vector resistance, which constitute a major obstacle to vector control, particularly considering that resistance concerns all the classes of insecticides used at present [9-12].
In order to overcome these various difficulties facing vector control, one option is to research and develop new control products that, respect the environment [13]. The use of plants in folk medicine and against harmful insects in sub-Saharan Africa is well known in rural areas [14]. Previous research has shown the efficacy of plant extracts and essential oils against depredators

of stored products and vectors [15-18]. Some extracts showed larvicidal, adulticidal and oviposition-deterrence properties against disease vectors [19-22]. Hexane, chloroforme and ethyl acetate extracts of *Breynia vitis-idaea* leads to high death rate of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles maculipennis* larvae [15]. These natural plant extracts offer the advantage of inducing very low resistance compared to synthetic insecticides, because their efficacy comes from the synergic actions among their deferent organic compounds [13, 23, 24].

The larval stages of the *Anopheles gambiae* are ideal targets for reducing a large number of mosquitoes, and they are also easier to control [25, 26]. Larvicide has the greatest control impact on mosquito populations because the larvae are concentrated and easily accessible due to their reduced mobility. The current study aims to contribute to the research and development of new insecticide compounds. *Lantana camara* L., *Ocimum canum* Sims, *Hyptis suaveolens* Poit. and *Hyptis spicigera* Lam. have been harvested for extractions of polar compounds using ethanol and acetone, and the non-polar hexane compound, in order to evaluate their larvicidal and oviposition -deterrence effects on *Anopheles gambiae* s. l. These plants have been chosen because of their repellent effect against *Anopheles gambiae* malaria vectors [27] and the depredators of stored products [17].

2. Materials and Methods

2.1 Collection of plants

Plants were harvested in Gampela at east (12°25'12"N, 1°24'04"W), Loumbila (12°29'30"N, 1°24'06"W) and Kamboinse (12°27'54"W) villages at north of Ouagadougou in 2010-11 (Fig. 1). These villages are situated at an average of 20 km in average far from Ouagadougou in the sudano-Sahelian zone. *Lantana camara* was harvested in Kamboinse, *Ocimum canum* was harvested around the national museum in Ouagadougou, *Hyptis spicigera* was harvested in Loumbila and *Hyptis suaveolens* was harvested in Gampela in 2011. The taxonomic identification of plants was carried out in the "Laboratoire de Biologie et d'Ecologie Végétales" (University of Ouagadougou, Burkina Faso), and voucher specimens OUA are kept at the "infobio" of the University. The voucher specimen numbers are 6818/OUA, 6819/OUA, 6820/OUA and 6821/OUA respectively for *L. camara*, *H. spicigera*, *H. suaveolens* and *O. canum*.

2.2 Soxhlet extraction and phytochemical screening

The collected plants, after drying and being sheltered from the sun, were spayed using a mixer. Acetone, ethanol and Hexane were used for the extractions. For the Soxhlet extraction, 25g of vegetal powder was used with 250 ml of solvent for 5-6 hours. After extraction, the extracts were concentrated with a rotavapor. The obtained concentrates were placed in stoves to be dried.

Qualitative phytochemical screening of the extracts was carried out to determine chemical groups contained in the extracts, following the analysis method described by Ciulei [28].

2.3 Strains and breeding of *An. gambiae* s. l.

The *Anopheles gambiae* s. l. used, comes from a local strain (Goden) that was established in the "Centre National de Recherche et de Formation sur le Paludisme" (CNRFP), a medical entomology laboratory since 2004, from mosquitoes that were caught in the locality of Goden (12°25'N,1°20'O), a village located at 20 kms east from Ouagadougou. This local strain is composed of *Anopheles gambiae* s. s. and *Anopheles coluzzii*.

The sensitive Kisumu strain used for testing the plant extracts was established in 2012 at the insectaries of CNRFP. This strain was used as a control because of it sensitivity to four classes of chemical insecticide.

Mosquitoes breeding of was done in the CNRFP's insectariums at 27°± 2 °C, 75 ± 5% humidity and a photoperiod of 12 hours/12 diurnal and nocturnal. Female mosquitoes were periodically blood-fed on a restrained male rabbit for egg production. Larvae were fed using dog biscuits and the pupae were withdrawn daily using a plastic cup. For emergence, pupae were put in cages covered with mosquito nets. Adult mosquitoes were fed with a 10% glucose solution.

2.4 Oviposition-deterrence test

Oviposition deterrence activity was tested using Xue *et al.* [29] method. Ten gravid females (5-10 days old) of the *Anopheles gambiae* (local strain) were put to oviposit in the covered cages, and provided with a 10% glucose solution. A serial dilution of the plant extracts was made in ethanol to obtain three extract solutions at concentrations of 100, 500, and 1000 ppm. In each case, one product in these different concentrations was used. Two plastic cups, one treated with the extract and the other not treated but containing a 1% dilution of the solvent, were placed in a cage (45 X 45 X 30 cm) at opposite angles. The plastic cups' position were alternated randomly to avoid the effect of position on oviposition. Four repetitions were done for each concentration and for each test. Mosquitoes were maintained in conditions of 27° ± 2 °C and 75 ± 5% humidity. The laid eggs were counted 24 h after the egg-laying by removing the filter paper.

The effective repellency or (ER) was calculated using the following formula:

$$ER = \frac{NC-NT}{NC} \times 100$$

ER= effective repellency; NC= number of eggs in control; NT= number of eggs in treatment

The oviposition activity index (OAI) was calculated using the Kramer and Mulla [30] formula. The index varies between -1 for highest oviposition-deterrence effect to 1 for lowest effect. Positive values indicates that's there is more oviposition in the treated laying plastic cups than in the controls, which means that the correspondent does not inhibit the oviposition. Negative values indicates that there is more oviposition in the control laying plastic cup than in the treated laying plastic cup, which means the extract is repellent to the gravid females and inhibits oviposition. The calculation of the oviposition activity index was done using the following formula:

$$OAI = \frac{NT - NC}{NT + NC}$$

Three categories of substances can be identify according to the oviposition activity index:

Strong oviposition inhibit substance (strong oviposition deterrence) -1< OAI <-0.5

Moderated oviposition inhibit substance (moderate oviposition deterrence) - 0.5< OAI < 0

No oviposition deterrence or attractant substance OAI > 0

2.5 Larvicidal tests

The larvicidal tests were carried out following the standard WHO protocol [31].

The extracts were removed from the Petri dish and diluted in bottles to make stock solutions. One gram of each extract was taken and melted in 100 ml of acetone. The Dimethyl-sulfoxid (DMSO) was used as an emulsifier with a 0.1% content. From a stock solution of 10,000 ppm, serial dilution was made to obtain concentrations ranging from 10 to 320 ppm in 300 ml plastic cups. The final volume was 250 ml, containing 20 third to fourth instar mosquito larvae. For every concentration, five replications were made. In the control cups, 20 larvae were introduced in 249 ml of distilled water (0.1% DMSO) and one ml of acetone. Larvae mortality was assessed after 24 h. The tests were conducted in the same conditions of humidity, temperature and of photoperiods as for the oviposition deterrence tests.

2.6 Data analysis

Data were analyzed using the software XLStat 7.5.2, using a significance level of 0.05. When larval mortality was higher than 10% in the control, the formula of Abbott [32] was applied to correct the mortality in the treatments:

$$\%M = \frac{\%\text{ test mortality} - \%\text{ control mortality x } 100}{100 - \%\text{ control mortality}}$$

The log-Probit model [33] was fitted to the data recorded in the larvicidal bioassay to assess the 50% lethal concentration (LC_{50}), the 90% lethal concentration (LC_{90}) and their 95% confidence limits. The LC by strain and the mean number of eggs in the control plastic cup and treatment plastic cup were compared using ANOVA following the LSD test of Fisher.

3. Results

The yields of extracts were 0.31, 0.71, 0.36 and 0.29% for hexane; 1.42, 1.04, 1.12 and 1.24% for ethanol; 1.18, 0.71, 0.61 and 1.21% for acetone respectively for *L. camara*, *H. spicigera*, *H. suaveolens* and *O. canum*. The yield was higher in the ethanol extracts than other solvents, irrespective of the plants. The qualitative screening of the extracts' composition reveals the presence of the steroids Ester and triterpene in all the extracts (Table 1). The aglycons flavonoids and the carotenoids were more or less present in the acetone and hexane extracts except in the ethanol extract. Tannins, saponosides and alkaloids were evident in the extracts of the polar solvents, except in the hexane extracts. Coumarins were detected in the ethanol extract of *Hyptis suaveolens.* Anthocyanosides were only present in acetone and ethanol extracts of *Hyptis spicigera*.

The oviposition activity index was negative at all concentrations for all plant extracts tested, which indicates an inhibition of *Anopheles gambiae* oviposition. The inhibition was concentration-dependent, when the dose was high, mosquitoes oviposition deterrence was also high. It is evident from the study that the rise in concentrations of the crude extracts caused an increase of effective repellency percentage (Table 2). The number of eggs laid in the control plastic cup was different from the number of eggs laid in treated plastic cups (P<0.05) for all the extracts, except at 100 ppm of the acetone extracts of *H. suaveolens*, *H. spicigera*, *O. canum* and hexane extract of *O. canun*. Effective repellency percentages varied from 18.4 to 100%. The acetone extract of *O. canum* at 100 ppm yielded the lowest percentage, and the acetone extracts of *H. suaveolens* and *L. camara* presented the strongest oviposition-deterrence at 1000 ppm. All the extracts revealed a strong oviposition deterrence from the concentration of 500 ppm with OAI varying from-0.90 for the acetone extract of *L. camara* to -0.22 for the hexane extract of *H. spicigera*. The mean number of eggs laid in the acetone extracts of *L. camara* and *H. suaveolens* at the three different concentrations 100 ppm, 500 ppm and 1000 ppm were 128 ± 16, 10 ± 1, 0 ± 0 eggs and 25 ± 24, 7 ± 2, 0 ± 0 eggs per cup respectively.

The least active extract was the ethanol extract of *H. spicigera* which inhibited the egg-laying of 76.00% at 1000 ppm (Table 2).

The larvicidal effect of plant extracts on *Anopheles gambiae* (local and sensitive strains) were dependent of the concentration of the extracts, and is presented in terms of percentage of mortality in table 3. No mortality was observed in the control. The sensitive strain was more susceptible to the different plant's extracts than the local strain, and the results are summarized in the table 4, 5 and 6 respectively for acetone, ethanol and hexane extracts. The hexane extract, regardless of the plant, presented the highest toxicity against larvae (Fig. 2). Among the four plants used, *L. camara* showed the lowest LC value regardless of the solvent with the LC_{50} ranging from 15.94 to 63.13 ppm for sensitive strain and LC_{90} ranging from 20.19 to 202.34 ppm for local strain (Fig. 2). Acetone and ethanol extracts of *H. spicigera* and *H. suaveolens* were least toxic against larvae (Table 3). *H. spicigera* acetone and ethanol extracts showed LC_{50} of 110.03 (95% CL=88.86-135.23) and 233.19 ppm (95%CL=224.14-374.94), and LC_{90} of 217.43 (95% CL=183.11-273.81) and 354.19 ppm (95% CL=334.64-435.65) respectively for the sensitive strain (Table 4 and 5). *H. spicigera* acetone extract was more toxic to *An. gambiae* larvae than *H. spicigera* ethanol extract. For ethanol and acetone extracts of *H. suaveolens*, LC_{50} for sensitive strain varied from 78.88 (95% CL=59.41-101.65) to 95.66 ppm (95% CL=77.73-118.07) and the LC_{90} varied from 193.49 (95% CL=158.99-253.50) to 196.76 ppm (95% CL=164.83-249.65). Similar trends were observed in larval mortality in case of local strain.

The lethal concentration (LC), which were obtained with the acetone extracts, varied depending on plants according to Fisher's Least Significant Difference (LSD) test ($P < 0.05$). LC_{50} and LC_{90} which were obtained with *L. camara* were the lowest and statistically different from the others LC. LC_{50} and LC_{90} of *L. camara* were respectively of 32.62 (95% CL=26.99-39.66) and 54.64 ppm (95% CL=44.85-69.59) for the sensitive strain and 106.09 (95% CL=88.82-128.59) and 180.29 ppm (95% CL=154.95-224.54) for the local strain (Table 4). *O. canum* was the second most toxic plant against the third and fourth instar larvae of the LC_{50} and $LC_{90,}$ which were respectively of 36.96 (95%CL=24.53-75.64) and 91.17 ppm (95%CL=72.98-127.95) for the sensitive strain and of 162.08 (95% CL=141.15-184.58) and 228.55 ppm (95% CL=202.88-272.46) for local strain (Table 4). Acetone extracts of *H. suaveolens* and *H. spicigera* were the least toxic against larvae of the two strains.

The results of the larvicidal tests with ethanol extract of the four plants are presented in tables 3 and 5. *L. camara* was the most toxic with LC_{50} and LC_{90} respectively of 29.23 (95% CL=22.00-37.43) and 63.13 ppm (95% CL=37.42-84.18) for the sensitive strain and of 122.82 (95% CL=103.77-146.68)

and 202.34 ppm (95% CL=173.65-247.59) for the local strain ($P < 0.05$). These value were different from the other extracts which were six or eight times higher regarding the ratio of the LC by strain and by plants. *H. spicigera* was the least toxic with LC_{50} and LC_{90} which are respectively of 233.19 (95%CL=224.14-374.94) and of 354.19 ppm (95%CL=334.64-435.65) for sensitive strain and of 265.66 (95% CL=203.21-271.83) and 367.80 (95% CL=308.23-433.40) ppm for local strain.

L. camara and *O. canum* hexane extracts did not show any difference ($P < 0.05$). *L. camara* and *O. canum* LC_{50} were respectively 15.94 (95 CL=10.01-21.34) and 20.69 ppm (95% CL=13.22-27.81) for the sensitive strain and of 20.19 (95% CL=14.35-26.67) and 43.22 ppm (95% CL=32.86-55.97) for the local strain (Table 6). These LCs illustrates the potential of the hexane extract in the control of *An. gambiae* larvae.

4. Discussion

In this study, the oviposition deterrence of the gravid females and the larval mortality of two strains of the malaria vector *An. qambiae* were measured in response to different plant extracts of varying concentrations.

All the OAI were negative, showing that all the tested extracts inhibit the oviposition of *An. gambiae*. This replicates observations made by Elango *et al*. [21] who tested the ethyl acetate, acetone and methanol extracts of *Andrographis paniculata* Burm, *Eclipta prostrata* L. and *Tagetes erecta* L. on *Anopheles subpictus*. Their results showed that the inhibition was dose-dependent as shown by the current study. The extracts of petroleum ether and ethyl acetate of *Eugenia jambolana*, *Solidago canadensis*, *Euodia ridleyi* and *Spilanthes mauritiana* leads to a dose-dependent inhibition of the egg-laying of *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [34]. The averages of the eggs deposited in the treated plastic cups were different ($p < 0.05$) at the 500 and 1000 ppm concentrations. This can be explain by the fact that *An. gambiae* prefer egg-laying in clean waters rather than in organic polluted waters[35] or waters containing potential insecticide substances, which may be due to changes induced in the physiology and behavior of the adult mosquito and reflected by their egg-laying capacity[34]. Oviposition, which involves the sensory receptors of the mosquito for it to find the ideal oviposition site is an important step in the life cycle of mosquitoes [29, 34, 36]. Thus, reducing egg-laying in breeding sites could significantly advance the control of *An. gambiae*.

Larvicidal tests showed that all the extracts have a larvicidal effect on the two strains of *An. gambiae* with a remarkable variation in sensitivity. All extracts show a larvicidal activity increasing from the lowest to the highest concentrations. The bioactivity of plant-based-insecticides against mosquito larvae varies significantly according to the solvent used in the extraction and the mosquito species tested [23]. The sensitive strain was less resistant to the extracts than the local strain. This variation of sensitivity between two strains of *An. gambiae* has also been shown by Azokou *et al*. [37]. These authors showed that the ethanol extracts of *Cochlospermum planchonii*, *Phyllanthus amarus*, *Heliotropium indicum*, *Cissus populnea* and *Vitex grandifolia* act differently on sensitive strain and the wild strain of *Anopheles gambiae* and *Culex quinquefasciatus*. Hexane, chloroforme, ethyl acetate, acetone and methanol extracts of *Annona squamosa*, *Chrysanthemum indicum* and *Tridax procubems* leads to strong mortality of *An. subpictus* [38]. The larvicidal effects that we have observed differ from those obtained by Pierre *et al*. [39] regarding the LC. The hexane extracts of *Callistemon rigidus* R.Br. presents LC_{50} and LC_{90} respectively of 17.11 ppm and 82.71ppm on four instar larvae of *An. gambiae* after an exposition of 24 hours [39]. This could be linked to the plants' ecology and the solvent used for the extraction. However, in both cases, the hexane extracts were more toxic towards the *An. gambiae* larvae. Among all the used plants, *L. camara* showed the highest toxicity against larvae regarless the kind of solvent. The insecticidal properties of this plant have already been shown through use of its essential oils on the adults of *Ae. aegypti*, *Cx. quinquefaciatus, An. culicifacies*, *An. fuviatilis* and *An. stephensi* [40] and its methanol and ethanol extracts[41]. This plant seems to have a greater insecticide potential than the other plants studied here.

Phytochemical screening of the extracts revealed the presence of triterpenes, tannins, saponosides, alkaloids, flavonoids and phenols. The same chemical groups have been found in the extracts of *Ipomoea cairica* (L.), *Ageratina adenophora* (Spreng.), *Pistia stratiotes C*, *Leucas martinicensis*, *Cynodon dactylon* and *Nymphaea lotus* [42, 43] which possess larvicidal properties. From one extract to another, the chemical composition varies for the same extract. This may explain the variability of the larvicidal and oviposition-deterrence's effects according to the nature of the solvent. In fact, the larvicidal and oviposition-deterrence activities could be due to secondary metabolites extracts, of which insecticide effects have been proved by some authors [39, 44, 45]. The high larvicidal activity of the hexane extracts could be due to the presence of terpenes, known to have insecticidal activity properties [46], and steroids that add themselves to the other compounds to manifest their larvicidal effects.

These results attest to the effective larvicidal and oviposition-deterrence properties against *An. gambiae* of plant extracts use in this study against *An. gambiae*. Among the tested effects, those obtained from the hexane and above all those of *L. camara*, presented a high toxicity against larvae and strongly inhibit the egg-laying of *An. gambiae*. Further studies will permit the identification of these extracts' active compounds and their residual effects in aquatic areas.

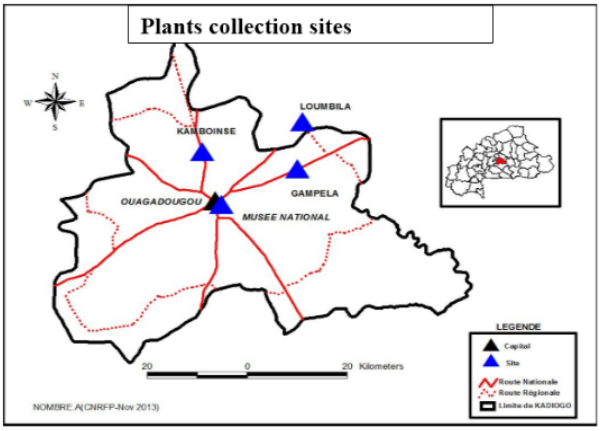


Fig 1: Map illustrating the harvest sites of the plants

Table 1: Qualitative phytochemical compounds highlighted in the extracts

Compounds	*Lantana camara*			*Hyptis suaveolens*			*Hyptis spicigera*			*Ocimum.canum*		
	Acetone	**Ethanol**	**Hexane**	**Acetone**	**Ethanol**	**Hexane**	**Acetone**	**Ethanol**	**Hexane**	**Acetone**	**Ethanol**	**Hexane**
Steroids Esters and Triterpenes	+	+	+	++	++	++	+	+	+	++	++	+
Flavonoid Aglycons	-	-	+	-	-	-	-	-	+	-	-	-
Carotenoids	+/-	-	+/-	+	-	++	+	-	++	+/-	-	+/-
Chlorophylls	++	+/-	+	++	+/-	+	++	+	+	+	-	+/-
Tannins & Phenolic compounds	+	+	-	+	+	-	++	++	-	+	+	-
Saponosids	+/-	++	-	+	++	-	+	+/-	-	+/-	++	-
Alkaloids	+/-	+/-	-	+/-	+	-	+/-	+/-	-	-	-	-
Reducing compounds	+	+	-	+	+	-	+	++	-	+/-	+	-
Flavonoids	+	+	-	-	-	-	+	+	-	++	++	-
Coumarins	-	-	-	-	+	-	-	-	-	-	-	-
Anthocyanosides	-	-	-	-	-	-	+	+	-	-	-	-

(++)= Present at high concentration**; (+) =** Present at low concentration **;(+/-)** = Traces ;(**-)** = Not pre

Table 2: Oviposition-deterrent activity of *L. camara, H. suaveolens*, *H. spicigera* and *O. canum* extracts against *Anopheles gambiae* s.l.

		Acetone				**Ethanol**				**Hexane**			
Plants	**Concentrations (ppm)**												
		Number of eggs ±SD				**Number of eggs ±SD**				**Number of eggs ±SD**			
		Treated	**Control**	**ER%**	**OAI**	**Treated**	**Control**	**ER%**	**OAI**	**Treated**	**Control**	**ER%**	**OAI**
Lantana camara L.	1000	$0±0^{a}$	258±36*	100	-1	$5±4^{a}$	200±12*	98.20	-0.96	$27±5^{a}$	163±8*	83.42	-0.71
Verbenacae	500	$10±1^{a}$	224±55*	94.70	-0.90	$13±3^{ab}$	300±45*	95.80	-0.91	$35±7^{a}$	130±6*	73.57	-0.58
	100	$128±16^{b}$	256±31*	50.00	-0.33	$39±6^{b}$	109±12*	65.00	-0.50	$77±5^{b}$	174±38*	54.00	-0.37
Hyptis. suaveolens	1000	$0±0^{a}$	116±7*	100	-1	$8.6±5^{a}$	265±47*	96.80	-0.93	$12±2^{a}$	102±15*	87.20	-0.77
Poit (Lamiaceae)	500	$7±2^{b}$	62±7*	88.20	-0.78	$15±6^{a}$	225±21*	90.60	-0.82	$24±8^{a}$	194±62*	85.50	-0.75
	100	$25±24^{c}$	117±60	77.00	-0.62	$49±9^{b}$	200±49*	75.20	-0.60	$138±14^{b}$	186±18*	25.90	-0.14
Hyptis. spicigera	1000	$27±1^{a}$	266±29*	87.70	-0.78	$41±8^{a}$	173±45*	76.00	-0.61	$21±6^{a}$	260±79*	91.8	-0.84
Lam (Lamiaceae)	500	$56±2^{a}$	218±9*	74.90	-0.60	$88±34^{ab}$	259±48*	66.48	-0.50	$94±13^{b}$	149±29*	36.7	-0.22
	100	$79±22^{a}$	158±35	51.60	-0.36	$67±6^{b}$	106±3*	37.00	-0.22	$126±11^{c}$	181±10*	30.00	-0.17
Ocimum. canum	1000	$32±6^{a}$	162±42*	78.66	-0.65	$15±3^{a}$	254±50*	93.70	-0.88	$1±4^{a}$	113±11*	99.35	-0.98
Sims (Lamiaceae)	500	$75±2^{a}$	207±46*	64.50	-0.47	$18±4^{a}$	183±29*	89.70	-0.81	$112±3^{b}$	232±7*	51.68	-0.34
	100	$181±29^{b}$	224±41	18.40	-0.10	$42±13^{b}$	101±19*	57.70	-0.41	$101±39^{b}$	156±6	33.90	-0.20

Means of 4 repetitions; OAI= Oviposition Active Index, ppm= parts per million; SD: Standard Deviation d-; ER= effective repellency;*=Significant differences between treated and control by LSD test of Fisher ($P<0.05$), eggs mean for the same plant and the same solvent follow by the same letter doesn't shown statistical differences by Turkey test ($P<0.001$)

Table 3: Result of larval mortality of different concentrations of extracts on third to four instar larvae of *Anopheles gambiae* s.l.

		Mean mortality (%±SD)					
Plants	**Concentra. (ppm)**	***An. gambiae* (Local)**	***An. gambiae* (Sens.)**	***An. gambiae* (Local)**	***An. gambiae* (Sens.)**	***An. gambiae* (Local)**	***An. gambiae* (Sens.)**
H. spicigera		Hexane	Hexane	Ethanol	Ethanol	Acetone	Acetone
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00	0.00 ± 0.00
	10	8.66 ± 1.41	25.81 ± 0.69	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	40	56.70± 2.06	39.75 ± 1.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	35.43 ± 3.25
	80	55.93 ± 7.54	95. 43 ± 7.87	1.44 ± 0.25	10.00 ± 4.08	2.50 ± 0.28	79.99 ±0.08
	160	84.08 ± 1.06	100 ± 0.00	3.63 ± 0.48	30.00 ± 4.08	26.25 ± 3.58	80.71 ±1.14
	240	81.04 ± 5.04	100 ± 0.00	38.87 ± 2.67	56.66 ± 10.00	51.25± 5.07	88.33 ± 2.03
	320	100 ± 0.00	100 ± 0.00	45.22 ± 0.21	77.55 ± 6.45	82.53 ± 7.33	100 ± 0.00
H. suaveolens		Hexane	Hexane	Ethanol	Ethanol	Acetone	Acetone
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	10	0.00 ± 0.00	15.03 ± 1.54	0.00 ± 0.00	23.43 ± 3.06	0.00 ± 0.00	1.33 ± 0.23
	40	16.13 ± 0.75	28.54 ± 4.10	7.50 ± 0.5	33.35 ± 3.01	12.55 ± 6.45	49.71 ± 1.24
	80	28.39 ± 1.79	47.33 ± 3.05	16.25 ± 0.6	61.10 ±1.10	25.75 ± 6.29	64.07 ± 1.73
	160	76.37 ± 0.72	98.71 ± 1.22	43.75 ± 7.5	73.92 ± 1.46	38.75 ± 4.78	67.49 ± 3.30
	240	86.20 ± 2.33	100 ± 0.00	65.00 ± 5.2	95.44 ± 4.05	62.50 ±1.04	75.33 ± 4.27
	320	100 ± 0.00	100 ± 0.00	78.75± 4.7	100 ± 0.00	82.50 ± 5.00	100 ± 0.00
L. camara		Hexane	Hexane	Ethanol	Ethanol	Acetone	Acetone
	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	10	25.92 ± 3.70	49.82 ± 1.49	0.00 ± 0.00	30.88 ± 1.18	0.00± 0.00	11.22± 0.53
	40	80.54 ± 9.51	97.20 ± 0.25	10.26 ± 4.09	75.77 ± 7.11	16.25 ± 2.50	82.18 ± 0.82
	80	98.03 ± 3.39	100 ± 0.00	34.53 ± 5.85	95.88 ± 4.00	37.50 ± 1.65	95.69 ± 0.46
	160	100 ± 0.00	100 ± 0.00	74.48 ± 10.18	98.54 ± 2.51	80.00 ± 4.07	100 ± 0.00
	240	100 ± 0.00	100 ± 0.00	90.83 ± 7.89	100 ± 0.00	100 ± 0.00	100 ± 0.00
	320	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
O. canum		Hexane	Hexane	Ethanol	Ethanol	Acetone	Acetone
	0	0,00 ± 0,00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	10	22.65 ± 1.65	56.63 ± 3,52	0.66± 0.57	0.00 ± 0.00	0.00 ± 0.00	28.40 ± 3.23
	40	43.82 ± 2.68	76.40 ± 0.73	9.01 ± 0.71	59.81 ± 5.36	0.00 ± 0.00	68.38 ± 1.58
	80	74.50 ± 1.97	100 ± 0.00	17.13 ± 5.10	66.82 ± 1.52	7.50 ± 0.64	73.41 ± 1.47
	160	100 ± 0.00	100 ± 0.00	24.54 ± 1.11	88.90 ± 2.04	50.00 ± 4.08	100 ± 0.00
	240	100 ± 0.00	100 ± 0.00	58.14 ± 8.31	95.88 ± 4.04	91.25 ± 8.53	100 ± 0.00
	320	100 ± 0.00	100 ± 0.00	77.50 ± 1.12	100 ± 0.00	100 ± 0.00	100 ± 0.00

Means of 5 repetitions; ppm = parts per million; SD: Standard Deviation; Sen.= sensitive strain

Table 4: Median and 90% lethal concentrations (LC_{50} and LC_{90}) of acetone extract of *L. camara, H. suaveolens*, *H. spicigera* and *O. canum* against L3 to L4 larvae of *Anopheles gambiae* local strain (Goden) and sensitive strain (Kisumu) 24 h post-treatment

Plants	Solvent	Strain	LC_{50} (ppm)	LLC-ULC	LC_{90} (ppm)	LLC-ULC	χ^2
H. suaveolens	Acetone	Sensitive	95.66^{c}	77.73-118.07	196.76^{d}	164.83-249.65	51.67
		Local	201.66^{d}	173.15-238.13	341.00^{d}	393.91-415.83	58.54
H. spicigera	Acetone	Sensitive	110.03^{c}	88.86-135.23	217.43^{d}	183.11-273.81	51.71
		Local	233.48^{e}	204.65-269.51	349.83^{d}	305.99-428.98	37.31
L. camara	Acetone	Sensitive	32.62^{b}	26.99-39.66	54.64^{b}	44.85-69.59	36.49
		Local	106.09^{b}	88.82-128.59	180.29^{b}	154.95-224.54	52.52
O. canum	Acetone	Sensitive	36.96^{b}	24.53-75.64	91.17^{c}	72.98-127.95	34.10
		Local	162.08^{c}	141.15-184.58	228.55^{c}	202.88-272.46	34.48
Cypermethrin		Sensitive	0.03^{a}	0.02- 0.04	0.13^{a}	0.11- 0.16	17.04
		Local	0.08^{a}	0.06-0.11	0.31^{a}	0.26-0.46	26.01

Means of 5 repetitions; LC: lethal concentration; LCL: Lower confidence limit; UCL: Upper confidence limit;χ2=Chi-square The LC values of the same column for the same strain followed identical alphabetic letters are not statistically different (Fisher's LSD test p <0. 05).

Table 5: Median and 90% lethal concentrations (LC_{50} and LC_{90}) of ethanol extract of *L. camara, H. suaveolens*, *H. spicigera* and *O. canum* against L3 to L4 larvae of *Anopheles gambiae* local strain (Goden) and sensitive strain (Kisumu) 24 h post-treatment

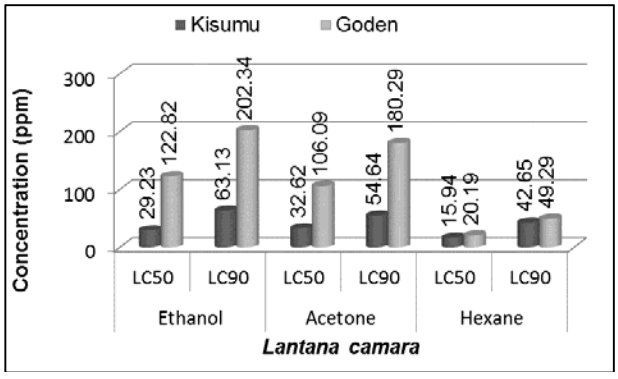
Plants	Solvent	Strain	LC_{50} (ppm)	LLC-ULC	LC_{90} (ppm)	LLC-ULC	χ^2
H. suaveolens	Ethanol	Sensitive	78.88^{c}	59.41-101.65	193.49^{c}	158.99-253.50	42.50
		Local	206.49^{c}	178.44-241.85	339.97^{c}	294.34-411.03	58.87
H. spicigera	Ethanol	Sensitive	233.19^{d}	224.14-374.94	354.19^{d}	334.64-435.65	16.28
		Local	265.66^{d}	203.21-271.83	367.80^{c}	308.23-433.40	43.50
L .camara	Ethanol	Sensitive	29.23^{b}	22-37.43	63.13^{b}	37.42-84.18	32.57
		Local	122.82^{b}	103.77-146.68	202.34^{b}	173.65-247.59	55.53
O. canum	Ethanol	Sensitive	70.23^{c}	54.2-88.48	153.49^{c}	127.87-195.76	47.64
		Local	209.84^{cd}	179.39-249.96	359.61^{c}	300.15-433.40	51.87
Cypermethrin		Sensitive	0.03^{a}	0.02- 0.04	0.13^{a}	0.11- 0.16	17.04
		Local	0.08^{a}	0.06-0.11	0.31^{a}	0.26-0.46	26.01

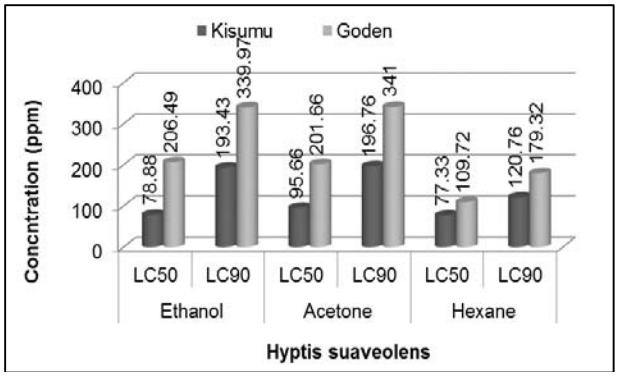
Means of 5 repetitions; LC: lethal concentration; LCL: Lower confidence limit; UCL: Upper confidence limit; χ2=Chi-square; the LC value of the same column for the same strain followed identical alphabetic letters are not statistically different (Fisher's LSD test p <0.05).

Table 6: Median and 90% lethal concentrations (LC_{50} and LC_{90}) of hexane extract of *L. camara, H. suaveolens*, *H. spicigera* and *O. canum* against L3 to L4 larvae of *Anopheles gambiae* local strain (Goden) and sensitive strain (Kisumu) 24 h post-treatment

Plants	Solvent	Strain	LC_{50} (ppm)	LLC-ULC	LC_{90} (ppm)	LLC-ULC	χ^2
H. suaveolens	Hexane	Sensitive	77.33^{d}	66.18-93.33	120.76^{d}	102.68-151.90	40.02
		Local	109.72^{d}	94.4-129.53	179.32^{d}	154.65-218.68	56.20
H. spicigera	Hexane	Sensitive	29.77^{c}	22.86-38.76	58.87^{c}	48.41-82.63	30.65
		Local	60.71^{c}	40.39-75.81	211.29^{d}	173.01-282.64	39.33
L. camara	Hexane	Sensitive	15.94^{b}	10.01-21.34	42.65^{b}	31.83-53.97	28.54
		Local	20.19^{b}	14.35-26.67	49.29^{b}	37.13-65.40	27.25
O. canum	Hexane	Sensitive	20.69^{b}	13.22-27.81	48.38^{bc}	38.85-68.39	29.00
		Local	43.22^{bc}	32.86-55.97	93.02^{c}	75.34-127.19	42.22
Cypermethrin		Sensitive	0.03^{a}	0.02- 0.04	0.13^{a}	0.11- 0.16	17.04
		Local	0.08^{a}	0.06-0.11	0.31^{a}	0.26-0.46	26.01

Means of 5 repetitions; LC: lethal concentration; LCL: Lower confidence limit; UCL: Upper confidence limit; χ2=Chi-square; The LC values of the same column for the same strain followed identical alphabetic letters are not statistically different (Fisher's LSD test p <0.05





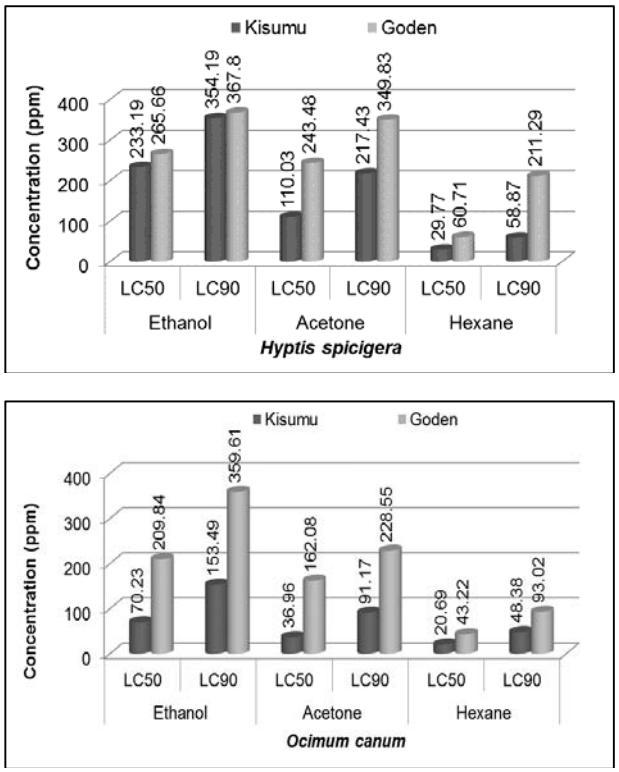


Fig 2: Lethal concentrations (LC_{50} and LC_{90}) of acetone, ethanol and hexane extracts of four plants against both strains of *Anopheles gambiae* larvae

5. Conclusion

The present study clearly demonstrated larvicidal and oviposition-deterrence activities efficacy of extracts against *An. gambiae*. Among the tested effects, those obtained with hexane and above all plants, *L. camara*, present a strong toxicity towards larvae and strongly inhibit the egg-laying of mosquitoes. The usage of these extracts against larvae is promising and could help for developing of new natural product from plants.

6. Acknowledgements

This work was partly supported by the grant IFS F/5017-1 from International Foundation for Science to Athanase Badolo. The authors are grateful to West African Agricultural of productivity Programme (WAAPP/2015) for the scholarship given to Dimitri Wangrawa. We also thank Dr. Nabéré Ouattara from LABIOCA for his help during the compounds extraction and the technicians of CNRFP for their support during the biological tests. Thank you to Charlotte L. R. Payne for the language edition.

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