



Mosquitocidal Properties of Three Essential Oil Plants Tested against *Anopheles gambiae* Sensu Stricto, Giles 1902 (Diptera: Culicidae, Anophelinae), Main Malarial Vector

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ABSTRACT

Objective: The efficacy of essential oils of *Lantana camara*, *Ricinus communis* and *Securidaca longepedunculata*; three medicinal plants from Northern Cameroon has been assessed against malarial vector, *Anopheles gambiae* Sensu Stricto (*An. gambiae* s.s.). The synthetic chemicals Delthamethrin (DEET), was used as positive control, and water and hexan were used as negative control.

Methods: The insecticidal effect of the essential oils from leaves was tested in old adult *An. gambiae* s.s. aged three to four days at five concentrations (35, 45, 55, 100 and 150 ppm). The adult mortality was observed after 24 hours under the laboratory conditions according to WHO (2020) bottle method.

Results: All of bioassay; the maximum efficacy was recorded in the three essential oils after 24 hours exposure. The LC50 values against adults of *An. gambiae* s.s. were 66.72 ppm, 72.67 ppm, 79.63 ppm and 59.49 ppm, respectively for *Securidaca longepedunculata*, *Lantana camara*, *Ricinus communis* and T+ (DEET). However there was no significant difference between these letal concentrations ($P \leq 0.05$). Elsewhere, the LC95 values were 111.40 ppm, 113.4 ppm 1, 105.35 ppm and 99.08 ppm for *L. camara*, *R. communis*, *S. longepedunculata*, and DEET respectively. Six percent (6%) mortality was observed in negative control (hexan). The HC50 were 3h40, 4h38 and 7h34 for *S. longepedunculata*; *L. camara* and *R. communis* respectively. The HC50 of positive control was 1h49. Between DEET HC50 and HC50 of all essential oils, difference was significant.

Conclusion: At the end of our experiments, we realized that there are local means to fight against vector agent of malaria. Essential oils of *Lantana camara*, *Ricinus communis* and *Securidaca longepedunculata* are potential for controlling malarial vector.

Keywords: Essential oil; Vector mosquitocidal effect; Efficacy; *Anopheles gambiae*

INTRODUCTION

Malaria is the main parasitic disease which caused the most mortality in all over the world particularly in Africa. It is transmitted by the *Anopheles gambiae* mosquito's complex [1]. Mosquitoes do not only cause malaria but others borne diseases. They are the main vectors which contribute to the major burden in Africa, particularly in Cameroon [2]. Since there is no effective vaccine available for the control of malaria disease, prevention of mosquito bites is one of the main strategies to control or minimize incidence of this disease and the others borne diseases [3]. The malaria control strategies in Africa include the combination of insecticide treated nets and

indoor residual spraying that was found to be effective as a control measure [4]. Although effective, the repeated use these synthetic insecticides has disrupted natural biological control systems and sometimes resulted in widespread development of physiological resistance [5]. These problems have warranted the need for developing alternative strategies using eco-friendly products [6]. Plant products (extracts, essential oils or vegetal oils) appeared safer for non-target organisms including man. They are cost-effective and biodegradable. They not only can play an important role in the interruption of the transmission of malaria disease at the individual as well as at the community level but also to others vectors (Dengue, yellow fever). Various reports on the plant's extracts or essential oils

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like pesticide, larvicide or adulticide against insects vectors have been published [7]. Cameroon has wide vegetation which contains many medicinal plants. From North to South, East to west, these plants are employed in the traditional healthcare and that some of them said to be indigenous to have healing potentials [8]. Example, the results of the study conducted by Saotoing Pierre showed that vegetable oils of *Khaya senegalensis* and *Azadirachta indica* are widely used against malaria pathogen, *Plasmodium falciparum* [9]. Essential oils of *Occimum canum* are adulticidal against *Anopheles funestus* [10]. For preventing malaria and other diseases it is commonly practiced by indigenous people using traditional insecticide plants. In the present investigation, essential oils from indigenous people from Farth-Nord Cameroon plants have been tested to assess their adulticidal properties when applied to the adults of *Anopheles gambiae* sensu stricto, a main malaria vector. The essential oils from leaves of *Lantana camara*, *Ricinus communis* and *Securidaca longepedunculata* have been tested in adults under the laboratory conditions. The overall purpose of the present study is to value the adulticidal properties of three essential oils in order to contribute to malarial vector prevention.

MATERIALS AND METHODS

Selection and collection of plant materials

Three different plant species were selected in the town of Maroua, the Farth north region of Cameroon. Plant selection in the study was based on interviewing traditional members of the community to specify the indigenous plant species known for their use in the regular control of mosquitoes and other insects in their localities. In the many villages, the most used plants by the members of the community were *Lantana*, *Ricinus*, *Securidaca*, *Hyptis* and *Leucas*, then through analysis of the relevant literature. Plant materials of the three indigenous plant species listed in Table 1 were collected from different localities in Maroua. Leaves were collected from different quarters of Maroua town during the growing season (October-September and April respectively) in 2019. Bulk samples were air-dried in the shade at room temperature and after drying the sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared and identified with the help of national Herbarium of Yaounde.

Extraction of essential oils

One thousand grams of leaf powder of each plant species was

used separately for essential oil extraction. The plant powder was subjected to hydro distillation 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 following the method [11].

Mosquito rearing

Anopheles gambiae s.s. colonies were maintained in the laboratory of Entomology of the University of Ngaoundere. The *Anopheles*' Eggs provided came from Coordination Organization for the fight against Endemics in Central Africa. (OCEAC) of Yaounde-Cameroon. Eggs were placed in white enamel trays each containing tap water and kept at room temperature (28 ± 2°C). After 48 hours larvae were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast. The trays with pupae were maintained in separate mosquito cages for emerging mosquitoes. Cotton soaked in 10% aqueous sucrose solution admixed with few drops of multivitamin drops in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. Adulticidal test was carried out on adults aged from three to four days.

Preparation of stock solutions and coating of bioassay bottles

The essential oils were dissolved in 98% hexane. The choice of this chemical solvent was about the ability of this solvent to evaporate very fast. Pipeman Gilson P 200M8 RL. 0.02-2 ml was used to sample the concentration. To obtain the concentrations of 30, 40, 55, 100 and 150 mg/bottle of essential oils, products were dissolved in adequate quantity of hexane to make 10 ml of total solution. Each 1 mL of this solution would contain 30, 40, 55, 100 and 150 mg of the essential oils. 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% hexane was added to the negative control bottle and 1 ml of Deltamethrin for positive control. 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 12 hours on their sides and covered with aluminum foil that will keep them protected from light.

Table 1: Plant materials.

Scientific names	Vernacular names	Family names	No. Identification	Locality
<i>Lantana camara</i>		Verbenaceae	61845 HNC	Pitoaré
<i>Ricinus communis</i>	Kélééré'e (Tupuri)	Euphorbiaceae	14741SRF/Cam	Zilling
<i>Securidaca longepedunculata</i>	Gwerga (Tupuri)	Polygalaceae	10411SRF/Cam	Mt. makabayé

Adulticidal test using CDC bottles

Adulticidal bioassay was performed following the CDC, OMS method [12,13]. Protocol was followed to prepare and coat bottles. 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 150, 100, 55, 40 and 30 mg/bottle to identify the lowest concentration that inhibits the mosquito adults. Delthamethrin 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 LC50 and LC95 [14]. Using a mouth aspirator, 25 adults of *An. gambiae* s.s. were introduced into each test bottle including the control bottle. At start time, the bottles were examined to count the number of dead and live mosquitoes. The number of mosquitoes dead or alive was subsequently recorded every 20 minutes up to 24 hours or in a shorter time if all the mosquitoes died. However, data were grouped such that mortality counts were reported for 1, 6, 12, 18 and 24 hours post-treatment. The mortality in the control bottle was taken into consideration at the end of the bioassay (24 hours) 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 15%. The bioassay results were discarded, if mortality in the

control bottle at the end of the test was >15%. Mosquitoes were considered dead if they can no longer stand or move.

Data analysis

The adults' percent mortality was calculated and when control mortality ranged from 5%-20% it was corrected using Abbott's formula [15]. Graph pad version pris 5 was used for drawing graphs. LC50, LC95, HL50 and HL95 values were calculated using Finney formula [16]. Mortality data was subjected to ANOVA procedure and significantly different means were separated using Turkey's test (P=0.05) (SPSS version 16.0).

RESULTS

Yield

The yields of the essential oils were 0.78, 0.80 and 1.00 for *Securidaca longepedunculata*; *Lantana camara* and *Ricinus cominus* respectively. The yield varies from one plant to another. This fluctuation may be due at the nature of plant species, at the condition of individual plant; at the age of leaves and the moment of collection, in the flower time or not. The yields of essential oils of the three plants are confined in Table 2.

Table 2: Yields of essential oils of the three plants.

Species	Quantity of powder (g)	Wight (mL)	Yield (%)
<i>Lantana camara</i>	1000	8.7	0.87
<i>Ricinus cominus</i>	1000	10	1
<i>Securidaca longepedunculata</i>	1000	7.8	0.78

Phytochemical screenings

The three local plant essential oils contained all organic classes compound targeted and supposed to have insecticidal effects. The quantity of phytochemicals varied among plants compound. Tannins were lowly concentrated in essential oils of the three

plants while saponoids were highly concentrated in *R. cominus* and low in the others two. Flavonoids were moderately in the three essential oils. Terpenoids were highly concentrated in *L. camara* and *S. longepedunculata* but lowly in *R. cominus*. The results of phytochemicals screening are presented in Table 3.

Table 3: Qualitative phytochemical screening of essential oils.

Secondary metabolits	<i>Lantana camara</i>	<i>Ricinus cominus</i>	<i>Securidaca longepedunculata</i>
Flavonoïds	++	++	++
Saponins	+	+++	+
Tannins	+	+	+
Terpenoids	+++	+	+++

Adulticidal activity

The three plant essential oils were studied for their use as natural insecticides instead of organic phosphorous materials or other synthetic agents. Results on the adulticidal *An. gambiae* s.s. effects were reported in the present study, confirming their potential for control malarial vector. Results, after 1, 6, 12, 18 and 24 hours post-treatment are confined in Figures 1-3.

The results of adulticidal activity from essential oils of the three plants showed that the mortality increases with concentrations applied and time post-exposure. Until 16 hours post-treatment, there was no mortality observed in the negative control group, while in positive control adult mortality significantly varied from 63.33% to 100.00% after 1 hour and 6 hours post-exposure respectively. After 24 hours post-exposure, the lowest concentration (30 ppm) recorded 56.66%, 60.00% and 80.00% of mortality respectively for *R. communis*, *L. camara* and *S. longepedunculata* essential oils. For the three plants, 100% mortality was observed at the 100 ppm concentration. However, 55 ppm leads 100% of mortality after 24 hours post-exposure for the essential oils of *L. camara* and *S. longepedunculata*, and 86.66% of mortality for *R. communis*.

All the oils tested were toxic to adults of *An. gambiae* s.s. *Securidaca longepedunculata* oil showed strongest activity and was equitoxic to the commercial insecticide (Deltamethrin). The low dose that killed 50% (LC50) mosquito adults was obtained after 24 hours post-treatment. They were: T+ (Deltamethrin) 59.97 ppm; *S. longepedunculata* 66.72 ppm; *L. camara* 72.67 ppm and *R. communis* 79.63 ppm. The CL 95 was T+ (Deltamethrin) 99.08 ppm; *S. longepedunculata* 105.35 ppm, *L. camara* 111.75 ppm and *R. communis* 113.40 ppm. Essential oils often consist of thousand to two thousands (1000-2000) complex mixtures of active compounds. Advances of using complete mixture may act synergistically, they may show greater overall bioactivity compared to the individual constituents. In the present study, the activity of these essential oils would be attributed to the complex mixture of active secondary metabolites like terpenoids, flavonoids, tannins and saponins. Therefore, terpenoids are always the main compound which toxically nervous induce insect's mortality. Certainly it is the reason why essential oil of *S. longepedunculata*, the most contained terpenoids is the most efficient. The difference observed between toxicity of Deltamethrin and essential oils may be explained by the fact that Deltamethrin is a synthetic product. The LC 50, LC 95 and LH 50, LH95 are presented in Table 4.

Table 4: LC 50, LC 95 and LH 50, LH 95 values of the three plant essential oils.

Plant species	LC50 (ppm)	LC95 (ppm)	LH50 (h)	LH95 (h)
<i>Lantana camara</i>	72.67A*	111.75C*	4.38E*	6.33G*
<i>Ricinus communis</i>	79.63A*	113.40C*	7.34E*	10.02G*
<i>Securidaca longepedunculata</i>	66.72A*	105.30C*	3.40E*	5.13G*
T+ (Deltamethrin)	59.97B	98.08D	1,50F	3.57H
Between CL50 of H.E. $ddl=2$; $\alpha=0.05$; $Chi^2_{obs}=5.99$	$Chi^2_{cal}=1.14$	$Chi^2_{cal}=0.32$	$Chi^2_{cal}=1.65$	$Chi^2_{cal}=1.85$

Note: Same letter in the same colonne means no significant difference and different letters means difference is significant. A*=no significant; B=significant; C* and D=significant; E* and F=significant and G*and H=significant.

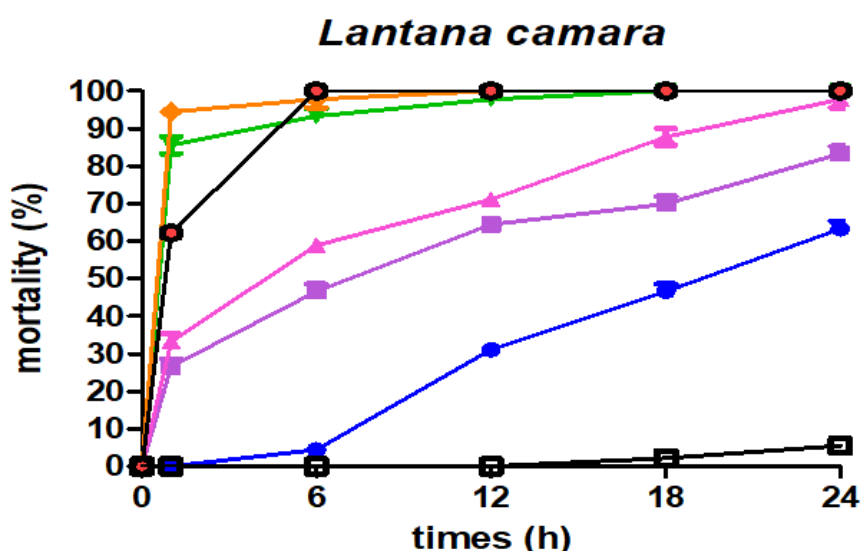


Figure 1: Anopheles mortality according to the various concentrations of essential oils of *Lantana camara*. Note: (●) 30; (■) 40; (▲) 55; (▼) 100; (◆) 150; (●) T+ (Deltamethrin 55); (■) T-.

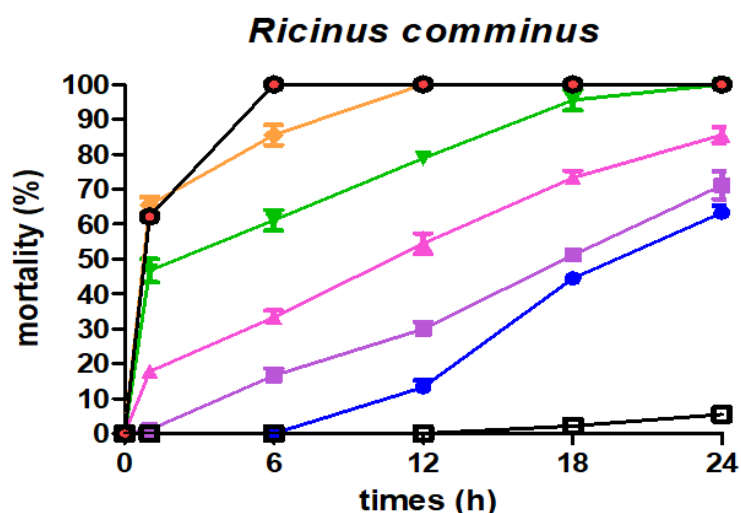


Figure 2: Anopheles mortality according to the various concentrations of essential oils of *Ricinus communis*. Note: (—●—) 30; (—■—) 40; (—▲—) 55; (—▼—) 100; (—◆—) 150; (—●—) T⁺ (Deltamethrin 55); (—■—) T.

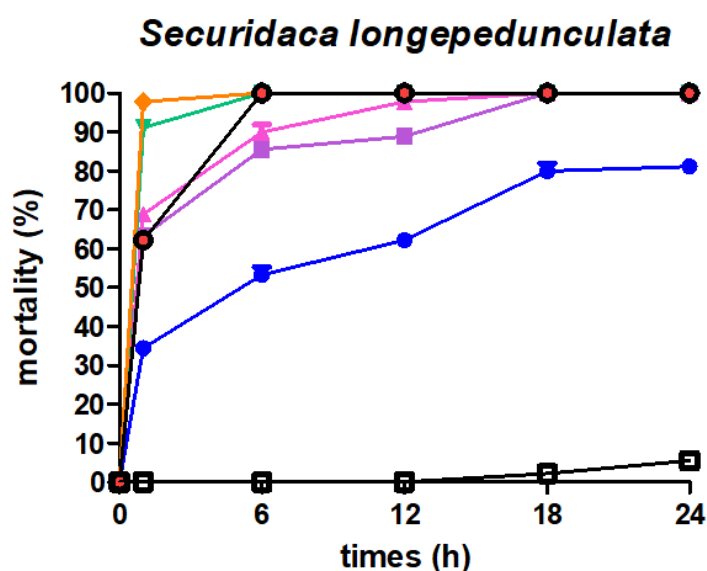


Figure 3: Anopheles mortality according to the various concentrations of essential oils of *Securidaca longepedunculata*. Note: (—●—) 30; (—■—) 40; (—▲—) 55; (—▼—) 100; (—◆—) 150; (—●—) T⁺ (Deltamethrin 55); (—■—) T.

DISCUSSION

The quality of essential oils, such as yield, chemical constituents and physical properties depends on many factors. Factors affecting the quality of essential oils include plant species, climate conditions, ground nature and maturation of harvested plants, plant storage, plant preparation and methods of extraction Tia, et al. [17]. They report the fluctuation in the yield of *Cymbopogon* and *Ocimum* genera in Gold Cost. Yield of the *R. communis* essential oil is the highest, while for *S. longepedunculata* is the lowest. It sticks out that the plant which had the highest yield was inefficacy and the plant that had the lowest yield was more efficient. These results are also corroborated at those of Bossou, et al. [18] who reported the insecticidal activity of plant essential oils from Benin against *Anopheles gambiae*. In their case, the essential oil of *S. longepedunculata* was extracted with a yield of 0.7% comparatively with the essential oil of *Cymbopogon citratus* (1.7%), *Cymbopogon giganteus* (1.4%) *Cymbopogon ambrosioides* (1.3%) *Eucalyptus tereticornis* (1.0%) *Cymbopogon tinctorium* (0.2%) and then *Cymbopogon tinctorium* was the most efficient against

Anopheles gambiae. *Anopheles gambiae*. Mohini, et al. [19] also studied the bioactivity of some essential oils of local plants against adult *Anopheles arabiensis* in India and their results showed that the plant that had the less yield of oil was the most toxic. Different essential oils contain secondary metabolites which are thought to be due toxic. In our report the presence of flavonoids, saponoids, tannins and terpenoids varied from plant to plant. Aviello, et al. also reported that the essential oils of *Lantana trifolia* and *Lantana fucata* from Brazilian presented a high content of sesquiterpenes, and monoterpenes respectively [20]. Similar results were obtained by Blythe who identified 78.9% triterpenes in the essential oil of *Lantana montevidensis* and its efficacy against *Anopheles aegypti* [21]. In our results, essential oils of *L. camara*, *R. communis* and *S. longepedunculata* have shown significant adulticidal activity against *An. gambiae* s.s. with LC 50=72.67; 79.63 ppm and 66.72 ppm respectively. These results are comparable with an earlier report by Dua, et al. who reported that the maximum adulticidal activity was observed with the essential oil of *L. camara* against *i* after 24 hours

[22]. Ntonga, et al. have also reported that at 200 ppm, essential oils of *Ocimum canum* and 250 ppm of *Ocimum basilicum* 100% of mortality have been induced in adult *An. funestus* s. s. at Douala town (Cameroon) [10]. Comparatively, Ahmad, et al. reported that oils of *R. comminus* have a pharmacological action, marketed as medicinal products [23]. Furthermore, Patrick, et al. revealed that the root bark of *S. longepedunculata* has pesticidal properties against grain weevil and large grain borer in stored grains [24]. Root extracts has properties against molluscs. Roots of *S. longepedunculata* are poisonous and traditionally used as arrow poison. The active compound, securinine, has been shown to have activity against the causative agent of malaria *Plasmodium falciparum*.

The efficacy of these essential oils are comparable at the results of Abagli, et al. who have shown that essential oil of *Hyptis suaveolens* is as effective as deltamethrin against Fekadu mosquitoes [25]. In their study, Fekadu also reported the toxicity of essential oils of some plants against Massebo adult *Anopheles arabiensis* [26]. Comparatively with results of Elumalai the efficacy of the extract of *Oxystelma esculentum* against India's *Ades aegypti* has been also shown [27]. This establishment has also been shown out by Govindarajan [28] who have carried out the adulticidal properties of *Cardiospermum halicacabum* (LC50=264.13; LC90=487.07 ppm) plant extracts against three important vector mosquitoes in Tamil Nadu (India).

CONCLUSION

The current study has dealt with the insecticidal properties of essential oils of three plant species traditionally used in Northern Cameroon for their repellency against flies and mosquito bites. The present study showed that the adulticidal activities of these essential oils varied with concentrations against *Anopheles gambiae* *Sensu Stricto*, malarial vector. *Securidaca longepedunculata* oil was the most effective with LC50 and LC95 values 66.72 ppm and 105.35 ppm respectively. Its LH 50 was 3H40 while the LH50 for the synthetic insecticide was 1H49. Sommary the activity was found in the order: *S. longepedunculata*>*L. camara*>*R. comminus*. The present study will be quite helpful in developing plant based anti-malarial vector and others borne diseases.

DATA AVAILABILITY

All the data supporting the results of this study have been included in the manuscript.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest regarding the publication of this paper.

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