



# Insecticidal Potential of *Eucalyptus citriodora* Hook and *Hyptis suaveolens* (L.) Poit Ethanol Leaves Extracts against Malaria Mosquito (*Anopheles gambiae* Giles)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Adult mosquitoes are known to transmit diseases, including malaria and filariasis. The larvicidal, pupacidal and mosquitocidal properties of the leaves ethanol extracts of *E. citriodora* and *H. suaveolens* against the larva, pupa and adult *Anopheles gambiae* were examined. Phytochemical screening was also conducted on the selected plant species to determine the secondary metabolites. Ethanol was the solvent used for the extraction of the leaves using Soxhlet extractor at 60°C to determine the secondary metabolites. The larvae, pupa and adult mosquito were exposed

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to 0.2, 0.4, 0.6, 0.8 and 1.0% of the extract at  $28 \pm 2^\circ\text{C}$ ,  $75 \pm 5\%$  RH, 12L:12D photoperiod. Results showed that the leaves extracts of both plants caused high mortality of larvae, pupae and adults of *An. gambiae* at 1.0% concentration level. The most effective extract was *H. suaveolens* which caused 100 % mortality of larvae and pupae at 1.0% concentration level within 48 h, while 100% mortality of adult *An. gambiae* was observed at 1.0% concentration within 4 h. Results also showed that *E. citriodora* leaves contains saponins, tannins, flavonoid, phenol, quinones and alkaloids, while that of *H. suaveolens* leaves contains saponins, tannins, flavonoid, phenol, alkaloids and sterols. As the extracts of both plants caused mortality at every stage of *An gambiae*, it can be suggested that the two plants could be integrated into vector control program of malaria and filariasis.

**Keywords:** *Eucalyptus citriodora* and *Hyptis suaveolens*; *Anopheles gambiae*; larvicidal; pupacidal, mosquitocidal and phytochemicals.

## 1. INTRODUCTION

Mosquitoes are rural- urban insect which had been noted for their high commonness in many under-developing and developing countries where insect pest management is still minimal. Mosquitoes of different species including *Aedes aegypti*, *Anopheles dirus*, *Culex quinquefasciatus*, *Aedes albopictus*, *Anopheles funestus*, *Anopheles arabiensis*, *Anopheles annularis*, *Anopheles culicifacies*, *Anopheles stephensi* and *Anopheles gambiae*, among others have been noted to be vector of different types of diseases among which malaria is the most prevalent in developing nations [1-5] (Roll Back Malaria 2014;) [6-8]. The high incidence of malaria disease has been reported and it is the leading cause of death in most countries of the world [9,10].

*Anopheles gambiae* is a principal malaria vector in most countries of the world where the females were responsible for the transmission of the malaria parasite called Plasmodium. There are other malaria vectors such as *Anopheles arabiensis*, *Anopheles pharoensis*, *Anopheles funestus*, *Anopheles nili*, and *Anopheles stephensi* which also transmit malaria in some countries of the world [11-13]. Various species of mosquitoes are abundant in more than 100 countries infecting over 700 million people every year globally [6,14]. Presently there are over three hundred species in the world grouped in 39 genera and 135 subgenera [15]. *Anopheles gambiae* is one of the notorious species which have been incriminated for transmitting malaria parasites an estimated 3.3 billion people in 97 countries (Roll Back Malaria 2015) [16].

Indiscriminate and rampant use of the chemical insecticides in the control of mosquito vectors in order to prevent diseases vectored by them has

resulted in causing adverse effects on the environment (potential toxicity), depletion of the ozone layer, high operational cost, some of the mosquitoes develop resistance to the chemical insecticides, poor handling by peasant farmers and community acceptance are affected. The factor mentioned above prompted the search for new methods of control. Use of plant extracts, essential oils and phytochemicals with larvicidal, mosquitocidal, and pupacidal potential are recognized as alternative to replace the chemical insecticides in mosquito control program. These botanicals and their derivatives also have excellent oviposition inhibition, insect repellence or insect growth regulatory effects, and they are found environmentally to be safe, bio-degradable and target specific. Several authors, from different part of India, timely reported the potential role of various plant extracts [17-20]. Since these contain multiple active ingredients with different mode of action, and thus lessening the chance of resistance development by the mosquito population.

The larvicidal, pupicidal and mosquitocidal activities of the botanicals may be the effect of the toxic phytochemicals present in the plants. This research was carried to analyze the phytochemicals present in the leaves of *E. citriodora* and *H. suaveolens* and to evaluate the toxicity of their extracts on all the developmental stages of *An gambiae*.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

The fresh leaves of *E. citriodora* and *H. suaveolens* were obtained from the campus of Ekiti State University, Ado Ekiti, Nigeria. The authentication of the leaves were carried out by the herbarium curator in the Department of Plant

Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria.

## 2.2 Preparation of Ethanol Extracts from the Plant Materials

The leaves of *E. citriodora* and *H. suaveolens* were washed separately in clean water, cut into small pieces and air-dried for ten days on the laboratory tables. The dry leaves were pulverized into powders using a Binatone, electric grinder (Model BL-400). The powders were kept in a black polythene bag and stored at the ambient temperature of  $28 \pm 2^\circ\text{C}$ . Two hundred grams (200 g) of each of the ground leaf material was weighed into a thimble and their oil was extracted with absolute ethanol at the temperature of  $60^\circ\text{C}$ , using a Soxhlet extractor. The resulting extract was concentrated using rotary evaporator and then air-dried to remove any trace of ethanol. The extracts were poured into brown bottles, labeled and stored until needed for the experiment.

## 2.3 Rearing of Mosquito Larvae and Pupae

Two black (opaque) plastic water-bath with large surface area were filled with rain water. The importance of the rain water was to simulate the natural breeding environment of mosquito and to attract adult mosquitoes for the purpose of breeding. Twenty grams (20 g) of yeast was sprinkled on the surface of the water and allowed to dissolve in order to feed the mosquitoes larvae. The containers filled with rain water and dissolved yeast also served as the bait for the adult mosquitoes. The containers were arranged under shade trees outside the laboratory. Adult mosquitoes were able to visit the bait to lay their eggs which later undergo metamorphosis to develop into larvae and pupae. Afterward, the containers were moved to the laboratory. One of the containers was kept in a large netted cage for complete metamorphosis to take place and there was emergence of numerous adult mosquitoes which were exposed to the extracts of the test plants.

## 2.4 Toxic Effect of the Ethanol Extracts of *E. citriodora* and *H. suaveolens* on the Larvae and Pupae Stages of *An. gambiae*

Serial concentrations of the extracts of *E. citriodora* and *H. suaveolens* leaves were

prepared by adding 2.0 µg of extracts to 100 mL of distilled water to prepare, 0.2, 0.4, 0.6, 0.8 and 1.0 %. Five grams (5 g) of yeast powder was added to the extracts to serve as food for the larvae. Afterward, 20 larvae or pupae of *An. gambiae* were introduced into the beakers containing each of the extracts concentration (0.2, 0.4, 0.6, 0.8 and 1.0 %). Untreated and water treatment control were also set-up. Larvae and pupae mortality were observed 48 h after treatment, by introducing the larvae and pupa into distilled water to notice recovery. Larvae and pupae were considered dead when they failed to come to the surface for gaseous exchange and when they did not respond to probing on the abdomen with a sharp pin.

## 2.5 Fumigant Effect of the Ethanol Extracts of *E. citriodora* and *H. suaveolens* on Adult *An. gambiae*

Ten adults mosquito were introduced into a test tube which was later plugged with cotton wool. Stripes of Whatman No. 1 filter paper (90 mm diameter) soaked in different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 %) of extracts were suspended into each test tube with the aid of a thread. Each treatment and the control was replicated 4 times and arranged in Complete Randomization Design (CRD) Adult mortality was observed, counted and recorded after 4 h of application

## 2.6 Qualitative Phytochemical Screening of Leaves Ethanol Extracts of *Eucalyptus citriodora*

The phytochemical composition was determined following the procedure of Harborne, J.B. [21].

### 2.6.1 Saponins test (foam test)

Two milliliters (2 mL) of extract was dissolved in 3 mL distilled water and shaken vigorously. The formation of a stable layer of foam indicated the presence of saponins in the sample.

### 2.6.2 Tannins test

Half milliliter (0.5 mL) of extract was dissolved in 1.0 mL of water and 1-2 drops of ferric chloride ( $\text{FeCl}_3$ ) solution was added. The appearance of blue colour indicated the presence of tannins

### 2.6.3 Flavonoid test

Two milliliters (2 mL) of the filtrate was pipetted into a test tube and 5 drops of concentrated

hydrochloric acid (HCl) and 2.0 g of magnesium filings were added to it. The appearance of red colour indicated the presence of flavonoids.

#### 2.6.4 Phenol test

A pinch of ferric chloride ( $\text{FeCl}_3$ ) was added to 2 mL of the extract and the appearance of green colour indicated the presence of phenol.

#### 2.6.5 Quinone test

A few drops of concentrated  $\text{H}_2\text{SO}_4$  was added to 2 mL of the extract. Appearance of red colour indicated the presence of quinones.

#### 2.6.6 Test for alkaloids

The method of Harborne [21] was used to determine the presence of alkaloid in the tested plants. A drop of Mayer's reagent was added by the side of the test tube to a few quantity of the filtrate. A creamy or white precipitate indicates the presence of alkaloid.

### 2.7 Data Analysis

Data obtained were subjected to Analysis of Variance (Anova), while Turkey's test was used in separating the means.

## 3. RESULTS

### 3.1 Effect of Leaves Ethanol Extracts of *E. citriodora* and *H. suaveolens* on the Mortality of Larvae of *An. gambiae* within 48 h Post-Treatment

The Mortality of the larvae of *An. gambiae* treated with the leaves extracts of *E. citriodora* and *H. suaveolens* is presented in Table 1. The mortality of insect larvae varied with the species of plant used as well as the concentration of the extracts used. Extracts of *H. suaveolens* was able to achieve 100 % mortality within 48 h of treatment with 0.8 % concentration and it is significantly ( $P < 0.05$ ) higher than the mortality recorded in other concentration levels of the extracts. Moreover, all extracts concentrations of *H. suaveolens* achieved above 50 % larvae mortality. *E. citriodora* was only able to achieve 97.30 % larva mortality when treated with 1.0 % concentration level of extracts within 48 h of exposure. All levels of concentrations recorded significantly higher larvae mortality than the control experiments.

### 3.2 Effect of Leaves Ethanol Extracts of *E. citriodora* and *H. suaveolens* on the Mortality of Pupae of *An. gambiae* at 48 h Post-Treatment

The mortality of the pupa of *An. gambiae* treated with the leaves extracts of *E. citriodora* and *H. suaveolens* is presented in Table 2. The mortality of the pupae varied with the species of plant used as well as the concentration levels of the extracts used. Extracts of *H. suaveolens* was able to cause 100 % mortality within 48 h of exposure to 1.0 % concentration and it is significantly ( $P < 0.05$ ) higher than the mortality recorded in other concentrations of extracts. Moreover, not all extracts concentration levels of *H. suaveolens* achieved above 50 % larvae mortality because, only 44.50 % pupae mortality was achieved on exposure to 0.2 % concentration level. *E. citriodora* was only able to achieve 95.25 % larva mortality when treated with 1.0 % concentration level of extracts during the 48 h of exposure. All levels of concentrations recorded showed significantly higher pupae mortality than the control experiments.

### 3.3 Effect of Ethanol Extracts of *E. citriodora* and *H. suaveolens* on Mortality of adult *An. gambiae*

The fumigant effect of extracts of *E. citriodora* and *H. suaveolens* on mortality of adult *An. gambiae* within 4 h after treatment is presented in Table 3. Insect mortality increased with increased concentration level of the extracts and varied with the species of plants used. Generally, *An. gambiae* adults exposed to extracts of *E. citriodora* and *H. suaveolens* showed significantly higher ( $P < 0.05$ ) mortality values than the control experiments. Extracts of *H. suaveolens* at concentration level of 0.8 % was able to cause 100 % adult mortality within 4 h of exposure. *E. citriodora* leaf extract was also able to attain 93.25 % adult mortality on exposure to 1.0 % concentration level during the 4 h post-treatment.

### 3.4 Phytochemical Screening of Ethanol Leaves Extracts of *E. citriodora* and *H. suaveolens*

The results of the qualitative phytochemical screening of *E. citriodora* showed the presence of saponins, tannins, flavonoid, phenol, quinones and alkaloids in the leaves of *E. citriodora* while that of *H. suaveolens* showed the presences of saponins, tannins, flavonoid, phenol, alkaloids and sterols. (Table 4).

**Table 1. Percentage mortality of the larva of *An. gambiae* during 48 h of exposure to different concentration of leaves ethanol extracts of *E. citriodora* and *H. suaveolens***

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
<i>E. citriodora</i>	35.30 ± 0.75 <sup>b</sup>	46.10 ± 1.25 <sup>b</sup>	68.50 ± 2.40 <sup>b</sup>	86.20 ± 3.14 <sup>b</sup>	97.30 ± 3.25 <sup>b</sup>
<i>H. suaveolens</i>	52.50 ± 1.37 <sup>a</sup>	66.10 ± 2.13 <sup>a</sup>	87.30 ± 2.21 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
Water treatment	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
Untreated	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>

Each value is mean ± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at  $p < 0.05$  using Tukey's test

**Table 2. Percentage mortality of pupae of *An. gambiae* at 48 h of exposure to different concentration of leaves ethanol extracts of *E. citriodora* and *H. suaveolens***

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
<i>E. citriodora</i>	38.25 ± 0.68 <sup>b</sup>	45.22 ± 2.18 <sup>b</sup>	59.25 ± 2.33 <sup>b</sup>	74.50 ± 3.21 <sup>b</sup>	95.25 ± 4.54 <sup>b</sup>
<i>H. suaveolens</i>	44.50 ± 1.14 <sup>a</sup>	56.30 ± 2.11 <sup>a</sup>	78.55 ± 2.60 <sup>a</sup>	85.15 ± 2.17 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
Water treated	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
Untreated	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>

Each value is mean ± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at  $p < 0.05$  using Tukey's test

**Table 3. Fumigant effect of *E. citriodora* and *H. suaveolens* on the mortality of Adult *An. gambiae* at 4 hours post-treatment**

Plant material	Percentage Concentration				
	0.2	0.4	0.6	0.8	1.0
<i>E. citriodora</i>	42.20 ± 1.44 <sup>a</sup>	52.25 ± 1.31 <sup>b</sup>	64.30 ± 2.41 <sup>b</sup>	79.25 ± 3.33 <sup>b</sup>	93.25 ± 3.32 <sup>b</sup>
<i>H. suaveolens</i>	46.25 ± 2.37 <sup>a</sup>	68.22 ± 2.10 <sup>a</sup>	85.50 ± 3.77 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
Water treated	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>
Untreated	0.00 ± 0.00 <sup>b</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>	0.00 ± 0.00 <sup>c</sup>

Each value is mean ± standard error of four replicates. Means in the same column followed by the same alphabet(s) are not significantly different at  $p < 0.05$  using Tukey's test

**Table 4. Qualitative phytochemical composition of *E. citriodora* and *H. suaveoles* leaves ethanol extracts**

S/N	Phytochemical	<i>E. citriodora</i>	<i>H. suaveolens</i>
1	Saponins	+	+
2	Tannins	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Sterols	+	+
6	Alkaloids	+	+
7	Quinone	+	-

Key: + = detected, - = not detected

#### 4. DISCUSSION

The hazards caused by mosquitos and the conventional chemicals insecticides used in controlling them have necessitated the needs for development of new the management strategies for the management of mosquitos and other insects. Mosquito-borne diseases such as malaria fever, filariasis, dengue hemorrhagic fever, yellow fever, among others have created huge impact on human all over the world. The chemical insecticides used in controlling mosquitoes in order to prevent the diseases vectored by them, have created problems such as environmental pollution, poor application by illiterate farmers have led to the development of resistance by the vectors, also ozone depletion potentials of some chemical insecticides containing chloro-floro carbon is part of the problems [22]. These setbacks have necessitated the search for underutilized potential botanicals that could serve as alternatives to the active and hazardous chemical insecticides [23,24].

The results obtained from this work showed that the essential oils of the leaves of *E. citriodora* and *H. suaveolens* possess the potential to control the developmental and adult stage of *An. gambiae*. The ability of these essential oils to control the insects depends on the concentration level. Both the larva pupa and the adult stages were susceptible to the essential oils of *E. citriodora* and *H. suaveolens*. Moreover, the oil of *H. suaveolens* was more potent on the developmental and the adult stage of *An. gambiae* because it caused 100% mortality of the larvae and pupa when treated with 1.0% concentration level of the essential oils of *H. suaveolens* during 48 h of exposure to the essential oils, while the adult *An. gambiae* also recorded 100% mortality on exposure to 1.0% concentration level of the essential oils within 4 h of exposure.

The high larvae and pupa mortality caused by the essential oils of *H. suaveolens* is similar to the result obtain by Okumu *et al.* (2007) who obtained 95% larvae mortality on exposure to extract of *Azadirachta indica*. The 100% mortality recorded when the adult *An. gambiae* was exposed to 1.0% essential oils of *H. suaveolens* is similar to the result obtain by Massebol *et al.* (2013) who obtained 90 % adult mortality of *A. arabiensis* on exposure to ethanol leaf extract of *Ocimum lamiifolium*. The ability of the essential oils of *E. citriodora* and *H. suaveolens* to cause high mortality of the pupa and larvae is due to

the blockage of the respiratory apparatus by the oils thereby, preventing gaseous exchange and the consequent suffocation and death of the developmental stages [6].

The phytochemical analysis of the oils of *E. citriodora* and *H. suaveolens* revealed secondary metabolites such as alkaloids, flavonoids, tannins, saponnin, sterols, quinones and phenols which have insecticidal activities. All these phytochemical have been reported to cause reduction in growth and reduction in the larvae and pupa survival as well as disrupting the life cycle of the insects (Yang *et al.*, 2006). These phytochemicals could have contributed to the high effectiveness of the essential oils of *E. citriodora* and *H. suaveolens* against *An. gambiae*. These plants have also been used as insecticides against stored beetles [25,26,27-30].

#### 5. CONCLUSION

In the present research the leaves extracts of *E. citriodora* and *H. suaveolens* have shown a great insecticidal potential against the larva, pupa and adult mosquito. Hence, it could be integrated into malaria vector strategies to replace the hazardous chemical insecticides

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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