

## **Insecticidal potential of essential oils of leaves of *Eucalyptus citriodora* Hook and *Hyptissuaveolens*(L.) Poit against Malaria mosquito (*Anopheles gambiae* Giles)**

### **Abstracts**

Adult mosquitoes are known to transmit diseases, including malaria and filariasis. The larvicidal, pupacidal and mosquitocidal properties of the essential oil extracts of the leaves 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 to 0.2, 0.4, 0.6, 0.8 and 1.0 % of essential oil extract at 28 ± 2°C, 75 ± 5 % RH, 12L:12D photoperiod. Results showed that the essential oil extracts of both plants caused high mortality of larvae, pupae and adults of *An. gambiae* at 1.0 % concentration level. The most effective essential oil 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 contain saponins, tannins, flavonoid, phenol, quinones and alkaloids, while that of *H. suaveolens* leaves contain saponins, tannins, flavonoid, phenol, alkaloids and sterols. As the essential oil 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 *Hyptissuaveolens*, *Anopheles gambiae*, larvicidal, pupacidal, mosquitocidal and phytochemicals.

### **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 gambiae*, *Culex*

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*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 (Tawastinet *et al.*, 2001; Joy *et al.*, 2003; Van Geertruyden *et al.*, 2004; Aina *et al.*, 2009; WHO 2010; Roll Back Malaria 2014; Akinkulere *et al.*, 2011; Shankar *et al.*, 2013; Ileke *et al.*, 2014). The high incidence of malaria disease has been reported and it is the leading cause of death in most countries of the world (Federal Ministry of Health 2012; Onwujekwe *et al.*, 2000).

*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 (Kloss and Zein 1993; Zein 2021; Balkew *et al.*, 2021). Various species of mosquitoes are abundant in more than 100 countries infecting over 700 million people every year globally (Akinkulere *et al.*, 2011; Rahuman 2011). Presently there are over three hundred species in the world grouped in 39 genera and 135 subgenera (Remia and Logaswamy, 2010). *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; WHO 2015).

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~~ [chemical insecticides](#) in mosquito control program. These botanicals and their derivatives also have excellent ~~oviposition inhibition~~ [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 (Govidaranjan *et al.*, 2011; Prabhu *et al.*, 2011; Kumar *et al.*, 2011;

Mandal 2011). 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 phytochemical present in the plants. This research was carried to analyzing the phytochemicals present in the leaves of *E. citriodora* and *H. suaveolens* and to evaluate the toxicity of their essential oils on all the developmental stages of *An gambiae*.

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## Materials and methods

### 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.

### Preparation of essential oils 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 pulverised 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 (200g) 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 essential oils were poured into brown bottles, labeled and stored until needed for the experiment.

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### 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 essential oil of the test plants.

#### **Toxic effect of the essential oil extracts of *E. citriodora* and *H. suaveolens* on the larvae and pupae stages of *An. gambiae***

Serial concentrations of the essential oil extracts of *E. citriodora* and *H. suaveolens* leaves were prepared by adding 2.0 µg of essential oils 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 essential oil to serve as food for the larvae. Afterward, 20 larvae or pupae of *An. gambiae* were introduced into the beakers containing each of the essential oil 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 48h 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.

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#### **Fumigant effect of the essential oil of *E. citriodora* and *H. suaveolens* on adult *An. gambiae***

Ten adult mosquitoes were introduced into a test tube which was later plugged with cotton wool. Strips 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 essential oil 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 4h of application.

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#### **Qualitative phytochemical screening of leaf extracts of *Eucalyptus citriodora***

The phytochemical composition was determined following the procedure of Harborne, J.B. (1973).

**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.

**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

**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.

**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.

**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.

**Test for alkaloids:** The method of Harborne (1973) 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

### Data Analysis

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

### Results

**Effect of leaves essential oils 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 leaf essential oils of *E. citriodora* and *H. suaveolens* presented in Table 1. The mortality of insect larvae varied with the species of plant used as well as the concentration of the essential oils used. Essential oil 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 essential oils. Moreover, all essential oils concentrations of *H. suaveolens* achieved above 50 % larva mortality. *E. citriodora* was only able to achieve 97.30 % larva mortality when treated with 1.0 % concentration level of essential oil within 48 h of exposure. All levels of concentrations recorded significantly higher larva mortality than the control experiments.

**Table 1: Percentage mortality of the larva of *A. gambiae* during 48 h of exposure to different concentration of leaves essential oils 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>
<b>Water treatment</b>	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>
<b>Untreated</b>	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

#### **Effect of leaves essential oils 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 essential oils of *E. citriodora* and *H. suaveolens* presented in Table 2. The mortality of the pupae varied with the species of plant used as well as the concentration levels of the essential oils used. Essential oil of *H. suaveolens* was able to **caused** 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

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essential oils. Moreover, not all essential oil 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 essential oil during the 48 h of exposure. All levels of concentrations recorded showed significantly higher pupae mortality than the control experiments.

**Table 2: Percentage mortality of pupae of *An. gambiae* at 48 h of exposure to different concentration of leaves essential oils 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>
<b>Water treated</b>	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>
<b>Untreated</b>	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

**Effect of essential oils of *E. citriodora* and *H. suaveolens* on mortality of adult *An. gambiae***

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

**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>
<b>Water treated</b>	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>
<b>Untreated</b>	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

#### Phytochemical screening of ethanol extracts of leaves essential oils 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 4: Qualitative phytochemical composition of *E. citriodora* leaf essential oils**

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

#### 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 (Shyamapada Mandal, 2011). These setbacks have necessitated the search for underutilized potential botanicals that could serve as alternatives to the active and hazardous chemical insecticides. (Ogunbiteet *et al.*, 2014; Begum *et al.*,2003).

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 *A. 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 *A. 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 48h of exposure to the essential oils, while the adult *A. gambiae* also recorded 100% mortality on exposure to 1.0% concentration level of the essential oils within 4h 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 *A. gambiae* was exposed to 1.0% essential oils of *H. suaveolens* is similar to the result obtain by Massebolet *et al.* (2013) who obtained 90 % adult mortality of *A. arabiensis* on exposure to ethanol leaf extract of *Ocimumlamiifolium*. 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. (Adedireet *et al.*, 2011)

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The phytochemical analysis of the oils of *E. citriodora* and *H. suaveolens* revealed secondary metabolites such as alkaloids, flavonoids, tannins, saponin, sterols, quinones and phenols which have insecticidal activities. All these phytochemicals 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 *A. gambiae*. These plants have also been used as insecticides against stored beetles (Ilekeet *et al.*, 2014; Obembe and Ogunbite 2017). In the present research the essential oils 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 (Ilekeet *et al.*, 2015).

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