



Effect of Methanolic Leaf Extract of Pignut (*Hyptis suaveolens* L.) against *Aedes aegypti* Mosquitoes

¹Aina, O. A., ²Etudaye, U., ¹Usman, F. O., ¹Upahi, L., and ¹Oludare, T. T.

¹Department of Biology,
Confluence University of Science and Technology, Osara, Kogi state, Nigeria

²National Biotechnology Research and Development Agency,
Abuja, Nigeria

ABSTRACT

Mosquito-borne diseases are the most significant public health risks globally. This study evaluates the potency of methanolic leaf extracts of Pignut (*Hyptis suaveolens*) as mosquito larvicides under laboratory conditions. The leaf extracts of test plants were obtained using cold extraction. Different test concentrations of 5, 4, 3 and 2 mg/ml (control) were prepared from stock solution. Twenty five larvae of *Aedes aegypti* were exposed to various concentrations and observations were made for the period of 24 hour. The data obtained from this were subjected to descriptive statistics, Analysis of Variance (ANOVA) coupled with Duncan Multiple Range Test (DMRT) were used to compare result within doses and among treatment and control group. The result with $p < 0.05$ was considered statistically significant. The result indicates that the mean larval mortality of *A. aegypti* recorded highest concentration of 5 mg/ml (25.00 ± 0.00) mortality at 12hrs followed by 4 mg/ml (20.25 ± 1.50) hence, no mortality was recorded in the control. Also, the qualitative phytochemical screening of the plant revealed metabolites like tannins, saponins, alkaloid, flavonoid and phenol except for anthraquinone, glycosides and terpenoid which were absent. For the quantitative screening, phenol revealed highest significant content of metabolite (299.19 ± 0.99) while alkaloids revealed the least metabolite content (20.27 ± 0.30). This result indicates that methanolic leaf extract of *H. suaveolens* is an excellent alternative to synthetic compounds. Therefore, the use of Bio-insecticides derived from plants should be implemented for the control of mosquitoes rather than the use of synthetic insecticides because bio-insecticides derived from plants is eco-friendly, less expensive and target species.

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INTRODUCTION

Mosquitoes are major vectors for a number of diseases, including dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis, and other fevers (Webb, 2008). According to Beatty *et al.* (2007), at least 500 million people worldwide are thought to be afflicted with mosquito-borne diseases (Madhumathy *et al.*, 2007), and the medical significance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality,

economic loss, and social disruption cannot be overstated. These diseases have been responsible for significant economic losses, low productivity, social discrimination, and mortality in many developing nations (Adeleke *et al.*, 2010). and according to Mgbemena (2010), depending on the species, mosquitoes are vectors of protozoa (e.g. *Plasmodium spp*) that cause malaria, the nematode worms that causes filariasis (e.g. *Wuchereria bancrofti*) and a large number of arboviruses including two which are of

Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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great impact in the tropical and subtropical regions (yellow fever and dengue viruses). The species of *Anopheles*, *Culex* and *Aedes* are important vectors in the transmission of malaria, filariasis, dengue, yellow and Chikungunya fevers respectively (Prajapati *et al.*, 2005). WHO (2014), implicates *Aedes* mosquito as vectors of diseases,

Aedes aegypti is responsible for transmission of Dengue, yellow fever, Chikungunya and Zika virus (Okigbo *et al.*, 2010). In the absence of an effective preventive vaccine against the majority of mosquito-borne diseases, it is desirable to find compounds that can effectively control mosquitoes with minimal damage to the environment. According to Okigbo *et al.* (2010), one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission, by killing or preventing mosquitoes from biting humans. Larval control (source reduction or suppression) has been identified as one of the most effective methods for the control of mosquito-borne diseases (Singh *et al.*, 2006).

Experience has shown that, aerial toxicants for the eradication of this mosquito are not effective, since it is highly domesticated and many adults rest indoors in hidden places such as closets (Senthilkumar *et al.*, 2008). Mosquito control has become increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb the ecological balance (Nganjiwa *et al.*, 2015).

Repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organisms (Govindarajan *et al.*, 2013). Due to the severe effects of synthetic pesticides, there is serious need to emphasis on natural, economical, biodegradable and environmental friendly pesticide in the control of pest especially mosquitoes and that is the essence of this research. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate

biodegradability. Plants are considered as a rich source of bioactive chemicals (Kamaraj *et al.*, 2008) and therefore an ideal control method is thus the system that uses the natural plant products as alternative to synthetic substances, as they are effective and compatible with human, animal life and the environment (Chaithong *et al.*, 2006).

Hyptis suaveolens is a medium aromatic weedy shrub found in the tropics and sub-tropical region (Ngozi *et al.*, 2014). The plant has been reported to possess repellent activity against insect pests of stored grains (Devan *et al.*, 2013). Methanolic extract of *Hyptis suaveolens* possess insecticidal activity against *Sitophilus maculatus* (Olotuah, 2013). Fumes of the dried leaves are also used locally to repel mosquitoes and to control insect pests of stored grains. It was also reported by (Seyoum *et al.*, 2002) that placing *H. suaveolens* branches or whole plants in houses was one of the most effective methods to repel malaria vector. This study aims to develop a plant-based insecticide formulation as an alternative to synthetic insecticides, leveraging the eco-friendly, biodegradable, and cost-effective properties of plant-derived compounds. Specifically, this research investigates the larvicidal potential of Pignut leaves (*Hyptis suaveolens*) against mosquito larvae of *Aedes aegypti* species.

MATERIALS AND METHODS

Description of Study Area

The study was carried out in Osara, Adavi Local Government, Kogi-state. Osara populace are well known for their rich agricultural activities including farming and cultivation of crops. The town is also a home to Osara Dam which serves as a major water source for nearby communities. The town is part of the larger Okene Local Government Area. It has latitude 7°40'52.7N (7.6813000°) and longitude 6°25'43.0E (6.4286000°).

Plant Collection and Extraction

Fresh mature leaves of *Hyptis suaveolens* were collected from Osara village in the North Central region of Nigeria (Adavi Local Government Area, Kogi state). The leaves were

Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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shade-dried in the Biology Department Laboratory at Confluence University of Science and Technology, Nigeria, for 14 days at room temperature. The plant specimen was identified by a Botanist at Confluence University, Osara, Kogi-state.

The plant extract was prepared using the maceration method. Dried leaves were pulverized using a mortar and pestle, after which, a blender was used to get a fine powder. 100g of the powder was soaked in 400ml of methanol in a 500ml conical flask for 72 hours, with intermittent stirring. The mixture was filtered using a sieve and filter paper, and the residue was rinsed and filtered multiple times with fresh solvent. The filtrate was evaporated to dryness using a water bath at 70°C. The extract was stored in a sample container in the refrigerator.

Collection, Identification and Maintenance of Aedes Mosquito

Collections of larvae were carried out at permanent site of Confluence University of Science and Technology Osara, Kogi State. The ovitraps were set under vegetation in five strategic point, larvae were also collected from the natural sites such as plastic containers, used tires and stagnant water, the equipment used includes scoop- spoons, bowls, nets and specimen bottles. The eggs were collected from ovitraps, both the eggs and the larvae were sent to the laboratory, for sorting and identification. The larvae were reared under room temperature (Ahmad *et al.*, 2004). The larvae stage of *Aedes species* were distinguished from those of *Culex species* using morphological differences.

Stock Solution

The stock solutions of *H. suaveolens* were prepared by dissolving 1g of each crude extract in 100ml of methanol, yielding a concentration of 10mg/ml. Subsequent serial dilutions of the stock solutions produced test concentrations of 10, 8, 6, 4, and 2mg/ml for plant extract.

Bioassay of Larvae with Plant Extract

In this present experiment, treatment of larvae with ethanol extracts was conducted in

accordance with WHO standard method (WHO, 1986). Twenty-five (25) larvae of *A. aegypti* were placed in transparent plastic cups, 100 ml of distilled water was added into the cups and was replicated four times. The larvae were then exposed to concentrations of *H. suaveolens*. Control medium was also maintained with twenty-five 25 larvae in 100ml of distilled water for each concentration in which 1ml of ethanol was added. Mortality rates were recorded at 0min, 5mins, 10mins, 15mins, 20mins, 25min, 30min, 1hr, 2hrs, 3hrs, 6hrs, 12hrs, 18hrs, and 24hrs in the laboratory at a room temperature. Each of the test samples and standard were replicated 4 times. This method was modified from Okigbo *et al.* (2010).

PHYTOCHEMICAL SCREENING AND ANALYSIS OF LEAF EXTRACT

Total Phenol Determination

Senthilkumar *et al.*, (2008) method was used to determine total phenol content of extract. 0.01g of the extract was dissolved in 10mL of distilled water, and 0.5mL was oxidized by 2.5mL of 10% Folin-Ciocalteu's reagent which was then neutralized by 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40minutes. Absorbance was read at 765nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard gallic acid was used to prepare the calibration curve.

Total Flavonoids Determination

Total flavonoid content of the extract was determined using the method of (Chaithong *et al.*, 2006). 0.5ml of the extract was added to a test tube containing 1.5ml of absolute methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M sodium acetate and 2.8ml of distilled water and incubated at ambient temperature for 30minutes. The absorbance was read at 415nm with double beam Shimadzu UV-spectrophotometer, UV-1800. Standard quercetin was used to prepare the calibration curve.

Total Alkaloid Determination

Total alkaloid of the extract was determined using the method of Onyido, (2018).

Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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0.5g of the crude extract was weighed and dissolved in 5ml of mixture of 96% ethanol:20% H_2SO_4 (1:1) and then filtered. 1ml of the filtrate was then added to a test tube containing 5ml of 60% H_2SO_4 and allowed to stand for 5minutes. Thereafter, 5ml of 0.5% formaldehyde was added and allowed to stand at room temperature for 3hours. The absorbance was read at wavelength of 565nm. Vincristine extinction coefficient (E_{296} , ethanol {ETOH}= $15136\text{M}^{-1}\text{cm}^{-1}$) was used as reference alkaloid.

Determination of Tannins

Tannin content of the crude extract was determined using the method of (Okigbo *et al.*, 2010). 0.2g of the extract was weighed into a 50ml beaker and 20ml of 50% methanol was added to it and covered with para film and heated in water bath at 80°C for 1hour. The reaction mixture was shaken thoroughly to ensure uniformity. The extract was then filtered into a 100ml volumetric flask, and 20ml of distilled water, 2.5ml of Folin-Denis' reagent, and 10ml of sodium carbonate were added and mixed properly. The reaction mixture was then allowed to stand for 20minutes at room temperature for the development of bluish-green coloration. The absorbance was taken at 760nm using double beam shimadzu UV-spectrophotometer, UV-1800. Standard tannic acid was used to prepare the calibration curve.

Determination of Saponins

Saponins content of the extract was determined using the method of (Onyido, 2018). 0.5g of the crude extract was weighed and dissolved in 20ml of 1N HCl and boiled in water bath at 80°C for 4hours. The reaction mixture was cooled and filtered. 50ml of petroleum ether was added and the ether layer was collected and evaporated to dryness. Thereafter, 5ml of acetone-ethanol (1:1), 6ml of ferrous sulphate and 2ml of concentrated sulphuric acid were added and allowed to stand for 10minutes. The absorbance was taken at 490nm. Standard saponins was used to prepare the calibration curve.

Data Collection and Statistical Analysis

The data obtained from this study was subjected to descriptive statistics, Analysis of Variance (ANOVA) coupled with Duncan Multiple Range Test (DMRT) were used to compare result within doses and among treatment and control group. For calculation of LC50 & LC90 values, the data was subjected to Probit Regression Analysis (PRA). The result with $p < 0.05$ was considered statistically significant.

RESULTS

Larvicidal efficacy of methanol extract of *H. suavelons* on fourth instar larvae of *Aedes aegypti* mosquitoes

The larvicidal efficacy of the methanol extract of *H. suavelons* on fourth instar larvae of *A. aegypti* mosquitoes is shown in Figure 1. There was significant effect in mortality; the higher the concentration the higher the mortality with time. The highest mortality was recorded by 0.5 mg/L with 100 % (25.00 ± 0.00) mortality at 12 hrs, followed by 0.4 mg/L with 81 % (20.25 ± 1.50) mortality after 24 hrs, while 0.2 mg/L recorded the least mortality of 32 % (8.00 ± 2.16) after 24 hrs. Both positive and negative controls recorded no mortality.

Qualitative Phytochemical Constituents of *H. suaveolens*

Table 1 shows the result of phytochemicals of the leaves of *H. suaveolens*. The results revealed the presence of bioactive metabolites like flavonoids, phenols, alkaloids, tannins, saponins in the methanolic plant extracts while cardiac glycoside, terpenoids and anthraquinone were absent.

Quantitative Phytochemical Components of *Hyptis suaveolens*

Table 2 shows the result of the quantitative phytochemical constituents of *H. suaveolens*, phenols (299.19 ± 0.99) content was significantly higher than other phytochemicals metabolites. This was followed by saponins content. Also, the plant species recorded a lower content of Alkaloids (20.27 ± 0.3) and it was significantly lower than all the other phytochemical

constituents recorded in *H. suaveolens* in this study.

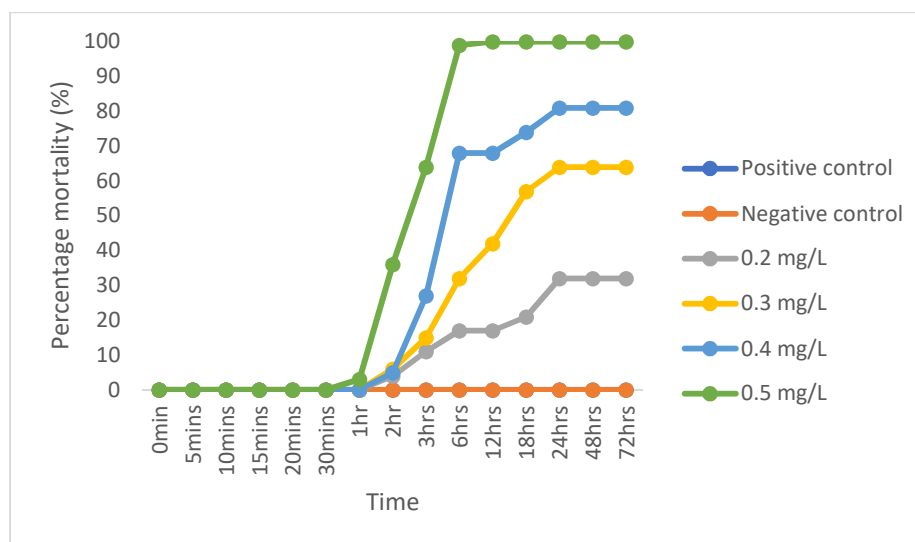


Figure 1: Larvicidal Efficacy of Methanol Extract of *Hyptis suaveolens* on Fourth Instar larvae of *Aedes aegypti* Mosquitoes

Table 1: Qualitative Phytochemical Screening of *Hyptis Suaveolens*

Phytochemicals <i>suaveolens</i>	<i>Hyptis</i>
Flavonoids	+
Tannins	+
Saponins	+
Alkaloids	+
Phenols	+
Terpenoids	—
Cardiac glycosides	—
Anthraquinones	—
KEY: += Present - = Absent	

Table 2: Quantitative Phytochemical Screening of *Hyptis Suaveolens*

Phytochemical (mg/100g)	<i>Hyptis suaveolens</i>
Flavonoids	41.91±1.82 ^d
Tannins	75.90±0.83 ^c
Saponins	133.38±0.95 ^b
Alkaloids	20.27±0.30 ^e
Phenols	299.19±0.99 ^a

Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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DISCUSSION

The result of the phytochemical screening shows that *H. suaveolens* contains significant phytochemicals like Flavonoid, Tannin, Alkaloid, Saponin, Phenols in methanolic extract of *H. suaveolens* but absent in Terpenoids, Glycosides. Nazifi *et al.* (2020) identified Flavonoid, Tannin, Alkaloid, Saponin and Phenol component in *Eucalyptus spp* and *Tamarindus indica* using methanol as solvent when tested against *Aedes aegypti*. These secondary metabolites are known to be effective against a wide range of insect pests as well as mosquito vectors (Sukontason *et al.*, 2008). The most reported insecticidal group of compounds from plants with larvicidal activity against various species of mosquitoes are Steroids, flavonoids, phenol, tannins, saponins. These phytochemicals may be responsible for their insecticidal properties (Kabar *et al.*, 2001).

The data on the quantitative phytochemical screening revealed that Phenol was the highest in quantity followed by Saponin while Alkaloid had the least quantity. The presence of different phytochemical in this study suggest that *H. suaveolens* has biological activities. According to Bobbo *et al.* (2016), different plant extract may contain different phytochemicals each of which has its peculiar biological activities. The presence of this various phytochemicals also reveal that *H. suaveolens* is of medicinal importance as Alkaloid, Flavonoid, Tannin, Phenol and Saponin are known to have antibacteria, antifungi, antiviral, antioxidant and larvicidal properties (Chaithong *et al.*, 2006.)

Also, the bioassay findings revealed that the methanolic extracts of *H. suaveolens* showed promising larvicidal efficacy against aedes species. At 24 hours' larval exposure to methanol extracts, the results demonstrated progressive increase in percentage mortality as concentrations increased. After 48 hours, methanol extract demonstrated significant larvae mortality progressively as the concentrations increased as well. A mortality of 100% was recorded in the highest concentration. This study is in conformity with findings of Murugasen (2015) who reported 100% mortality of acetone aerial extract of *Hyptis*

suaveolens at the highest concentration against the larvae of filarial vector *Culex quinquefasciatus* after 48 hours bioassay.

In comparing this result to other similar studies, the result of Mean Larvicidal Mortality of *A. aegypti* exposed to various concentrations of *A. conyzoides* revealed that the larval mortality of *A. aegypti* vary in a dose dependent manner thus, there was increase in mortality as concentration increased, with significant difference ($p < 0.05$) between mortality in concentrations and control. This finding is in agreement with the present study. Also, the result of this study is in line with the preliminary findings by Dawet *et al.* (2016) using methanol leaf extract of the same plant on Anopheles Mosquito Larvae. There was also increase in mortality as concentration increased.

Finally, the findings of this study is in agreement with the previous findings of Okigbo *et al.* (2010), when *Azadirachta indica* (Juss) and *Ocimum gratissimum* (L.) were compared with *H. suaveolens*. The percentage mortality of Leaf extract of *Azadirachta indica* (Juss), and *Ocimum gratissimum* (L.) were greater than that of *H. suaveolens*. However, there was no significant difference ($P > 0.05$) between mortality of *A. aegypti* on exposure to *A. conyzoides* and *H. suaveolens*. This indicates that the two leaf extracts have similar effect on larval mortality of *A. aegypti*.

In conclusion, this study showed that, the methanolic leaf extracts of *H. suaveolens* can be used as larvicides against *Aedes aegypti*. Also, the study revealed that the crude extracts of *H. suaveolens* contain bioactive insecticidal compounds including phenols, flavonoids, saponins, tannins and alkaloids. The larvicidal activities of the plant extracts increases with increase in extract concentration and exposure period.

In view of these findings, it was recommended that the use of Bio-insecticides derived from plants should be implemented for the control of mosquitoes rather than the use of synthetic insecticides. Synthetic insecticides are harmful to humans and the ecosystem by killing non-target species but bio-insecticides derived



from plants is eco-friendly, less expensive and target species.

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Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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Corresponding author: Aina, O. A.

✉ ainaoa@custech.edu.ng

Department of Biology, Confluence University of Science and Technology, Osara, Kogi State.

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