ISSN: 2714-3643
October, 2023

THE LARVICIDAL EFFECT OF ETHANOL LEAF EXTRACT OF *Hyptissuaveolens* AGAINST MOSQUITO LARVAE

Adewumi Bolorunduro Lanre
Department of Science Laboratory Technology,Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria**.**

Abstract
The larvicidal effect of Hyptissuaveolens leaf extract on mosquito larvae was determined using the bioassay technique. Anopheles stephensis larvae in their third and fourth instars were exposed in vitro to larvicidal tests at the concentrations of 2, 4, 8, and 10 grams of the plant leaf extracts per 100 ml of distilled water respectively. The efficacy of the plant leaf extract was dose-dependent and the LC_{50} was 1.71 ppm. The phytospray produced from the extract also resulted in 100% mortality at 5% concentration in 30 minutes and compared favorably with commercial Bagon used as the positive control. This suggests that the leaf of H. suaveolens could be exploited in the production of effective mosquitocides. However, further research could be done to isolate, identify, and test for the phytoconstituents of the plant leaf oil responsible for its larvicidal properties.
Keywords: Hyptissuaveolens, Anopheles stephensi,Larvicidal, Mosquito larvae, Mosquitocides

Introduction

Mosquitoes play a significant role in spreading deadly diseases such as dengue and yellow fever globally (Ngwamah and Naphtali, 2019). Every 30 seconds, one infant dies from malaria, killing three million people annually. The emergence of pesticide resistance complicates efforts to contain these diseases. Malaria is caused by five species of the eukaryotic protozoan genus Plasmodium: *Plasmodium malaria, Plasmodium knowlesi, Plasmodium falciparum, Plasmodium vivax,* and *Plasmodium ovale*(Moola, Ayyadurai, Balasubramani, Vignesh, Mohan, Sathish and Diana, 2023). The disease is so prevalent in Nigeria that it accounts for an average of 13.2% of all fatalities and 22% of deaths among children under five.

There are 15,344 reported cases of malaria per 100,000 people and 70 malaria-related fatalities per 100,000 people of all ages. Malaria is prevalent in Sub-Saharan Africa, South Africa, Southeast Asia, the Pacific archipelago, India, Central and South America, and even the Arctic and Antarctic (Adelaja *et al*., 2022).

In most endemic regions, *Plasmodium falciparum* predominates, whereas *Plasmodium vivax* predominates in India and South America. Plasmodium ovale, the malaria parasite, is endemic to West Africa (Hemen and Hadiza, 2019). The emergence of pesticide resistance complicates efforts to contain these diseases. Malaria prevention requires a series of interdependent actions, each of which must be effective. Since its inception in the 2000s, Nigeria's National Malaria Control Programme (NMCP) has effectively fought the disease and brought it closer to elimination as a public health concern (Adelaja, Oduola, Ande, Abiodoun and Adelaja, 2022).

Adult mosquitoes were treated with a residual pesticide, mass chemoprophylaxis with pyrimethamine-treated salt was used, and drainage systems were upgraded. The objective of the "roll back malaria" (RBM) initiative in 2009 was to halve malaria-related fatalities by 2010. In Nigeria, Artesunate/Amodiaquine, part of the global malaria control plan, is frequently the initial treatment for uncomplicated malaria (Ngwamah and Naphtali, 2019).

Patients who cannot tolerate Artesunate/Amodiaquine may benefit from Artemether /Lumefantrine or Dihydroartemisinin / Piperaquine as an alternative first-line treatment. To control adult mosquito populations, it is necessary to seal entry points to human dwellings, cover windows and doors, wear protective garments, and use adult pesticides (Moola *et al*., 2023).

The development of mosquito larvae can be halted as part of source reduction measures. In rural areas, the Nigerian plant *Hyptissuaveolens* is frequently used as a biotoxic agent against mosquito larvae. As a result of feeding on these secondary metabolites, insects may be exposed to toxic chemicals with relatively non-specific effects on a broad array of molecular targets, which are the active toxic components that

plants have evolved to defend themselves against herbivores (Sonibare, Okorie, Aremu and Adegoke, 2015). However, only some of these extracts have been commercially synthesised and extensively utilised in vector control operations. Although the insecticidal properties of plant leaves have been extensively studied, more is needed to know about *H. suaveolens*. As a result, this study aims to determine if *H. suaveolens* leaf extracts have insecticidal effects on mosquito larvae. The objectives are to develop *H. suaveolens* as a mosquito repellent for malaria control and evaluate its larvicidal activity.

Materials and Method

Collection of Mosquito Larvae

Anopheles stephensi larvae were collected from stagnant water in the students' dormitory at Rufus Giwa Polytechnic, Owo, and preserved in a plastic container before bioassay testing (Ileke and Ogungbite, 2015).

Sterilization of Materials

Petri dishes, beakers, conical flasks, test tubes, and pipettes were cleaned with soap solution, rinsed with sterile distilled water, enveloped in aluminium foil, and dried at 160°Cfor one hour in the oven. After being heated in a flame, 70% ethanol was used to sterilise inoculating loops and forceps. Before and after experimentation, workbenches were cleaned with 70% ethanol-soaked cotton swabs. (Akinnibosun and Adewumi, 2019).

Collection and Preparation of plant leaf extract

The leaves of*Hyptissuaveolens* were harvested from an uncultivated farmland in the campusand deposited at the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo forauthentication purposes. The verified leaves were thoroughly cleaned with faucet water and then air-dried in the shade for eight weeks. After desiccating the samples, they were crushed into a coarse powder and stored in clean, airtight containers in a cool, dry location until required (Akinnibosun and Adewumi, 2019).

A 100g portion of the pulverized sample was immersed in 300 mls of ethanol for 72 hours while stirred with a sterile spatula. A rotary evaporator model was used to condense the plant extracts in a vacuum after filtering them through Whatman No. 1 filter paper into miniature flasks. 0.10g, 0.20g, 0.30g, 0.40g, and 0.50g of extracts were diluted in 100ml of 0.01% Tween-20 to produce 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, and 50mg/ml, respectively (Opawale, Oyetayo and Agbaje, 2015).

Production of Larvicidal Agent

Taxopon (50 g) and Phenol (200 mL) were combined in a 2-litre container and stirred until completely dissolved and particles remained; menthol was added to facilitate dissolution. The mixture was properly mixed, 200g of *H. suaveolens*leaf extract was added, and the solution was homogenised. The mixture was poured into new spray vials without allowing the solvent to escape for analysis and comparison with the control (Ileke and Ogungbite, 2015).

Larvicidal Tests

Anopheles stephensi larvae in their third and fourth instars were subjected to larvicidal assay at test concentrations of 2, 4, 8, and 10 g of the plant extract from *H. suaveolens* leaves by dissolving each in 100ml of distilled water. Another 100ml of distilled water was collected in a 200ml beaker which served as a negative control. In the *in vivo* test,ten (10) larvae were used for each concentration (Ezihe *et al.*, 2017). A Completely Randomised Design (CRD) was used to establish three replicates of the plant extract concentrations. After twenty-four hours, the total number of dead larvae wasdetermined. The mortality rate wascalculated as a percentage.

$$\text{Percentage Mortality} = \frac{\%\ \text{mortality in treated} - \%\ \text{mortality in control X } 100}{100 - \%\ \text{mortality in control}}$$

Bio-LarvicidalSpray

Third- and fourth-instar *Anopheles stephensi* larvae were collected and reared in a beaker of water with a muslin cover until they were pupated and sprayed with *H. suaveolens* leaves extract mixture. After 5 minutes of spraying through muslin cloth, the fatality rate was determined (Ezihe *et al*., 2017). The positive experimental control used was the commercial Baygon.

Results

Table 1: Percentage mortality of mosquito larva at different concentrations of *H. suaveolens*leaf ethanolextract

Ethanol extract				
Dosage (ppm)	**Initial larvae**	**No. of survivors**	**No. of deaths**	**% mortality**
1000	20	0	20	100
100	20	3	17	85
10	20	6	14	70
1	20	9	11	45
LC_{50}				**1.71**

Table 2: Percentage mortality of mosquito's larvae exposed to *H. suaveolens*leaf extract spray (phytospray)

Concentration (%)	**5**	**10**	**15**	**20**	**30**
	Minutes after Treatment (MAT)				
1	0.00±0.00	0.00±0.00	10.33±0.00	16.67±0.00	16.67±0.00
2	0.00±0.00	6.67±0.04	16.33±0.00	36.67±0.00	66.67±0.00
5	31.67±0.00	52.67±0.00	84.33±0.00	100.00±0.00	100.00±0.00
Unexposed	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Baygon	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Discussion

The extract of *Hyptissuaveolens* is widely used in the treatment of malaria. It is efficacious against chloroquine-sensitive and -resistant strains of Plasmodium falciparum under laboratory conditions. In addition, diterpenoid 13alpha-epi-dioxiabiet-8(14)-en-18-ol from petroleum ether extract of *H. suaveolens* leaves isalso known to be an antiplasmodial activity (Peniche *et al*., 2022). This study confirms the effectiveness of *H. suaveolens* as a larval mosquito repellents against *Anopheles* mosquitoes.

The effectiveness of *H. suaveolens* extracts against mosquito larvae is shown in Table 1. The larvicidal efficacy of *H. suaveolens* extract had a dose-response relationship with the mortality of mosquito larvae; at higher concentrations, the extract was more effective. At 1ppm of the plant ethanol leaf extract, 45% mortality was observed, and the LC_{50} was determined to be 1.71 ppm. The effectiveness of thephytospray made from *H. suaveolens* ethanol leaf extract against adult mosquitoes is presented in Table 2. The result demonstrates that after 30 minutes of exposure to the extract, 100 percent of the mosquitoes were deceased, making it comparable to the test commercial insecticide (Baygon).

These results demonstrated the astonishing effect of *H. suaveolens* ethanol leaf extract on anopheles mosquito larvae. However, it was revealedthat the efficacy of plant extract was concentration dependent. Similarly, Ileke and Ogungbite (2015) reported that the oil and extract of *A. boonei*had a significant larvicidal activity on mosquito larvae respectively.

The phytospray produced from*H. suaveolens* leaf extract's was more effective against mosquito's larvae at concentrations of 2% and 5%, eliminating more than 50% of the larvae after 30 minutes of exposure. Within 10 minutes of exposure to the ethanol extract of the leaves at a concentration of 5%, 50% of the insects were killed. The activity levels are encouraging compared to a commercially available insecticide (Baygon). According to Adelaja *et al*. (2022), a 30% concentration of *H. suaveolens* essential oil repelled 70% of mosquitoes, while a 30% concentration of *Sennahirsuta* essential oil repelled 50%.

This study demonstrated that extract of *H. suaveolens* effectively repelled mosquitoes larvae, when diluted to a concentration of 50%. Throughout the experiment, no dermatological or cutaneous reactions were observed. It has been previously demonstrated that the polarity of the solvents used to extract active

biochemical from plants is crucial (Abok, Ombugadu and Angbalaga, 2018).

This study also confirmed previous findings that *H. suaveolens* extracts derived from acetone and petroleum ether are larvicidal against Anopheles mosquitoes. This research paves the way for future studies to evaluate, identify, and isolate the bioactive component(s) of *H. suaveolens* extracts and its systemic effects on target mosquitoes, which could lead to the extract's application as a larvicidal in low-volume aquatic habitats or around human dwellings to control vector mosquitoes effectively (Hemen and Hadiza, 2019). The transparent, pale yellow liquid oil is identical to all other instances. Duniya, Joseph and Sokoato (2022) reported that the terpene content of the essential oil of *H. suaveolens* leaves was related to its adult mosquito-killing activity.

Abok *et al*. (2018), Aremu *et al*. (2022), and Muema *et al*. (2017) demonstrated that the essential oil from *H. suaveolens* leaves has insecticidal properties against multiple insect species and larvicidal activity against certain insect larvae. In less than fifteen minutes, 97% of mosquitoes were repelled. The 50% lethal concentration(LC_{50}) of the plant extract were 3,336 and 0.878 ppmrespectively. Ezihe *et al*. (2017) demonstrated that plant placement, burning, a sprinkling, and sticks are all effective insect repellents. Muema *et al*. (2017) determined no statistically significant difference in death rates between 250 and 350 ppm, the lowest quantity they examined. Arivoli and Tennyson (2013)have also demonstrated that the larvicidal activity of *H. suaveolens* essential oil against mosquito larvae is concentration-dependent. During the study, it was discovered that *H. suaveolens* plant parts had a toxic effect on the larvae, but a significantly greater proportion of mosquito larvae died when the extract wasadministered at higher concentrations.

Conclusion

Hyptissuaveolens is one plant species utilised as a repellent for biological mosquito control in rural areas with minimal difficulty. The stated objective of the study was to determine whether or not *H. suaveolens* is fatal. The study revealed that mosquito vectors had a strong affinity for the concentration of *H. suaveolens*, resulting in a high mortality rate at all times of the testing period. The high mortality of mosquitolarvae at modest doses suggests that *H. suaveolens* extract could be used to develop effective mosquitocides and this may be anatural alternative to synthetic insecticides for regulating vectors. This study supports the long-held notion that *H. suaveolens* can be used as a repellent and insecticide due to its larvicidal properties. It has been discovered that the leaf extract of *H. suaveolens* has larvicidal and effective repellent properties against adult mosquitoes. Extracts of the plant's leaves have been shown to eliminate mosquito larvae, indicating that terpenes in the plant are likely responsible for these effects.

In addition, most important information presented in this article pertains to the insecticidal properties of *H. suaveolens* leaves, which can produce detergent, perfume, and mosquito repellent. Additional research could be conducted to isolate the essential oil's compounds and identify the ones responsible for the larva-killing, and repellent properties.

Due to resistance to chemical pesticides, the harmful effects of chemical insecticides on non-target organisms, and human health issues, indigenous people have learned to combat insect infestations with plant extracts. Developing an effective mosquito repellent derived from *H. suaveolens* will assist in meeting the urgent demand for novel botanical and synthetic mosquitocides.

References

Abok, J., Ombugadu, A., &Angbalaga, G. (2018). *Hyptissuaveolens* Extract Exhibits Larvicidal Activity Against Anopheles gambiae Larvae. *Tropical Journal of Natural Product Research*, *2*(5), 245–249. https://doi.org/10.26538/tjnpr/v2i5.8.

Adelaja, O. J., Oduola, A. O., Ande, A. T., Abiodun, O. O. &Adelaja, A. R. (2022).*Toxicity potentials of selected insecticidal plant oils against Anopheles gambiae (Diptera: Culicidae) under laboratory conditions* (p. 2022.08.14.503910). bioRxiv. https://doi.org/10.1101/2022.08.14.503910.

Akinnibosun, F.I. &Adewumi, L.B. (2019). Properties and potentials of *Cola milleni*iK .

Aremu, H. K., Adekale, I. A., Azeez, L. A., Busari, H. K., Adebisi, O., Iwalewa, Z.

O., Alle, O. E., & Musa, D. A. (2022). Assessment of larvicidal and genotoxic potentials of extracts of Hyptissuaveolens against Culexquinquefasciatus based on enzyme profile and RAPD-PCR assay. *ActaTropica*, 229, 106384. https://doi.org/10.1016/j.actatropica.2022.106384.

Arivoli, S. & Tennyson, S. (2013). Screnning of plant extracts for oviposition activity against Spodopteralitura (Fab). (Lepidoptera: Noctudae). International Journal Fauna and Biological Studies, 1: 20-24.

Duniya, S. V., Joseph, B. C., &Sokoato, M. I. (2022). Insecticidal and mosquito repellency properties of essential oil from *Hyptissuaveolens* leaves. *Open Access Journal of Science,* 5(1), 77–83. https://doi.org/10.15406/oajs.2022.05.00179.

Ezihe, E. K., Egbuche, C. M., Nwankwo, E. N., Onyido, A. E., Uzochukwu3, I. M., Joseph, A. U., Anthony, C. O., &Ikechukwu, O. O. (2017). The Larvicidal and Repellent Effect of Bush Mint Plant (*Hyptissuaveolens*) and Hairy Sicklepod (Sennahirsuta) Extracts against the Larvae and Adult of Culexquinquefasciatus. *Journal of Mosquito Research*, 7(8), Article 0.

Hemen, T. J., &Hadiza, A. M. (2019). Bio-Control of Fresh and Dried Portion of *Hyptissuaveolens* Plant on Mosquitoes Population during Raining Season in Nigeria Police Academy, Kano*. Journal of Biomedical Science and Engineering*, 12(11), Article 11. https://doi.org/10.4236/jbise.2019.1211038

Ileke, K., &Ogungbite, O. (2015). Alstoniaboonei De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *Journal of Coastal Life Medicine*, 3(7), 557–563. https://doi.org/10.12980/JCLM.3.2015J5-74

Moola, A. K., Ayyadurai, T., Balasubramani, S., Vignesh, R., Mohan, P. K., Sathish, S. & Diana, R. K. B. (2023). Chemical composition and larvicidal activity against Aedesaegypti larvae from *Hyptissuaveolens* (L.) Poit essential oil. *Journal of Natural Pesticide Research*, 3, 100018. https://doi.org/10.1016/j.napere.2022.100018

Muema, J. M., Bargul, J. L., Njeru, S. N., Onyango, J. O., &Imbahale, S. S. (2017). Prospects for malaria control through manipulation of mosquito larval habitats and olfactory-mediated behavioural responses using plant-derived compounds. Parasites and Vectors, 10(1), 184. https://doi.org/10.1186/s13071-017-2122-8

Naphtali, R., &Ngwamah, J. s. (2019). Phytochemical Screening and Larvicidal Activities of Some Ethnobotanicals from North Eastern Nigeria against Culicine (*Dipera: Culicidae*) Mosquito. International *Journal of TROPICAL DISEASE and Health*, 1–9. https://doi.org/10.9734/ijtdh/2019/v37i430171

Ngwamah, J.S. & Naphtali, R. (2019). Activities of Some Ethnobotanicals from North East Nigeria, against Culicine Mosquitoes. *Asian Journal of Research in Infectious Diseases*, 2(4), 1–11. https://doi.org/10.9734/ajrid/2019/v2i430108

Opawale, B. O., Oyetayo, A. &Agbaje, R. (2015). Phytochemical Screening, Antifungal and Cytotoxic Activities of TrichiliaheudelotiiPlanc (Harm). *International Journal of Sciences: Basic and Applied Research (IJSBAR),* 24(6),: 267-276.

Peniche, T., Duarte, J. L., Ferreira, R. M. A., Sidônio, I. A. P., Sarquis, R. S. F. R., Sarquis, Í. R., Oliveira, A. E. M. F. M., Cruz, R. A. S., Ferreira, I. M., Florentino, A. C., Carvalho, J. C. T., Souto, R. N. P., &Fernandes, C. P. (2022). Larvicidal Effect of *Hyptissuaveolens* (L.) Poit. Essential Oil Nanoemulsion on *Culexquinquefasciatus (Diptera: culicidae).* Molecules, 27(23), Article 23. https://doi.org/10.3390/molecules27238433.

Sonibare, M. A., Okorie, P. N., Aremu, T. O., &Adegoke, A. (2015). Ethno-medicines for Mosquito Transmitted Diseases from South-western Nigeria. *Journal of Natural Remedies,* 15(1), 33–42. https://doi.org/10.18311/jnr/2015/470.