

Evaluation of the Bio-Pesticide Efficacy of the Leaf Extracts of *Hyptis suaveolens* on *Culex quinquefasciatus* (Diptera: Culicidae)



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Abstract

The urgent need for novel mosquitocidal agents, due to the limitations of synthetic chemicals, prompted this study to assess the biopesticide potential of *Hyptis suaveolens* extracts against *Culex quinquefasciatus*, an intermediate host of filariasis. Phytochemical screening of the aqueous and methanol extracts was performed using standard protocols. Graded concentrations of both extracts, ranging from 1.0 to 5.0 mg/L, were tested against 4th instar larvae of *Culex quinquefasciatus*. Mortality rates were recorded at regular intervals up to 24 hours. Probit regression analysis was utilized to determine the concentrations required to achieve 50% (LC50) and 90% (LC90) larval mortality. The results revealed that both aqueous and methanol extracts contained bioactive components, including phenols, alkaloids, flavonoids, saponins, and tannins. Larvicidal activity increased significantly ($P < 0.05$) with higher extract concentrations and longer exposure periods. Notably, the methanol extract achieved 100% mortality at 5.0 mg/L, indicating greater potency compared to the aqueous extract. The methanol extract exhibited the highest efficacy with an LC50 of 0.94 mg/L, whereas the aqueous extract had an LC50 of 2.84 mg/L. These findings suggest that *H. suaveolens* has substantial larvicidal properties and supports its traditional use as a repellent or insecticide, highlighting its potential as a natural alternative to synthetic chemicals.

Keywords: Biopesticide; *Hyptis suaveolens*; Larvicidal; *Culex quinquefasciatus*

Abbreviations: WHO: World Health Organization; HPLC: high performance liquid chromatography

Introduction

Mosquitoes are well-documented vectors for a range of debilitating diseases, including dengue fever, yellow fever, filariasis, encephalitis, malaria, and other febrile illnesses Ayange-Kaa et al. [1]. In tropical and sub-tropical regions, mosquitoes are categorized into various genera such as *Anopheles*, *Aedes*, and *Culex* Ayange-Kaa et al. [1]. The World Health Organization WHO [2] identifies *Aedes aegypti* as a critical species due to its role in transmitting the dengue virus, which leads to dengue fever and yellow fever in humans Ayange-Kaa et al. [1]. Additionally, *Culex* mosquitoes, often referred to as house mosquitoes, are significant vectors for West Nile fever, encephalitis, and other viral diseases affecting birds and horses. Among them, *Anopheles gambiae* is

notable for its efficiency in transmitting *Plasmodium falciparum*, the causative agent of malaria, due to its preference for humans as hosts and its indoor-feeding behavior CDC [3]. This species also plays a role in transmitting *lymphatic filariasis*, commonly known as elephantiasis Wendy et al. [4]. *Lymphatic filariasis*, caused by filarial worms, often presents without symptoms but can lead to severe conditions such as swelling and thickening of the skin in various body parts, impacting an individual's social and economic circumstances. Preventative measures include using bed nets and mass deworming programs Amer and Mehlhorn [5].

The control of mosquito populations has become increasingly challenging due to the rise of resistance to synthetic insecticides

and environmental concerns arising from their persistent use Wendy et al. [4]. The limitations of traditional vector control strategies have underscored the need for alternative approaches Radhika et al. [6], Ramirez-Lepez & Ramirez-Suero [7]. Current mosquito control methods include chemical treatments (e.g., insecticides and repellents), physical methods (e.g., treated bed nets and protective clothing), and biological methods (e.g., the use of *Bacillus* species, genetic manipulation of mosquitoes, and mosquito-eating fish such as *Gambusia*) Chandra et al. [8].

Hyptis suaveolens, commonly known as bush mint, is a weedy herb native to tropical America and now widespread in tropical and sub-tropical regions Devi [9]. Belonging to the *Lamiaceae* family, *H. suaveolens* is renowned for its insecticidal properties and has been traditionally used to treat various ailments, including respiratory infections, uterine infections, and skin diseases Devi [9]. In Nigeria, the plant is valued for its mosquito-repellent properties, with its leaves often burned to produce smoke that repels mosquitoes Jeremiah et al. [10].

Despite numerous efforts to control mosquito populations, the medical and economic impacts of mosquito-borne diseases continue to escalate Wendy et al. [4]. The challenges associated with existing control measures and increasing insecticide resistance emphasize the need for innovative solutions Achs and Malaney [11]. While traditional methods such as water management and chemical insecticides have been employed, their effectiveness is often compromised by resistance and environmental concerns Okonta et al. [12]. Plant-derived natural pesticides offer a promising alternative due to their potential environmental benefits Hang [13]. Essential oils from plants possess natural insecticidal properties, making them viable candidates for developing eco-friendly mosquito control solutions Amer & Mehlhorn [5]. This study aims to evaluate the bio-pesticide efficacy of *Hyptis suaveolens* leaf extracts against *Culex quinquefasciatus*, an intermediate host of filariasis. Such an evaluation will contribute to identifying sustainable and effective mosquito control measures that address the challenges posed by insecticide resistance and environmental sustainability.

Materials and Methods

Plant Collection and Identification

Fresh leaves of *Hyptis suaveolens* were collected from the Biological Garden of the Federal University of Technology, Minna, Bosso Campus, Nigeria. Identification and authentication of the plant were carried out by a botanist in the Department of Plant Biology at the same institution. The selection of the leaves was based on their traditional use in treating various ailments such as diabetes, fever, cancer, hepatitis, dysentery, hypertension, and malaria, as well as their recognized organoleptic properties Abdullahi et al. [14].

Preparation of Plant Powder

Following identification, the fresh leaves were thoroughly washed first in tap water and then in distilled water to remove any contaminants Abdullahi et al. [14]. The leaves were air-dried under shade at room temperature ($28\pm 2^\circ\text{C}$) for two weeks until completely dehydrated. The dried leaves were then ground into a coarse powder using a wooden mortar and pestle. This powdered sample was used to prepare the crude extracts.

Preparation of Crude Extracts

The total weight of the powdered sample was 200 grams. Extraction was performed using the cold maceration method.

Preparation of Aqueous Extract

One hundred grams of the dried powdered leaves were weighed and placed in a round-bottom flask. Four hundred milliliters of distilled water were added, and the mixture was shaken thoroughly. The flask was allowed to stand for 48 hours, after which the mixture was filtered through a clean muslin cloth into a sterile beaker. The resulting solution was concentrated using a water bath at 40°C and subsequently weighed. The extract was stored in a clean universal container and kept in a refrigerator at 4°C to protect it from light and moisture Sutharson et al. [15].

Preparation of Methanol Extract

Similarly, one hundred grams of the powdered leaves were placed in a round-bottom flask, and 400 milliliters of 100% methanol were added. The mixture was shaken and left to stand for 48 hours. After filtration through muslin cloth into a sterile beaker, the solution was concentrated using a water bath at 40°C . The methanol extract was stored in a clean universal container and refrigerated at 4°C until use Sutharson et al. [15].

Phytochemical Analysis of the Leaf Extracts

Phytochemical screening of the leaf extracts was conducted to identify the presence of phenols, flavonoids, alkaloids, tannins, and saponins. The analyses were performed according to methods described by Singleton et al. [16] and Sofowora [17].

Entomological Investigation

Collection of Mosquito Larvae

Mosquito larvae were collected from stagnant water sources in Bosso market, Nigeria, three times a week. Collection was carried out between 6:00-9:00 AM and 5:00-6:00 PM. A yellow bowl or scoop was used to collect water samples, which were transported to the biology laboratory. After allowing the water to settle, larvae were separated using a pipette. Only larvae in the third and fourth instar stages were preserved in a rubber container for rearing, as per WHO guidelines WHO [2]. The larvae were kept at room temperature ($28\pm 2^\circ\text{C}$) and fed with powdered yeast.

Preparation of Stock and Working Solutions

Stock and working solutions of the plant extracts were prepared according to protocols developed by the Department of Biological Sciences for testing mosquito larvicides. A 1-gram sample of the crude extract was dissolved in 10 mL of the extraction solvent to prepare a 100% concentrated stock solution. To create a 10% concentration of the original extract, 1 mL of the stock solution was diluted with 99 mL of distilled water. For test concentrations of 0.1, 0.5, 1.0, and 2.0 mg/L (equivalent to 1, 5, 10, and 20 ppm, respectively), graded volumes of the working solution were added to 99, 95, 90, and 80 mL of distilled water, respectively.

Bio-Assay of Extracts against Mosquito Larvae

The bio-assay was conducted in Biological Laboratory II at the Federal University of Technology, Minna, under controlled laboratory conditions. The assay followed the WHO [2] protocol with minor modifications. Twenty-five late third instar and early fourth instar larvae of *Culex quinquefasciatus* were transferred to 250 mL bowls containing 100 mL of distilled water mixed with various concentrations of plant extract solutions ranging from 0.1 to 2.0%. For each concentration, four replicates were set up, along with a control group with four replicates. Observations were made at various intervals: 0, 5, 10, 15, 20, 30 minutes, 1, 2, 3, 6, 12, 18,

and 24 hours. Dead or moribund larvae were identified by their lack of movement upon being pricked with a sharp object and were removed using a rubber pipette.

Bio-Assay Details

Each bowl containing the working solution received 25 mosquito larvae. Mortality rates were observed at regular intervals. For the positive control, 1 mL of solvent was added to 99 mL of distilled water, and 1 mL of this solution was further diluted in 99 mL of distilled water. Twenty-five larvae were added to this mixture. For the negative control, 25 larvae were added to 100 mL of distilled water with no added substances.

Results

Phytochemical Components of the Aqueous and Methanol Extract of *Hyptis suaveolens*

Table 1 presents the phytochemical analysis results for the aqueous and methanol extracts of *Hyptis suaveolens*. Both extracts contained phenols, flavonoids, tannins, and saponins. However, alkaloids and saponins were absent in the methanol extract. These findings highlight differences in phytochemical profiles between the two extraction solvents, with the methanol extract lacking certain compounds present in the aqueous extract (Table 1 & 2).

Table 1: Qualitative Phytochemical Components of Aqueous and Methanol Extract of *Hyptis suaveolens*.

Phytochemicals	<i>H. suaveolens</i>	<i>H. suaveolens</i>
Aqueous Methanol	+	+
Phenol	+	-
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Saponins	+	+

Note: Key: + = Present, - = absent

Table 2: Media (LC₅₀) and Upper (LC₉₀) Lethal concentration of the Larvicidal Potency of Aqueous and Methanol Extract of *H. suaveolens*.

Extract	LC ₅₀ (mg/L)	LC ₉₀ (mg/L)	R ²	Regression Equation
Aqueous	2.84	6.88	0.9486	Y=9.9x+21.9
Methanol	0.94	4.65	0.8967	Y=10.8x+39.8

Mosquito larval mortality caused by aqueous and methanol extract of *H. suaveolens* against *Culex quinquefasciatus*

The larvicidal activity of the aqueous extract of *Hyptis suaveolens* is summarized in Table 3. The data show a clear pattern of increased mosquito larval mortality with higher extract concentrations and longer exposure times. At lower concentrations (1.00 and 2.00 mg/L), the mortality rate was relatively low, but

it increased significantly at higher concentrations (3.00 and 4.00 mg/L). Notably, the mortality rate continued to rise with extended exposure, indicating a time-dependent effect. At the highest concentration (4.00 mg/L), the mortality rate was notably higher, reaching 16.00% after 24 hours. This suggests that the aqueous extract is effective against *Culex quinquefasciatus* larvae, with efficacy improving both with higher concentration and longer exposure time.

Table 4 presents the results for the methanol extract of *Hyptis suaveolens*. Similar to the aqueous extract, there was an increase in larval mortality with higher concentrations and longer exposure periods. However, the methanol extract demonstrated superior effectiveness compared to the aqueous extract. For instance, at a concentration of 4.00 mg/L, the methanol extract achieved 20.00% mortality after 24 hours, which is significantly higher

than the maximum mortality observed with the aqueous extract (16.00%). The methanol extract also exhibited a faster onset of action, with notable mortality occurring as early as 5 minutes for some concentrations. This suggests that the methanol extract has a higher potency and faster acting larvicidal effect than the aqueous extract (Table 3 & 4).

Table 3: Mosquito larvicidal activities of the aqueous extract of *H.*

Extract Conc. (mg/L)	Exposure period							
	5mins	10mins	30mins	1hr	3hrs	6hrs	12hrs	24hrs
1	0.00±0.00 ^a	2.00±0.71 ^b	3.75±0.48 ^c	5.00±0.71 ^c	7.00±0.71 ^c	7.75±0.63 ^b	9.00±0.75 ^b	9.00±0.41 ^b
2	0.00±0.00 ^a	2.25±0.63 ^b	2.50±0.50 ^b	3.25±0.75 ^b	3.75±1.03 ^c	8.25±1.65 ^b	9.25±1.93 ^b	9.25±1.65 ^b
3	0.00±0.00 ^a	3.00±0.71 ^b	4.75±0.85 ^c	5.75±1.49 ^d	7.25±2.02 ^c	8.75±2.02 ^b	11.25±0.60 ^c	12.25±1.31 ^c
4	0.00±0.65 ^b	2.75±1.55 ^b	4.75±1.65 ^c	7.00±1.00 ^c	8.00±1.35 ^c	9.00±1.55 ^b	13.00±2.38 ^d	16.00±1.91 ^d
Positive control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Negative control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Table 4: Mosquito larvicidal activities of the methanol extract of *H. suaveolens* against 4th instar larva of *Culex quinquefasciatus*.

Extract Conc. (mg/L)	Exposure period							
	5mins	10mins	30mins	1hr	3hrs	6hrs	12hrs	24hrs
1	0.00±0.00 ^a	0.00±0.00 ^a	1.75±0.03 ^b	5.00±1.47 ^b	7.75±1.11 ^b	9.25±0.95 ^b	10.50±0.96 ^b	13.75±0.11 ^c
2	0.00±0.00 ^a	1.50±0.50 ^b	2.00±0.91 ^b	7.75±1.18 ^c	10.00±1.29 ^b	10.75±1.31 ^b	11.50±0.96 ^b	15.50±0.76 ^b
3	0.00±0.00 ^a	2.25±0.95 ^c	2.00±1.08 ^b	8.50±0.96 ^c	10.25±1.31 ^b	11.75±0.38 ^b	12.25±1.70 ^b	16.00±1.08 ^c
4	0.75±0.85 ^b	3.50±1.04 ^d	4.75±0.75 ^c	9.75±0.03 ^d	12.25±1.38 ^c	14.75±1.89 ^d	15.50±1.10 ^c	20.00±0.25 ^d
Positive control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Negative control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

Percentage mortality caused by aqueous and methanol extract of *H. suaveolens* after 24 hours exposure

Figure 1 illustrates the percentage mortality of larvae exposed to the aqueous and methanol extracts of *Hyptis suaveolens* after 24 hours. The graph confirms that both extracts increase larval mortality with higher concentrations, but the methanol extract is

significantly more effective. This is evidenced by the consistently higher mortality rates observed with methanol extracts across all concentrations compared to aqueous extracts. This enhanced efficacy of the methanol extract could be attributed to the higher solubility and extraction efficiency of phytochemical compounds in methanol, which may contribute to its superior larvicidal properties (Figure 1).

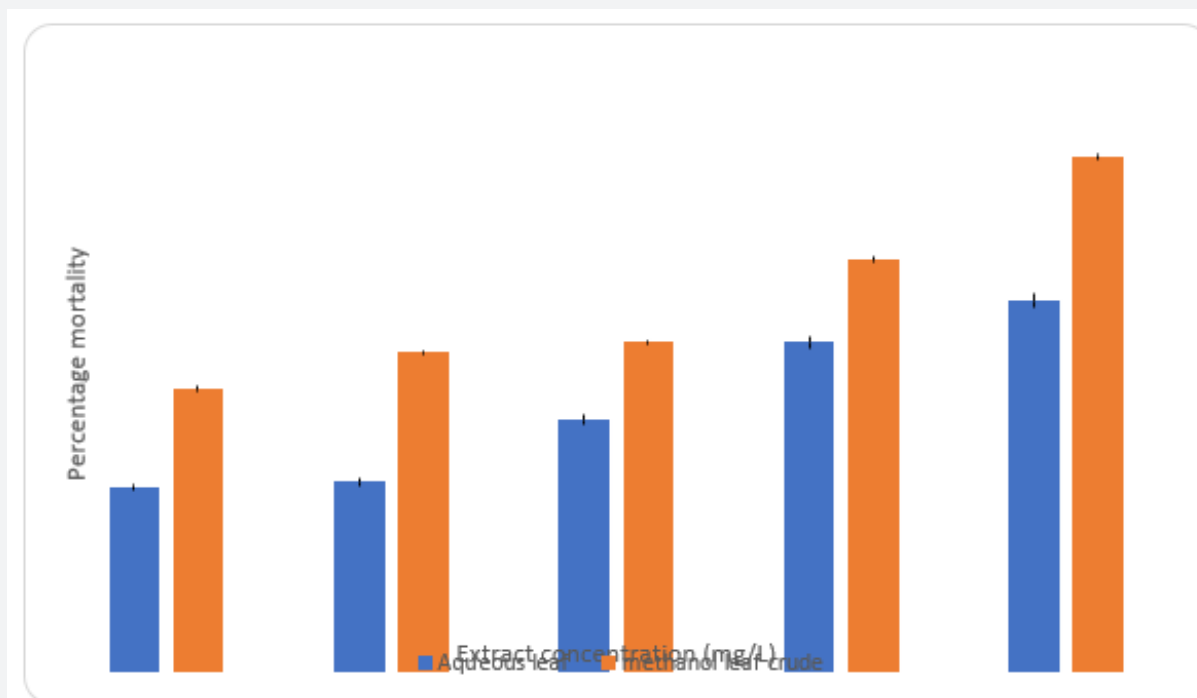


Figure 1: Percentage mortality caused by aqueous and methanol extract of *H. suaveolens* after 24 hours exposure.

Discussion

Plants have long been recognized as sources of bioactive compounds with potential applications in pest control, particularly in the development of environmentally friendly insecticides (Vasudevan et al., 2009). This study focuses on *Hyptis suaveolens*, a plant known for its traditional uses and medicinal properties, and examines its potential as a larvicidal agent against *Culex quinquefasciatus*. The phytochemical analysis revealed the presence of several bioactive components, including phenols, alkaloids, flavonoids, tannins, and saponins, which are known for their diverse biological activities Kamalakannan et al. [18]. The variation in phytochemical composition between the aqueous and methanol extracts of *H. suaveolens* was notable, reflecting the different solubility profiles of the bioactive compounds. Methanol, being a polar solvent, is more effective in extracting a wider range of phytochemicals compared to water Olayemi et al. [19]. This difference in extraction efficiency is likely responsible for the observed disparity in larvicidal activity between the two solvents. The enhanced larvicidal efficacy of methanol extracts over aqueous extracts corroborates findings from other studies where methanol extracts of various plants exhibited higher insecticidal activity Quevedo et al. [20].

The observed increase in larval mortality with higher concentrations and extended exposure periods underscores the concentration-dependent nature of the larvicidal activity of *H. suaveolens* extracts. Methanol extracts demonstrated a more

potent effect compared to aqueous extracts, which aligns with the results of Patel et al. [21], who found that methanol extracts of *Azadirachta indica* were more effective against mosquito larvae than aqueous extracts. The progressive increase in larval mortality with higher concentrations of methanol extract suggests a dose-dependent response, which is consistent with the principles of chemical toxicity and efficacy Singh et al. [22].

The differential larvicidal activity observed between the two solvent extracts could be attributed to the specific phytochemicals extracted by each solvent. For instance, methanol is known to extract a higher concentration of flavonoids and alkaloids, which have been documented to exhibit potent insecticidal properties Kamalakannan et al. [18]. Flavonoids, in particular, are known to disrupt the physiological processes of insects, while alkaloids can affect the nervous system, leading to increased mortality Prabakar and Jebanesan [23]. The presence of these compounds in higher concentrations in the methanol extract may explain its superior larvicidal activity. In addition to the phytochemical composition, the efficacy of *H. suaveolens* extracts may also be influenced by the method of application and the environmental conditions during testing. The laboratory conditions under which the bioassays were conducted may not fully replicate field conditions, where factors such as temperature, humidity, and competition from other organisms can affect larvicidal efficacy Quevedo et al. [20]. Therefore, while the laboratory results are promising, further field studies are necessary to validate the effectiveness of *H. suaveolens* extracts in real-world scenarios.

The LC50 and LC90 values obtained for the methanolic extract of *H. suaveolens* in this study were lower than those reported for other plant species, such as *Azadirachta indica* and *Vitex negundo* Prabakar & Jebanesan [23]. This indicates that *H. suaveolens* may possess a higher intrinsic toxicity towards mosquito larvae compared to these well-known larvicides. The relatively high toxicities observed suggest that *H. suaveolens* could be a valuable source of potent insecticidal agents, particularly for mosquito vector control. This aligns with recent research that emphasizes the potential of plant-based insecticides as sustainable alternatives to synthetic chemicals Singh et al. [22].

The promising results of this study contribute to the growing body of evidence supporting the use of *H. suaveolens* as a natural insecticide. However, there are several considerations that should be addressed in future research. Firstly, the safety and environmental impact of using *H. suaveolens* extracts as insecticides need to be thoroughly evaluated. This includes assessing potential toxicity to non-target organisms and the impact on ecological balance. Secondly, the stability and shelf-life of the extracts should be investigated to ensure their practical application in vector control programs. Moreover, the isolation and characterization of the specific active compounds responsible for the larvicidal activity of *H. suaveolens* are crucial for understanding the mechanism of action and optimizing the extract's efficacy. Advances in analytical techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry, can facilitate the identification of these compounds and their interactions with mosquito larvae Patel et al. [21].

Conclusion

In conclusion, *Hyptis suaveolens* demonstrates significant potential as a source of insecticidal agents, particularly in methanol extracts, which exhibited superior larvicidal activity against *Culex quinquefasciatus*. The study highlights the importance of solvent selection and concentration in determining the efficacy of plant extracts. These findings support the continued exploration of *H. suaveolens* for sustainable mosquito control solutions and emphasize the need for further research to optimize extraction methods and assess the environmental impact of using this plant in vector control programs.

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