



Evaluation of Chemical Composition of *Mesosphaerum suaveolens* as a Potential Insecticide for *Anopheles gambiae*

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ABSTRACT

Introduction: *Mesosphaerum suaveolens* (Lamiaceae), commonly found in tropical regions, is traditionally used for its medicinal and insect-repelling properties. Increase in resistance of *Anopheles gambiae* to synthetic insecticides coupled with growing concern of mammals' health and the environment has pose a great need for safer insecticide(s). Thus, the aim of this study was to evaluate the chemical composition of *M. suaveolens* as a potential natural alternative for mosquito control. **Methodology:** Aerial parts of the plant were collected, shade-dried, pulverized, and extracted using n-hexane in a Soxhlet extractor. This was followed by the preliminary phytochemical screening, and GC-MS analysis of the crude extract at the Multipurpose Lab, ABU Zaria, Nigeria. Insecticidal efficacy of GC-MS identified compounds was evaluated using the CDC bottle bioassay on adult *A. gambiae* at concentrations of 2000, 4000, and 6000 µg/bottle. **Findings:** The preliminary qualitative phytochemical analysis revealed the presence of high concentration of terpenoids, with minute concentrations of alkaloids, tannins, saponins, and glycosides. From GC-MS analysis, eight potential insecticidal compounds were identified, including caryophyllene, caryophyllene oxide, phytol, and spathulenol. Results of insecticidal efficacy showed a dose-dependent increase in mortality: 44%, 83%, and 100% respectively within 24 hours. Probit regression gave $LC_{50} = 666.59$ µg/bottle and $LC_{95} = 1,571.95$ µg/ bottle. Although ANOVA showed no statistically significant differences among treatment groups ($P = 0.125$), the consistent mortality trend confirms the biological relevance of the extract. **Conclusion:** This study highlights *M. suaveolens* as a promising candidate for developing plant based, eco-friendly insecticides in mosquito vector control programs.

Keywords:

Mesosphaerum
Suaveolens;
Anopheles gambiae;
Soxhlet extraction;
Phytochemical
Screening;
Gas chromatography-
Mass spectroscopy.

INTRODUCTION

Weeds are commonly considered as unwanted herbs, however; many of them possess medicinal as well as toxic properties. The toxic characteristics of weeds can be used for insecticidal purposes (Lawal *et al.*, 2025). The development of resistance by the mosquito vector against the synthetic inorganic insecticides such as DDT (organochlorine) and the environmental hazard caused by the insecticide resulting from the persistent usage are making mosquito control extraordinarily cumbersome (Abdulahi & Muhammad, 2025). The Lamiaceae family is one of the most diverse and widespread in terms of the ethno-medicinal value and variety of plant with biological applications (Uritu *et al.*, 2018).

In terms of chemical and economic scope, the Lamiaceae family has the potential for the extraction of essential oils, which are produced from hairs and glandular trichome. In Northeast Brazil, the use of plant species as therapeutic resources is widespread, and one species present in this region is *Mesosphaerum suaveolens* (L.) Kuntze, known as "bamburral," "erva-canudo," or "alfazema-brava" (Bezerra *et al.*, 2017). This plant is commonly called bush mint, bush tea, pignut, or chan, and its known as 'vilayatituls' in Hindi; 'kondathulasi' in Telugu; 'bhustrena' in Sanskrit; 'daddoya-ta-daji' in Hausa; 'efiri' in Yoruba; 'nchuanwu' in Igbo; and 'tanmotswangi-eba' in Nupe.

M. suaveolens is an aromatic plant known for its unique chemical profile, such as phenolics, steroids, di- and tri-terpenoids, flavonoids, saponins and tannins (Joseph & Jeeva, 2016). Its leaves are mainly used to treat respiratory diseases (asthma, bronchitis, colds, and flu) and diseases related to the gastrointestinal tract (Almeida-Bezerra *et al.*, 2022). Such medicinal uses are related to the chemical heterogeneity arising from the secondary metabolism of the species, a recurrent characteristic in species of the Lamiaceae family. Recent studies have shown that the non-polar extracts from *Mesosphaerum suaveolens* exhibit potent mosquito repellent activity against *Aedes aegypti* and *Anopheles gambiae* (Ndiath *et al.*, 2012). Besides causing mortality in insects, the products of *M. suaveolens*, especially the hydrocarbon components of the leaves, can repel insects and arachnids of public health and economic interests. The bioactive compounds extracted from essential oil which were obtained from *M. suaveolens* leaves showed larvicidal and repellent properties against *A. albopictus*, the most invasive insect in the world (Ganesan *et al.*, 2019). Isolated chemical component such as β -caryophyllene (8.0%), terpinen-4-ol (12.31%), sabinene (41.0%), and β -pinene (10.0%), from the leaves of *M. suaveolens*, were used to assess the plant's insecticidal properties against pests. *Mesosphaerum suaveolens* has been traditionally used in medicine for its insecticidal and repellent properties (Joseph & Jeeva, 2016; Almeida-Bezerra *et al.*, 2022).

Of approximately 60 genera of mosquitoes identified, members of the *Anopheles*, *Culex*, *Aedes*, *Hemagogus* and *Mansonia* complexes play a significant role as pests in Nigeria (Oyewole *et al.*, 2007). *Anopheles* mosquitoes, the primary carriers of malaria, go through a full metamorphosis that includes four different developmental stages: egg, larva, pupa, and adult (Takken *et al.*, 2024). A mosquito's life cycle lasts roughly one month. Changes in the target locations and faster insecticide metabolism are key contributors to insecticide resistance in mosquito vectors ((Ibrahim *et al.*, 2014; Ndiath *et al.*, 2012). In order to determine the quality and quantity of secondary metabolites that are biologically and pharmacologically essential, such as phenolics, alkaloids, terpenoids, glycosides, tannins, saponins, steroids, derivatives of carbohydrates, gums, essential oils, fatty acids, and other chemical compounds, it is necessary to analyze the metabolic profile of this significant plant through GC-MS. Thus, this research targeted the GC-MS identification of secondary metabolites in n-hexane extract of *M. suaveolens* as a potential natural alternative for adult mosquito control, especially *A. gambiae*, in Kaduna State, Nigeria.

MATERIALS AND METHODS

Instrument: Soxhlet Extractor (ZDHW), GC-MS (Shimadzu QP2010 Plus), Analytical Weighing, Balance

(ME204E), Microscope (Labomed), Oven (Eurosonic), and Digital thermo hygrometer (HTC-1).

Reagents: All reagents were of analytical grade. They include n-Hexane (PHD), Acetone (JHD), Mayer's Reagent, Hydrochloric Acid (JHD), Magnesium Tuning (LOBA Chemie), Ferric Chloride (LOBA Chemie), and Distilled Water.

Biological Materials: Aerial part of *Mesosphaerum suaveolens*, Baker's Yeast, *Anopheles* Mosquito, and Sugar.

Study Area: This study was conducted within the premises of Air Force Institute of Technology, Kaduna and Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Plant and Mosquito Collection: The aerial parts of *Mesosphaerum suaveolens* was collected randomly from the back of Air Force Institute of Technology Clinic, Kaduna State in the month March, 2025. *Anopheles* mosquito larvae were collected from different breeding site in prison yard, number 11 Kofan Doka road, Zaria city with the Longitude 7.706419°, Latitude 11.081839° and Altitude 615.76m. The mosquitoes were reared in entomology laboratory in the department of Zoology at the Ahmadu Bello University, Zaria, Kaduna state.

Plant Identification and Authentication: The plant was identified and authenticated at the Herbarium section of the biological science laboratory, Department of Biological Science, Nigerian Defense Academy, Kaduna, with the Voucher number NDA\BIOH\202518.

Extraction of Plant Material: The procedure as described by Tesfaye & Tefera, (2017) was employed.

A total of 130g of the dried pulverized aerial extract of *Mesosphaerum suaveolens* was weighed on an analytical balance and it was poured into a beaker. Furthermore, it was well packed in a clean handkerchief and it was taken for extraction using a Soxhlet Extractor. The crude n-hexane extract from the plant was taken for GC-MS analysis. The percentage yield for the extract was determined using the formula;

$$\text{Percent (\%)} \text{ of Extracts yield} = \frac{(L2-L1)}{L0} \times 100$$

Where: L2 = Mass of the extract + Mass of the beaker

L1 = Mass of the empty beaker

L0 = Mass of the pulverized plant sample

Phytochemical Screening of the Aerial Plant Extract of *M. suaveolens*. The qualitative phytochemical constituents of *M. suaveolens* were analyzed using the standard qualitative methods comprising tests for Alkaloids (Mayer's Reagent) (Evans, 2009), Saponins

(Faizal & Geelen, 2013), Flavonoids (Edeoga, Okwu & Mbaebie, 2005), Terpenoid (Parekh & Chanda, 2007), and Glycosides (Ayoola *et al.*, 2008)

Characterization using GC-MS Technique:

The dried n-hexane aerial extract of *M. suaveolens* was dissolved again in an appropriate solvent, vortexed, and was then filtered through a 0.45 µm syringe filter. A 1 µl aliquot of the filtered solution was injected into the GC 7890B system using Agilent Automated Liquid Sampler (ALS) G4513A. A DB - 5 MS (5% phenyldimethylsiloxane) fused silica capillary column 30 m, 320 µm which is connected to a 5977A mass selective detector (MSD) for analysis on a GC-MS instrument with helium gas (99.999% purity) as carrier gas at flow rates of 1.5ml/min. The Inlet temperature was set at 350°C, MS Source at 235°C and MS Quad at 150°C. The analysis was run for a total of 50 minutes. Data were acquired by GCMSD/Enhanced Mass Hunter Software and processed GCMSD Data analysis software incorporated with 2017 Version of NIST Library.

Mosquito collection and rearing:

The larvae of mosquitoes were collected from a breeding sites in Kofan Doka Road, Zaria city, Kaduna State Nigeria. The collected larvae were transported inside a container to the laboratory of the Department of Zoology, ABU Zaria. The larvae were further poured in a rearing tray and it was sorted, quantified and classified. The collected *Anopheles* mosquitoes were further reared to adult mosquitoes in cage for 7 days at the temperature 28.63°C and humidity 75.5% with 12-hour light and 12-hour dark cycle to mimic dawn and dusk. While it emerges it was feed on 10% sugar solution.

Testing Mortality rate of *Anopheles gambiae* using CDC Bottle bioassay. The method as described by Alhaji *et al.* (2023) was employed.

Procedure for Testing the Mortality rate of *Anopheles gambiae*.

A concentration of 2000micrograms/ bottle, 4000 micrograms/ bottle and 6000 micrograms/bottle of the n-hexane extract samples of the plant were dissolved in adequate quantity of acetone to make 10ml of total solution respectively. One ml of the solution of each concentration of the prepared stock solution was coated to the test CDC bottle, and 1ml of acetone was coated to the negative control CDC bottle. Afterward the bottles were capped and swirled in the bottom and turn to the side and tilt back and front; in other to coat the bottle efficiently on the side. The cap was then removed, lay on their sides and roll them on the table until its observed that all visible signs of acetone are gone. The bottles were then left for 1hr in a dark place. Twenty-five mosquitoes were introduced into each test bottles and the replicate bottles including the control bottle using an aspirator. At start time (Time 0), the bottles were examined to count the number of dead and live mosquitoes. Mortality was assessed at 30min, 1hr, and 24hrs after the mosquito were introduced. The mosquito was considered dead if they could no longer stand or move Afterward the dead mosquitoes were identified using dissecting microscope.

RESULTS AND DISCUSSION

The percentage yield of the n-hexane extract of *M. suaveolens* Aerial parts is 22.71% as detailed in Table 3.1.

Table 3.1 Percentage Yield and Appearance of *M. suaveolens* n-hexane Extract

Solvent	Weight taken (g)	Amount after drying (g)	Percentage yield	Appearance of the extract
n-Hexane	130	29.53	22.71	dark-green

The phytochemical constituents of the extract are as listed in Table 3.2 with terpenoids/terpenes showing highest concentration.

Table 3.2 The phytochemical constituents of the aerial part of n-hexane extract of *M. suaveolens*

Test	Inference
Flavonoids	-ve
Terpenoids	++ve
Glycoside	+ve
Phytosterols	+ve
Saponins	+ve
Alkaloids	+ve
Tannins	+ve

Key Note: ++ve Present, -ve Absent, and + slightly present

The result of Table 3.3 presented the compounds identified in *M. suaveolens* n-Hexane Extract by GC-MS analyses with potential insecticidal activities.

Table 3.3: Compounds Identified from GC-MS Analyses of *M. suaveolens* Extract with Insecticidal Activities

Peak No	Retention Time (min)	Compounds Name	Molecular Formula	Molecular Weight (mg/mol)	Peak Area
52	11.943	Caryophyllene	C ₁₅ H ₂₄	204.35	0.66
53	12.091	α -Bergamotene (trans)	C ₁₅ H ₂₄	204.35	0.06
55	12.388	α -Humulene	C ₁₅ H ₂₄	204.35	0.10
61	13.979	Spathulenol	C ₁₅ H ₂₄ O	220.35	0.16
62	14.052	Caryophyllene oxide.	C ₁₅ H ₄₀ O	220.35	0.28
66	15.275	α -Bergamotene (trans)	C ₁₅ H ₂₄	204.35	0.19
71	16.771	Neophytadiene	C ₂₀ H ₃₈	278.52	0.08
84	19.525	Phytol	C ₂₀ H ₄₀ O	296.53	0.23
105	25.565	Squalene	C ₃₀ H ₅₀	410.72	0.88

The mortality rate of *Anopheles gambiae* Using CDC bottle bioassay indicated that the highest % mortality is at a concentration of 6000 μ g/L (Table 3.4) within 24 hours. Probit analysis yielded an LC₅₀ of 666.59 μ g/bottle,

indicative of high potency, and an LC₉₅ of 1,571.95 μ g/bottle.

Table 3.4: The Percentage and Mean Mortality after 24 hrs of Exposure of *A. gambiae* s.l to *M. suaveolens* n-Hexane Extract

Conc. (μ g/bottle)	Log Conc.	Number Tested	Percentage Mortality	Mean mortality	Probit Mortality	LC ₅₀	LC ₉₅
Negative control	0.00	100.00	0.00	0.00 $\pm$ 0.00	0.00	666.59 μ g/bottle	1,571.95 μ g/bottle
2000.00	3.30	100.00	44.00	28.00 $\pm$ 9.87	4.85		
4000.00	3.60	100.00	83.00	32.33 $\pm$ 25.34	5.95		
6000.00	3.78	100.00	100.00	31.75 $\pm$ 10.16	8.09		

Conc.= concentrations, LC₅₀= lethal concentration at which half (50%) of the mosquito has been killed, and LC₉₅ = lethal concentration at which 95% has been killed.

The data obtained from the analysis of variance (ANOVA) revealed that there was no statistically significant difference in the mean mortality of *Anopheles gambiae* across the tested concentrations of *M. suaveolens* (F = 2.598, P = 0.125) @ P $\leq$ 0.05.

The gas chromatography-mass spectroscopy (GC-MS) analysis of the n-hexane extract of *M. suaveolens* aerial parts revealed the presence of various active ingredient. The bioactivity of the plant can be principally attributed to the high presence of terpenoids (Table 3.2) which

interfere with the insect behaviour and physiology. A total of eight compounds with known insecticidal activities were identified in the GC-MS chromatogram; these include caryophyllene, humulene, spathulenol, caryophyllene oxide, neophytadiene, phytol, trans- α -Bergamotene and squalene (Table 3.3).

Spathulenol: Spathulenol is a sesquiterpene alcohol widely found in essential oils. It has demonstrated insecticidal and repellent activities, particularly against *Aedes aegypti*, and *Anopheles stephensi*. It is believed to act by inhibiting neurotransmission and disrupting metabolic processes in insects (Almeida-Bezerra *et al.*, 2022).

Phytol: Phytol is a diterpene alcohol commonly derived from the hydrolysis of chlorophyll. It has been shown to exhibit potent larvicidal activity against mosquito species such as *A. stephensi* and *A. aegypti*. Its mechanism involves disruption of cell membranes and mitochondrial functions in larvae (Pushpanathan *et al.*, 2008).

Neophytadiene: Neophytadiene is a diterpenoid hydrocarbon often detected in aromatic plants. It has shown moderate insecticidal activity, particularly when combined with phytol. The combination has enhanced larvicidal effects against *Culex quinquefasciatus* and other mosquito species (Olagundoye *et al.*, 2023).

Alpha-Humulene: α -Humulene is a monocyclic sesquiterpene that has demonstrated larvicidal and antifeedant activity. It is known to be effective against mosquitoes such as *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*. Its mode of action includes interference with respiration and nervous coordination (Govindarajan and Benelli, 2016).

Squalene: Squalene is a naturally occurring triterpene found in the unsaponifiable fraction of plant oils, though it is not conventionally considered a primary insecticidal agent. In plant-based formulations, squalene enhances the permeability of the insect cuticle, allowing more potent compounds to act effectively. Some studies suggest it may function as a synergist in essential oil-based insecticides (Joshi *et al.*, 2025).

Caryophyllene: Caryophyllene is a bicyclic sesquiterpene found in many medicinal plants and spices, including clove, cannabis, and *Mesosphaerum suaveolens*. It is a well-documented insecticidal compound, with strong contact toxicity and feeding deterrent effects on mosquito larvae and other insects (Xu *et al.*, 2020). In addition to being larvicidal, it may exert antifeedant and neurotoxic effects, disrupting the development of pest larvae.

Caryophyllene Oxide: Caryophyllene oxide is the oxidized derivative of β -caryophyllene, commonly found in aged essential oils. It has received much attention for its broad-spectrum bioactivity, especially as a mosquito larvicide and insect repellent. It disrupts insect physiology, particularly at the neuromuscular junction, and is often cited for its toxicity against mosquito larvae in both laboratory and semi-field trials (Xu *et al.*, 2020).

Trans- α -Bergamotene: Trans- α -Bergamotene is a sesquiterpene hydrocarbon often found in essential oils from *Ocimum*, *Cymbopogon*, and *Mesosphaerum suaveolens*. Though typically present in minor amounts, it exhibits repellent properties against mosquitoes such as *A. aegypti* and *A. stephensi*. Its mechanism of action is primarily through interference with mosquito olfactory

receptors, making it a useful additive in natural repellent formulations (Osei-Owusu *et al.*, 2023).

These findings corroborate with the study by Almeida-Bezerra *et al.* (2022), which also detected β -caryophyllene, phytol and spathulenol as the major active ingredient in the essential oil of *Mesosphaerum suaveolens*, and associated their presence with mosquito repellent and insecticidal activity. Also, the traditional uses of *M. suaveolens* as a mosquito repellent in rural African communities further validate the bioactivity observed (Kweka *et al.*, 2008). In another related study on *Poncirus trifoliata* by Rahman *et al.* (2018), spathulenol and caryophyllene were identified as the bioactive compounds which contribute to the strong insecticidal activity. This supports the assertion that these compounds, when present together, may exert synergistic effects leading to enhanced mosquito toxicity.

With respect to insecticidal bioassays conducted according to the CDC bioassay method, the adulticidal effect of *M. suaveolens* extract was investigated on *Anopheles gambiae* mosquitoes. The results demonstrated a dose-dependent increase in mortality of adult *A. gambiae*, with mortality rising from 0% at 0 $\mu\text{g/L}$, to 44% at 2000 $\mu\text{g/L}$, 83% at 4000 $\mu\text{g/L}$, and reaching 100% at 6000 $\mu\text{g/L}$ after 24 hours of exposure (Table 3.4). This pattern highlights the potent biological activity of the plant extract and supports its potential as a natural insecticide. The increase in mosquito mortality with higher concentrations indicates a dose-response relationship. This indicates that the bioactive compounds in *M. suaveolens* interact with the target mosquito species in a concentration-dependent manner. Such a relationship is crucial in toxicology and provides a foundation for determining the lethal concentration-50 (LC_{50}) and lethal concentration-90 (LC_{90}) values, which are key indicators of insecticidal efficacy. The probit regression analysis further confirmed the concentration-dependent insecticidal effect of *M. suaveolens*. The calculated LC_{50} value of 666.59 $\mu\text{g/ bottle}$ indicates that the extract is highly effective, requiring less than 700 $\mu\text{g/bottle}$ to kill half the adult mosquito population. Meanwhile, the LC_{95} value of 1,571.95 $\mu\text{g/ bottle}$ reflects the concentration necessary for near-total mortality, accounting for natural tolerance variation within the population. These findings align with John *et al.* (2022), who reported LC_{50} values ranging from 3.22 to 5.68 mg/bottle for *Hyptis suaveolens* oils. Similarly, Joseph *et al.* (2024) observed moderate-to-high efficacy against resistant *A. gambiae* strains, highlighting the potential of this plant in overcoming insecticide resistance. The data obtained from the analysis of variance (ANOVA) revealed that there was no statistically significant difference in the mortality of *A. gambiae* across the tested concentrations of *M. suaveolens* ($F = 2.598$, $P = 0.125$). This result suggests that although a dose-dependent trend in mortality was

visually observed, the variation in mortality among the treatment groups was not strong enough to be statistically confirmed at the $P \leq 0.05$ confidential level. Nevertheless, the results of the Probit analysis ($LC_{50} = 666.59 \mu\text{g}/\text{bottle}$; $LC_{95} = 1,571.95 \mu\text{g}/\text{bottle}$) confirmed that *M. suaveolens* exhibits biologically relevant insecticidal activity even if the variation in response between concentrations was not statistically significant in the current test.

CONCLUSION

This study has demonstrated that the n-hexane extract of *M. suaveolens* exhibits biologically relevant insecticidal activity toward *A. gambiae* species of mosquito of which 8 bioactive compounds with reported insecticidal activities were identified through the GC-MS analysis. Thus, this highlights that *M. suaveolens* is as a promising candidate for developing plant based, eco-friendly insecticides in mosquito vector control programs. The research was limited to single solvent extraction due to paucity of fund.

Recommendations:

1. Ecotoxicity profiling should be conducted to evaluate safety concentrations of the extract on non-target organisms such as pollinators, aquatic species, and mammals, ensuring environmental friendliness and biosafety.
2. Activity-guided fractionation of the extract should be done in order to identify the specific pure compound(s) with highest natural insecticidal activity.

Combination of different bioassay methods such as WHO Tube Bioassay, Cone Bioassay and CDC bottle Bioassay methods for comprehensive elucidation of the insecticidal mechanism of the plant extract fraction should be carried out.

Conflict of Interest: Authors declared no conflict of interest of any form.

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