# Chemical Composition and Larvicidal Activity of Hyptis spicigera Volatile oils against Mosquito Larvae: Anopheles gambiae and Culex quinquefasciatus Say.

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**Abstract -** The study reflects the chemical composition and larvicidal potency of volatile oil obtained from *Hyptis spicigera* against the larvae of *Anopheles gambiae* and *Culex quinquefasciatus*. The analysis of the oil showed that the major constituents of the oil as β- Caryophyllene (25.7%), Caryophyllene oxide (11.56%), Sabinene (9.60%) 2- Carene (8.78%), α-Pinene (6.52%) and 1-Octen-3-ol (4.91%). Larvicidal activity of demonstrated that both the mosquito larvae were susceptible to the volatile oil with 45.18 and 53.87 µg/mL for *Anopheles gambiae* and *Culex quinquefasciatus* respectively. This high activity could be attributed to one or several volatile compounds present. The results suggest that *Hyptis spicigera* essential oil has potential to control the two mosquito species therefore can be considered as a candidate for the development of a new larvicide.

**Index Terms** - *Anopheles gambiae*, *Culex quinquefasciatus*, *Hyptis spicigera*, larvae

## 1 INTRODUCTION

Lymphatic filariasis and malaria are the two mosquito borne parasitic infections that cause severe economic burden in several parts of the world, especially, the tropical regions [1], [2]. These diseases are caused by mosquito of *Culex quinquefasciatus* and *Anopheles species*. Currently there is no vaccine available to prevent these infections in human and as the parasites are continually developing resistance to the available drugs, the best option left is to control the vector [3], [4]. Control strategies against the vectors in sub-Saharan Africa should concentrate on stopping the mosquito at larval stage since the larval mosquito breeding sites can be identified and are relatively small in an area. Many synthetic larvicides have been used in several countries for decades [5]. However, the vector resistance to pesticides and the toxicity of synthetic larvicides to other non-target organisms including human beings has guided research to develop new methods of controlling mosquitoes [6], [7]. Moreover, the synthetic insecticides are poisonous and harmful to the environment because they pollute the soil, water and air. Recently natural product alternatives are being search as replacement for the hazardous synthetic analogues. Plant-based larvicides appear to have no ill effects on non-target populations and are biodegradable, in addition to being available in many parts of the world.

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Essential oils from plant sources been reported by many researchers as larvicides, insect repellent, ovipositor attractant and growth regulators. Volatile oils components such as allyl isothiocyanate, (+)-limonene, (-)-limonene, *α*-terpinene, *p*-cymene, γ-terpinene, and (*E*)-nerolidol were reported be responsible for ≥ 95% mortality at 0.1 mg/mL [8].

*Hyptis spicigera* Lam. is an erect, aromatic, annual herb growing up to 1 metre tall [9]. Literature survey has revealed that the plant is used as expectorant, febrifuge and to treat malaria, wounds, bronchial troubles and skin diseases. The plant is commonly used to repel insects, e.g. mosquitoes and termites [10], [11], [12]. Some of the biological activities reported includes: insecticidal activity [13], [14], [15], mosquito repellency activity [16], larvicidal activity [17] and acetylcholinesterase activity [17]. Essential oil composition of the plant from various part of the globe has also been reported [18], [19], [20], [21] (Tchoumbougnang *et al*., 2005; Conti *et al*., 2011; Sidibe *et al*., 2001; Bogninou *et al*., 2013). We now report larvicidal activity of *H. spicigera* essential oil against two mosquito species: *Anopheles gambiae* and *Culex quinquefasciatus* Say.

## 2 MATERIALS AND METHODS

### 2.1 Plant Collection

The aerial parts of *Hyptis spicegera* was collected at their flowering stage in September, 2014 from Gombe State University campus. The plant was identified and authenticated by Mr. Daniel Zigla of Biological Science Department, Gombe State University, Gombe and a herbarium copy is kept in the same Departmental Herbarium.

### 2.2 Mosquito Larvae

Two mosquito larvae species (*An. gambiae* and *Cx. Quinquefasciatus)* were used for this study. *An. gambiae* mosquito larvae were collected from pools of stagnant water in Hinna village, Yelmatu/Deba Local Government Area of Gombe State while *Cx. quinquefasciatus* was collected from Malam-Inna, Gombe metropolis. The larvae were identified by Dr. Kennedy Yoriyo of Biological Science Department, Gombe State University, Gombe.

### 2.3 Essential Oil Extraction

The aerial part of the *H. spicegera* (400 g) was subjected to hydrodistillation using a modified Clevenger-type apparatus for 3 hrs. according to the British Pharmacopoeia (1980) specification. The average yields were taken over four experiments and calculated in a manner corresponding to dry weight of the plant materials. The oils were stored in small vials at 4°C until needed for bioassay and the analysis was carried out by gas chromatography fitted with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS).

### 2.4 Gas chromatography-mass spectrometry

The volatile oil components were determined using Agilent 6890N GC with an FID and GC–MS 5973 equipped with a DB-5MS column (30 m x 0.25 mm i.d., 1µm film thickness, Agilent Technologies). The GC settings were as follows: the temperature of the oven was kept at 50° C for 5 min, and then raised to 300 °C at the rate of 10°C/ min. The flow rate of helium conveying gas was 1.2 ml/min. The temperature of injector was maintained at 230 °C. The samples were injected neat with split ratio of 1:10. The recording of the mass spectra were carried over the range of 40-450 amu at one scan per second with 70 eV ionization energy and 230°C ion source temperature. The retention indices were calculated for all volatile oil constituents using a homologous series of n-alkanes C on the DB-5MS column. The percentage composition of each component of the oils was determined on the basis of GC peak area (FID response) without correction. Their relative retention indices and NIST MS database search was used to identify the various components (Adams, 2001; Giamakis *et a*l., 2001).

### 2.5 Bioassay

Larvicidal activity was carried out according to the WHO larval susceptibility test methods (WHO, 1981). Stock solution was prepared in ethanol by measuring 200 mg of the essential oil and dissolving it in 2 mL of ethanol to produce 10,000 µg/mL crude essential oil solution. This stock solution was further diluted to obtain the final concentrations of 400, 200, 100, 50, and 25 µg/mL. The tests were performed in plastic containers, each containing 20 larvae of the third and fourth instar larvae in 100 mL of each test concentration. The larvae were fed with cabin biscuits and baker's yeast at ratio 3:1. Series of concentrations for the essential oil were replicated five times. Deltamethrin at 5 µg/mLwas used as positive control. A plastic container to which was added 1 mL of 5% (v/v) ethanol solution constituted the control. The plastic containers were left on the laboratory table for 24 hrs. The larvae that had pupated during the test were discarded. Mortality was assessed by direct observation of larval movements.

### 2.6 Statistical Analysis

Percentage mortality of the larvae was ascertained using Student MiniTab software with significant level of 0.05. The lethal concentrations ($LC_{50}$ and $LC_{90}$), defined as the concentration at which at 50% and 90% of the larvae were killed by the essential oil was calculated using Log-Probit and their 95% confidence limits.

## 3 RESULTS AND DISCUSSION

The hydrodistillation of the aerial part of *H. spicigera* afforded essential oil whose chemical composition is given in **table 1**. The plant sample yielded 0.26% of the oil per sample dry weight and 21 compounds have been identified which accounted for 97.57% of the total composition. The main constituents of the essential oil extracted from *H. spicigera* were β- Caryophyllene (25.7%), Caryophyllene oxide (11.56%), Sabinene (9.60%) 2- Carene (8.78%), α-Pinene (6.52%) and 1-Octen-3-ol (4.91%).

TABLE 1: PERCENTAGE COMPOSITION OF THE ESSENTIAL OIL OF *H. SPICIGERA*.

| Compound | KI | % Composition |
|---|---|---|
| α-Thujene | 924 | 0.68 |
| α- Pinene | 938 | 6.52 |
| Octen-3-ol | 979 | 4.91 |
| β - Pinene | 973 | 2.40 |
| Sabinene | 978 | 9.60 |
| Ocimene | 993 | 3.80 |
| 2- Carene | 998 | 8.78 |
| α- Phellandrene | 1003 | 0.87 |
| Limonene | 1028 | 2.36 |
| cis-β- Ocimene | 1030 | 0.94 |
| γ-terpinene | 1056 | 1.34 |
| α- Limonene diepoxide | 1128 | 2.71 |
| Mentha-1,8-dien-7-ol | 1282 | 3.96 |
| β -Caryophyllene | 1386 | 25.71 |
| α-Humulene | 1459 | 4.05 |
| γ- Elememine | 1465 | 1.74 |
| Germacrene D | 1486 | 1.02 |
| Caryophyllene oxide | 1586 | 11.56 |
| Trans-Z-α-bisaboline epoxide | 1820 | 1.52 |
| Hexadecanoic acid | 1968 | 3.64 |
| phytol | 2045 | 1.16 |
| **Total** | | **99.27** |

The composition of the essential oils extracted from aerial part of *H. spicigera* showed some differences with respect to previous studies reported in the literature. Many different chemical compositions for essential oil of *H. spicegera* have been reported. For instance, the yields of essential oils by the hydrodistillation of *H. spicegera* observed in our experiment was 0.26%) as compared to those obtained from Cameroon (0.12%), Italy (0.30%), Mali (0.1-0.3%), Togo (1.2%) and Benin (0.2%) (Tchoumbougnang *et al*., 2005; Conti *et al*., 2011; Sidibe *et al*., 2001; Koba *et al*., 2007; Bogninou-Agbidinoukoun *et al*., 2013). Several chemotypes have also been identified from different parts of the globe, for instance α- pinene/sabinene and β- pinene have been identified as major components of the oil from Pisa, Italy, β-caryophyllene/1, 8-cineole from Benin, and α- pinene, α-phelladrene and β- pinene as the major component the plant from Zaria (Conti *et al*., 2011; Bogninou-Agbidinoukoun *et al*., 2013; Ladan *et al*., 2011). These differences could be attributed to the geographical characteristics of the ecological zone, the times of harvest and the vegetative state of the plant species.

Larvicidal activity of *H. spicegera* essential oil at various concentrations against the filarial carrrier, *Cx. quinquefasciatus* and malarial carrier *An. gambiae* is given in **Table 2**. After exposure to different concentrations of essential oil of the plant for 24 h, the mortality rate of the larvae varies according to the concentrations of essential oils. At 150 µg/L, the crude essential oil of *H. spicigera* was more active than deltamethrin against *An. gambiae* larvae. This result in agreement with the report of Dorta *et al*., 1993 which depicted that the $LC_{50}$ of deltamethrin was greater than 5 µg/L for *An.stephensi*.

**TABLE 2**: PERCENT MORTALITY (MEAN ± SE; N=5) OF LARVAE OF *AN. GAMBIAE* AND *CX. QUINQUEFASCIATUS* EXPOSED TO VARYING CONCENTRATIONS OF *H. SPICIGERA* ESSENTIAL OILS.

| Species | Concentration (µg/mL) | % Mortality | Larvicidal activity (95% CL, µg/mL) | |
|---|---|---|---|---|
| | 25 | 28±0.84 | $LC_{50}$ | $LC_{90}$ |
| | 50 | 56±1.08 | | |
| *An. gambiae* | 100 | 75±2.34 | 44.42(43.21 - 46.86) | 143.67(136.11 - 149.82) |
| | 150 | 93±1.41 | | |
| | 200 | 98±0.89 | | |
| | 400 | 100±0.20 | | |
| Deltamethrin | 5 | 91±2.18 | - | - |
| *Cx. quinquefasciatus* | 25 | 17±2.11 | | |
| | 50 | 45±0.98 | 53.87(49.63-56.23) | 151.90 (147.56-153.78) |
| | 100 | 62±2.03 | | |
| | 150 | 89±0.24 | | |
| | 200 | 97±0.56 | | |
| | 400 | 99±0.21 | | |
| Deltamethrin | 5 | 100±0.43 | - | - |

LC50; LC90 = 50% and 90% lethal concentration expressed in µg/mL; 95% CL = 95% confident limit.

*An. gambiae* was more susceptible to the plant essential oils with $LC_{50}$ of 44.42 µg/mL. This result corroborates with the report published by Wangrawa *et al*., 2015 who evaluated the larvicidal activities of the young leaves of *H. spicigera* essential oil with the $LC_{50}$ of the plant essential oil 45.18. The oil of the plant also demonstrated a good activity against *Cx. quinquefasciatus* $LC_{50}$ and $LC_{90}$ of 53.87 and 151.90 respectively. This is the first report of the *H. spicigera* essential oil against the vector *Cx. quinquefasciatus.* This study showed that *H. spicigera* volatile oil could play a key role in the search of new plant-based larvicides to control *An. gambiae* and *Cx. quinquefasciatus* mosquitoes thereby helping to eliminate malaria and filariasis from West Africa.

## 4 CONCLUSION

Our study reflects the larvicidal potency of essential oil obtained from *Hyptis spicigera* against *Anopheles gambiae* and *Culex quinquefasciatus* larvae. This will go a long way in the development of new larvicides that will replace the toxic and non biodegradable synthetic ones to rid our communities of malarial and filarial infections.

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