Bima Journal of Science and Technology, Vol. 6 (2) Aug, 2022 ISSN: 2536-6041

# CHEMICAL CONSTITUENTS AND LARVICIDAL ACTIVITY OF VOLATILE OIL FROM *Solenostemon monostachyus* (P Beauv.) Briq. AGAINST *Anopheles gambiae*

ATIKO, R.*[1], KWAJI, A.[1] and MUSA, A. A.[1]

[1]Department of Chemistry, Faculty of Science, Gombe State University, Gombe. Nigeria.

*Corresponding Author E-mail: r_atiko@yahoo.co.uk

## ABSTRACT

Synthetic larvicides have been used to control malarial vector. However, increasing resistance of mosquito larvae to the synthetic larvicides poses great problem in the vector control. Natural control with plant essential oil may serve as an alternative. This study investigates the chemical constituents and larvicidal activity of volatile oil obtained from *Solenostemon monostachyus* against the larvae of *Anopheles gambiae*. The volatile oils were extracted using hydro-distillation in a Clevenger type apparatus and analyzed using GC-MS. The larvicidal assay was carried out against *Anopheles gambiae* larvae at concentration range of 12.5- 400 µg/mL. The analysis of the volatile oil showed that the major constituents of the oil were Caryophyllene oxide (21.6%), β-Caryophyllene (19.6%), β-Pinene (9.8%) and Germacrene D (7.3%). Results showed that larval mortality increases with increasing exposure period to the volatile oils from *S. monostachyus*. The larvicidal activity demonstrated that the mosquito larvae were susceptible to the volatile oil with $LC_{50}$ of 23.44 µg/mL. The results suggest that *S. monostachyus* essential oil has potentials for the control of *Anopheles gambiae.* Therefore the plant may serve as a potential source of raw material for a new and eco-friendly larvicide.

**Keywords**: *Anopheles gambiae*, larvae, mortality, *S. monostachyus,* Volatile oil

## INTRODUCTION

Malaria is a deadly parasitic disease caused by *Plasmodium* parasite, and is mainly spread through the bites of adult female *Anopheles* mosquitoes. It is one of the most significant public health problems in the world (WHO, 2021). In 2020, there were an estimated 241 million cases of malaria worldwide and sub-Saharan Africa alone was responsible for 95% of malaria cases and 96% of malaria deaths. Nigeria accounted for the highest proportion (31.9%) of deaths worldwide (World Malaria Report, 2021). Two strategies were used to reduce the global burden of malarial infection which include the use of preventive antimalarial drugs and effective vector control via mass insecticide treated nets (ITN) distribution campaign and pocket indoor residual sprays (Feng *et al*., 2022)

Effective malaria treatment and prevention of infection using antimalarial preventive drugs (primaquine and pyrimethamine) had been employed for decades for management of the disease in malarial endemic areas. However, the development of resistance to drugs by the malarial parasites poses one of the greatest threats to malaria control and results in increased malaria morbidity and mortality. Resistance to currently available antimalarial drugs such as chloroquine, sulfadoxine–pyrimethamine (Roux *et al*., 2021) and artemisinin (Asley *et al*., 2014) has been confirmed in two of human malaria parasite species *Plasmodium falciparum* and *P. vivax*. This drug resistance compelled scientists to opt for vector control methods.

Vector elimination strategy is a vital component of malaria control as it is highly effective in preventing infection and reducing disease transmission. Many

synthetic insecticides are cytotoxic to other non-target organisms including human beings. This has necessitated researchers to develop other methods of controlling malaria transmission (N'Guessan *et al*., 2006; Atiko *et al*., 2016). In general, control strategies against malaria that are geared towards eradicating the mosquito larvae seems to be the most effective alternative (N'Guuessan *et al*., 2007). This is because it is easier and more efficient to control the delicate larvae that are relatively immobile and more concentrated in their aquatic breeding sites than the adult mosquitoes that are mobile (Intirach *et al*., 2012). Some of the conventional larvicides in use worldwide includes synthetic organochlorines, organophosphates, carbamates, and pyrethroids.

Unfortunately, there has been increasing reports of resistance of larval populations of malarial vectors to one or more commonly used synthetic larvicides and its toxicity to other non-target organisms including human beings (Conti *et al*., 2012). One of the most promising ways of minimizing pesticides resistance and reducing its negative impact on the environment is by employing the use of biopesticides (Intirach *et al*., 2012). Phytochemicals are highly biodegradable, environmentally friendly, less toxic to non-target organisms and have lower side effects compared to synthetic pesticides. Essential oils from plant sources have been reported by many researchers as alternative to synthetic larvicides, insect repellent, ovipositor attractant and growth regulators (Khan *et al*., 2020).

*S. monostachyus* (P Beauv.) Briq. (Hausa name: "tumukun biri") is an aromatic plant which belongs to Lamiacae family. The Lamiaceae had been used traditionally to repel mosquitoes in many culture worldwide (Atiko *et al*., 2016; Conti *et al*., 2012; Asadollahi *et al*., 2019). Chukwura and Iheukwumere (2013) evaluated the larvicidal activity of *S. monostachyus* leaf extract and reported $LC_{50}$ for the acetone and water extracts to be 28 and 200 ppm respectively. There is dearth of information on the larvicidal activity of the volatile oil of plants. This study reports the chemical constituents and larvicidal activity of *S. monostachyus* aerial parts essential oil.

## MATERIALS AND METHODS

### Plant Collection and Extraction of Essential Oil

The aerial parts of *S. monostachyus* were collected in May, 2019 from Gombe State University Staff Quarters. The plant was identified and authenticated by Dr. Daniel Zigla of Biological Science Department, Gombe State University, Gombe. The plant sample was air-dried, weighed, pulverized and stored in an airtight container in a refrigerator at 4°C until required for use. The aerial part of the *S. monostachyus* (380 g) was subjected to hydrodistillation using a Clevenger-type apparatus for 3 hrs according to the British Pharmacopoeia (1980) specification. The average yields were taken over three experiments and expressed as percentage dry weight per volume. The resulting essential oils were dried over anhydrous sodium sulphate and stored in small amber-coloured vials at 4°C until needed for bioassay and GC-MS analysis.

### Gas Chromatography-Mass Spectroscopic Analysis

The volatile oil components was 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.

**Larvicidal Assay**

Larvicidal activity was carried out according to the WHO larval susceptibility test methods (WHO, 2005). 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, 25 and 12.5 µg/mL. The oils were dissolved in 1 mL of ethanol, then diluted to 100 mL in deionized water to obtain the desired concentrations. The tests were performed in 500 mL plastic containers each containing 20 of the third and fourth instar larvae. The larvae were fed with cabin biscuits and baker's yeast at ratio 3:1. These experiments were replicated three times. Control test was carried out using 1 mL of ethanol in 99 mL of water. The test containers were kept for 24 hrs with a photoperiod of a 12:12 hrs light/dark cycle. Mortality was assessed by direct observation of larval movements and probing the cervical region with a needle after 24 hrs.

**Statistical Analysis**

The percentage mortality of the larvae was ascertained using Probit analysis at $p \leq 0.05$. The lethal concentrations ($LC_{50}$ and $LC_{90}$), was calculated using Log-Probit at 95% confidence limits.

## RESULTS AND DISCUSSION

The essential oil extracted from S. *monostachyus* aerial part afforded a yellow oil with percentage yield of 0.28% per sample dry weight. This result corroborates the work reported by Essien *et al*. (2017) (0.2%) from the same plant in Uyo, Nigeria but showed much differences with one reported by Mve-Mba *et al*. (1994) (0.02%) from Cameroon. Preliminary GC analyses of the oil samples revealed that the composition of the essential oil was a complex mixture. Table. 1 shows the composition of the essential oil, their percentage composition and retention indices listed in order of elution. A total of 29 compounds representing 90.80% of the oil have been identified.

The main constituents of the essential oil extracted from *S. monostachyus* were Caryophyllene oxide (21.6%), β-Caryophyllene (19.6%), β-Pinene (9.8%), Germacrene D (7.3%) and α-Farnesene (4.1%). This result agree with previous studies reported by Essien *et al.* (2017) and contradicts the result reported by Mve-Mba *et al*. (1994). Mve-Mba *et al*. reported that the major components of the essential oils of the plant leaf were α-Pinene (13%), Oct-1-en-3-ol (12.6%), β- Caryophyllene (6.9%), Octan-3-ol (6.8%), α-Farnesene (6.2%) and Germacrene D (5.2%). In the case of Essien *et al.,* the major components of the plant leaf oils observed were β- Caryophyllene (27.43%), α-Caryophyllene (12.90%), Caryophyllene oxide (24.83%), β-Pinene

(6.96%) and Germacrene D (2.2%). Although there are variations in percentage composition of the oil constituents, β-Caryophyllene, β-Pinene, Germacrene and α-caryophyllene are common to all. The difference in percentage composition could be due to difference in geographical location, climate condition, time of harvest and method of extraction (Sanli and Karadogan, 2016). Furthermore, the aerial part of the plant was used in this study. This may also account for the observed variations in their chemical composition and percentage yield.

**Table 1:** Chemical composition of *S. monostachyus* essential oil

| S/N | Compounds | *RI Calculated* | RI Literature | % Composition |
|---|---|---|---|---|
| 1 | α-Thujene | 925 | 925 | 0.1 |
| 2 | α- Pinene | 940 | 933 | 2.3 |
| 3 | β-Pinene | 973 | 973 | 9.8 |
| 4 | Myrcene | 980 | 983 | 0.6 |
| 5 | Octen-1-ene-3-ol | 965 | 968 | 2.9 |
| 6 | δ-3-carene | 1014 | 1013 | 1.1 |
| 7 | *p*-Cymene | 1026 | 1027 | 0.9 |
| 8 | Limonene | 1023 | 1024 | 1.2 |
| 9 | (E) 2-octen-1-ol | 1067 | 1067 | 1.8 |
| 10 | Linalool | 1087 | 1103 | 2.3 |
| 11 | Thujol | 1096 | 1097 | 0.1 |
| 12 | α-Terpineol | 1182 | 1177 | 0.1 |
| 12 | Thymol | 1278 | 1276 | 2.1 |
| 14 | Limonene oxide | 1135 | 1134 | 0.1 |
| 15 | Myrcenyl acetate | 1306 | 1327 | 1.3 |
| 16 | α-Copaene | 1374 | 1378 | 1.2 |
| 17 | β-Caryophyllene | 1420 | 1421 | 19.6 |
| 18 | Aromadendrene | 1440 | 1443 | 0.2 |
| 19 | α-Himachalene | 1447 | 1445 | 0.1 |
| 20 | α-Humulene | 1453 | 1455 | 3.3 |
| 21 | Germacrene D | 1476 | 1480 | 7.3 |
| 22 | α-Selinene | 1493 | 1495 | 0.7 |
| 23 | α- Farnesene | 1501 | 1500 | 4.1 |
| 24 | δ-Cadinene | 1522 | 1518 | 1.2 |
| 25 | E-Nerolidol | 1554 | 1550 | 1.4 |
| 26 | Caryophyllene oxide | 1584 | 1586 | 21.6 |
| 27 | Globulol | 1590 | 1592 | 1.5 |
| 28 | δ-Cadinol | 1642 | 1645 | 2.2 |
| 29 | Phytol | 1949 | 2022 | 0.5 |
| | **TOTAL** | | | **90.8** |

Larvicidal activity of *S. monostachyus* essential oil at various concentrations against the larvae of *An. gambiae* is given in Table 2. After 24 hrs exposure, the mortality rate of the larvae varied according to the concentrations of essential oils. At 25 µg/mL, the *S. monostachyus* volatile oil mortality rate was 57%. The $LC_{50}$ and $LC_{90}$ were found to be 23.44 and 186.21 µg/mL respectively. The high activity of the oil could be due to the presence of an essential oil constituent or their synergistic effect. Caryophyllene oxide and phytol have been reported to have high activity against *An. sinensis* with $LC_{50}$ of 39.09 and 16.03 µg/mL respectively (Luo *et al*., 2022).

Furthermore, Hung *et al*. (2019) reported mosquito larvicidal activity of β-caryophyllene and α-pinene with $LC_{50}$ of 41.66 and 32.09 µg/mL respectively.

**Table 2:** Percent mortality of larvae of *An. gambiae* exposed to varying concentrations of *S. monostachyus* Volatile oil.

| Species | Conc. (µg/mL) | % mortality | Larvicidal activity (95% CL) | |
|---|---|---|---|---|
| | | | $LC_{50}$ (µg/mL) | $LC_{90}$(µg/mL) |
| Essential oil | 12.50 | 38 ± 0.73 | | |
| | 25.00 | 57±1.12 | | |
| | 50.00 | 64±0.98 | 23.44 | 186.21 |
| | 100.00 | 77±2.01 | (22.42-24.46) | (185.87-186.55) |
| | 200.00 | 86±1.88 | | |
| | 400.00 | 98±2.33 | | |
| Ethanol | 0.00 | 0±0.10 | | |

This result is similar to the report published by Chukwura, and Iheukwumere (2013) who evaluated the larvicidal activities of the leaf extract of *S. monostachyus* with $LC_{50}$ of 28 ppm.

## CONCLUSION

The study showed that *S. monostachyus* essential oil exhibited strong activity against *Anopheles gambiae* larvae. The plant may serve as a new source of raw material for the control of malarial parasite by breaking the vector life cycle. The *S. monostachyus* volatile oil could serve as a new plant-based larvicides to control *An. gambiae* mosquitoes. The availability and accessibility of the plant will go a long way in assisting the indigenous populace to meet their healthcare needs at little or no cost.

## REFERENCES


Asadollahi, A., Khoobdel, M., Zahraei-Ramazani, A. Z, Sahar Azarmi, S. and Mosawi, S. H. (2019). Effectiveness of plant-based repellents against different Anopheles species: a systematic review, *Malaria Journal*, 18(1):436-455

Ashley, E.A., Dhorda, M., Fairhurst, R.M., Amaratunga, C., Lim, P., Suon, S., Sreng, S. *et al*. (2014). Spread of Artemisinin Resistance in *Plasmodium falciparum* Malaria. *The New England Journal of Medicine*, 371:411-23.

Atiko, R., Kwaji, A., Yoriyo, K. P. and Onocha, P. A. (2016). Chemical composition and larvicidal activity of *Hyptis spicigera* volatile oils against mosquito larvae: *Anopheles gambiae* and *Culex quinquefasciatus* Say.*International Journal of Scientific & Engineering Research*, 7(7):888-891.

British Pharmacopoeia II (1980). Her Majesty Stationary Office London, UK. pp 109-123

Chukwura, E. I. and Iheukwumere, I. (2013). Larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves on *Anopheles gambiae*. *Journal of Scientific & Industrial Research,* 72: 577-580.

Conti, B., Benelli, G.,Flamini, G., Cioni, P. L., Profeti, R., Ceccarini, L., Macchia, M and Canale, A. (2012). Larvicidal and repellent activity of *Hyptis suaveolens* (Lamiaceae) essential oil against the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae) *Parasitology Resource,* 110(5):2013–2021.

Essien, E. E., Thomas, P. S. and Choudhary, M. I. (2017).Aroma profile of essential oils of *Solenostemon monostachyus* P.

Beauv from Nigeria. *UK Journal of Pharmaceutical and Biosciences*, 5(2): 34-37.

Feng, X., Feng, J., Zhang, L., Tu, H. and Xia, Z. (2022). Vector control in China, from malaria endemic to elimination and challenges ahead. *Infectious Disease of Poverty* 11(54) Doi: 10.1186/s40249-022-00971-3).

Hung, N. H., Satyal, P., Hieu, H. V., Chuong, N. T. H., Dai, D. N., Huong, L. T., Tai, T. A. and Setzer, W. N. (2019). Mosquito larvicidal activity of the essential oils of *Erechtites* species growing wild in Vietnam. *Insects*, 10(2): 47-50

Intirach, J., Junkum,A., Tuetun,B, Choochote, W., Chaithong, U., Jitpakdi, A. *et al*. (2012) Chemical constituents and combined larvicidal effects of selected essential oils against *Anopheles cracens* (Diptera: Culicidae). *Psyche*, 2012:1-11.

Khan, S., Uddin, M. N., Rizwan, M. Khan, W., Farooq, M., Shah, A. S. *et al*. (2020). Mechanism of insecticide resistance in insects/pests. *Polish Journal of Environmental Studies,* 29(3): 2023-2030.

Luo, D-Y., Yan, Z-T., Che, L-R., Zhu, J. J. and Chen, B. (2022). Repellency and insecticidal activity of seven Mugwort (*Artemisia argyi*) essential oils against the malaria vector *Anopheles sinensis*. *Scientific Reports*, 12:5337-5346.

Mvé-Mba, C. E., Menut, C., Lamaty, G., Zollo, P. H. A., Tchoumbougnang, F. and Bessière, J. M. (1994). Aromatic plants of Tropical Central Africa. Part XIX. Volatile components from Leaves of two Lamiaceae from Cameroon: *Leucas deflexa* Hook and *Solenostemon monostachyus*, (P. Beauv.) Briq. *Flavour and Fragrance Journal*, 9(6):315-317.

N'Guessan, R., Rowland, M., Moumouni, T-L., Kesse, N. B. and Carnevale, P. (2006). Evaluation of synthetic repellents on mosquito nets in experimental huts against insecticide-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 100(12):1091– 1097.

N'Guessan, R., Corbel, V., Akogbeto, M. and Rowland, M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance, *Emerging Infectious Diseases*. 13(2):199-206.

Roux, A. T., Maharaj, L., Oyegoke, O., Akoniyon, O. P., Adeleke, M. A., Maharaj, R. and Okpeku, M. (2021). Chloroquine and Sulfadoxine–Pyrimethamine Resistance in Sub-Saharan Africa—A Review. *Frontiers in Genetics*, DOI: 10.3389/668574.

Şanli, A. and Karadoğan, T. (2016). Geographical impact on essential oil compositionof endemic *Kundmannia anatolica* Hub.-Mor. (Apiaceae). *African Journal of Traditional, Complementary and Alternative Medicine*, 14(1):131-137.

WHO (2005).Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization. https://apps.who.int/iris/handle/10665/69101.

WHO: World malaria report 2021.Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO. 6 December 2021 Global report.