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# LARVICIDAL AND SYNERGISTIC ACTIVITIES OF *LEMON EUCALYPTUS* STEM EXTRACTS AGAINST *AEDES AEGYPTI*

**By**

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**Abstract**

*Aedes aegypti* is the main vector mosquito for transmitting Zika virus, which causes congenital microcephaly and other debilitating conditions. This study assessed the larvicidal and synergistic activities of *Lemon eucalyptus* stem extracts against the 4$^{th}$ instar larvae of *Ae. aegypti*. Stem samples were collected, dried, powdered, and extracted using methanol, hexane, ethyl acetate, and acetone. About 64.3% of the extracts caused moderate larvicidal activity after 24 hours. Hexane and acetone extracts showed the highest activity at 1000ppm. The extracts contained saponins, tannins, steroids, flavonoids, alkaloids and resins. Synergistic studies revealed moderate larvicidal activity for all combinations of extracts at different concentrations. *Lemon eucalyptus* stem extracts have promising larvicidal and synergistic activities against *Ae. aegypti* as a safe effective way to stop Zika virus transmission.

**Keywords**: Nigeria, Phytochemical, *Aedes aegypti*, Zika virus, larvicidal, synergistic.

## Introduction

*Aedes aegypti* mosquitoes are the main vectors of four viral hemorrhagic fevers including Zika posing a substantial threat to global public health (El-Bahnasawy *et al*, 2014). *Ae. aegypti* originated in Africa, where ancestral populations still exist, with larvae developing in water-filled tree holes (Jeffery and Powell, 2018). Engorged female laid an average of 100-200eggs per batch up to 5 batches with a total of 500-1000 eggs (Zettel and Kaufman, 2019). *Aedes aegypti* also laid desiccation-resistant eggs, which greatly increased the amount of time a virus could persist (Sota and Mogi, 1992). *Ae. aegypti* is the main Zika virus vector (Sajadi and Paluzzi, 2021). The arthropod-borne viruses, or arboviruses as *Aedes* species, can become routinely transmitted in any of the geographic region through three mechanisms, including 1) regular viral reintroduction and circulation across geographies, 2) establishment of mosquito and human or nonhuman reservoir transmission cycles, or 3) virus maintenance in the vector population (Weaver and Reisen, 2010). Moreover, occupational or nosocomial or hospital acquired transmission by an instrument and/or needle-stick injury can occur (Saleh *et al*, 2016), or even via blood transfusion from an infected donor (Morsy *et al*, 2022).

Consequently, the maintenance in the *Aedes* vector population sustains viruses when there are few human or reservoir hosts, such as between outbreaks (Lequime *et al*, 2016).

Many people infected with Zika virus may be asymptomatic or only have mild symptoms for several days up to a week (Morsy, 2016). Zika infection during pregnancy can cause serious birth defects and other pregnancy problems, but rarely, Zika infection can cause Guillain-Barré syndrome (GBS) or severe disease affecting the brain (CDC, 2025).

WHO (2022) reported that there was no specific treatment yet available for Zika virus infection or disease. They added that patients with symptoms such as rash, fever or joint pain should get plenty of rest, drink fluids, and treat symptoms with antipyretics and/or analgesics, and that non-steroidal anti-inflammatory drugs must be avoided until dengue virus infections are ruled out because of bleeding risk. If symptoms worsen, patients should seek medical care and advice. Pregnant women living in areas with Zika transmission or who develop symptoms of Zika virus infection should seek medical attention for laboratory testing, information, counseling and other clinical care. No vaccine is yet available to prevent or to treat Zika virus, and thus effective control remains the cruci- al strategy (Singh *et al*, 2018).

Chemical pesticides or plant-extracted ones crucial protected human public health and economy (James *et al*, 2016). However, exposure to over dose of chemical pesticides caused bad side effects on man, animal and the environment in general (Polyxeni *et al*, 2016). The prolonged use of synthetic pesticides led to the development of resistance, which indicated the need for eco-friendly alternatives to manage vector populations and protect the environment (Anupam *et al*, 2012). Exploring natural mosquito larvicides as a substitute for synthetic chemicals offers a promising solution from the natural sources help to stop harmful environmental and health impacts associated with traditional mosquito controls (Amin *et al*, 2019). Plant extracts are rich in bioactive compounds and biodegradable properties, paving the way to develop natural, eco-friendly mosquito control solutions (Ajaegbu *et al*, 2022). *Lemon eucalyptus* stem extract offers a natural solution for mosquito larval control (Sengul and Canpolat, 2022). This fact of lemon extracts were documented by many authors (Al Dakhil and Morsy, 1999; Ajaegbu *et al*, 2014; Abdel-Hady *et al*, 2014; Lame *et al*, 2015; Danga *et al*, 2020; Ibe *et al*, 2020; Onah *et al*, 2022; Nwaso *et al*, 2024).

The present study aimed to evaluate the larvicidal activities of *Lemon eucalyptus* stem extract against *Aedes aegypti* larvae.

## Materials and methods

Plant collection: Fresh *Lemon eucalyptus* stems were collected from their natural habitat at Lakokari Farm, Billiri, Gombe State, Nigeria in June 2021. Collected stems were cleaned, identified, processed, and stored according to protocols (Ezeh *et al*, 2020).

Plant extraction and fractionation: The extraction procedure was done following the method of Ezeagha *et al.* (2021). In the Federal College of Dental Technology and Therapy laboratory, Trans-Ekulu, Enugu State, powdered plant material of 100g was cold maceration extraction for 48 hours. Extracted solvents were methanol, ethyl acetate, hexane, and acetone (800ml each). The mixture was agitated three times daily. Suspension was filtered through Whatman® filter paper No. 1, and crude extracts were concentrated to dryness by a rotary vacuum evaporator at 40±5°C (RE300, ROTAFLO, England).

Phytochemical analysis identified bioactive larval toxicity compounds; alkaloids, flavonoids, saponins, tannins, steroids, and resins (Ujam *et al*, 2024).

Test organisms: Larvae were obtained from the WHO/National Arbovirus and Vector Research Centre, Enugu and colonized in the Federal College of Dental Technology, and Therapy laboratory. Larvae were reared in tap water and fed a 3:1 mixture of fish feed and chicken grower. Culture water was changed every 48 hours until 4$^{th}$ larval instar emerged and tested (Ajaegbu *et al*, 2016).

Larvicidal bioassay: The bioassay was carried out after the WHO standard techniques. Stock solutions were prepared using 1gm of each extract and fraction mixed with 2ml of Tween 80, and diluted with tap water to 100 ml. Serial dilutions of each stock solution gave concentrations of 125 to 1000ppm. A negative control was 1ml Tween 80 in 99ml tap water for each test. For each bioassay, 25 starved 4$^{th}$ larval instars were released into 250ml beakers with100ml of each tested extract. Larval mortality percentage was recorded after 24 hours, if they didn't respond to gentle probing with a thin needle. All bioassays were carried out at 26±2°C & relative humidity (8±2%) with four experimental replicates side by side with their corresponding controls.

*L. eucalyptus* stem extracts were combined in various ratios to assess their joint larvicidal activity. Each combination was mixed in a 50:50ratio. Synergistic factor (SF) determined the interaction between extracts. An SF value > 1 was considered synergism, but < 1 was antagonism.

Statistical analysis: Data were collected, reviewed, tabulated and statistically analyzed. The mortality rates were corrected by using the Abbott's formula (1925). Mortality percent was subjected to one-way analysis of variance (ANOVA) by using the Statistical Package for Social Science (SPSS 17.0). Data means were analyzed by the Student-New-man-Keels (SNK) test and considered significant at P< 0.05. Both $LC_{50}$ & $LC_{90}$ were determined 24 hours post-exposure and parameters included slope, Chi-square, and 95% upper & lower confidence limits.

## Results

*L. eucalyptus* extractions were methanol (3.099g), ethyl acetate (0.881g), hexane (0.440g) and acetone (1.423g), with 6.198%, 1.762%, 0.88% &2.846% respectively. Stem analysis showed saponin, tannins, alkaloids, steroids, flavonoids, steroids, and resins.

Larval mortality, ELS was12% at 125, 250 & 500ppm, and at 1000ppm, showed 20%. HLS were 20% mortality at 250 & 500 ppm, and 32% at 1000ppm, but zero at 125ppm. ALS didn't show mortality at 125 & 250ppm and 12% & 20% mortality at 500 & 1000 ppm respectively. MLS didn't show mortality at 125, 250& 500ppm, but 12% at concentration 1000 ppm.

Synergistic assay: ALS: HLS gave high mortality between 125-1000ppm with $LC_{50}$ of 1353.710, and ELS showed least mortality with $LC_{50}$ of 66419.621 at 500 & 1000 ppm respectively. Mortality 20% was at 500 & 1000ppm respectively. None was at 125 & 250 ppm of ALS: HLS; ALS: MLS; ALS: ELS, MLS: HLS & MLS: ELS formulations Details were given in tables (1, 2, 3 & 4).

Table 1: Yield of plant extract

| Code | Weight (g) | Solvent (ml) | Yield (g) | Yield (%) |
|---|---|---|---|---|
| IALS | 40 | 400 | 1.423 | 2.846 |
| IMLS | 40 | 400 | 3.099 | 6.198 |
| IELS | 40 | 400 | 0.881 | 1.762 |
| IHLS | 40 | 400 | 0.440 | 0.88 |

Table 2: Phytochemical screening

| Extracts | Tannins | Alkaloids | Flavonoids | Resins | Steroids | Saponins |
|---|---|---|---|---|---|---|
| ALS | + | + | + | - | + | + |
| MLS | + | + | + | + | + | + |
| ELS | + | - | + | + | + | - |
| HLS | - | - | - | - | - | + |

Table 3: *Aedes aegypti* $4^{th}$ stage larvicidal assay

| Extracts | Conc.(µg/ml) | Mortality% | F-value | $LC_{50}$ (UCL-LCL)ppm | $LC_{90}$ (UCL-LCL)ppm | M ± SE | $X^2$ |
|---|---|---|---|---|---|---|---|
| ALS | 125<br>250<br>500<br>1000 | 0.00±0.00<br>0.00±0.00<br>12±1.15<br>20±0.57 | 230.40<br>(***) | 3433.782<br>(***) | 21045.413<br>(***) | 1.628±0.73 | 5.961 |
| MLS | 125<br>250<br>500<br>1000 | 0.00±0.00<br>0.00±0.00<br>0.00±0.00<br>12±1.15 | 108.00<br>(***) | 2175.560<br>(***) | 4936.213<br>(***) | 3.602±2.476 | 0.296 |
| MLS | 125<br>250<br>500<br>1000 | 0.00±0.00<br>20±1.15<br>20±1.52<br>32±0.57 | 176.00<br>(***) | 1910.940<br>(939.374-70110.272) | 16053.311<br>(3500.509-<br>10142313.0) | 1.386±0.502 | 3.473 |
| AECS | 125<br>250<br>500<br>1000 | 12±0.57<br>12±1.15<br>12±1.00<br>20±0.57 | 21.333<br>(***) | 410285.046<br>(***) | 1670943.537<br>(***) | 0.355±0.464 | 0.336 |

Means within by same letter insignificantly=0.05, *p<0.05; $LC_{50}$ & $LC_{90}$ lethal concentration killed 50% & 90% larvae respectively; ppm: parts per million; LCL: lower confidence limit; UCL: upper confidence limit; (**): value too large; $x^2$: chi square

Table 4: Synergistic activities of the extracts

| Extracts | Conc. (µg/ml) | Mortality% | F-value | $LC_{50}$ ppm | $LC_{90}$ ppm | M ± SE | $X^2$ |
|---|---|---|---|---|---|---|---|
| ALS : HLS | 125 | 0.00±0.00$^{a}$ | 248.727 (***) | 1353.710 (959.514-4589.971) | 3474.328 (1801.211-58418.52) | 3.131±0.101 | 0.657 |
| | 250 | 0.00±0.00$^{a}$ | | | | | |
| | 500 | 12±2.64$^{b}$ | | | | | |
| | 1000 | 32±2.00$^{c}$ | | | | | |
| ALS : MLS | 125 | 0.00±0.00$^{a}$ | 230.400 (***) | 2049.229 (1151.657-99386.48) | 7108.313 (***) | 2.372±0.935 | 1.372 |
| | 250 | 0.00±0.00$^{a}$ | | | | | |
| | 500 | 12±1.00$^{b}$ | | | | | |
| | 1000 | 20±2.00$^{c}$ | | | | | |
| ALS : ELS | 125 | 0.00±0.00$^{a}$ | 200.000 (***) | 2034.117 (***) | 8425.013 (***) | 2.076±0.767 | 3.943 |
| | 250 | 0.00±0.00$^{a}$ | | | | | |
| | 500 | 20±2.00$^{b}$ | | | | | |
| | 1000 | 20±2.00$^{b}$ | | | | | |
| MLS : HLS | 125 | 0.00±0.00$^{a}$ | 576.00 (***) | 2049.229 (1151.657-99386.48) | 7108.313 (***) | 2.372±0.935 | 1.372 |
| | 250 | 0.00±0.00$^{a}$ | | | | | |
| | 500 | 12±1.00$^{b}$ | | | | | |
| | 1000 | 20±2.00$^{c}$ | | | | | |
| HLS : ELS | 125 | 12±2.00$^{a}$ | 19.692 (***) | 66419.621 (***) | 52536848.49 (***) | 3.375±0.682 | 0.241 |
| | 250 | 12±2.00$^{a}$ | | | | | |
| | 500 | 20±2.00$^{b}$ | | | | | |
| | 1000 | 20±1.00$^{b}$ | | | | | |
| MLS : ELS | 125 | 0.00±0.00$^{a}$ | 576.00 (***) | 2049.229 (1151.657-99386.48) | 7108.313 (***) | 2.372±0.935 | 1.372 |
| | 250 | 0.00±0.00$^{a}$ | | | | | |
| | 500 | 20±1.00$^{b}$ | | | | | |
| | 1000 | 20±1.00$^{b}$ | | | | | |

## Discussion

In the present study, the extracts showed maximum value was with methanol extract (6.198%), followed by acetone (2.846%) but ethyl acetate and hexane extractions gave 1.762% and 0.88% respectively, and steroids were in hexane extracts. This agreed with hexane's ability to extract steroids from the *Phyllanthus amarus* (Zubair *et al*, 2016).

The presence of tannins, alkaloids, flavonoids, steroids, and saponins was demonstrated by acetone extract. The conducted experiments on phytochemical screening of *Datura metel* leaves using acetone support the findings of the current study. The outcome showed that tannins, alkaloids, flavonoids, steroids, and saponin were present (Dhawan and Gupta, 2017). The presence of tannins, alkaloids, flavonoids, steroids, and saponin was demonstrated using methanol extracts. The outcome supports earlier scientific studies on the ability of methanol to extract tannins, alkaloids, flavonoids, and saponin from lemon grass oil (Ekpenyong *et al*, 2014).

The present results indicate that the optimal method for extracting a variety of active phytochemicals from plants is to use methanol as an extraction solvent (Dhawan and Gupta, 2017). Flavonoids, saponins, and tannins were discovered by ethyl acetate examination for tannins and other phytochemicals *Samanaea saman* pods (Ukoha *et al*, 2011).

Inhibiting proteins and nucleic acids is a known property of flavonoids, which also has pharmacological, antibacterial, and insecticidal effects. As treatments, they are useful in medical, and as insecticides, in agriculture. Besides, it serves as a signal molecule, protects plants from biotic and abiotic stress, was responsible for the colors and scents in flowers, defends against plaques in vegetables, and attracts pollinators to fruits (Panche *et al*, 2016). However, some flavonoids prevent an enzyme from forming a crucial hormone in *Ae. aegypti*, powerful to kill *Ae. aegypti* larvae and tamper with the physiology and development of insects (Inaba *et al*, 2022). Numerous plants contain tannins, also referred to as tannic acid, particularly the bark of pine trees. They perform a variety of tasks, including acting as antimicrobial agents by preventing the development of several bacteria, viruses, fungi, and yeasts (Chung *et al*, 1998). By creating complexes with proteins, carbohydrates, cellulose, and minerals, these substances precipitate proteins (Pasiakos *et al*, 2015). Tannins are harmful to insects due to their binding to their salivary proteins and digestive enzymes (trypsin and chymotrypsin), which results in the inactivation of the proteins, they are regarded undesirable in humans since they cause indigestion. Besides, herbivorous insects inject large amounts of tannins to die young (Abubakar *et al*, 2020).

Saponins are plant-derived heterosides structurally contain one or more sugar molecules, which might be a pesticide (El-Hela *et al*, 2013). Due to their capacity to form when dissolved in water, they are utilized industrially in the production of soap. By reacting with cholesterol to create insoluble compounds, they repel insects. They primarily impede insects' ability to feed by preventing food intake, slowing the metabolism of dry food (which results in weight loss in larva), disrupting the length of developmental phases, and preventing insects from molting (Chaieb, 2010). In *Spodoptera littoralis*, several saponins that were introduced during the larval stage prolonged both the larval and pupal stages, increased mortality, slowed growth, and decreased fertility. These are brought on by saponin's capacity to exacerbate digestive issues by prolonging food retention in gut (Adel *et al*, 2000). Plants produce phytoecdysteroids (PEs), a subclass of steroid hormones that act as secondary metabolites and provide defense against phytophagous insects. They resemble the hormones produced during insect molting structurally. They have control over the insect molting and metamorphosis processes. Insects that consume plants containing these compounds molt, suffer metallic degradation, and ultimately perish (Das *et al*, 2021). The largest class of secondary metabolites, alkaloids, is found in many different plant families. Alkaloids are toxic substances that serve as a plant's defensive mechanism against predators. To protect the plant, alkaloid buildup is sparked by unfavorable environmental conditions, rapid aggressor perception, and effective and direct signal transduction. The mechanism for toxicity varies and may involve suppression of DNA synthesis and repair mechanisms, central nervous system effects, or enzymatic changes that disrupt physiological processes (Matsuura and Fett-Neto, 2015). The nature of resins is heterogeneous. They are made up of a variety of organic substances, including waxes, resin acids, fatty acids, and esters of these acids, sterols, and alcohols. These shield the plant from infections and insects. Most resins come from dipterocarpaceae, legume groups, and panacea plants (Pallardy, 2008). The majority of resins are antibacterial and aid in wounds' healing in plants and animals (Chinnasamy *et al*, 2021).

Different mixtures *L. encalyptus* stem extracts (ALS:HLS), (ALS:MLS), (ALS:ELS), (MLS:HLS), (HLS:ELS) and (MLS:ELS) at varied concentrations of 125, 250, 500, and 1000 ppm were used in synergistic research of larvae mortality of *Ae. aegypti*. The ALS: HLS combination shown the most toxicity and had the lowest $LC_{50}$ value (1353.710), whereas the HLS: ELS combination demonstrated the lowest toxicity and had the highest $LC_{50}$ value (66419.621 ppm). This agreed with (Ajaegbu *et al*, 2022b).

A study was conducted to evaluate the larvicidal activity of *Citrus limon* and *Bacillus thuringiensis* against the dengue vector, Aedes aegypti. The methanol leaf extract of *Citrus limon* and *Bacillus thuringiensis* were tested at concentrations of 100, 200, 300, 400, and 500mg/L against third-instar larvae of *Aedes aegypti*. The results showed that *C. limon* exhibited $LC_{50}$ values of 285100 and 219500 ppm after 24 and 48 hours, respectively. *Bacillus thuringiensis* displayed $LC_{50}$ values of 1900 and 1400ppm after 24 &48 hours, respectively. The combination of *Citrus limon* and *Bacillus thuringiensis* demonstrated a synergistic larvicidal effect, with $LC_{50}$ values of 158500 and 109900 ppm after 24 and 48 hours, respectively (Ajaegbu *et al*, 2022).

The present study showed a notable difference in larvicidal activity among the various solvents employed; acetone and methanol extracts exhibited the highest larvicidal activity, ethyl acetate extract showed moderate activity and hexane extract displayed the lowest activity. These results went with the conclusion that the plant bioactive compounds present in *L. eucalyptus* stem extracts have potential as natural larvicides against *Ae. aegypti* (Corzo-Gómez *et al,* 2024).

## Conclusions

*Lemon eucalyptus* stem contains flavonoids, resins, steroids, tannins, alkaloids, and saponins. The study also demonstrated that the Zika virus vector mosquito *Aedes aegypti's* $4^{th}$ instar larvae were susceptible to the larvicidal effects of *L. eucalyptus* stem extracts.

The outcome results may be helpful in managing the *Ae. aegypti* field population. Applying these extracts to *Ae. aegypti* breeding habitats could have a positive impact on the effectiveness of Zika virus control programs by smothering the oral symptoms or outcomes of the virus.

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