



Insecticidal Activity of *Azadirachta indica*, *Lantana camara* and *Citrus aurantifolia* Extracts Against The Malaria Vector Mosquito (*Anopheles Gambiae s l.*) in Bauchi State, North-Eastern Nigeria

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ABSTRACT

Malaria is a leading cause of morbidity and mortality worldwide, with millions of deaths recorded annually. In Africa, it poses a significant epidemiological burden, hindering economic development. In Nigeria, malaria is the most prevalent parasitic disease, affecting 90% of the population (over 140 million people). It accounts for 63% of reported cases in healthcare facilities, 30% of childhood deaths, 25% of deaths in children under one year, and 11% of maternal deaths. The economic impact is substantial, with estimated annual losses of 132 billion Naira due to treatment costs, prevention, and lost productivity. This study investigates the potential of selected plant extracts to control mosquito larvae in breeding sites. The results show that different concentrations of the extracts have varying effects on the larvae, indicating their potential as larvicides. The *Azadirachta indica* extract demonstrated the highest mortality rate, with an Lc50 of 0.98mg/L. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, and terpenoids in the extracts. The study concludes that these extracts are highly effective against mosquito larvae and warrants further exploration as botanical pesticides to harness their potential.

Keywords: Malaria, plant extract, Adulticidal

INTRODUCTION

Malaria control programs in Nigeria and beyond face a growing challenge: increasing insecticide resistance in major mosquito vectors (Shililu, J. I, 1998). Synthetic insecticides, while effective, pose environmental and health risks, and their overuse has contributed to resistance development (Charlwood *et al*, 1995; Omar *et al*, 2022). The need for alternative insecticides is pressing, given the limited chemical arsenal available and the lack of new insecticides anticipated until 2020 (Olayemi *et al*, 2009). Moreover, the absence of available alternative insecticides for vector control has exacerbated the issue (Onyido *et al*, 2009). Botanical insecticides offer a promising solution, as they comprise complex blends of chemical

compounds that act on both behavioral and physiological processes, making resistance less likely (Sibina *et al*, 1984; Lehane M. J., 2005).

The aim of this research is to explore botanical insecticides as a sustainable alternative for mosquito control. We seek to identify plant-derived compounds that can effectively control mosquito populations without the environmental and health risks associated with synthetic insecticides, Charlwood *et al*, 1995. This study investigates the potential of selected plant extracts as larvicides against mosquito larvae, with the goal of harnessing their potential as botanical pesticides for malaria control.



MATERIALS AND METHODS

Study Area

Bauchi State, situated in northern Nigeria, covers an area of 49,119 km², representing 5.3% of the country's total landmass (NPC, 2006). It lies between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east, bordered by seven states. The state spans two distinctive vegetation zones: the Sudan savannah and the Sahel savannah.

Vegetation and Climate

The Sudan savannah covers the southern part of the state, with richer vegetation towards the south, especially along water sources (Ndams, I. S., 2004). The Sahel savannah, also known as semi-desert vegetation, dominates the middle and northern parts of the state, characterized by isolated stands of thorny shrubs. Climatic factors condition the vegetation types, with rainfall ranging from 1300 mm per annum in the south to 700 mm per annum in the extreme north.

Hydrology

Bauchi state is watered by several rivers, including the Gongola and Jama'are rivers, and has a substantial part of the Hadeja-Jama'are River basin (Ndams, I. S., 2004). Various fadama (floodplain) areas and dams, such as the Gubi and Tilde-Fulani dams, support agricultural activities.

Research Design

This study adopted a Completely Randomized block design (CRD) and involved both field and laboratory assessments. The research included:

- i. Collection and preparation of plant extracts (*Azadirachta indica*, *Lantana camara*, and *Citrus aurantifolia*).

- ii. Identification of mosquito breeding sites and collection of larvae
- iii. Rearing of collected *Anopheles* mosquito larvae to adulthood
- iv. Morphological identification of adult mosquitoes
- v. Susceptibility tests to 4% Dieldrin, 0.05% Lambdacyhalothrin, 0.1% Bendiocarb, and 5% Malathion
- vi. Adulticidal effects assessment of plant extracts against *Anopheles gambiae* s.l.

Sampling Procedure

A multi-stage sampling procedure was used, involving purposive sampling and simple random sampling techniques to select four Local Government Areas in the Bauchi-North Senatorial District.

Phytochemical Screening

Preliminary screening of the major phytochemical groups was conducted to determine the active toxic compounds in the plant extracts, using methods by Ciulci (1994), Adeleke (2001), and Abdulkadir *et al.* (2001).

Mosquito Larval Collection and Rearing

Immature stages of *Anopheles* mosquitoes were collected from naturally occurring breeding sites and reared in the laboratory, following methods by Shilulu (2001).

Adulticidal Bioassay

The adulticidal activity of crude extracts was assessed using the WHO protocol (1998) with modification.

RESULTS

Results obtained from this study shows that some chemical constituents are presence while some are absence in the selected three plant extracts, this can be seen from the table 1 below.

**Table 1:** Qualitative analysis of the phytochemicals content of plant extracts.

Phytochemicals	<i>Azadirachta indica</i>	<i>Lantana camara</i>	<i>Citrus aurantifolia</i>
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phlobatannins	-	-	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	-
Reducing Sugar	-	-	-
Limonoids	+	+	+
Sterols	+	+	-
Gallic acid	+	-	-
Catechins	+	-	-
Triterpenoids	-	+	-
Vitamin C	-	-	+
Phenolic compounds	-	-	+
Essential oils	-	-	+

+ = Presence of constituent - = Absence of constituent

The results also presents the percentage composition of various phytochemicals in three different plant species: *Azadirachta indica*, *Lantana camara*, and *Citrus aurantifolia*. Phytochemicals, which include alkaloids, saponins, flavonoids, tannins, limonoids, and terpenoids, are compounds produced by plants that have potential health benefits and are often studied for their medicinal properties.

Azadirachta indica, commonly known as neem, shows the highest concentration of flavonoids at 6.6771%, followed by alkaloids at 5.1660%, saponins at 3.9670%, terpenoids at 3.0460%, tannins at 3.4440%, and limonoids at 2.8890%. The high levels of flavonoids and alkaloids suggest significant antioxidant and antimicrobial properties, making neem a valuable plant in traditional medicine and pharmacology.

Lantana camara, on the other hand, has a different phytochemical profile. Its highest concentration is of flavonoids at 3.4563%,

closely followed by terpenoids at 3.1212%, alkaloids at 3.4470%, limonoids at 2.9100%, tannins at 2.3232%, and saponins at 2.1462%. The presence of a relatively balanced distribution of these phytochemicals indicates its potential use in a variety of therapeutic applications, although the lower percentages compared to *Azadirachta indica* suggest it may be less potent in some aspects.

Citrus aurantifolia, known as lime, contains the highest number of flavonoids at 4.5121%, followed by alkaloids at 4.111%, saponins at 3.1620%, terpenoids at 3.0054%, tannins at 2.9099%, and limonoids at 1.9987%. The notable concentration of flavonoids and alkaloids points to its potential antioxidant and anti-inflammatory benefits. The lower percentage of limonoids in *Citrus aurantifolia* compared to the other plants suggests a different balance of phytochemical benefits.

In conclusion, each plant exhibits a unique profile of phytochemical concentrations, with *Azadirachta indica* showing particularly high

levels of flavonoids and alkaloids, suggesting strong medicinal properties. *Lantana camara* presents a more balanced but lower concentration of these compounds, and *Citrus aurantifolia* stands out for its high flavonoid

and alkaloid content, indicating potential health benefits, particularly in antioxidant and anti-inflammatory applications. This result can be seen the table 2 and figure 1 below.

Table 2: Quantitative phytochemicals analysis of the three plant extracts.

Phytochemicals	<i>Azadirachta indica</i> (%)	<i>Lantana camara</i> (%)	<i>Citrus aurantifolia</i> (%)
Alkaloids	5.1660	3.4470	4.111
Saponins	3.9670	2.1462	3.1620
Flavonoids	6.6771	3.4563	4.5121
Tannins	3.4440	2.3232	2.9099
Limonoids	2.8890	2.9100	1.9987
Terpenoids	3.0460	3.1212	3.0054

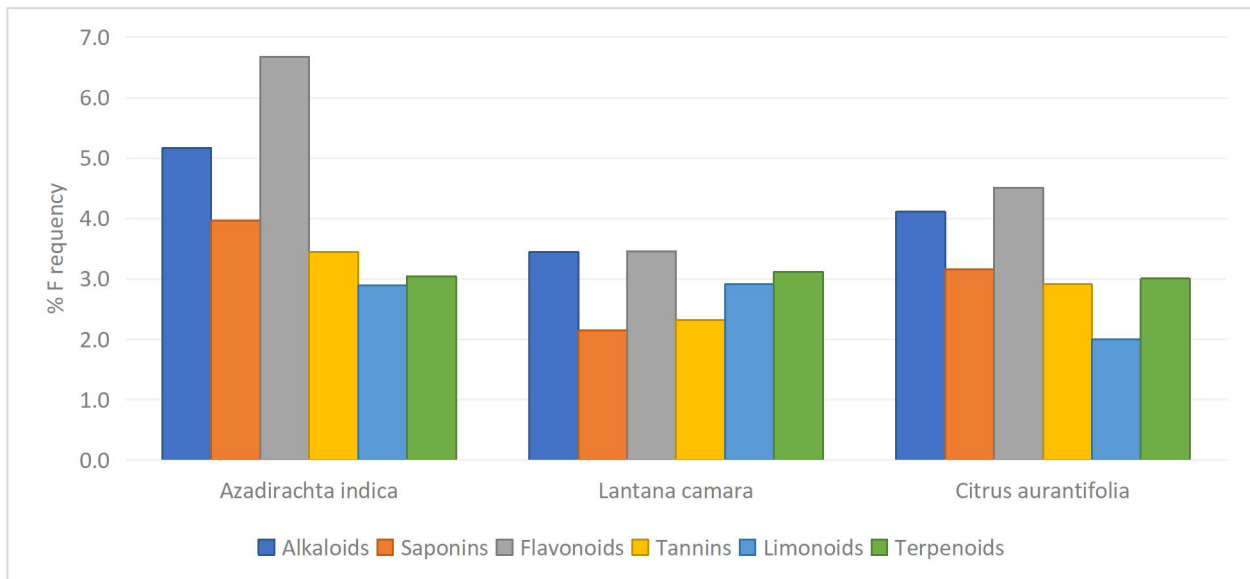


Figure 1: Quantitative phytochemicals analysis of the three plant extracts

The tables 3, 4 and 5 and figure 2 below presents data on the adult mortality rates at different concentrations of *Azadirachta indica*, *Lantana camara*, and *Citrus aurantifolia* over a period of 192 hours. The data include both the percentage mortality and the corrected mortality values, with the lethal concentration 50 (LC_{50}) values specified where applicable.

Table 3 illustrates the adult mortality rates induced by varying concentrations of *Azadirachta indica*. At a concentration of 5 mg/L, the percentage mortality is recorded at

53.43±2.85%, with a corrected mortality of 51.4±0.67%. Increasing the concentration to 10 mg/L results in a mortality rate of 60±3.512% and a corrected mortality of 58.3±1.58%, with an LC_{50} value determined to be 8 mg/L. At the highest concentration of 15 mg/L, the mortality rate is 71.6±3.16%, and the corrected mortality is 50.7±2.23%. The control group exhibits a mortality rate of 6.3±0.00%, with a corrected mortality of 51.4±3.37%. Each value represents the mean of four observations over the 192-hour period.

Table 3: Adult mortality at different concentration of *Azadirachta indica* from 24h to 192h

Conc. (mg/L)	% Mortality	Corrected mortality	Lc ₅₀
5	53.43±2.85	51.4± 0.67	
10	60± 3.512	58.3± 1.58	8 mg/L
15	71.6±3.16	50.7± 2.23	
Control	6.3±0.00	51.4± 3.37	

Each value (X ± S.E.) represents mean of four values for the period of 192 h.

In Table 4, the mortality rates for *Lantana camara* are shown. At 5 mg/L, the percentage mortality is 23.3±1.92%, with a corrected mortality of 43.6±0.29%. At 10 mg/L, the mortality rate rises to 55±2.31%, and the corrected mortality is 50±1.98%, with an LC₅₀ value of 5 mg/L. A concentration of 15 mg/L

results in a mortality rate of 82.6±2.33% and a corrected mortality of 77.6±2.46%. The control group shows a mortality rate of 0.51% and a corrected mortality of 41.3±1.69%. These values are also the mean of four observations over 192 hours.

Table 4: Adult mortality at different concentration of *Lantana camara* from 24 to 192h.

Conc. (mg/L)	% Mortality	Corrected mortality	Lc ₅₀
5	23.3±1.92	43.6± 0.29	
10	55±2.31	50 ±1.98	5mg/L
15	82.6±2.33	77.6±2.46	
Control	0.51	41.3± 1.69	

Each value (X ± S.E.) represents mean of four values

Table 5 below presents the mortality rates for *Citrus aurantifolia*. At the lowest concentration of 5 mg/L, the percentage mortality is 71.5±2.77%, which matches the corrected mortality of 71.5±2.75%. At 10 mg/L, the mortality rate increases to 85.5±1.23%, with a corrected mortality of 64.9±3.13% and an LC₅₀ value of 1.98 mg/L.

The highest concentration of 15 mg/L results in a percentage mortality of 81±1.66% and a corrected mortality of 97.5±0.88%. The control group's mortality rate is 2.8±1.25%, with a corrected mortality of 81±0.15%. These results are averaged from four values over the 192-hour period.

Table 5: Adult mortality at different concentration of *Citrus aurantifolia* from 24 to 192h.

Conc. (mg/L)	% Mortality	Corrected mortality	Lc ₅₀
5	71.5±2.77	71.5±2.75	
10	85.5±1.23	64.9±3.13	1.98 mg/L
15	81±1.66	97.5± 0.88	
Control	2.8±1.25	81±0.15	

Each value (X ± S.E.) represents mean of four values.

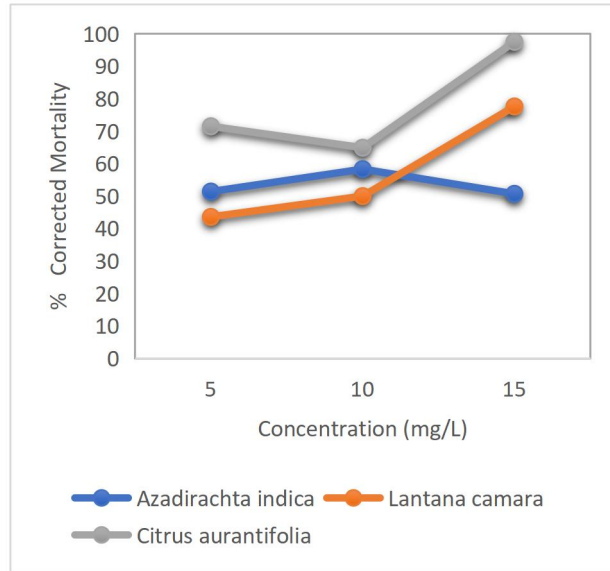


Figure 2: Adult mortality for different Concentrations for three different.

DISCUSSION

The exploration of plant extracts with insecticidal properties against malaria vector mosquitoes is crucial in mitigating the challenges associated with malaria transmission in endemic areas. This study investigated the insecticidal potential of *A. indica*, *L. camara*, and *C. aurantifolia* against malaria vector mosquitoes, and the results are promising. The findings corroborate previous research that has widely reported the chemotherapeutic properties of these extracts, not only as malaria herbs but also for other medicinal uses (Umar *et al.*, 2007).

The phytochemical screening revealed that the extracts of these plants are rich in alkaloids, flavonoids, tannins, and saponins, which are likely responsible for their insecticidal properties. These phytochemicals have been previously reported to possess larvicidal and insecticidal abilities (Molta, 2004). Specifically, neem crude extract or oil has been shown to inhibit metamorphosis, preventing pupation or adult emergence of mosquitoes (Smits *et al.*, 1995). This study's

findings align with those of Lehane M. J. (2005), who reported that neem is highly toxic to mosquitoes and delays pupation.

Furthermore, exposure of *A. gambiae* larvae to sub-lethal doses of neem and catnip leaves extract in the laboratory prolonged larvae development and pupation (Murray *et al.*, 2012). This is consistent with the present study's findings. The probit analysis of percentage mortality of the three extracts at Lc50 shows a moderate to average level of concentration, which agrees with the findings of Oduoala *et al.* (2013).

The insecticidal properties of these plant extracts offer a potential alternative to synthetic insecticides, which are often associated with environmental and health concerns. The use of botanical insecticides could provide a sustainable and eco-friendly approach to managing malaria vector mosquitoes. However, further research is needed to fully explore the potential of these extracts and to determine their efficacy in field settings. Additionally, investigations into the optimal concentrations and application methods for these extracts are necessary to ensure their effective use in malaria control programs.

CONCLUSION

This study's findings demonstrate the differential efficacy of *Citrus aurantifolia*, *Azadirachta indica*, and *Lantana camara* plant extracts in inducing mortality among adult malaria vector mosquitoes, with varying concentrations and exposure times yielding distinct outcomes. Notably, *Citrus aurantifolia* exhibited the highest mortality rates at relatively low concentrations, indicating a superior potency compared to *Azadirachta indica* and *Lantana camara*. This suggests that *Citrus aurantifolia* may be a more effective and efficient botanical pesticide for malaria vector control. The three plant



extracts have shown remarkable potential as environmentally friendly and sustainable alternatives to synthetic pesticides, offering a promising solution for malaria control and reduction of disease burden in Africa. The widespread availability of these plants in Africa provides an affordable and readily accessible source of raw materials for the production of botanical pesticides. Leveraging this potential could significantly contribute to the reduction of malaria transmission, alleviation of disease burden, and improvement of public health outcomes in Africa, aligning with global health initiatives aimed at controlling and eliminating malaria. Furthermore, the use of botanical pesticides could also help mitigate the environmental and health risks associated with synthetic pesticides, promoting a more sustainable approach to malaria control.

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