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Laboratory-based evaluation of larvicidal potential of six plant extracts against larval stages of Anophelines and culicine mosquitoes in Abeokuta, Nigeria

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ABSTRACT

Background: Mosquito-borne diseases remain a major global public health challenge. In Nigeria, *Anopheles, Aedes*, and *Culex* mosquitoes transmit several parasitic and arboviral diseases. Vector control strategies such as long-lasting insecticidal nets (LLINs) and larval source management (LSM) using larvicides are widely employed. However, growing concerns about the environmental and health impacts of synthetic larvicides have spurred interest in eco-friendly, plant-based alternatives. This study evaluated the larvicidal efficacy of crude aqueous extracts from six plant species against mosquito larvae.

Methods: Crude aqueous extracts of six plants (*Polyscias balfouriana*, *Bougainvillea x buttiana*, *Acalypha wilkesiana* (tricolor), *Acalypha wilkesiana* (marginata), *Codiaeum variegatum*, and *Duranta erecta*) were tested against third and fourth-instar larvae of *Anopheles, Aedes*, and *Culex* mosquitoes collected from Odeda Local Government Area, Ogun State, Nigeria. Two concentrations (0.25 g/ml and 0.50 g/ml) were prepared and tested in four replicates. Larval mortality was recorded after 24 hours according to the WHO larvicide testing guidelines. Data were analyzed using one-way analysis of variance (ANOVA) with significance set at $p < 0.05$.

Results: Larval mortality increased with extract concentration across all treatments. The aqueous extract of *P. balfouriana* produced the highest larvicidal activity against *Anopheles* larvae, achieving 100% mortality at 0.50 g/ml, while *D. erecta* produced 98% mortality at the same concentration ($p < 0.05$). At the lower concentration (0.25 g/ml), *P. balfouriana* also demonstrated strong activity with 61% mortality after 24 hours. Among Culicine mosquitoes (*Aedes* and *Culex*), *D. erecta* showed the highest efficacy, producing 84% mortality at 0.50 g/ml and 56% mortality at 0.25 g/ml ($p < 0.05$). In contrast, the extracts of *B. x buttiana*, *A. wilkesiana* (tricolor), A. *wilkesiana* (marginata), and *C. variegatum* showed comparatively lower larvicidal activity, generally producing mortality values below 60%. No mortality was observed in the control group.

Conclusion: The findings indicate that aqueous extracts of *Polyscias balfouriana* and *Duranta erecta* possess strong larvicidal activity against mosquito larvae. These plants are widely available in Nigeria and may serve as environmentally friendly alternatives to synthetic larvicides for small-scale larval source management programs.

Keywords: Larvicidal, Plant extracts, Mosquito larvae, Nigeria

1.0 INTRODUCTION

Malaria is a major public health issue, affecting a vast majority of people living in tropical countries [1]. The malaria parasite is transmitted through the bite of an infective female *Anopheles* mosquito. In addition to malaria, other mosquito-borne arboviral diseases, such as yellow fever and dengue fever, transmitted by *Aedes* mosquitoes, are also prevalent in the country. Arboviral diseases are of great public health concern and are currently receiving increased global attention [2], particularly in Africa. This is due to the widespread distribution of *Aedes* mosquitoes and the significant burden and risks associated with these diseases [3]. *Culex* mosquitoes are also known to transmit deadly arboviral diseases, including West Nile virus and Japanese encephalitis [4].

These examples highlight the critical role of mosquitoes in transmitting various deadly diseases. In Nigeria, malaria is primarily transmitted by members of the *Anopheles gambiae* complex, while yellow fever is mainly spread by *Aedes aegypti* and *Aedes albopictus* [5]. Currently, the main mosquito control strategies in Nigeria are the use of insecticide-treated bed nets (ITNs) and, to a lesser extent, indoor residual spraying (IRS). However, resistance to most insecticides used in malaria vector control has emerged, necessitating alternative control methods such as larviciding [6].

Organophosphate larvicides (e.g., temephos and pirimiphos-methyl), which target the nervous system of immature larvae, have been widely used with high success rates [7]. Alternatively, naturally occurring microbes, such as *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*), kill larvae by ingestion of toxins [7, 8]. However, environmental concerns and resistance to chemical larvicides have driven the search for natural, plant-derived insecticides [9, 10]. Botanical larvicides have gained attention for their broad-spectrum larvicidal properties and environmental friendliness. For instance, extracts from *Azadirachta indica* (neem) have been extensively studied for their insecticidal, pesticidal, and larvicidal potential [11, 12].

Studies have demonstrated the efficacy of plant extracts against mosquito larvae. For example, *Codiaeum variegatum* at 6.25% achieved 100% mortality in 30 hours against *Culex* larvae [13]; *Syzygium aromaticum* at 0.25 mg/ml caused 93.33% mortality in 60 minutes against *Culex quinquefasciatus* [14]; and *Rosmarinus officinalis* showed significant mortality at low concentrations against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* larvae [10].

In Nigeria, efforts to combat malaria have been ongoing since the 1950s, and yellow fever control has been a focus for over five decades. Despite continuous distribution of long-lasting insecticidal nets (LLINs) across the country, studies report limitations in their usage [15]. Vector control, through scaling up LLINs and larval source management (LSM) with larviciding and small-scale environmental management, remains a critical strategy [8, 16]. However, increasing insecticide resistance in *Anopheles, Aedes,* and *Culex* mosquitoes highlights the need for alternative approaches.

This study explores the potential of using simple, readily available, and environmentally friendly plant-based larvicides to control immature mosquito stages, particularly in rural communities where vector-borne diseases are most prevalent. The plants were selected based on their local availability and prior consultations with community members about traditional non-chemical mosquito-control practices. Here, we present preliminary findings on the efficacy of crude extracts from six plants (*Polyseias balfouriana, Bougainvillea x buttiana, Acalypha wilkesiana* [tricolor], *Acalypha wilkesiana* [marginata], *Codiaeum variegatum*, and *Duranta erecta*) against the third and fourth instar larvae of *Anopheles, Culex,* and *Aedes* mosquitoes.

2.0 METHODOLOGY

2.1 Mosquito larval collection sites

The mosquito larvae were collected directly from their breeding sites in the Odeda Local Government Area, Abeokuta, Ogun State, Nigeria (Figure 1). The collections were conducted in two communities: Kofesu (Latitude

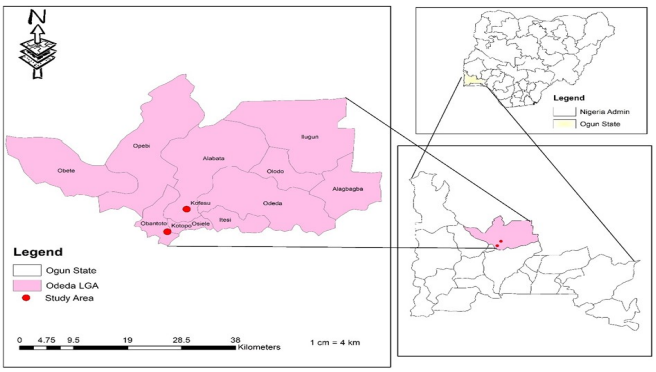

Figure 1. Map of the study area in Odeda Local Goverment Area of Ogun State Nigeria

7.227858042, Longitude 3.454305683) and Kotopo (Latitude 7.18310769, Longitude 3.423761384) during the rainy season in June 2022. Third and fourth instar mosquito larvae were specifically targeted, while first and second instar larvae that were collected were reared in the laboratory until they developed into later instars.

2.2 Mosquito larval collection

Mosquito larvae were collected using the WHO standard dipper and transferred into a transparent, ten-liter plastic bucket with a handle and a net cover to allow air circulation. The bucket was used to handle and transport the larvae. The scooped larvae were filtered using clean cheesecloth prepared for this purpose and then transferred to the bucket. Approximately 1–1.5 liters of water, along with plant debris from the natural breeding sites, was added to the bucket to provide food for the larvae until they reached the insectary facility [17]. *Anopheles* species were obtained from ditches and blocked drainages, while *Aedes* and *Culex* species were collected from used tires and discarded containers. *Anopheles, Culex*, and *Aedes* larvae were identified based on their characteristic resting position and respiratory structures in water. *Anopheles* larvae lack a respiratory siphon and lie parallel to the water surface, whereas *Culex* larvae possess a long respiratory siphon and hang obliquely from the water surface. A short, stout respiratory siphon distinguishes *Aedes* larvae and hangs at an angle from the water surface.

2.3 Rearing of mosquitoes

The mosquito rearing laboratory was maintained at 27 ± 2 °C and 75 ± 10% relative humidity. Field-collected mosquito larvae were sorted into Anopheles and Culicine groups and transferred into a white enamel plastic tray. They were fed with a diet of baker's yeast. After 5 minutes, the tray was gently swirled to distribute the powder evenly and prevent suffocation from undiluted or accumulated powder [18]. Larvae were fed twice daily, and trays were regularly checked for leftover food. In cases where remnants were observed, no additional food was added. The third and fourth larval stages of the mosquitoes were used for the bio-efficacy studies.

2.4 Plant collection, Identification, and Preparation of crude extracts

A total of six plants from four families (Table 1) were collected from a botanical garden in Odeda LGA, Ogun State. The plants were identified and deposited in the herbarium of the Department of Pure and Applied Botany, with assigned voucher numbers. The aqueous extracts of the plants were prepared by grinding 1 g and 2 g of the leaves in 4 ml of water to produce diagnostic concentrations of 0.25 g/ml and 0.50 g/ml of the crude aqueous leaf extracts for each plant, which were later used for the efficacy study.

Table 1. Description of the plant used for the experiment

S/N	Name	Family	Voucher No.
	Acalphya wilkesiana Mull.		
1	Arg. Var. *tricolor* *Acalphya wilkesiana* Mull.	Euphorbiaceae	FHA-4267
2	Arg. Var. *maginata* *Cardiaeum variegatum* (L.)	Euphorbiaceae	FHA-4268
3	A. Juss.	Euphorbiaceae	FHA-4269
4	*Duranta erecta* Linn.	Verbenaceae	FHA-4271
5	*Polyscias balfouriana marginata* (Andre) L. H. Boiley *Bougainvillea xbuttiana*	Araliaceae	FHA-4273
6	Holttum & Standi	Nyctaginaceae	FHA-4274

2.5 Efficacy of the plant extracts against the mosquitoes

To expose the larvae to the different plant extracts, late third- to early fourth-instar larvae of *Anopheles* and Culicine mosquitoes were sorted into petri dishes containing a mixture of 12.5 ml distilled water and 1 ml of each extract concentration, using pipettes. Batches of 25 larvae were exposed per petri dish. The test was conducted in four replicates, with an equal number of negative controls set up simultaneously using deionized water. During the efficacy tests of the plant extracts, larval mortality was recorded after 1 hour and 24 hours [17]. Larvae that sank to the bottom of the water or floated on the surface with swollen and blackened bodies were considered dead. According to WHO guidelines for laboratory and field testing of larvicides, the test should be rejected if the control mortality exceeds 20% or if pupation exceeds 10%.

2.6 Data analysis

The data were recorded using the WHO larvicide efficacy evaluation results recording form [19]. All replicates were pooled and entered into an Excel spreadsheet for analysis using STATA version 14.0. If the control mortality was between 5 and 20%, the mortalities of treated groups were corrected using Abbott's formula. Differences in mortality among the extracts were analyzed using one-way analysis of variance (ANOVA).

3.0 RESULTS

3.1 Collection of *Anopheles, Aedes*, and *Culex* mosquitoes

Mosquito larvae and pupae were abundant at the survey sites. Over 2,000 Anopheline and Culicine mosquitoes (*Aedes* and *Culex*) were collected from the study locations. Larvae and pupae of Anopheles species were found in potholes, clogged drainages, and dishes. In contrast, *Culex* and *Aedes* were found in used tires, discarded plastic and metal containers, and domestic water reservoirs.

3.2 Efficacy of the plant extracts against the mosquitoes

3.2.1 *Anopheles* species

Except for two of the plant extracts (*D. erecta* and P. *balfouriana*), which showed some level of mortality (6% and 9% at 0.25 g/ml, and 18% and 20% at 0.50 g/ml, respectively) after 1 hour of exposure, none of the other plant extracts killed the *Anopheles* mosquito larvae at 1 hour of exposure (Figure 2a). However, mortality increased significantly ($p<0.05$) after 24 hours of exposure, with mortality ranging from 10% at 0.25 g/ml and 15% at 0.50 g/ml (*A. wilkesiana* tricolor) to 61% at 0.25 g/ml and 100% at 0.50 g/ml (*P. balfouriana*). The result was significantly different ($p<0.05$) from that of the control group, which showed no mortality (Figure 2b). Two of the plant extracts, *P. balfouriana* and *D. erecta*, killed up to 100% and 98% of the larvae at 24 hours, respectively. Additionally, mortality increased with concentration

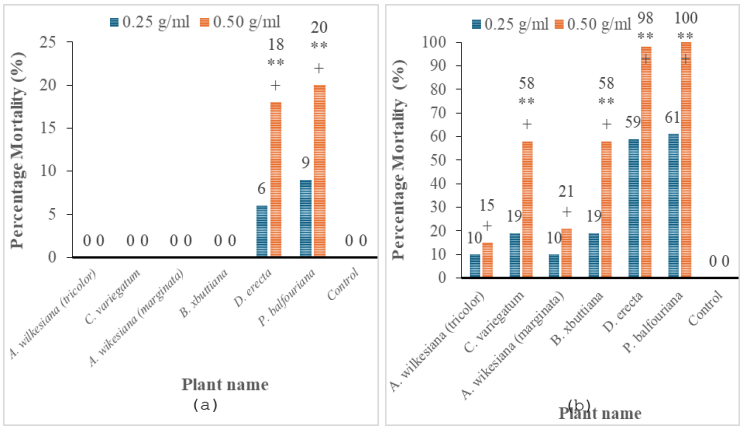

Figure 2. Percentage mortality of Anopheles mosquito larvae in aqueous extract of six plants after 1 and 24 hours of exposure time

** *Connotes significant difference in mortality ($p<0.05$) between concentrations of extracts of the same plant, +connotes significant difference in mortality ($p<0.05$) across all plant extracts, including control.*

across all the plant extracts.

3.2.2 Culicines

Only the *D. erecta* crude extract showed some mortality (6% at 0.50mg/ml) at 1h (Figure 3a). However, morality increased at 24h, ranging from 9% to 8% for *C. variegatum* and from 56% to 84% for *D. erecta* at 0.25g/ml and 0.50g/ml, respectively (Figure 3b). Furthermore, a reduction in efficacy of these plant extracts was observed against Culicines compared with *Anopheles*. The result was significantly different ($p<0.05$) compared with the control group (with no mortality (Figure 3b). Though mortality increased with concentration across all the plant extracts (except *C. variegatum*), only *D. erecta* extract produced up to >70% (up to 84%) mortality against

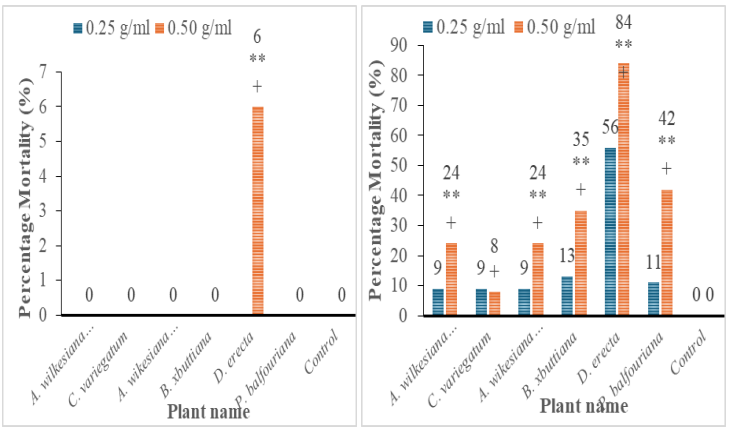

Figure 3. Percentage mortality of six plant extracts on *culicines* after 1 hour and 24 hours of exposure

** *Connotes significant difference in mortality ($p<0.05$) between concentrations of extracts of the same plant, +connotes significant difference in mortality ($p<0.05$) across all plant extracts, including control.*

the Culicines mosquitoes (Figure 3 b)

4.0 DISCUSSION

The In this study, larvicide bioassays with crude extracts of *P. balfouriana* and *D. erecta* at 0.50 g/ml demonstrated remarkable larvicidal properties against *Anopheles* mosquitoes collected from two locations in Odeda Local Government Area. The crude extracts of *P. balfouriana* and *D. erecta* were efficient in killing 100% and 98% of exposed *Anopheles* larvae at a concentration of 0.50 g/ml. Mozaffari *et al.* [20] also studied the larvicidal effect of essential oil extract from the root of *D. erecta* against *Anopheles stephensi*. Their study showed that applying the essential oil extract at a concentration of 0.00025 g/ml (lower than our minimum concentration of 0.25 g/ml) killed 90% of *An. stephensi* at 24 hours, indicating that this plant has good larvicidal potential. However, as in our study, mortality increased with concentration in their work [20]. The differences observed in our results may be due to the plant part and extraction method used [21].

The larvicidal properties of *D. erecta* have been attributed to certain phytochemicals such as cedren-9-one, alpha

-cadinol, eugenol, safranal, 3-hexenyl benzoate, heneicosane, linalool, tricosane, and alpha-muurolene [20, 22], and the extractable amount is dependent on the type of extraction method utilized [21]. It is essential to clearly identify the potential effects of the extraction method as an important factor when assessing plant extracts for larvicidal properties. Studies have shown that the extraction of active biochemicals from plants depends on the polarity of the solvent used [23, 24]. It has been reported that solvents with lower polarity, such as hexane, petroleum ether, and essential oils, often yield better results than more polar solvents like water, which was used in our study [21]. This suggests that the solvent may have significantly influenced the efficacy of the plant extract used in this study.

The results also indicated that the top-performing plant extracts (*P. balfouriana* and *D. erecta* at 0.50 g/ml) were more potent against *Anopheles* mosquitoes compared to Culicines. Apart from the extraction method, another factor that may significantly affect the efficacy of plant extracts as larvicides is the mosquito species. Sukumar *et al.* [25] and Ghosh *et al.* [21] emphasized that variations in the effectiveness of phytochemical compounds may be directly related to species responses and developmental stages to the specific extract.

One major limitation of this study is that we did not vary the solvents or plant parts. This will be considered in future studies. Although the other plant extracts (*Bougainvillea xbuttiana*, *Acalypha wilkesiana* (tricolor), *Acalypha wilkesiana* (marginata), and *Cardiaeum variegatum*) showed some level of larvicidal properties, the mortality rates produced were significantly lower against both Anopheline and Culicine mosquitoes compared to the top-performing extracts. It is, however, possible that their performance might be enhanced by using extraction methods other than water.

The present study revealed that aqueous extracts of *P. balfouriana* and *D. erecta* are effective larvicides against *Anopheles* mosquito larvae collected from two areas of Odeda LGA in Ogun State, Nigeria. Furthermore, only *D. erecta* showed significant larvicidal properties (84% mortality) when applied to Culicine (*Aedes* and *Culex*) mosquito larvae. These plants are readily available in the country. Locals could use them as alternatives to chemical larvicides for small-scale larval source management (LSM), where breeding sites are few, identifiable, and manageable. Further studies could also involve isolating bioactive compounds from these plants, with a view to developing and synthesizing compounds for LSM.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

OAI conceived and designed the study, contributed to data analysis tools, and manuscript writing. **OCP** contributed to data collection, data analysis tools and, manuscript writing. **ASB** contributed to data analysis tools, analysis of data and, manuscript writing. **IOO, JKF, OMM, NF** contributed to data collection and manuscript writing. All authors approved the final copy of the manuscript.

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