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ORIGINAL RESEARCH ARTICLE

Evaluation of *Alchornea cordifolia* Extracts for Larvicidal Activity against Anopheles Mosquitoes: Implications for Malaria Control

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ABSTRACT

Alchornea cordifolia is a botanical specimen renowned for its medicinal benefits. The phytochemicals present in this plant boast antioxidant, anti-inflammatory, antimicrobial, and analgesic properties, making it a versatile and effective remedy. This plant has been utilized for several decades in treating various ailments like; malaria, diarrhoea, and respiratory infections. To evaluate its efficacy in eradicating mosquito larvae, extractions from both the plant's bark and leaves were obtained using various methods, including crude methods, hot water, and ethanol. The plant was macerated in alcohol and hot water, while the bioassay was carried out using a static non-renewal test. The results of the bioassay indicated that the LC50 values for the various extracts of the plant. The ethanol extract was the most active against the mosquito larvae, with an LC50 value of 8.37 ppm. It was followed by the crude stem-bark extract, which had an LC50 value of 13.18 ppm, and the hot water extract, which had an LC50 value of 110.00 ppm. The plant's diverse range of phytochemicals may have played a role in its potency in eliminating mosquito larvae. This activity of the plant strongly suggests that *Alchornea cordifolia* has insecticidal properties as a larvicide against malaria.

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INTRODUCTION

Alchornea cordifolia is a predominant African shrub in the Euphorbiaceae family that is found around the coastal parts of West Africa. It was described in the literature as a straggling, upright-climbing shrub leaf having stationary glands near its petiole (Nyananyo, 2006). *Alchornea cordifolia* is a plant known for centuries for its remarkable medicinal properties. It is particularly noted for its high levels of antioxidants (Kumar *et al.,* 2013; Qadir *et al.,* 2012). Oxidative radical is a contributing factor to several health conditions, such as Alzheimer's disease, Parkinson's disease, and cancer. In addition to its antioxidant properties, *Alchornea cordifolia* has anti-inflammatory, antimicrobial, and analgesic properties (Okokon *et al.,* 2013). These properties have made it a popular natural remedy for various health conditions. For instance, it has been traditionally employed to combat illnesses like malaria, diarrhoea, and respiratory infections (Djimeli *et al.,* 2017). It is also believed to be effective in treating skin conditions such as eczema and psoriasis (Hu *et al.,* 2020). The leaves and bark of the *Alchornea cordifolia* plant are commonly used to prepare herbal remedies, which can be taken orally or applied topically (DeFilipps & Krupnick, 2018).

A parasitic disease transmitted by infected mosquitoes causes malaria. Over 3 billion people are affected globally (Ohimain *et al.,* 2014; Mace & Arguin, 2017), especially in sub-Saharan Africa. Symptoms include fever, chills, headaches, muscle pain, and fatigue. Malaria can lead to anaemia, respiratory distress, and organ failure in a hundred countries on an annual basis (Okumu *et al.,* 2007). The various species of mosquito that host the plasmodium parasite range from 30 - 40 species in number, according to Ghosh *et al.* (2007). However, several authors have identified *Anopheles gambiae* and *Anopheles arabiensis* as the primary malaria transmitters in tropical countries (Angaye *et al.,* 2014a; Hamza *et al.,* 2014). In many countries where malaria is prevalent, there have been reports of resistance to drugs and the high cost of treatment. As a result, there is a growing interest in developing herbal remedies that can effectively treat malaria with minimal or no side effects (Ankrah *et al.,* 2023; Kojom Foko et al., 2023).

The most common method to combat insect-borne diseases involves administering insecticides or medications to those infected. However, innovative approaches such as manipulating the mosquito life cycle to hinder their reproduction and impede maturation through larvicidal measures are gaining momentum.

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These techniques have been thoroughly investigated and developed by experienced experts. For instance, Angaye's (2015) and Angaye et al.'s (2017a; 2017b) studies provide compelling evidence of the effectiveness of these methods. The Silver nanoparticles of *Alchornea cordifolia* have been synthesized and characterized for their antiplasmodial, hemocompatibility, and larvicidal potential against malaria (Kojom Foko *et al*., 2023).

The current method of mitigating malaria involves administering insecticides or medication to individuals already afflicted by the disease. However, this approach has proven challenging, as it requires identifying all infected individuals and providing them with timely treatment. In many countries where malaria is prevalent, there have been reports of resistance to drugs and the high cost of treatment. As a result, there is a growing interest in developing herbal remedies that can effectively treat malaria with minimal or no side effects (Ankrah *et al*., 2023). To overcome these challenges, new approaches are being explored, such as using larvicides to prevent mosquito maturation and manipulating their reproductive cycle (Okumu *et al.,* 2007; Dibua *et al.,* 2013). Larvicides are chemicals used to kill mosquito larvae before they mature into adults. This method has shown promising results, as it can significantly reduce the number of adult mosquitoes and, consequently, the incidence of malaria (Angaye *et al.,* 2017a). Comparatively, Kojom Foko *et al.* (2023) used fresh leaves of A. *cordifolia* to create an extract and silver nitrate to synthesize AC-AgNPs. Their results showed significant antiplasmodial and larvicidal activity against *Plasmodium falciparum*, *Culex quinquefasciatus*, *Aedes aegypti* mosquitoes, and *Anopheles stephensi* mosquitoes. AC-AgNPs also showed high compatibility with blood. The urgent need for new malaria control strategies in the face of the emergence of artemisinin-resistant Plasmodium falciparum parasites in Africa beckons us to rise to the challenge and develop innovative solutions that will save countless lives (Kojom Foko *et al*., 2023). There is a need to expedite the discovery and development of new antimalarial drugs due to the growing resistance to artemisinin-based combination treatments; herbal medicines are crucial for the innovation of novel drugs (Ocan *et al*., 2023). The emergence of insecticide resistance in sub-Saharan Africa represents a significant challenge to managing malaria-transmitting insects (Kumala *et al*., 2022). To maintain their efficiency, looking into other techniques, including using plant-based pesticides like *Alchornea cordifolia,* has become necessary.

MATERIALS AND METHOD

Study Area

The research was carried out at the Niger Delta University's Biological Science Research Laboratory in Wilberforce Island, Bayelsa State. The research area has a tropical climate with two distinct seasons: dry and wet. Interestingly, the wet season lasts from April to October, with much precipitation - around 2000mm per year. From November through March, the study area is typically dry and dusty, with an elevation of 45m above mean sea level (Angaye, 2015).

Plant Collection

Leaves and stem bark of *Alchornea cordifolia* were collected from the bush around the Niger Delta University and transported to the Research Laboratory of the Department of Biological Sciences for identification and extraction. The plant leaves and bark were washed with tap water and air-dried at room temperature. The leaves and stem bark of the plant were separated into three portions for solvent extraction (crude and ethanolic) and proximate composition analysis.

Plant Extraction Procedure

Three hundred grams of the leaves and stem bark were weighed and pounded. Afterward, the juice of the pounded leaves was squeezed and filtered into a clean conical flask using a muslin cloth. Prior to the bioassay, the obtained juice of the plants was distinctly labelled and preserved at room temperature (Angaye *et al.,* 2017a). For 72 hours, 300 g of the leaves and stem bark were macerated in 500 ml of each of the three solvents (water, hot water, and ethanol (BHD Chemical Ltd. Poole England). The extraction filtrates were concentrated further using a rotary evaporator set to 60°C. The obtained extract was deemed suitable for bioassay and was thus used for evaluation at room temperature.

Mosquito Culture

The study used mosquito larvae cultured from the wild using culture baits. This process involves placing a formulation around areas with vegetation cover and stagnant water, such as bushes, and using conspicuous breeding sites like vehicle tyres, cans, and plastic containers half-filled with stagnant water (Angaye *et al.,* 2014; Ohimain *et al*., 2015; Angaye *et al.,* 2017a). The development and emergence of mosquito larvae were closely monitored to prevent adult emergence, and the larvae were subsequently brought to the laboratory and transferred to a plastic enamel tray filled with water.

Larva screening test

The process for assessing larvicidal bioassay involved two distinct phases: the rapid screening and final screening stages, as documented by Agboola *et al.* (2011) and Angaye (2015). The plant extracts were prepared in various concentrations and placed in perforated petri dishes. The rapid screening stage established the range of activity (ROA), while the final screening phase identified the minimum dose required to wholly eliminate a larva within 24 hours. Results indicated that 400 and 200 ppm were appropriate for the rapid screening stage. Only plant extracts that caused 100% mortality at 200 ppm during the rapid screening were deemed active and thus approved for the final screening phase.

Larvicidal Experimental Setup

With some modifications, a larvicidal screening was conducted according to the protocols set forth by the World Health Organisation (WHO, 1965; Agboola *et al*., 2011). The screening utilized a static non-renewal test, with each solvent extract being tested on ten larvae at varying concentrations in a 500-ml solution. The number of dead larvae was recorded after 24 hours. Agboola *et al.* (2011) recommended a two-phased (rapid and final) screening process. To evaluate the effectiveness of the

screening process, a known pesticide, Dipex pesticide, was utilized as the positive control. At the same time, a mixture of 2.5 ml of 10% dimethyl sulfoxide (DMSO) and 500 ml of distilled water with a pH level of 7.5 was used as the negative control (Dibua et al., 2013).

Statistical Analysis

The bioassay samples analysed in triplicatee. The resulting data were presented with standard deviation. Statistical mean separation was performed using Analysis of Variance (ANOVA), followed by post hoc analysis with Duncan's Multiple Range Test ($P < 0.05$) to identify significant differences. Using alphabets to indicate differences were employed, and mean values were used to evaluate the dose-response relationship, ultimately determining the median lethal dose (LC50).

RESULT AND DISCUSSION

Biolarvicidal Bioassay for the Stem-Bark Extract

The mortality rates for the stem-bark extracts of *A. cordifolia* are presented in Table 1. The bioassays showed that the positive control resulted in total mortality when the concentration was 10.00 ppm., while no mortality was observed in the negative control. Upon increasing the concentration of the crude extract, the mortality rate also significantly increased ($p<0.05$). The crude stem-bark extract had mortality rates that ranged from 370.00 – 100%, with significant ($p<0.05$), lowest and highest mortality rates observed at 10 and 70 ppm, respectively. The dose-response analysis further revealed that the crude stem-bark extract had an LC50 value of 13.18 ppm (Figure 1). These findings demonstrate that the stem-bark extracts of *A. cordifolia* have significant potential in controlling the spread of harmful larvae.

Table 1: Result of Mortality rates for the Stem-Bark Extracts of *Alchornea cordifolia*

Concentration	Mortality Rates (%)				
	Crude Extract	Hot-water Extract	Ethanol Extract	Positive Control	Negative Control
0 ppm	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
10 ppm	370.00±10.00b	38.67±0.58b	50.33±5.03b	100.0±00.00e	00.00±00.00a
20 ppm	46.33±1.15c	62.67±1.53c	680.00±1.73c	100.0±00.00e	00.00±00.00a
30 ppm	540.00±10.00d	68.67±1.52d	86.67±2.08d	100.0±00.00e	00.00±00.00a
40 ppm	640.00±10.00e	77.67±1.15e	100.0±00.00e	100.0±00.00e	00.00±00.00a
50 pm	770.00±2.65f	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a
60 ppm	90.33±1.53g	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a
70 ppm	100.0±00.00h	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a
80 ppm	100.0±00.00h	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a
90 ppm	100.0±00.00h	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a
100 ppm	100.0±00.00h	100.0±00.00f	100.0±00.00e	100.0±00.00e	00.00±00.00a

Data expressed as Mean±Standard Deviation, differences in alphabetical subscripts indicate the level of significance.

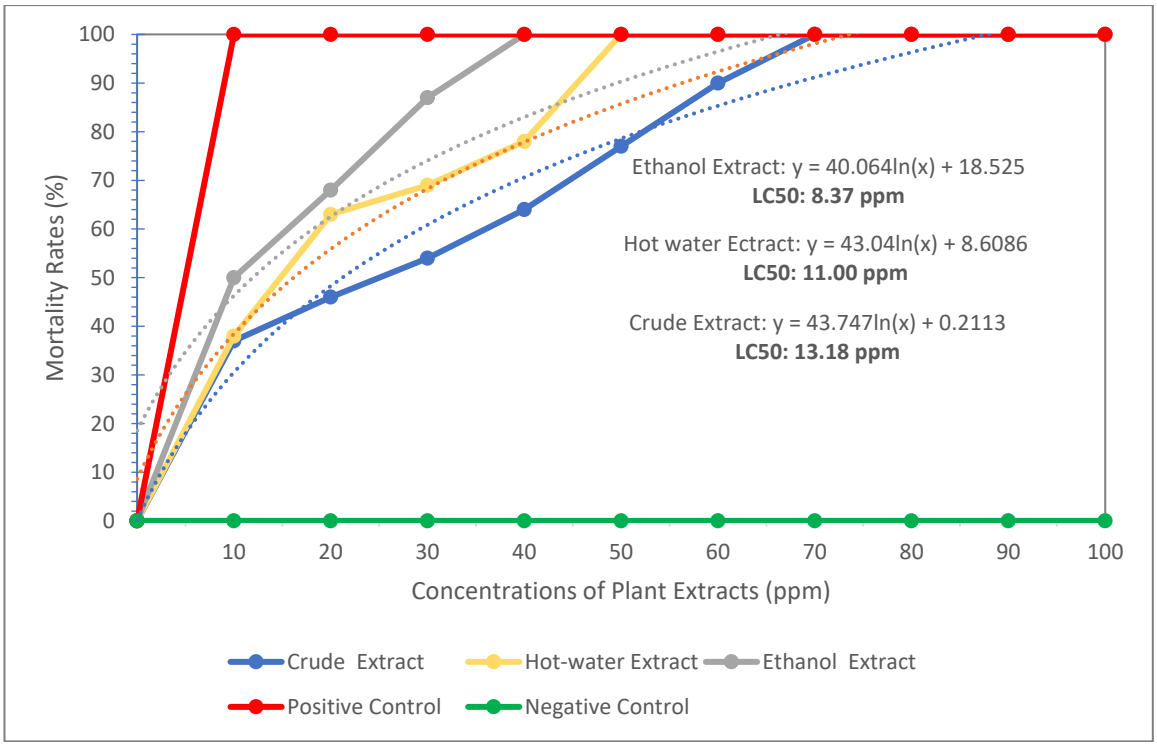

Figure 1: Dose-response graph and LC50 values for the stem-bark extracts

The results showed that the hot water and ethanolic extract of *A. cordifolia* stem bark effectively killed mosquito larvae ($p<0.05$). The results of the study showed that these extracts had a significant impact on the mortality rates of the larvae, which ranged from 38.67% to 100%. Interestingly, the concentration of 10 ppm resulted in the lowest mortality rate, while the highest mortality rate was observed at 70 ppm. These findings could be valuable in developing natural and effective methods for controlling mosquito populations and preventing diseases spread by them. The extracts were highly effective in killing the larvae, with the LC50 value being 110.00 ppm and 8.37 ppm for the stem-bark hot-water extract and ethanolic stem-bark extract, respectively. These results demonstrate the potential of these extracts as a natural way to control mosquito populations, which could be particularly useful in areas where mosquito-borne diseases are prevalent.

Table 2: Result of Mortality rates for the Leaf Extracts of *Alchornea cordifolia*

Concentration	Mortality Rates (%)				
	Crude Extract	Hot-water Extract	Ethanol Extract	Positive Control	Negative Control
0 ppm	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a	00.00±00.00a
10 ppm	110.00±00.00b	33.33±3.51c	47.67±1.15d	100.0±00.00e	00.00±00.00a
20 ppm	28.33±1.15b	48.67±0.58c	57.67±1.15d	100.0±00.00e	00.00±00.00a
30 ppm	42.33±1.53b	54.33±3.06c	73.67±1.52d	100.0±00.00e	00.00±00.00a
40 ppm	640.00±10.00e	77.67±1.15e	100.0±00.00e	100.0±00.00e	00.00±00.00a
50 pm	66.67±2.51b	84.33±2.31c	100.0±00.00d	100.0±00.00d	00.00±00.00a
60 ppm	84.33±2.31b	100.0±00.00c	100.0±00.00c	100.0±00.00c	00.00±00.00a
70 ppm	100.0±00.00b	100.0±00.00b	100.0±00.00b	100.0±00.00b	00.00±00.00a
80 ppm	100.0±00.00b	100.0±00.00b	100.0±00.00b	100.0±00.00b	00.00±00.00a
90 ppm	100.0±00.00b	100.0±00.00b	100.0±00.00b	100.0±00.00b	00.00±00.00a
100 ppm	100.0±00.00b	100.0±00.00b	100.0±00.00b	100.0±00.00b	00.00±00.00a

Data expressed as Mean±Standard Deviation, differences in alphabetical subscripts indicate the level of significance.

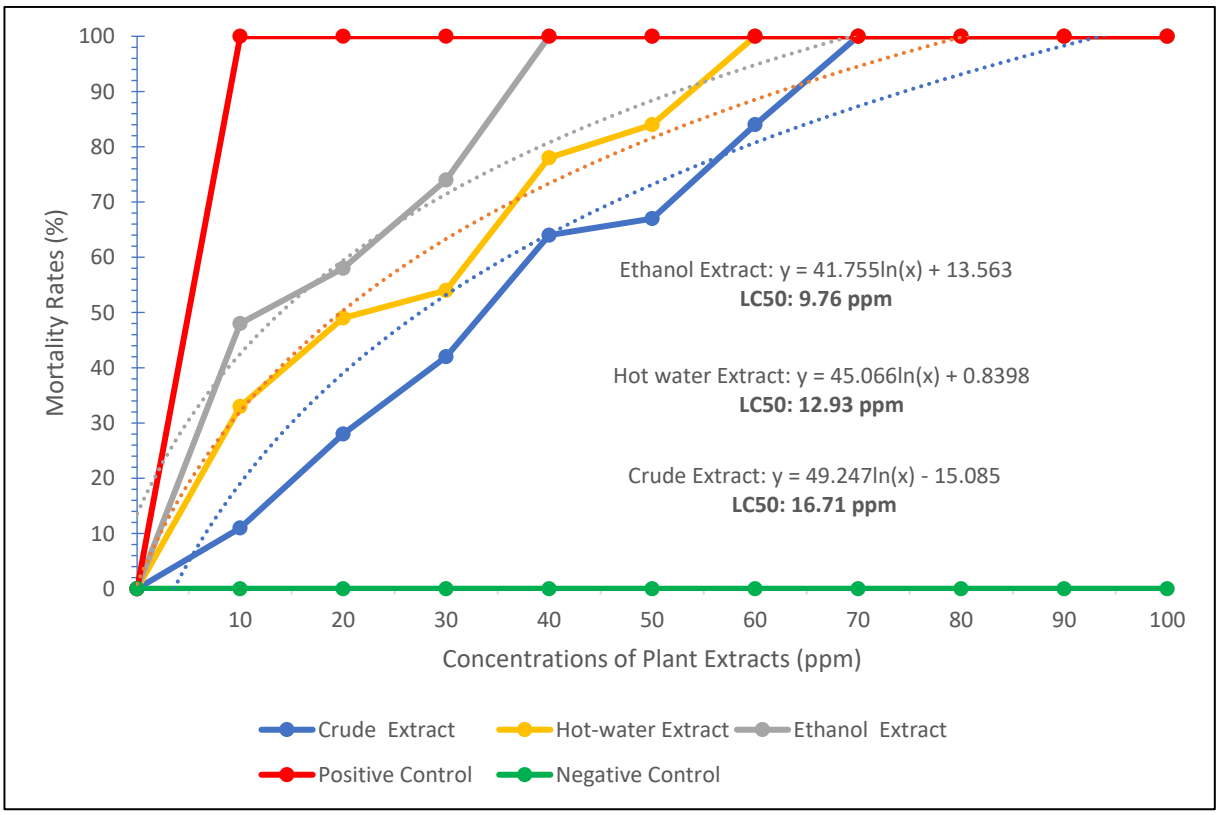

Figure 2: Dose-response graph and LC_{50} values for the Leaf extracts

Biolarvicidal Bioassay for the Leaf Extract

Table 2 presents the mortality rates for crude, hot-water, and ethanolic leaf extracts of *A. cordifolia*. A concentration of 10 ppm in the larvicidal bioassay resulted in total mortality for the positive control bioassay, while the negative control bioassay showed no mortality (Table 2). Looking at the results for the crude leaf extract, we observed a significant increase ($p<0.05$) in mortality rate as the concentration of the plant extract increased ($p < 0.05$), with mortality rates ranging from 110.00% to 100%

(Table 2). The minimum mortality rate was observed at ten ppm, with the total minimal mortality rate at 70 ppm (Table 1). Based on the dose-response, the crude leaf extract demonstrated larvicidal activity with an LC50 value of 16.71 ppm (Figure 2).

The plant's hot water leaf extract was tested for its effectiveness in killing larvae through a larvicidal bioassay, which produced noteworthy results, as indicated in Table 2. The study demonstrated that the mortality rate of larvae increased significantly with the concentration of the hot water leaf extract ($p<0.05$), ranging from 33.33% to 100%. The lowest mortality rate was observed at a concentration of 10 ppm, while the highest rate was recorded at 60 ppm ($p<0.05$). Figure 2 showed that the LC50 value of the extract was 12.93 ppm, indicating its potency in killing larvae. In the same way, the ethanolic leaf extract of A. cordifolia was tested to see how well it worked. The results showed that as the concentration increased, deaths went from 47.67% to 100% (Table 2). The LC50 value of the ethanolic leaf extract was recorded at 9.76 ppm (Figure 2), further confirming its effectiveness in killing larvae.

The efficacies and antioxidant properties of the *Alchonea* plant have been reported from the preceding; decoction and infusion of the Stem, bark, leaves, inflorescences, root were effective against hypoglycemia, jaundice; wounds and umbilical abscess have been reported by Iwu (1993). The leaves and bark were reported to be effective in remedying fertility problems (Gertrude, 2010) and sexually transmitted diseases (Magassouba *et al.,* 2007). The antibacterial activities of the methanolic leaf, stem bark, and root bark extracts were reported due to phytochemicals like alkaloids, phenolics, saponins, and terpenes (Ebi 2001). Also, Alchornea cordifolia leaf extracts' efficacy in combating parasites was evaluated, with particular attention paid to their anthelminthic, antimicrobial, and antioxidant properties. Findings revealed that the methanol extract was the most potent, successfully inducing paralysis and death in parasites at 12 mg/ml. The time to produce this effect was recorded at roughly 26.28 ± 0.575 and 57.30 ± 0.370 minutes, respectively (Akoto *et al.*, 2019).

According to a Djimeli *et al.* (2017) study, *Alchornea cordifolia* was toxic to mice, with LD50 values of 8.6 g/kg and 3.8 g/kg in male and female mice, respectively. The study also found that oral administration of the extract resulted in a decrease in bacterial load of E. coli in a dose-dependent manner. The extract eradicated the infection in 9, 11, and 13 days of treatment at 232 g/kg, 112 g/kg, and 58 g/kg, respectively. A study examined how six different types of polysaccharides from the leaves of *Alchornea cordifolia* affected the immune system. The type II arabinogalactan fraction was found to have the most immunomodulatory activity (Kouakou *et al.*, 2013).

Studies by Kojom Foko *et al.* (2023) have found that Alchornea cordifolia extracts exhibit strong antiplasmodial effects, with IC50 of 8.05 µg/mL and 10.31 µg/mL against Plasmodium falciparum strains 3D7 and RKL9. The extracts also killed many larvae of the *Culex quinquefasciatus, Aedes aegypti*, and *Anopheles stephensi* mosquitoes, with LC50 values of 18.41 µg/mL after 24 hours of exposure and 8.97 µg/mL after 48 hours of exposure. Additionally, the extracts are highly hemocompatible, with HC50 values exceeding 500 µg/ml. All these studies showed that the plant has antioxidants supporting its larvicidal activities. The antifungal activity of the extracts of *Alchornea cordifolia* was assessed *in vitro* and *in vivo* against Aspergillus flavus. Results showed that 56.38 – 68.22% and 67.245 – 80.01% inhibition rates at 100 mg mL^{-1} and 500 mg mL^{-1} respectively (Enyiukwu *et al.*, 2023). The aqueous extract of the leaves of *Alchornea cordifolia* has been found to have a huge diversity of phytochemicals that have antibacterial activity against tetracycline-resistant strains of the avian Escherichia coli (Bertin *et al.*, 2023).

CONCLUSION

This study aimed to evaluate the potential of *Alchornea cordifolia* plant extracts as a larvicide against mosquito larvae. The results indicated that the stem-bark extracts of *Alchornea cordifolia* had a significant impact on mosquito larvae, resulting in a mortality rate ranging from 37.00% to 100%. The dose-response analysis further revealed that the crude stem-bark extract had an LC50 value of 13.18 ppm. These findings suggest that *Alchornea cordifolia's* stem-bark extracts could effectively be a larvicide in malaria control programs. However, further research is necessary to determine the feasibility and effectiveness of these extracts in real-world scenarios. Additionally, the results of this study provide a foundation for the development of new larvicidal agents from *Alchornea cordifolia*. Overall, this study contributes to the growing body of literature on using natural products in malaria control and provides a potential new approach to controlling the spread of harmful mosquitoes.

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