International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(1): 56-60

www.dipterajournal.com
Received: 20-11-2021
Accepted: 22-12-2021

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Larvicidal and pupicidal potential of *Alchornea cordifolia* (Schum. & thonn.) leaf extract against the malaria vector *Anopheles gambiae* (Diptera: culicidae)

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DOI: https://doi.org/10.22271/23487941.2022.v9.i1a.581

Abstract
The *Anopheles* mosquito is one of the major medically important pests in Ghana. This research was to evaluate the larvicidal and pupicidal activities of *Alchornea cordifolia* leaf extract against *Anopheles gambiae* mosquitoes in the laboratory. Twenty 3rd/4th larval instar as well as two days old pupae were introduced into the treatments and control and replicated four times. The number of dead larvae and pupae were counted and recorded after 24 hours. Larvae and pupae mortality occurred in a dosage-dependent manner. There were significant differences in toxicity level of the leaf extracts on *An. gambiae* larvae and pupae at concentrations after 24 hours of exposure. LC_{50} and LC_{90} recorded after probit analysis for the larvae was 52.19mg/L and 180.10mg/L respectively and that for the pupae was 189.97mg/L and 857.00mg/L respectively. *A. cordifolia* leaf extract could serve as a potential larvicidal and pupicidal agent.

Keywords: *Alchornea cordifolia, Anopheles gambiae,* larvae, pupae, leaf extract.

1. Introduction
Malaria is the largest single component of disease burden and epidemic malaria in particular, remains a major public health concern in developing tropical countries such as Ghana. In many developing countries, and especially in Africa, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost [1].

One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. Mosquitoes alone transmit disease to more than 700 million people annually [2, 3]. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance [4], undesirable effects on non-target organisms and fostered environmental and human health concern [5], which initiated a search for alternative control measures.

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors [6]. However, more concerted efforts have been undertaken to make environment-friendly compounds viable for field use and for large-scale vector control operations [7] reported 99 families, 276 genera and 346 species to have insecticidal properties.

One of such natural plants is *Alchornea cordifolia* which is an important medicinal plant in African traditional medicine and much pharmacological research has been carried out into its antibacterial, antifungal, cytotoxic, hypotensive and antiprotozoal properties, as well as its anti-inflammatory activities, with significant positive results [8]. Koomson & Oppong (2018) [9], Koomson *et. al.* (2018) [10] and Koomson (2020) [11] found out the leaves, bark and roots of the plant was effective in controlling stored products insect pests through contact toxicity and repellency activities. There has however not been any study about its insecticidal activity against mosquitoes. Hence the objective the present study, is to evaluate the larvicidal and pupicidal potential of *Alchornea cordifolia* leaf powder against a potent malaria vector, *Anopheles gambiae*.

2. Materials and Methods

The research was carried out at the Biology Education Department laboratory of the University of Education, Winneba, Central Region, Ghana, at a temperature of 30±2°C and 75±5% relative humidity.

2.1 Collection and rearing of larva and pupa mosquito

Mosquito baits, consisting of shallow containers with a large surface area were established under a partial shade in an open field around the South Campus of the University of Education, Winneba. A clean transparent white bucket was filled with rain water to mimic mosquito natural breeding environment and to attract adult female for oviposition. Ten grams (10g) of yeast (Bakers' yeast) were sprinkled on the surface of the water and allowed to decompose slowly to nourish the developing larvae. Wild mosquitoes were allowed to freely visit the baits and to lay eggs. The water was monitored for 3–5 days for the development of the egg and first instar larva. These larvae were taken into the laboratory. In the laboratory, the larvae were identified into species level using the morphological keys [12]. The *Anopheles* larvae were separated from the mixed culture and transferred into another plastic container containing rain water. Some of the larvae were used for the larvicial tests. The *Anopheles* larvae were further nurtured to pupae for 3-5 days for pupicidal tests.

2.2 Collection and preparation of plant materials

Alchornea cordifolia plants were collected from the Gomoa Otapirow area of the Central Region of Ghana. Leaves were separated from the plant, rinsed in clean water to remove sand and other impurities, air dried at room temperature in the laboratory for 15 days, after which, ground into very fine powder using an electric blender. The powders were further sieved to pass through 1mm^2 perforations. The powders were packed in plastic containers with tight lids to ensure that the active ingredients are not lost and stored in the laboratory prior to use.

2.3 Extraction of plant materials

The extraction was carried out in the Chemistry Education laboratory of the University of Education, Winneba. About 400g of *Alchornea cordifolia* powders were soaked separately in an extraction bottle containing 500ml of absolute n-hexane for 3 days. The mixture was stirred occasionally with a glass rod and extraction was terminated after 3 days. Filtration was carried out using a double layer of Whatman No. 1 filter papers and solvent evaporated using a rotary evaporator at 30 to 40oC with rotary speed of 3 to 6 rpm for 8 hours [13]. The resulting extracts were air dried in order to remove traces of solvent. The extracts were kept in labeled plastic bottles till when needed.

2.4 Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 4g of the crude extracts in 1Litre of water. From these stock solutions, different concentrations of 25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L were prepared and these aqueous solutions were used for the various experiments.

2.5 Bioassay

2.5.1 Toxicity of Extracts on Larvae of Mosquito

One hundred millimeter (100mm) of aqueous solutions of the various plant extracts at various concentrations of 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L were each put in a labeled transparent bowl. Twenty (20) 3rd/4th larval instar obtained from the culture were introduced separately into the various plant extracts along with a set of control containing rain water without any test solution and all tested concentrations were replicated four times. The number of dead larvae were counted and recorded accordingly after 24 hours of treatment.

Dead larvae were those incapable of rising to the surface or without the characteristic diving reaction when the water was disturbed [14].

$$\text{Percentage larval mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced 1}} \times 100$$

The LC_{50} and LC_{90} were carried out by Probit analysis.

2.5.2 Toxicity of extracts on pupae of mosquito

Also, similar experiment as described above was carried out for pupae of *Anopheles gambiae*. One hundred millimeter (100mm) of aqueous solutions of the various plant extracts at various concentrations of 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L were each put in a labelled transparent bowl. Twenty (20) two (2) days old pupae of *Anopheles* mosquito were introduced separately into the various plant extracts. They were replicated four times and rain water was used as control. The number of dead pupae were counted and recorded accordingly after 24 hours of treatment.

$$\text{Percentage pupal mortality (\%)} = \frac{\text{no. of dead pupae}}{\text{no. of pupae introduced 1}} \times 100$$

The LC_{50} and LC_{90} were carried out by Probit analysis.

2.6 Statistical analysis of data

Data were subjected to analysis of variance (ANOVA), and means were separated using the new Duncan's multiple Range test. The log-Probit model analysis was done to the data recorded in the larvicidal and pupicidal bioassay to assess the 50% lethal concentration (LC_{50}), the 90% lethal concentration (LC_{90}) and their 95% confidence limits.

3. Results

3.1 Contact toxicity of *A. cordifolia* extract on *An. gambiae* Larvae

Effects of *A. cordifolia* leaf extract on percentage mortality of *An. gambiae* larvae is presented in table 1 and figure 1. Larvae mortality of *An. gambiae* occurred in a dosage-dependent manner. *A. cordifolia* at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L caused 42%, 58%, 71%, 94% and 100% mortality of *An. gambiae* larvae after 24 hours of exposure. Generally, *An. gambiae* larvae mortality increased with increase in hours of exposure.

3.2 Contact toxicity of *A. cordifolia* leaf extract on *An. gambiae* Pupae

Table 2 and figure 2 show the effects of contact toxicity of *A. cordifolia* extract on percentage mortality of *An. gambiae* larvae after 24 hours of exposure. Mortality of *An. gambiae* pupae occurred in a dosage-dependent manner. *A. cordifolia* leaf extract at concentrations 25mg/L, 50mg/L, 100mg/L, 150mg/L and 200mg/L caused 28%, 43%, 66%, 75% and 92% mortality of *An. gambiae* pupae after 24 hours of exposure, respectively. Generally, *An. gambiae* pupae

mortality increased with increase in hours of exposure.

3.3 LC_{50} and LC_{90} of *A. cordifolia* leaf extracts on *An. gambiae* larvae and pupae

LC_{50} and LC_{90} of *A. cordifolia* leaf extract on larvae and pupae of *An. gambiae* is shown on table 3. LC_{50} and LC_{90} of *A. cordifolia* leaf extract on larvae were 52.19 mg/L and 180.10 mg/L respectively. On pupae, LC_{50} and LC_{90} of *A. cordifolia* were 187.97 mg/L and 857.00 mg/L respectively.

Table 1: Effect of *A. cordifolia* leaf extract on % mortality of *An. gambiae* larvae after 24 hours of exposure.

	Concentration (mg/L)				
Plant extract	**25**	**50**	**100**	**150**	**200**
A. cordifolia leaf extract	42.00±2.22^{b}	57.50±2.50^{b}	71.00±2.22^{b}	94.00±2.22^{b}	100.00±0.00^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Mean ± Standard error followed by the same letters, superscript at the end of each value, down the column are not significantly different (p > 0.05) from one another using New Duncan's Multiple Range Test.

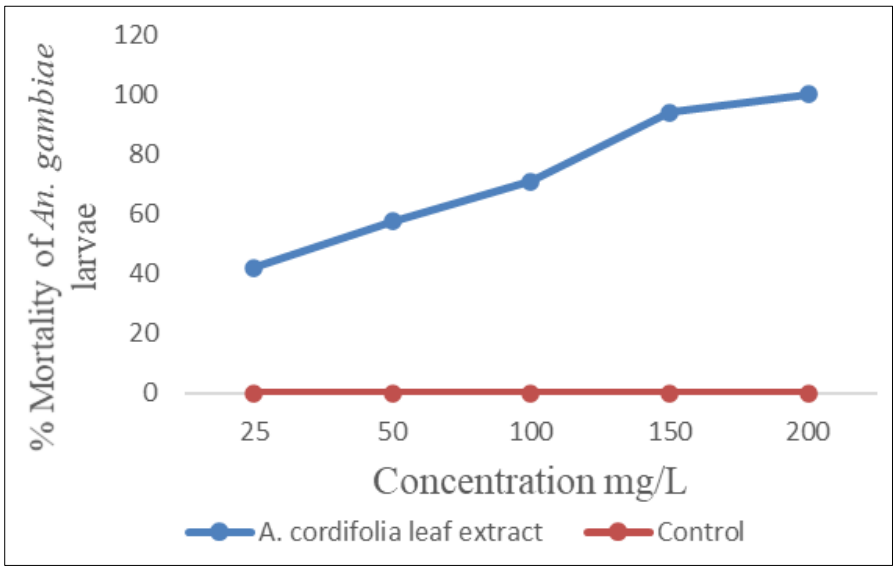

Fig 1: Effect of *A. cordifolia* leaf extract on % mortality of *An. gambiae* larvae after 24 hours of exposure

Table 2: Effect of *A. cordifolia* leaf extract on % mortality of *An. gambiae* pupae after 24 hours of exposure.

	Concentration (mg/L)				
Plant extract	**65**	**125**	**250**	**500**	**1000**
A. cordifolia leaf extract	28.00 ±2.22^{b}	43.00 ±2.25^{b}	65.50 ±2.50^{b}	74.50 ±2.50^{b}	92.50 ±2.50^{b}
Control	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Mean ± Standard error followed by the same letters, superscript at the end of each value, down the column are not significantly different (p > 0.05) from one another using New Duncan's Multiple Range Test

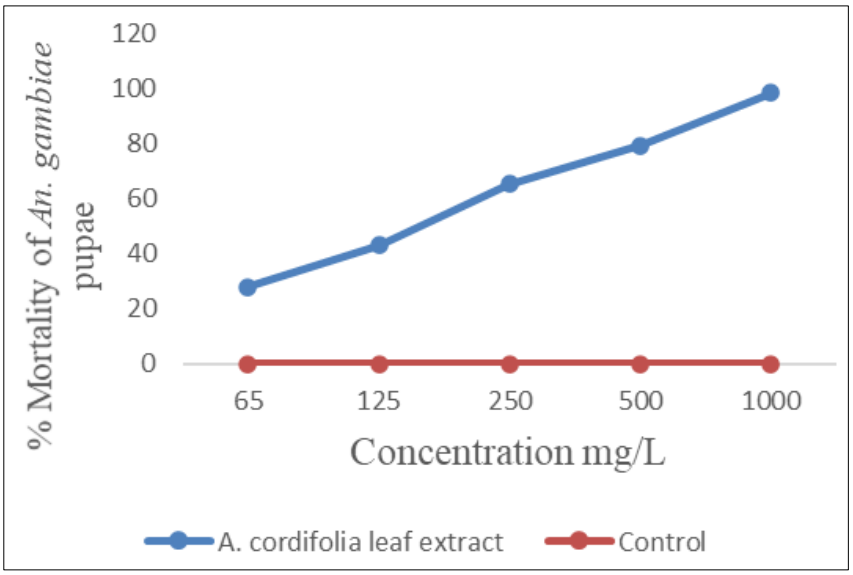

Fig 2: Effect of *A. cordifolia* leaf extract on % mortality of *An. gambiae* pupae after 24 hours of exposure

Table 3: LC_{50} and LC_{90} of *A. cordiflia* leaf extract on *An. gambiae* larvae and pupae.

An. gambiae	Concentration (mg/L)		
	Plant extract	**LC_{50} (Lower – Upper Limit)**	**LC_{90} (Lower – Upper Limit)**
Larvae	*A. cordiflia* leaf extract	52.19 (7.25 – 94.39)	180.10 (9.14 – 341.01)
Pupae	*A. cordiflia* leaf extract	189.97 (122.01 – 285.09)	857.00 (507.02 – 1213.04)

4. Discussion

Mosquito-borne diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries such as Ghana [15]. The direct and indirect contributions of such effects to treatment efficacy through reduced larval and pupal feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for of *A. gambiae* [16]. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future [17].

The aquatic immature larvae stage is recognized as the most vulnerable and best control strategy to effectively reduce mosquito population densities during infestations [18]. This study showed that botanicals extract can be suitable alternatives to the use of synthetic insecticide in the control of insect vectors of economic importance. The plant assessed had proven high insecticide ability against mosquito and this was in agreement with Ileke *et. al* (2014) [19], and Ileke & Adesina (2018) [20], who reported the insecticidal potential of botanicals like *Anacardium occidentale, Afromomum melegueta, Garcina kola, Citrus sinensis, Clerodendrum capitatum* and *Bridelia machranth* plants against malaria vector, mosquito, *Anopheles gambiae*.

Plants contain secondary metabolites and the effectiveness of these secondary metabolites such as alkaloids, isoflavonoids, saponine and steroids has potential mosquito larvicides and pupicides [21]. These essential oils could also affect the swimming ability of the mosquito larvae and pupae, thereby hinder their swimming to the surface for oxygen which could lead to their death. The strong choky odour of the plants may have also disrupted respiratory activity of the insect [22], blocking the spiracles thereby hinder the larvae breathe [20]. This could result into asphyxiation and death of the larvae [23, 24, 25].

The high mortality caused by the *A. cordifolia* leaves extract on the *Anopheles* mosquito larvae and pupae is in agreement with the findings of Koomson & Oppong (2018) [9], Koomson *et. al*, (2018) [10] and Koomson (2020) who tested the powder of the leaves, bark and roots respectively against insect pests in stored grains.

Higher concentrations of the leaf extract were needed to achieve higher mortality on pupae than on larvae. This indicates that the larvae is more susceptible to these plants than pupae and this could be attributed to the active feeding stage of the larvae since pupae stages of the insect are not feeding [26, [25, 20].

Natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability. With respect to resistance developed by the mosquito larvae and pupae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. The outcome of the present research obviously shows that *A. cordifolia* have astonished mosquito properties against *Anopheles gambiae* larvae and pupae. Further research is required to investigate the insecticidal potential of the *A. cordifolia* on the adult and eggs stages of the mosquito so as to integrate it into integrated pest management strategies in developing countries because they are locally available, potentially less expensive to the traditional farmer and relatively less harmful to human health and the environment.

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