Article

Adulticidal, ovicidal and repellent potencies of *Alchornea cordifolia* (Schum. & Thonn.) in the management of the malaria vector *Anopheles gambiae* (Diptera: Culicidae)

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Abstract
Malaria, which is transmitted by the mosquito *Anopheles gambiae*, has long been a major public health concern in the tropics. Chemicals used to control *A. gambiae* have caused significant harm to the environmental and non-target organisms. Furthermore, these mosquitoes have demonstrated a high level of resistance. This study evaluated the adulticidal, ovicidal and repellent potencies of leaf extracts of *Alchornea cordifolia* against *A. gambiae*. It was observed that 5 mg/ml of the leaf extract induced about 94% mortality in the adult *A. gambiae*, 0.8 gm/cm^3 of the leaf extract repelled 95% of the mosquitoes within 15 minutes and 0.6 mg/ml of the leaf extract completely inhibited hatching of mosquito ova. Evidently, *A. cordifolia* leaf extracts showed a good efficacy in the management of *A. gambiae* in this study. More research is needed to determine its mode of action, synergism with other products, and efficacy in actual field conditions.

Keywords *Alchornea cordifolia*; *Anopheles gambiae*; adulticidal; repellency; ovicidal, management.

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1 Introduction

The most important arthropod vector for medicinal purposes is the mosquito (Taubes, 1997). These dreadful insects are responsible for one of the greatest vector-borne diseases in the world, affecting the socio-economic status of many countries (Taubes, 1997) by exacting enormous toll in lives, in medical cost, and in days of labour lost (Lambert, 2009). In 2015, 90% of the malaria cases were reported in the sub-Saharan Africa and 92% of deaths were reported worldwide (Krishnappa et al., 2012).

Mosquito management is necessary to check the propagation of mosquito-borne diseases that in turn mend the quality of the environment and public health (Ghosh et al., 2012). Mosquito management programmes depend on a routine of chemical insecticides. These repeated uses of synthetic insecticides in mosquito management have destroyed natural ecosystems, environmental pollution, development of resistance and insect resurgence (DeSilva, 1997). These synthetic insecticides also cause serious health problems to applicators such as breathing problems, asthma, eye irritation, headache, sneezing and toxicity to the nervous and reproductive systems (Sharma, 2001).

This has necessitated the need to research into environmentally safe, biodegradable and inexpensive indigenous vector management methods that can be employed by poor resource people. The search for new management agents from natural products such as plant secondary metabolites have gained prominence among scientists in developing countries with a strong herbal tradition and large number of plants that have insecticidal properties (Komalamisra et al., 2005). Plant phytochemicals have the potency to act as larvicides, pupicides, adulticides, repellency and ovicides (Panneerselvam et al., 2012). Extracts of the flora may be a better choice to control populations of mosquito since they contain a variety of phytochemicals that are easily degradable and suitable for applications in their natural breeding environment (Rawani et al., 2013).

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 (Agbor, 2004). Extracts of the leaves have been found to be very effective in controlling the larvae and pupae of the *Anopheles gambiae* (Koomson et al., 2022).

This current research is aimed at assessing the adulticidal, ovicidal and repellency potency of the plant leaf extracts in the management of this dreadful malaria vector.

2 Materials and Methods

2.1 Location

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^{o}C and 75±5% relative humidity. The period of study was from February 2023 to May 2023.

2.2 Collection and rearing of mosquito ova and adults

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 rainwater to mimic mosquito natural breeding environment and to attract adult female for oviposition. Ten grams (10 g) 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 (Gillies et al., 1968). The *Anopheles* larvae were separated from the mixed culture and transferred into another plastic container containing rainwater. The *Anopheles* larvae were further nurtured to adult after eleven days. Some of the adults were used for the tests on the adults, repellency and others were made to lay eggs for test on the mosquito ova.

2.3 Collection and preparation of plant materials

A. 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 1 mm^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.

2.4 Extraction of plant materials

The extraction was carried out in the Chemistry Education laboratory of the University of Education, Winneba. About 400 g of *A. cordifolia* powders were soaked separately in an extraction bottle containing 500 ml 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 40^{o}C with rotary speed of 3 to 6 rpm for 8 hours (Udo, 2011). The resulting extracts were air dried in order to remove traces of solvent. The extracts were kept in labelled plastic bottles till when needed.

2.5 Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 4 g of the crude extracts in 1 litre of water. From these stock solutions, different concentrations of *A. cordifolia* were prepared and these aqueous solutions were used for the various experiments.

2.6 Bioassay

2.6.1 Adulticidal bioassay

The bioassay was performed using adult stages of *A. gambiae* following the protocol of WHO (1981). with slight modifications. Different concentrations such as 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, and 5 mg/ml of *A. cordifolia* leaf extract were used for the adulticidal activity. According to Dua et al., 2008, mentioned dose were spread on filter papers (size 12 cm × 15 cm). In the control set up, only distilled water applied on filter papers were used. Twenty-five adult female mosquitoes (blood starving 2-5 days old mosquitoes and glucose fed) were used for the bioassay. At first, they were smoothly moved into an elastic holding tube. Inside the tube they were kept for an hour for the acclimatization and after that they exposed to the treated paper (filter paper) for an hour. After the contact hour, adult female mosquitoes remain positioned inside the elastic holding tube and seized for 24 hours to recover. On the mesh screen a cotton plug drenched with 10% starch solution remained positioned for the feeding purpose. Mortality of mosquitoes was observed after the 24 h recovery period. Abbott's formula (Abbott, 1925) used for the correction of the percent mortality.

2.6.2 Ovicidal bioassay

For the ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs of *A. gambiae* laid during the experimental period were collected.Various concentrations of the leaf extracts ranging from 0.3 mg/ml to 0.6 mg/ml were used.The ova (100) were exposed to eachconcentration of the leaf extracts. After treatment, theova from each concentration were individually transferred todistilled water cups for hatching assessment after counting theova under microscope. Each experiment was replicated sixtimes along with appropriate control. The hatch rates wereassessed 48 h post-treatment by the following formula.

$$\text{mortality (\%)} = \text{No. hatched larvae} \times 100 / \text{Total No. eggs}$$

2.6.3 Repellent activity

Test of repellency of *A. cordifolia* leaf extract of leaves of plant was tested by the author himself. Repellent activity was implemented by using the methodology of Murugan et al. (2007). Three to five (3-5) days old blood starving female *A. Gambiae* numbering one hundred (100) were introduced in a mesh cage having dimension 45 cm × 30 cm × 45 cm. The hands were properly cleaned with water. 25 cm^3 area on the dorsal side of the skin on each arm was used for the experiment, and the rest of the part of the skin was covered with rubber gloves. The leaf extracts was applied with a concentration of 0.8 mg/cm^3 in the uncovered part of the hand. For control, water was used.Repellency against *A. gambiae*was tested between the hours of 16:00 to 18:00. Both the control arms and tested arms were inserted inside the mesh cage. The assessment was carried out by placing the processed arms and control arms in the similar cage for 120 minutes and the number of mosquitoes that bit the hand were noted every 15 minutes. The following formula calculated the percentage of repellency.

Repellency (%) = [(Ta-Tb) / Ta] × 100

where Ta denotes the number of mosquito bites in the control set, and Tb indicates the number of mosquito bites in the tested set.

2.7 Statistical analysis

The hatching rate and percentage of mortality data were subjected to a One-way analysis of variance (ANOVA) to compare the means. A post hoc test - Duncan test of multiple comparisons - was used to determine the significant differences between the treatments. Probit analysis was used to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality. All statistical analyses were done using the SPSS (Statistical Package of Social Sciences) software version 22. Results with $P<0.05$ were considered to be statistically significant.

3 Results

The mortality rates of the adults increased by the increase in toxicity of the*A. cordifolia* leaf extract with the 1 mg/ml concentration giving a 13% mortality and the 5 mg/ml concentration inducing the highest mortality of 94% after 24 hours (Table 1 and Fig. 1).

Table 1 Adulticidal activity of *A. cordifolia* leafon adults of *A. gambiae*.

Concentration (mg/ml)	**Mean mortality (%) after 24 hrs.**	**LC_{50} value (mg/ml)**
1.0	13.2±0.33	2.774
2.0	17.6±3.2	
3.0	42.4±3.3	
4.0	67.8±3.4	
5.0	94.3±3.3	
Control	0.47±0.16	

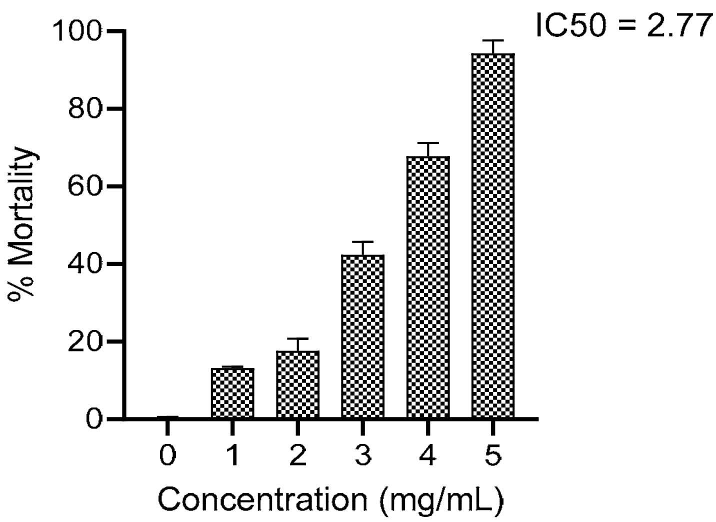

Fig. 1 Effect of *A. cordifolia* leaf extract on the mortality of adult *A. gambiae*.

From Table 2 and Fig. 2, it can be observed that all the concentrations of the leaf extracts used hadovicidal activities on the ova of *A. gambiae*. Complete ovicidalactivity occurred with the highest concentration of 0.6 mg/ml.

Table 2 Mean hatching rate of *A. gambiae ova* exposed to *A. cordifolia* leaf extracts.

Concentration (mg/ml)	Mean Hatching Rate ± SD
0.3	21.04 ± 4.89[b]
0.4	18.97 ± 2.76[b]
0.5	14.09 ± 2.75[b]
0.6	0.00 ± 0.00[a]
Control	56.34 ± 10.47[c]

Results with same letters in the column are not significantly different (P<0.05).

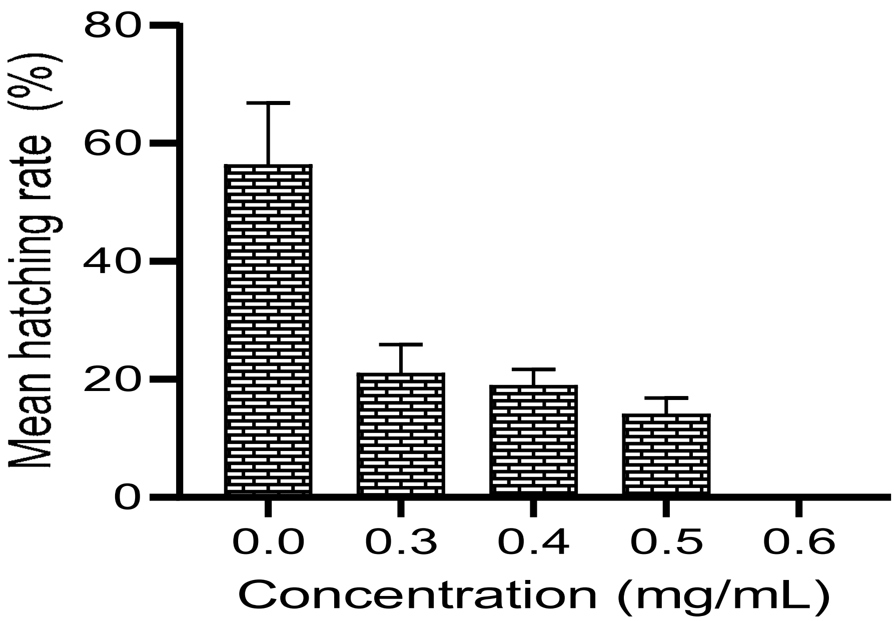

Fig. 2 Effect of *A. cordifolia* leaf extract on the hatching rate of *A. gambiae* ovids.

The biting repellency of adult *A. gambiae* wasobserved at a concentration of 0.8 gm/cm^3 (Table 3) with the highest repellent activity of *A. cordifolia* leaf extract observed within 15 minutes with only five bites in the processed hand which represents 95% repellency.

Table 3 Repellent activity of *A. cordifolia* leaf extract against *A. gambiae*.

Mosquito repellent product	Concentration	Observation time (4 pm - 6 pm)	Total No. mosquitoes	No. bites in the treated arm	Repellancy (%)
Leaf extracts of *A. cordifolia*	0.8 gm/cm^3	15 min	100	05	95
		30 min		11	89
		60 min		22	78
		90 min		38	62
		120 min		46	54

4 Discussion

Resistance of vector mosquitos to chemical insecticides has necessitated the need to the development of new insecticides (Gope and Rawani, 2022). There is a prompt awareness going on about the need to use natural, eco-friendly compounds such as plants for malaria vector management (Karmakar et al., 2023). Different plant species have been identified to contain various phytochemical constituents which are in form of secondary metabolites majorly for the protection of the plants (Egunjobi and Okoye, 2020). Various constituents such as saponins, phenols, alkaloids, flavonoids, terpenoids among others have been extracted from plants(Egunjobi and Okoye, 2020). These secondary metabolites exert varieties of physiological activity on pests, including larvicidal, pupicidal, adulticidal, ovicidal, repellent, etc. (Rawani et al., 2013).

The study revealed that the plant extracts induced complete ovicidal activity. The complete ovicidal activity might be as a result of the plant extract being able to block the micropyle region of the egg, thereby preventing the exchange of gases, which eventually killed the embryo in the egg. The disturbance with egg cytoplasm was reflected in the form of dead eggs with black spot stage due to the arrest of further development of embryo inside the egg (Agwu et. al., 2018). The number of eggs hatched into larvae was also found to be concentration dependent. The trend of hatching rate was inversely proportional to increase concentration ranges **(**Ateyim et al., 2022). The increase in the phytochemical constituents present in high concentrations of the plant extract played a remarkable role. Hence, the ability to inhibit hatching was accompanied by an increase in concentration (Egunjobi and Okoye, 2020). Consequently, as the concentration increases, the ovicidal potential of the extract also increases, resulting in an inverse proportionality between the percent hatchability of the eggs and the concentration of extract (Egunjobi and Okoye, 2020). Similar findings have been documented in *Boswellia dalzielii* leaf extractsagainst Anopheles species (Younoussa et al., 2016).

In current study, the plant extracts showed high adult mortality after 24 hours with the 5 mg/ml concentration producing the highest mortality of 94%. Thus, the entomocidal activityof the plant extract increased with increasing concentrations. Hence, the percent mortality and toxicity data is in accordance to theprevious findings of Odeyemi (2005), Sagheer et al. (2013) and Sultana et al. (2016) that the plant extracts becomemore toxic with increased dose and exposure time. Insecticidal property of the leaf extractcouldbe linked to it chemical constituents. The presence of thesephytochemical alters some biochemical functions oforganisms. Man (2013) reported that increase mortalityof *A. gambiae* rate which was reported in a studycould be attributed to phytochemical content of the leafextract. Studies have also shown that high dose of flavonoidwhich is common in leaves alters the normal body functioning of insects (Ileke et al., 2014).

The study on repellent activity of extract of leaves showed 95% repellency from biting of *A. gambiae* when tested at concentration of 0.8 g/cm^2 applied on the uppermost surface of the hand within 15 minutes of application.Many plant extracts and essential oils with highvolatility, such as alkanes, terpenoids, alcohols, andaldehydes are repellent to mosquitoes for periodsranging from 15 min to 10 hours (Rozendaal, 1997). A recent study revealed that *A. gambiae* is able to detect plant molecules by olfactory neurons in the antenna controlled by the TRPA1 gene, activated directly by the molecule with high potency (Kwon et al., 2010). These molecules interfere with olfactory receptors of mosquitoes (Alayo et al., 2015), and thus repel the mosquitoes.

5 Conclusion

Plant-based, environmentally friendly insecticides have grown in popularity in recent years.Because of their target specificity and readily biodegradable properties, they are nontoxic to other organisms.The findings of this current research indicate that extracts of leaves of *A. cordifolia* can serve as an effective adulticide, ovicidal and repellent agent against *A. gambiae*. It is profitable because it is native, easily degradable, and safe in comparison to synthetic chemical insecticides, which are hazardous to the environment as well as toxic to human and animal health, and its inclusion in an integrated mosquito pest management program is highly recommended. More research is needed to determine its mode of action, synergism with other products, and efficacy in actual field conditions.

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