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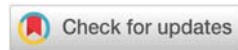
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## Research Article

# Mosquitocidal potential of *Chysophylum africanum* seed extracts against human vector mosquitoes *Anopheles stephensi* (Liston) (Diptera: Culicidae)

## Abstract

Mosquito borne diseases constitute a major public health problem in the list of communicable diseases, *An. stephensi* (Liston) is the primary vector of malaria in developing countries and improved methods of control are urgently needed. Larvicidal activity of *Chysophylum africanum* seed extracts was evaluated against *An. stephensi*. Fourth instar larvae were exposed to 100, 150 and 200µl concentrations of ethyl acetate, acetone and methanol extracts of *C. africanum* replicated thrice. Larval mortality was observed at 24 and 48 h after exposure. Results obtained show that the various extracts caused significant mortality effect with increased concentration and duration of exposure, with ethyl acetate extract invoking the maximum mortality (90%) within 24 hours of exposure at all concentrations tested, while acetone recorded the lowest mortality (33.85%) at 200µl. These findings indicate that seeds of *C. africanum* have larvicidal activity that can be exploited for the management of malaria vector. More studies are needed to determine phytochemical constituents and its mode of action for possible development of larvicides that are safe to use and environment friendly.

## Introduction

Vector-borne diseases are one of the paramount contributors to human death and ill health in the tropics and subtropics. Every year, more than one billion people are infected and more than one million people die from vector-borne diseases including malaria, dengue, yellow fever and lymphatic filariasis [1].

Mosquitoes disseminate more sicknesses than any other group of arthropods and present an immense threat to millions of people, since they act as vectors for important parasites and pathogens, causing millions of death annually across the world [2-3].

Vector control remains the most effective measure and is often the only way to avert disease transmission and outbreaks because there are no vaccines for many vector-borne diseases and drug resistance is an increasing threat. One of the approaches to manage these diseases is to control the vectors for bringing about interruption in disease transmission. The control of mosquitoes at larval stage is considered as an efficient way in integrated vector management [4]. Therefore,

the ideal method for controlling mosquito influx and its attendant diseases is the prevention of mosquito breeding by using appropriate larvicides or application of insecticides to larval habitats, destroying unwanted containers, and educating the public [5-7]. Larval stages of the mosquitoes are attractive targets for pesticides to control mosquito populations [8]. Hence, the advantages of targeting the larval stages are that mosquitoes are killed before they disperse to human habitations and that larvae, unlike adults, cannot change their behaviour to avoid control activities and also to moderate overall pesticide use in control of adult mosquitoes [9-10].

Synthetic insecticides are the first line of action owing to their quick action, but their incessant and indiscriminate use led to the resistance development and lasting residual effect on the environment which can be injurious to animals including human and higher rate of biological magnification [11-13].

Hostile environmental effects and the need to maintain a sustainable environment have created the need for environmental-safe, degradable and target specific-insecticides which remain the top research issue for scientists associated with alternative vector control [14]. The pursuit has

been directed extensively to the plant kingdom. Many plant compounds have larvicidal, pupicidal and adulticidal activities, most being repellents, ovipositional deterrents and anti-feedants against both agricultural and medically important insect species [15]. In fact, many researchers have reported the efficacy of plant extracts or essential oils against mosquito larvae [16-17]. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticides. The present study was carried out to determine the larvicidal activity of *Chrysophyllum africanum* seed extracts against malaria vectors *An. stephensi*.

## Materials and Methods

### Experimental location

The experiment was carried out in the Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria under ambient laboratory conditions of  $30 \pm 2^\circ\text{C}$  temperature,  $75 \pm 5\%$  relative humidity and 12L:12D photoperiod.

### Collection of mosquito larvae

The mosquito larvae were collected from stagnant water samples from drainage canals and temporary water pools around student hostels in the polytechnic using sterile wide mouth container. The identified *An. stephensi* mosquito larvae were kept in plastic bowl containing rain water, sprinkled with powder yeast to serve as food. The culture was maintained and reared in laboratory.

### Plants collection and extraction

Seeds of *C. africanum* were collected from different locations in Owo metropolis and air dried for 4 weeks. Thereafter, the seeds were cracked to remove their endocarps which were further air dried for 5 days. The dried seeds were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with ethyl acetate, acetone and methanol in a soxhlet apparatus separately. The extracts were concentrated under reduced pressure of 22-26mm hg at  $45^\circ\text{C}$  using rotary evaporator in order to remove the extracting solvents [18].

### Larvicidal activity of *C. africanum* extracts

The larvicidal activity of *C. africanum* extract was assessed by using the standard method as prescribed by World Health Organization [19]. From the stock solution, four different test concentrations (100, 150 and 200  $\mu\text{l}$  with the control) were prepared and they were tested against third instar larvae of *An. Stephensi* based on its morphological features. Five larvae were introduced in 250ml plastic dishes cups containing 100 ml of rainwater and the required amount of plant extract was added. Controls were run simultaneously.

The larval mortality was observed and recorded after 24 and 48 h of post treatment. Larval percentage mortality was corrected using Abott formula [20]. For each experiment, three

replicates were maintained at a time. The average mortality data were subjected to probit analysis for calculating LD<sub>90</sub> [21].

### Statistical analysis

Data from all replicates were pooled for analysis. LC<sub>90</sub> values were calculated using SPSS software by probit analysis [22]. ANOVA was performed to determine the difference in larval mortality between concentrations. Results with  $P < 0.05$  level were considered to be statistically significant.

## Results

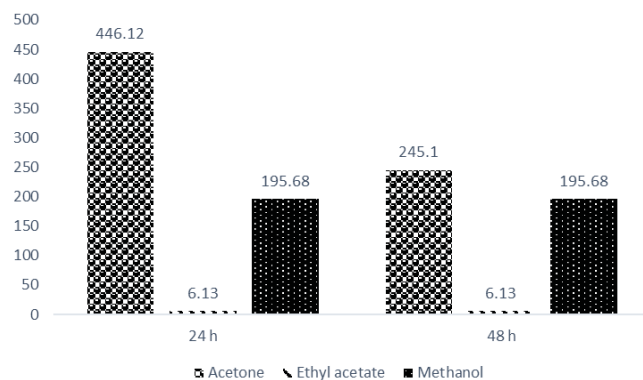
The seed extracts of *C. africanum* exhibited significant larvicidal activity in all the tested concentrations against *An. Stephensi*. Ethyl acetate extract evoked 90% larval mortality at all concentrations within 24 h of treatment, acetone and methanol extracts exhibited concentration and time of exposure dependent with the maximum mortality 33.85% and 51.93% recorded at 200 $\mu\text{l}$  respectively. Similar trend was observed 48 h after treatment (Table 1).

Lethal dose of the extracts was significantly influenced by the solvent used for the extraction. Results in figure 1 show that LD<sub>50</sub> was maximum with ethyl acetate extract at both 24 and 48 h exposure time (6.13), distantly followed by methanol extract (195.58).

**Table 1:** Larvicidal activity of *Chrysophyllum africanum* extracts against *An. Stephensi* larvae.

Exposure Time (hrs)	plant extract	Concentration ( $\mu\text{l}$ )		
		100	150	200
24	Acetone	21.93 $\pm$ 6.02b	28.77 $\pm$ 2.21c	33.85 $\pm$ 9.01ab
	Ethyl acetate	90.00 $\pm$ 0.00c	90.00 $\pm$ 0.00d	90.00 $\pm$ 0.00c
	Methanol	0.00 $\pm$ 0.00a	17.71 $\pm$ 4.81b	51.93 $\pm$ 19.38b
	Control	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
48	Acetone	25.78 $\pm$ 3.13b	45.00 $\pm$ 0.00c	33.85 $\pm$ 9.01ab
	Ethyl acetate	90.00 $\pm$ 0.00c	90.00 $\pm$ 0.00d	90.00 $\pm$ 0.00c
	Methanol	0.00 $\pm$ 0.00a	17.71 $\pm$ 4.81b	51.93 $\pm$ 19.38b
	Control	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a

Means with the same alphabet down the column are not significantly different using DNMRT at  $p > 0.05$



**Figure 1:** LD<sub>50</sub> ( $\mu\text{l}$ ) of *Chrysophyllum africanum* extract part at different time of exposure.

## Discussion

Mosquitoes are the oldest human enemy and controlling them is of prime importance in recent years because of the numerous diseases caused by them. Around the world, the medical and economic burden caused by vector-borne diseases continue to grow as current control measures fail. The results of the present study may contribute to a decline in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various medically important pests by botanical pesticides.

Results from the study clearly indicate that various *C. africanum* extracts significantly caused larvae mortality. This allude to the presence of phytochemicals, toxic to the survival of the larvae. The mortality recorded is directly proportional to increase in concentration level and period of exposure; also the larval mortality varies in relation to the extract used.

Extracts of the plants derived from specific solvents can influence the bioactivity, probably because of the active components present in large quantities [23]. It has been demonstrated that extraction of active biochemicals from plants confides upon the polarity of the solvents utilized. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules [24].

Ethyl acetate is a moderately polar solvent (polarity index of 4.4) that mainly extracts steroids, alkaloids, etc. Those biochemicals that were extracted using moderately polar solvents were seen to give good results as reported by a few bioassays. This corroborates with the findings of the present study. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species [25].

Plant extracts might have some complex mixture of biocidal active compounds, which may jointly or independently contribute to the mortality and delayed growth of larvae [26]. Rey *et al.* [27] and David *et al.* [28] found that botanical derivatives primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae.

## Conclusion

The results of this study indicate that *C. africanum* ethyl acetate extract has a promising effect to control mosquito larvae and may be an effective alternative to conventional synthetic insecticides. The ethyl acetate extract of this plant may be fractioned in order to locate the particular bioactive toxic agent responsible for larval toxicity.

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