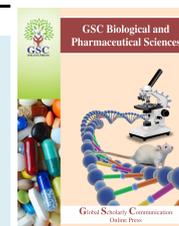


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(RESEARCH ARTICLE)



## Larvicidal activity of some tropical plants on the mortality of *Anopheles gambiae* s.l mosquitoes

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### Abstract

This study investigated the larvicidal activities of methanol leaf extracts of six tropical plants against *Anopheles gambiae* s.l. mosquitoes. 10 healthy laboratory stabilized larvae were treated with extracts of *Ocimum gratissimum*, *Chromolaena odorata*, *Terminalia catappa*, *Carica papaya*, *Vernonia amygdalina* and *Cymbopogon citratus* with different concentrations (0, 200, 400, 600, 800 and 1000 ppm) for 24, 48 and 72 hours after which the percentage mortality was calculated. All extracts tested were seen to possess moderate to good larvicidal effect against *An. gambiae* larvae in a concentration dependent manner with the highest mortality observed in *O. gratissimum* with 100%, *Cy. citratus* with 93%, *Ca. papaya* and *V. amygdalina* with 83%, *T. catappa* with 73% and the least being *Ch. odorata* with 63% at the end of the exposure period of 72 hours. These results showed that these plant extracts may be used as alternative insecticides against *An. gambiae* mosquitoes, with a further study on their phytochemicals, characterization and synergistic activities and their adaptability to field assay highly recommended.

**Keywords:** *Anopheles gambiae* s.l.; Mortality; Larvicidal; Tropical plants; Methanol extract.

### 1. Introduction

Insects, the most abundant and important group of organisms in the world found almost everywhere and able to thrive were others are absent transmit diseases that induce an enormous burden on the world's population [1], [2]. Mosquitoes, a group of insect transmit diseases than any other insect group and are well known vectors for most life threatening diseases affecting millions of people around the world such as malaria, Dengue fever, Lymphatic filariasis, Yellow fever, Japanese encephalitis, La Cross fever, Chikungunya fever, [3], [4], [5], [6], [7] and [8]. The genus *Anopheles* is the only vector for the transmission of malaria to man in the world especially in the tropics and subtropics, resulting in huge economic losses both in terms of health care cost and productivity [9]. According to World Health Organization [10], an estimate of 214million cases of malaria occurs resulting in deaths of 438,000 persons annually worldwide, of these 188 million new cases are shown to occur in Africa with an annual death of 395,000, accounting for 88% of the occurrence and deaths. It is more devastating in children and pregnant women and among the poor that do not have access to adequate prevention and treatment [11].

*Anopheles gambiae* Giles, is the most efficient vector of human malaria in the Afro tropical region because of the susceptibility to the *Plasmodium* parasite, preference for human host and their indoor feeding behaviour [12] and is commonly called the African malaria mosquito [13]. Interest in the control of *Anopheles* sp lies in the fact that it is the vector of malaria, which is the number one killer of children under 5years and pregnant women in tropical and subtropical regions [11], [10]; since there is no effective vaccine for malaria, the only effective approach to minimizing the incidence of this disease is to eradicate and control the vectors. The major tool has been synthetic insecticides, but the emergence of widespread insecticide resistance and the potential environmental issues associated with some of

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them (organochlorines and organophosphates) has necessitated the search for additional approaches in controlling the high spread of mosquito population [14], [5] and [8]. Botanicals have a wide spread insecticidal properties and are obviously suggestive of a new weapon in the arsenal of insecticides and in future may act as a suitable alternative product to fight against mosquito borne diseases [15], [9]. The secondary metabolites present in these botanicals constitute a defense system against insect pest attacks; their presence has been held responsible for the biological activity of plant extracts against target pests. The use of plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and the environment when compared to synthetic ones [16]. The efficacy of phytochemicals against the mosquito species have been shown to vary significantly depending on plant species, plant parts used, the nature of the plant (young, adult or senescent), a solvent used for extraction and the species of mosquito tested. These differential responses have been influenced by extrinsic and intrinsic factors such as the geographical location of the plant and mosquito species, plant parts used, extraction methodology adopted, the polarity of the solvent used during extraction [17-20], [5],[21-22] and [9]. The objective of this study was to determine the percentage larval effect and the effect of time of exposure and concentration of *O. gratissimum*, *Ch. odorata*, *T. catappa*, *Ca. papaya*, *V. amygdalina* and *Cy. citratus* on *An. gambiae* mosquito.

## 2. Material and methods

### 2.1. Study area

This study was conducted in the premises of University of Benin, Animal and Environmental Biology Department, Faculty of Life Sciences and the Department of Pharmacognosy, Faculty of Pharmacy.

### 2.2. Plant collection, preparation and extraction

Leaves of the test plants (Figure 1) were collected from within the University and Evbuomere community; the leaves were rinsed, shade dried, pulverized and extracted by maceration.



**Figure 1** The test plants (A) *Ocimum gratissimum* (B) *Chromolaena odorata* (C) *Terminalia catappa* (D) *Carica papaya* (E) *Vernonia amygdalina* (F) *Cymbopogon citratus*

### 2.3. Concentrate preparation

Stock solution was prepared by dissolving 1000 g in 100 ml distilled water, the test concentration (200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm) was prepared by serially diluting the stock according to the WHO protocol [23].

### 2.4. Mosquito collection and rearing

Larva forms were collected from rain pools within the University, transported to the laboratory, separated by instars into rearing pans, fed with yeast stabilized to produce adult which were fed with 10%w/v sugar solution and bloodfed with guinea pig; the rearing environment was  $27\pm 5^{\circ}\text{C}$  and 75-85%, the F2 generation were used for the assay.

## 2.5. Larval assay

Assay was carried out according to WHO, 2005 with slight modification, 10 healthy L3/L4 larvae were placed in each test bowl containing 100ml of distilled water with the appropriate concentration, mortality was observed in 24, 48 and 72 hours, respectively, the setup was made up of four replicates and a simultaneous control which contained 1ml of solvent and 100ml of water.

## 2.6. Data analysis

Data were analyzed using SPSS, version 21 and Microsoft office Excel 2007. Values less than 5% ( $P < 0.05$ ) were said to be statistically significant and PostHoc test determined by the Duncan Multiple Range Test.

## 3. Results

### 3.1. Mortality of *An. gambiae* larvae

The result of the determination of percentage mortality of larvae of *An. gambiae* exposed to methanol extract of the six plants is shown in Tables 1-6 and the trend of increase is observed in Figures 2-4. All the plants showed good to moderate larvicidal effect on the late third and early fourth instar of *An. gambiae*. At the end of 24 hours it was observed that none of the extract showed up to 90% mortality at 1000ppm while the least percentage was seen to be 25% at 200ppm from *Ch. odorata* and *Cy. citratus* extracts. At the end of 48 hours of exposure *O. gratissimum* showed 93% mortality while the others were less than 90%. At the end of 72 hours exposure only *O. gratissimum* registered 100% mortality; *Cy. citratus* registered 93% while the others showed less than 90% mortality rates.

**Table 1** Larvicidal effect of methanolic extract of the leaf of *O. gratissimum* on *An. gambiae* s.l larvae

Concn(ppm)	Larval mortality (Mean+CI)		
	24 hours	48 hours	72 hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	1.00 <sup>b</sup> (-0.30-2.30)	1.75 <sup>b</sup> (0.22-3.27)	2.75 <sup>b</sup> (1.95-3.55)
400	2.00 <sup>c</sup> (0.71-3.30)	3.00 <sup>b</sup> (0.75-5.25)	4.00 <sup>c</sup> (2.70-5.30)
600	3.00 <sup>d</sup> (1.21-4.30)	3.75 <sup>b</sup> (2.23-5.27)	5.00 <sup>c</sup> (2.70-6.30)
800	4.50 <sup>e</sup> (3.58-5.42)	6.50 <sup>c</sup> (3.74-9.26)	7.25 <sup>d</sup> (5.73-8.77)
1000	7.25 <sup>f</sup> (5.73-8.77)	9.25 <sup>d</sup> (9.73-10.77)	10.00 <sup>e</sup> (0.00-0.00)
F	50.67	34.64	117.04
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different ( $p > 0.05$ )

**Table 2** Larvicidal effect of methanolic extract of the leaf of *Ch. odorata* on *An. gambiae* s.l larvae

Concn(ppm)	Larval mortality (Mean+CI)		
	24 hours	48 hours	72 hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	0.50 <sup>a</sup> (0.42-1.42)	1.00 <sup>b</sup> (-0.30-2.30)	1.50 <sup>b</sup> (0.58-2.42)
400	1.00 <sup>b</sup> (0.30-3.0)	1.75 <sup>b</sup> (0.95-2.55)	2.75 <sup>c</sup> (1.95-3.55)
600	1.25 <sup>b</sup> (0.27-2.77)	2.25 <sup>c</sup> (1.45-0.46)	3.00 <sup>c</sup> (1.16-4.84)
800	2.25 <sup>c</sup> (0.73-3.77)	3.00 <sup>c</sup> (1.16-4.84)	5.00 <sup>d</sup> (3.16-6.84)
1000	2.50 <sup>c</sup> (0.45-4.55)	4.00 <sup>d</sup> (1.40-6.60)	6.25 <sup>d</sup> (3.86-8.64)
F	5.07	9.41	22.55
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different ( $p > 0.05$ )

**Table 3** Larvicidal effect of methanolic extract of the leaf of *T. catappa* on *An. gambiae* s.l larvae

Concn(ppm)	Larval mortality (Mean+CI)		
	24 hours	48 hours	72 hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	0.25 <sup>a</sup> (-0.55-1.05)	1.00 <sup>a</sup> (0.30-2.30)	1.50 <sup>b</sup> (0.58-2.42)
400	1.50 <sup>b</sup> (0.58-2.42)	2.75 <sup>b</sup> (1.23-4.27)	3.25 <sup>c</sup> (1.73-4.77)
600	2.00 <sup>c</sup> (0.70-3.30)	3.75 <sup>b</sup> (1.45-5.55)	3.50 <sup>c</sup> (1.45-5.55)
800	2.75 <sup>d</sup> (1.23-4.27)	5.26 <sup>c</sup> (3.73-6.77)	6.00 <sup>d</sup> (4.71-7.30)
1000	4.00 <sup>e</sup> (2.70-5.30)	5.75 <sup>c</sup> (4.23-7.27)	7.25 <sup>d</sup> (5.73-8.77)
F	19.48	24.48	39.02
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different (p>0.05)

**Table 4** Larvicidal effect of methanolic extract of the leaf of *Ca. papaya* on *An. gambiae* s.l larvae

Concn(ppm)	Larval mortality (Mean+CI)		
	24 hours	48 hours	72 hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	0.50 <sup>b</sup> (-0.42-1.42)	1.00 <sup>a</sup> (0.30-2.30)	1.50 <sup>a</sup> (0.55-3.53)
400	2.25 <sup>c</sup> (0.73-3.77)	3.75 <sup>b</sup> (1.75-5.75)	4.50 <sup>b</sup> (2.45-6.55)
600	2.50 <sup>c</sup> (0.99-40.91)	4.25 <sup>c</sup> (2.25-6.25)	5.50 <sup>b</sup> (3.45-7.55)
800	4.50 <sup>d</sup> (2.45-6.55)	5.50 <sup>d</sup> (3.45-7.55)	6.00 <sup>b</sup> (3.75-8.25)
1000	5.00 <sup>d</sup> (1.56-8.44)	6.00 <sup>d</sup> (3.75-8.25)	8.25 <sup>c</sup> (6.73-9.77)
F	11.49	18.77	28.07
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different (p>0.05)

**Table 5** Larvicidal effect of methanolic extract of the leaf of *V. amygdalina* on *An. gambiae* s.l larvae

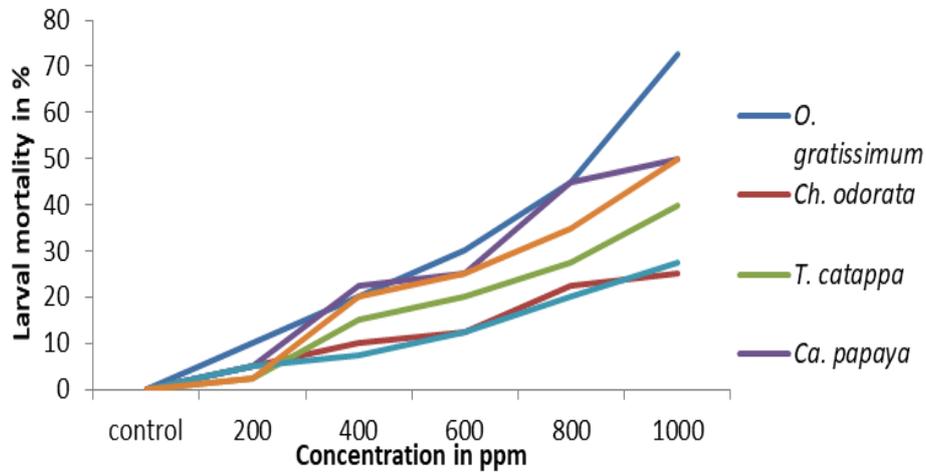
Concn(ppm)	Larval mortality (Mean+CI)		
	24hours	48hours	72hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	0.50 <sup>b</sup> (0.42-1.42)	1.00 <sup>a</sup> (-0.30-2.30)	1.75 <sup>b</sup> (0.95-2.55)
400	0.75 <sup>b</sup> (0.05-1.55)	2.00 <sup>b</sup> (0.71-3.30)	3.75 <sup>c</sup> (2.23-5.27)
600	1.25 <sup>c</sup> (0.45-2.05)	2.25 <sup>b</sup> (1.45-3.046)	4.75 <sup>d</sup> (3.23-6.27)
800	2.00 <sup>d</sup> (0.71-3.30)	3.00 <sup>c</sup> (1.71-4.30)	5.25 <sup>d</sup> (4.45-6.05)
1000	2.75 <sup>d</sup> (1.23-4.27)	4.00 <sup>d</sup> (2.71-5.30)	8.25 <sup>e</sup> (6.73-9.77)
F	10.28	16.54	61.00
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different (p>0.05)

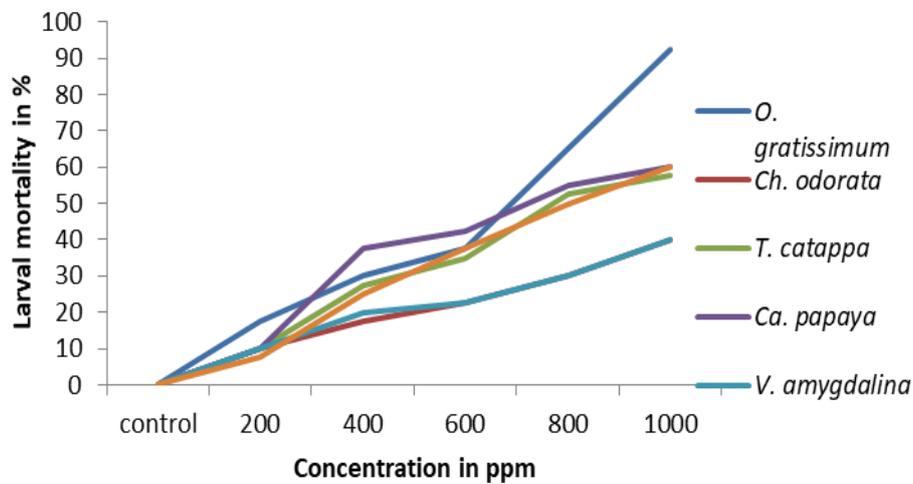
**Table 6** Larvicidal effect of methanolic extract of the leaf of *Cy. citratus* on *An. gambiae* s.l larvae

Concn(ppm)	Larval mortality (Mean+CI)		
	24 hours	48 hours	72 hours
Control	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)	0.00 <sup>a</sup> (0.00-0.00)
200	0.25 <sup>a</sup> (-0.55-1.05)	0.75 <sup>a</sup> (-0.05-1.55)	2.25 <sup>b</sup> (1.45-3.05)
400	2.00 <sup>b</sup> (0.71-3.30)	2.50 <sup>b</sup> (1.58-3.42)	4.50 <sup>c</sup> (3.58-5.42)
600	2.50 <sup>b</sup> (10.91-4.20)	3.75 <sup>c</sup> (2.23-5.27)	5.50 <sup>d</sup> (3.91-7.09)
800	3.50 <sup>c</sup> (1.45-5.55)	5.75 <sup>d</sup> (4.23-7.27)	7.00 <sup>e</sup> (5.71-8.30)
1000	5.00 <sup>d</sup> (3.72-6.30)	7.50 <sup>e</sup> (5.45-9.55)	9.25 <sup>f</sup> (8.45-10.05)
F	20.67	49.19	110.32
P-value	0.001	0.001	0.001

Mean (of four replicates) followed by same superscript in the same column are not significantly different (p>0.05)



**Figure 2:** Larval Mortality at 24hours



**Figure 3:** Larval Mortality at 48hours

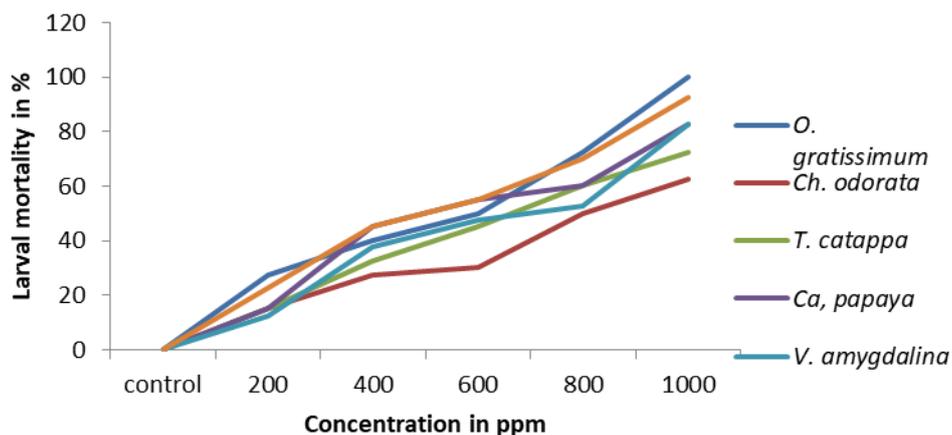


Figure 4: Larval Mortality at 72hours

### 3.2. Effect of Time of Exposure and Concentration on *An. gambiae*

The effect of the plant extracts and their interaction on the mortality of the larvae showed that there was statistically significant difference ( $P < 0.0005$ ) on the effect of time and concentration on the mortality from the six plant extracts on the larvae tested, viz:

Effect of time on *O. gratissimum*  $F_{2,71}=28.35185$ ,  $P = 3.8 \times 10^{-9}$ , *Ch. odorata*  $F_{2,71}= 24.1978$ ,  $P = 3.14 \times 10^{-8}$ , *T. catappa*  $F_{2,71}= 35.77852$ ,  $P = 1.28 \times 10^{-10}$ , *Ca. papaya*  $F_{2,71}= 16.80634$ ,  $P = 2.12 \times 10^{-6}$ , *V. amygdalina*  $F_{2,71}= 100.0777$ ,  $P = 6.87 \times 10^{-19}$  and *Cy. citratus*  $F_{2,71}= 66.6378$ ,  $P = 2.62 \times 10^{-15}$ .

Effect of concentration were *O. gratissimum*  $F_{5,71}=158.0296$ ,  $P = 5.93 \times 10^{-31}$ , *Ch. odorata*  $F_{5,71}=34.32527$ ,  $P = 1.3 \times 10^{-15}$ , *T. catappa*  $F_{5,71}=81.58792$ ,  $P=6.39 \times 10^{-24}$ , *Ca, papaya*  $F_{5,71}= 57.41404$ ,  $P = 2.15 \times 10^{-20}$ , *V. amygdalina*  $F_{5,71}=77.83107$ ,  $P = 1.95 \times 10^{-23}$  *Cy. citratus*  $F_{5,71}= 144.0803$ ,  $P = 6.03 \times 10^{-30}$ .

From the interaction of time and concentration all except *O. gratissimum*;  $P = 0.1262$  and *Ca. papaya*;  $P = 0.302765$  showed significant difference ( $P < 0.05$ ). The mortality rates were seen to increase steadily or nearly steadily according to the concentration and the exposure time of the extracts. The mean mortality of all the extracts showed highly significant effects, with the highest concentration having the highest values, thus depicting a dose dependent effect.

## 4. Discussion

The findings of this study showed that *O. gratissimum* was most effective being able to kill all test organisms at the end of exposure period, the percentage mortality of the different extract varied with time and concentration, there was no observed mortality in the control hence the solvent had no effect on the larvae and the conditions for the assay were according to the preferred standards, the effect of the plant extract increased with increasing concentration, showing a dose dependent mortality pattern, which agreed with those of [24], [25], [26],[27],[28], [29] and [7].

At the different time of exposure larvicidal effect of *O. gratissimum* was highest and followed a progressive trend being the only extract to register 100% mortality at the highest concentration of 1000ppm. *Cy. citratus* followed closely with 93% mortality at the end of the exposure time; followed by *Ca. papaya* and *V. amygdalina* with 83% each, followed by *T. catappa* with 73% and the least was *Ch. odorata* at 62.5%, this agrees with reports from [30] and [7] that *Ch. odorata* is not a very potent extract against vector mosquitoes.

## 5. Conclusion

The result from this study showed that the tested plant extracts were effective against the larvae and could serve as potential candidates against *Anopheles gambiae* mosquitoes for use as an alternative insecticides in Nigeria and worldwide. However, further studies on their phytochemicals, characterization and synergistic effects on the environment is suggested.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors wish to state that there is no conflict of interest between them or any other party.

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