



Activities of Crude, Acetone and Ethanolic Extracts of *Capsicum frutescens* var. *minima* Fruit Against Larva of *Anopheles gambiae*

Sylvester Chibueze Izah*

Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria

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Abstract

This study evaluated the activities of crude, acetone and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against *Anopheles gambiae* larva. The bioassay was carried out for 24 hours using *Anopheles gambiae* larva obtained from the wild. The *Anopheles gambiae* was identified following standard protocol. Results showed that the mortality rate increased statistically at $p < 0.05$ as the concentration of the plant extracts increased. The ethanolic, acetone and crude extracts had LC_{50} value of 115.24 ppm, 173.16 ppm and 265.19 ppm respectively, being apparently different. The efficacy of the *Capsicum frutescens* var. *minima* fruit were in the order aqueous < acetone < ethanol. Based on the findings of this study, there is the need for research to focus on the isolation and purification of the exact bioactive ingredients that enables *Capsicum frutescens* var. *minima* fruit confers insecticidal potentials.

Keywords: *Capsicum frutescens*, Malaria, Medicinal plants, Solvents, Vector borne disease

1 Introduction

Mosquito, a protozoan, is known to transmit vector-borne disease such as malaria, filariasis, yellow fever, dengue fever, encephalitis especially in the tropical countries [1-3]. According to Dash *et al.* [4], Ndiok *et al.* [3], mosquito belong to the Diptera Order and Culicidae Family which consist of three sub-families viz: *Anophelinae* (in this subfamily, the genus *Anopheles* is the most essential mosquito which has several species), *Culicinae* (consist of several genera including *Aedes*, *Coquillettia*, *Culex*, *Culiseta*, *Orthopodomyia*, *Psorophora*, etc) and *Toxorhynchitinae* (consisting of only one genus *Toxorhynchites* which occurs mainly in the tropics). Several species of mosquito abound in different region of the world. Studies have suggested that there are about 3,500 species of mosquitoes in the various region of the world which are distributed into several genera within each sub-families [1-5]. Among these sub-families some of the genera are not common such as *Orthopodomyia*, *Psorophora*, *Uranotaenia*, *Coquillettia*, *Orthopodomyia* and *Uranotaenia* in many part of the world compared to the genera such as *Aedes*, *Culex*, *Anopheles* and *Mansonia*.

These four genera are known to transmit some vital diseases that affect humans and other animals [1].

In many regions that malaria is endemic the genus *Anopheles* is the most important iniquitous dipteran fly that transmits the disease. Though, other mosquito such *Aedes aegypti* transmits chikungunya, yellow and dengue fevers; and *Culex quinquefasciatus* transmits lymphatic filariasis [1]. Malaria is transmitted through the bit of female *Anopheles* mosquito comprising of *Anopheles gambiae*, *Anopheles funestus*, *Anopheles arabiensis* and *Anopheles melas* [6]. Of these species, *Anopheles gambiae* and *Anopheles arabiensis* are the dominant malaria vectors in the sub-Saharan Africa [1, 7, 8].

For thousands of years, mosquitoes have co-existed with humans [9]. These mosquitoes in the tropical region are found in dirty environments (viz: stagnant water, slow flowing water, flowing water with several blockage and sand) [1]. Mosquito disturbs human during sleep through their noise, blood sucking and biting. The nuisance they constitute varies according to climate and weather conditions. Under Nigerian condition, mosquito biting is more intense between 6 to 7 a.m and optimal between 10 pm and 4 am [3, 10].

Iniquitous dipteran fly (mosquito) transmits diseases to a significant number of world population in different regions including Africa, South America, Central America, Mexico and Asia causing millions of deaths per annum. WHO [11] reported that of all disease-transmitting insects, mosquito causes the greatest menace, spreading malaria,

*Corresponding author: Sylvester Chibueze Izah, Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa state, Nigeria. E-mail: chivestizah@gmail.com.

dengue and yellow fever, which together are responsible for several million deaths and hundreds of millions of cases per annum. Other studies have indicated that malaria endemic in about 109 nations, infecting approximately 190-330 million people and causing about 1 million deaths annually [3, 7]. In a related study, WHO [11] reported that malaria is endemic in 91 countries, with about 40% of the global population at risk of infection, and up to 500 million cases occurs per annum. Of these 90% occurs in Africa. In addition, Nigeria Malaria Fact Sheet [12] reported that in 2010 about 216 million cases of malaria infection was reported, and of these, 81% occurred in Africa. This suggests that the global malaria burden is significantly higher in Africa. Studies have indicated that over 80% and 78% of cases and deaths respectively resulting from malaria infections occurs in 15 nations [13]. In addition, Nigeria Malaria Fact Sheet [12] reported that approximately 50% of global malaria burden occurs in Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda.

Probably due to the effects of these *Anopheles* mosquitos to human, research on their control have increased. Several synthetic based chemicals are used for the control of mosquito at their different developmental stages including eggs, larva, pupa and adult. This chemical has been reported to affect non target organisms. As such, the use of natural products has been advocated, and to this effect several plants has been studied.

Several plants have been reported to be effective for the control of larva of *Anopheles gambiae* including *Alstonia boonei* [14], *Anacardium occidentale* [15], *Annona senegalensis* [16], *Cassia mimosoides* [17], *Curcuma longa* [18], *Hyptis suaveolens* [19], *Datura stramonium* [7], *Spondias mombin* [20], *Xylopia aethiopica* [15]. Several plants parts (roots, stem, fruit and leaves) have been reported to have effective activities at varying concentration for the control of *Anopheles gambiae* larva. This could be due to the pharmacological potentials of these plants. Authors have reported that medicinal plants as plants in which one or more parts have therapeutic properties [21-31]. Again the choice of extraction solvent has shown to influence the mortality rate. Some of the commonly reported solvent for extracting plants includes ethanol, methanol, chloroform, n-hexane, dichloromethane, ethyl-Acetate, acetone, and petroleum ether and water. Therefore, this present study aimed at assessing the activities of crude, acetone and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against *Anopheles gambiae* larva.

2 Materials and Methods

2.1 Plant Collection and preparation

The fruit of *Capsicum frutescens* var. *minima* was obtained from a smallholder farmer in Ndemili, Delta state, Nigeria. The pepper was shade dried at room temperature before blending into powder with electronic blender.

2.2 Plant extracts

The dried powdered *Capsicum frutescens* var. *minima* were extracted by weighing 500g into 1000ml of the solvent (acetone, ethanol and water). The dried pepper was

soaked for 48 hours. Then after, the mixture was filtered using double layered muslin cloth. The resultant filtrate was concentrated using rotatory evaporator. The solid residue was reconstituted with distilled water to varying concentrations.

2.3 Culture of *Anopheles gambiae*

The larva of *Anopheles gambiae* used for this study was collected from the wild with the aid of baits in a plastic container and condemned tyre half filled with water, cotton wool and debris. The larva was obtained with aid of syringe without the needle section. Some larva of the mosquitoes used for study was allowed to develop into adult and identified using microscope. The resultant characteristics were compared with the ones presented by Gimba and Idris [32], Ahmed and Ahmed [33]. The abdomen is without laterally projecting tufts of scales. The generally scaling on the abdomen was scanty. There are speckles on the legs with tarsi 1-4 having conspicuous pale bands on the apices. There is third preapical dark area on vein 1 with a pale interruption. The larva was fed with biscuit and yeast at a ratio of 3:1 at room temperature ($27 \pm 3^\circ\text{C}$).

2.4 Larvicidal bioassay

The larvicidal bioassay was carried out based on the scheme of WHO [34] cited by Rathy et al. [35]. A total of 20 larvae were introduced into each of the experimental group containing 250ml of de-chlorinated and the plant extracts at 50, 100, 125, 150, 200 and 250 ppm. The mortality rate was determined at 24 hours. The larvae were considered dead if they settled and remain motionless or respond to repeated prodding with a soft brush. Then the percentage mortality was calculated.

2.5 Statistical Analysis

SPSS was used to carried out the mean, standard error, one way analysis of variance at $p=0.05$ and Duncan statistics (used to separate the means). The LC_{50} was calculated though probit analysis with the use of Finney's Table [36]. Regression analysis was carried out on the probit value against log concentration using Microsoft excel window 2010. The resultant equation from the chart was substituted with probit value of 5 and the anti-logarithm of the substituted equation values was taken as the LC_{50} .

3 Results and Discussion

Table 1 present Percentage concentration-mortality of *Anopheles gambiae* larva exposed to crude, acetone and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit for 24 hours. At 50 and 250 ppm of the extracts, the percentage mortality was 20.00% and 86.67%, respectively for ethanolic extracts; 10.00% and 76.67%, respectively for acetone extracts; and 5.00% and 55.00%, respectively for crude extracts. There was significant variations ($p<0.05$) among the concentration for each of the extracts. The mortality rate increased as the concentration of the extract increased. This occurred in all the extracts. Figure 1 shows the percentage mortality of *Anopheles gambiae* larva exposed to crude, acetone and ethanolic extracts of

Capsicum frutescens var. *minima* fruit at varying concentrations for 24 hours. The percentage mortality was significantly higher in for order ethanol > acetone > crude extracts for each of the concentrations. This trend is in consonance with previous works; where authors reported that different solvents have varying mortality rate on mosquito larva [37, 38].

The LC₅₀ values are presented in Figure 2 - 4. The ethanolic, acetone and crude extracts had LC₅₀ of 115.24ppm (Figure 2), 173.16ppm (Figure 3) and 265.19ppm (Figure 4) respectively.

Table 1: Percentage mortality of *Anopheles gambiae* larva exposed to crude, acetone and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit for 24 hours

Concentration, ppm	Ethanol	Acetone	Crude
50.00	20.00±5.77a	10.00±2.89a	5.00±2.89a
100.00	40.00±5.77b	23.33±3.33b	15.00±2.89a
150.00	46.67±1.67b	40.00±2.89c	31.67±4.41b
200.00	75.00±5.00c	53.33±1.67d	40.00±2.89b
250.00	86.67±4.41c	76.67±4.41e	55.00±2.89c

Data were expressed as mean± standard error; Different letters (a, b, c, d, e) along the column indicate significant difference at p<0.05 according to Duncan statistics

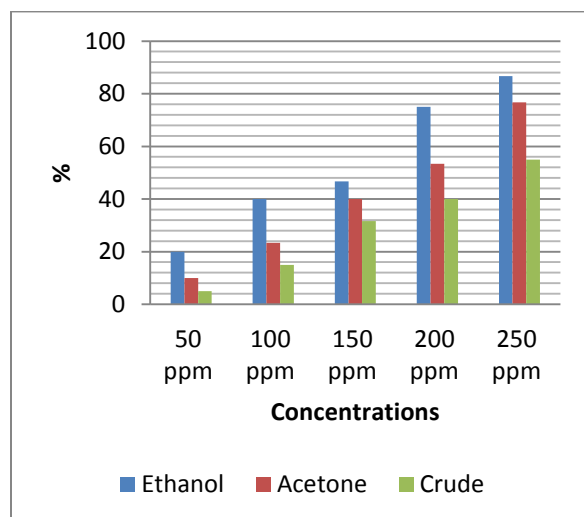


Figure 1: Percentage mortality of *Anopheles gambiae* larva exposed to crude, acetone and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit at varying concentrations for 24 hours.

The apparent variation could be associated to mortality and chemical composition of the solvents used. Previously studies have indicated that ethanol is superior solvent when compared to water [28-30]. In addition the mortality induced by the *Capsicum frutescens* var. *minima* fruit suggests its pharmacological properties probably due to the present of bioactive constituents. Studies have indicated that that some varieties of *Capsicum frutescens* fruit contain alkaloids, tannins, steroids, glycosides, saponins, flavonoids, phenol, carbohydrate, protein, reducing sugar and capsaicin [39 – 41]. Agu and Thomas [42] reported that the presence of alkaloids could account for the ability of some plant extracts to wade of pest.

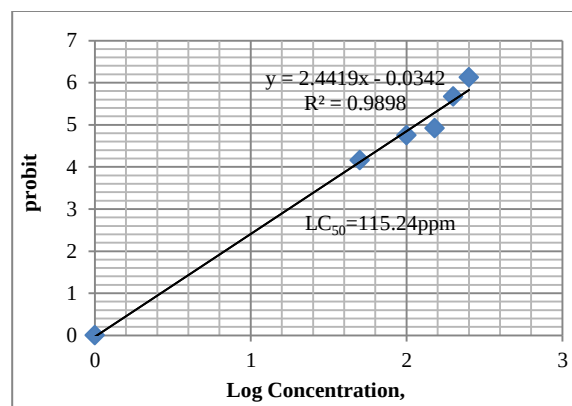


Figure 2: LC₅₀ value of *Anopheles gambiae* larva exposed to ethanolic extracts of *Capsicum frutescens* var. *minima* fruit for 24 hours

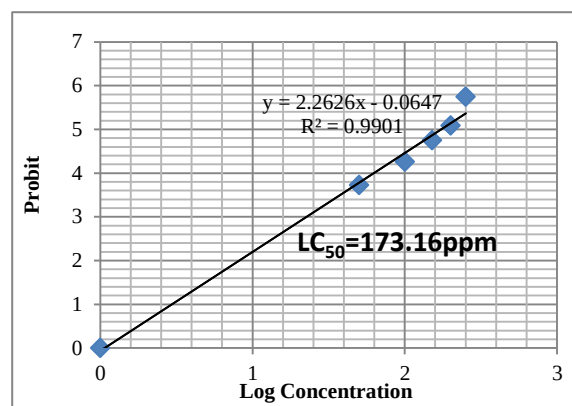


Figure 3: LC₅₀ value of *Anopheles gambiae* larva exposed to acetone extracts of *Capsicum frutescens* var. *minima* fruit for 24 hours

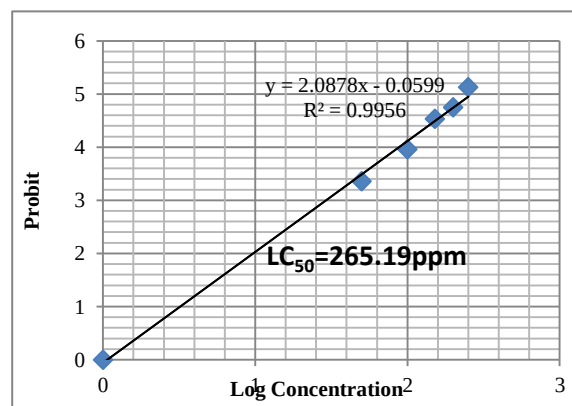


Figure 4: LC₅₀ value of *Anopheles gambiae* larva exposed to crude extracts of *Capsicum frutescens* var. *minima* fruit for 24 hours

Author have reported that chilli pepper contain Capsaicin which accounts for about 50 to 70% of the total capsaicinoids, which is responsible for its pungency [41]. Reyes-Escogido [28] reported that capsaicin is the main

capsaicinoid in chili pepper followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydro-capsaicin and homocapsaicin. Furthermore, capsaicin and dihydrocapsaicin made up of about 90% of capsaicinoids in fruits of chili pepper fruit [28]. Dadji et al. [22] also reported that capsaicinoids, such as capsaicin and dihydrocapsaicin accounts for 77 - 90% of secondary compounds found in pepper.

Barbehenn and Martin [43] reported that secondary metabolites such as polyphenols found in plants confers detrimental effects on the midgut epithelial barrier of Lepidoptera and Orthoptera larvae. Studies have also indicated that substances containing phenol have ovocidal and insecticidal properties against some species of insects [22, 44].

Polyphenol from plants have shown to confers insecticidal activity [45,46]. This suggest that the mortality observed in this study could be due to ingestion of phenolic compound associated with the pepper thereby causing toxicity which could have led to the death of the mosquitoes larva. *Capsicum frutescens* have been recognized as a plant that has therapeutic potentials in many regions of the world including its insecticidal capability [41].

The LC₅₀ values obtained in this study showed slight variation with the work of Alvarez et al. [38] that reported LC₅₀ value of *Capsicum frutescens* fruit as 231.59ppm and 300ppm for *Aedes aegypti* and *Aedes albopictus*, respectively using crude extract and 97.22ppm and 41.74 ppm for *Aedes aegypti* and *Aedes albopictus*, respectively using ethyl acetate fraction. Furthermore, Eze et al. [20] reported LC₅₀ value of 257.36 for *Anopheles gambiae* using acetone leave extract of *Spondias mombin* for 24 hours. Ivoke et al. [19] reported LC₅₀ value of 80.02 for *Anopheles gambiae* using aqueous leave extract of *Hyptis suaveolens*. The variations could be due to differences in the mosquito species and the extraction processes.

4 Conclusion

Anopheles mosquito is the vector of malaria parasite. Several mosquito species occurs, but among them *Anopheles gambiae* is the most frequently encountered in malaria endemic region like Nigeria. The control of mosquitoes is mainly carried out using chemical based insecticides which have been reported to have adverse environmental effects. Hence, the need for the development of sustainable alternative. Plants have emerged as a potential substitute to the chemical based insecticides. This study evaluated the activities of crude, ethanolic and acetone extracts of *Capsicum frutescens* var. *minima* fruit against larva of *Anopheles gambiae*. The study found that the mortality rate increased as the concentration increased. The study also found that the various extracts exhibited larvicidal potential against *Anopheles gambiae* in the order; ethanol > acetone > crude extracts. Hence there is the need for research to focus on the isolation and purification of the exact bioactive ingredients found in *Capsicum frutescens* var. *minima* fruit that enable it confers insecticidal potentials.

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