

Research

Larvicidal efficacy of aqueous extracts of *Zingiber officinale* Roscoe (ginger) against malaria vector, *Anopheles gambiae* (Diptera: Culicidae)

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Abstract

This study investigated the larvicidal efficacy of aqueous extracts of ginger, *Zingiber officinale* Roscoe against *Anopheles gambiae*, a malaria vector. The larvae of *Anopheles gambiae* used were obtained from the wild and the bioassay was carried for 24 hours. Results showed that mortality increased statistically ($p < 0.05$) as the concentration of the ginger extract increased. The cold water extracts, hot water extracts and boiled extracts had LC₅₀ value of 316.96 ppm, 176.20 ppm and 136.14 ppm respectively. The activities of the aqueous extracts were in the order; boiled extracts > hot water extracts > cold water extracts. From the findings of this study there is the need for study to focus on isolation and purification of exact bioactive compound that makes the plant confer larvicidal activity for possible development.

Keywords: Extracts; Malaria; Plants Vector Borne Disease; *Zingiber officinale*

Introduction

Plants have emerged as a credible alternative to many human health problems. Utilization of plant resources for human benefits can be traced back to the time immemorial. Weyder [1] reported that ginger has been used against stomach aches, nausea, and diarrhea far back fourth century BC. Till date plants have been recognized to play essential role in the treatment of several ailments. Authors have reported that about 70-80% of the world population rely on herbs for the treatment of several diseases especially in many rural regions in developing nations [2,3,4,5,6,7,8,9,10,11 12]. In addition, many of these plants are used as spices and food.

The distribution of plant resources depends of the climatic and soil requirements as well as the genetic composition of the individual plants. Some plant species have the capacity to grow in adverse environmental conditions while several others do not. This could be the reason why plants are diversely distributed in different

regions of the world. For instance, *Piptadeniastrum africanum* (Hook f) Brenan which belongs to the subfamily Mimosoideae are endemic to the tropical Africa. It's found in countries such as Central African Republic, Gabon, Ghana, Liberia, Nigeria, among others. But *Cathormion altissimum* (Hook f) Hutch. & Dandy which also belong to the Fabaceae family and subfamily Mimosoideae is the only representative of the genus *Cathormion* in Nigeria. It's also found in other tropical Africa countries such as Zambia, Sierra Leone, Sudan, Uganda, Cameroun etc and in tropical America [13].

In many regions of the world, several plants have been widely studied for possible use for the control of insects. Some of these plants have shown promising results. As such, bio-products from pyrethrum and neem have been well established for the control of insects [14]. Several other researchers have been documented in literature the potentials of plants for the control of insects such as mosquitoes [15,16].

Zingiber officinale Roscoe which belongs to the Zingiberaceae (commonly referred to as ginger) is a flowering herbaceous perennial plant whose roots (rhizomes) are used as spices and medicine against varying forms of diseases. Scientific studies have validated its anti-inflammatory [17,18,19], antimicrobial [20], anti-diabetic,

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hypolipideamic [21], antioxidants [22], antithrombotic[18] and larvicidal activities against dengue and filariasis vectors [23]. Ginger contain some chemicals that are responsible for their pharmacological properties. Weyder [1] that ginger contain many volatile oils (sesquiterpenes) and aromatic ketones (gingerols). The authors further reported that the gingerols could be the most active pharmacological constituents found in ginger. Similarly Kaur et al. [24], reported that the phytochemical constituents of ginger have a protective role against gastric ailments and irritations including ulcers, vomiting, nausea, dyspepsia, stomach ache, spasm, and gastrointestinal cancer.

Mosquito, an important iniquitous dipteran fly has the tendency to transmit several diseases including chikungunya, yellow, lymphatic filariasis, malaria and dengue fevers depending on the species of the mosquito. In tropical countries like Nigeria, Democratic Republic of the Congo, Mozambique and Uganda that malaria is endemic, the presence of *Anopheles* mosquito is high. Several species of *Anopheles* have been implicated in the transmission of the malaria parasite. The most common species are *Anopheles gambiae* and *Anopheles arabiensis* [25,26,16].

Worldwide, mosquito is mostly controlled using chemical based insecticides. But some of these chemical based insecticides have adverse effects on the environmental quality as well as on other non-target organisms. Furthermore, there is an indication that some mosquito species are beginning to develop resistance to some common insecticides. As such several studies have been carried out on the control of mosquito using bio-products especially plants [16]. Therefore, this study aimed at controlling the larvae of *Anopheles gambiae* mosquitoes using aqueous extracts of ginger, *Zingiber officinale*.

Materials and Methods

Plant Collection and Extraction

Ginger rhizome was purchased from Etegwe junction market in Yenagoa metropolis, Bayelsa state, Nigeria. The ginger rhizome was cut into pieces and then crushed using pestle and mortar and subsequently grinded into fine constituent using an electronic blender. Forty (40) g of the blended ginger was soaked in 200 ml of cold water (water at room temperature), hot water, and the same quantity was boiled in 200 ml of water as well. The boiled sample was allowed to cold at room temperature, and after 24 hours the mixtures were filtered using double muslin cloth. The filtrate was reconstituted with distilled water to different concentration.

Culture of *Anopheles Gambiae*

The larvae of *Anopheles gambiae* used for this study were obtained from wild with the use of baits in a plastic container and abandoned

tyre half filled with water, cotton wool and debris [15]. The water in the containers used to trap the larvae was removed and the larvae were transferred to the experimental set-up. Some of the *Anopheles gambiae* larvae were allowed to develop into adult and they were identified microscopically. The morphological characteristics were compared with the ones previously presented by [27,28]. The *Anopheles gambiae* larvae were fed with biscuit and yeast at a ratio of 3:1 at room temperature (27 ± 3 °C) [15].

Larvicidal Bioassay

The larvicidal efficacy of aqueous extracts of ginger, against *Anopheles gambiae* was carried out based on the experimental protocol of [29] cited by [30]. Varying concentrations of the cold water, hot water and boiled ginger extracts were made viz: 0, 50, 100, 150, 200 and 250 ppm with de-chlorinated. In each container of the experimental set-up (concentrations), 20 *Anopheles gambiae* larvae were added. After 24 hours, the possible effect of the extracts on the larvae was observed. The mortality rate was evaluated when the did not respond to repeated prodding with a soft brush. The percentage mortality was calculated based on the method previously used by [15].

Statistical Analysis

SPSS version 20 was used for mean, standard error and Analysis of variance analysis. The percentage mortality was expressed as mean \pm standard error. One way analysis of variance was used to show significant difference at $\alpha=0.05$. Duncan statistics was used to show the observed variations between the means. The LC50 value was calculated based on probit analysis using Finney's Table [31] and regression analysis in Microsoft excel as previously applied by [15].

Results and Discussion

The percentage -mortality of *Anopheles gambiae* exposed to cold water extracts, hot water extracts and boiled water extracts of ginger for 24 hours is presented in Table 1. At 50, 100, 150, 200 and 250 ppm the mortality was 10.00%, 13.33%, 18.33%, 38.33% and 45.00%, respectively (for cold water extracts), 15.00, 26.67%, 41.67%, 50.00% and 66.67%, respectively (for hot water extracts); and 25.00%, 38.33%, 46.67%, 56.67% and 76.67%, respectively (for boiled extracts). Statistically there was significant difference ($p<0.05$) among the concentrations for each of the extracts. However, multiple comparison showed that no significant difference ($p>0.05$) between 100ppm and 150ppm, and between 200 and 250ppm (for cold water extract), between 150 and 200ppm (for hot water extract), and 100 and 150ppm (for boiled extracts). The mortality rate increased as the concentration of the extract increased. This trend is in consonance with the findings of [15,32]. In addition, the degree of mortality were in the order; boiled extract > hot water extract > cold water extract.

This suggests that during boiling had a greater effect of leaching out active ingredients from the ginger. Studies have widely reported that extracts using different solvents have varying effects on test organisms [12,10,11,15].

The LC50 values are presented in Figure 1 - 3. The cold water extracts, hot water extracts and boiled extracts had LC50 of 316.96 ppm (Figure 1), 176.20 ppm (Figure 2) and 136 ppm (Figure 3) respectively. The apparent variation observed suggest heat has influence in determining the bioactive ingredients found in ginger. The findings of this study are higher than values of previous works Iqbal et al. [32] reported LC50 value of 7.48% and 0.55% at 24 and 48 hours respectively for the larvicidal efficacy of ginger against *Culex quinquefasciatus*. Kalu et al. [33] reported LC₅₀ values of activities of ethanolic extracts of *Allium sativum* (garlic bulb) against second, third and fourth larval instars of *Culex quinquefasciatus* as 144.54 ppm, 165.70 ppm and 184.18 ppm respectively at 24 hours. Rabha et al. [34] reported LC₅₀ of 15.8 (%v/v) and 21.8 (%v/v) of aqueous essential oil of ginger against larvae of *Aedes albopictus* and *Culex quinquefasciatus*. The variation could be due to age of the plant, plant parts, solvent used for extraction and even geographical origin of the plant materials. Again, moisture content of the ginger could also account for the observed variation.

The mortality observed suggests the presence of bioactive ingredients that are associated with ability to kill pests. Typically ginger have been reported to possess Borneol, β-Bisabolene, Cineole, α-Cedrene, α-Curcumene, β-Farnesene (E), β-Sesquihelladiene, β-Thujene and Zingiberene, and lemon grass contain Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-myrcene and β-Thujene in their essential oil [34]. Weyder [1]. reported that ginger contains many volatile oils (sesquiterpenes) and aromatic ketones (gingerols). Similarly Kaur et al. [24] reported that the phytochemical constituents of ginger including gingerols, zingerone, shogaols, and paradols. Of these gingerols could be the most active pharmacological active constituents [1]. In addition, authors have reported the presence of flavonoids, tannins, alkaloids, saponins, triterpenes and glycoside in ginger as the most pharmacological active constituent [35,36]. The presence of alkaloids could account for the ability of plants to ward off pests [37,15 2,3, 12].

Based on the findings of this study it can be deduced that plant extracts including ginger has the tendency to cause an alteration in the development stages of mosquito [16]. Sharma et al. [38], Iqbal et al. [32], Al-Mekhlafi [39] reported that phytoextracts can cause alterations to the midgut epithelium in mosquito vectors.

Table 1: Larvicidal efficacy of aqueous extracts of ginger, against *Anopheles gambiae* at 24 hours

Concentration, ppm	Cold water extract	Hot water extract	Boiled extract
0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a
50.00	10.00±2.89b	15.00±2.89b	25.00±2.89b
100.00	13.33±1.67bc	26.67±4.41c	38.33±1.67c
150.00	18.33±1.67c	41.67±1.67d	46.67±1.67c
200.00	38.33±1.67d	50.00±2.89d	56.67±1.67d
250.00	45.00±2.89d	66.67±4.41e	76.67±4.41e

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

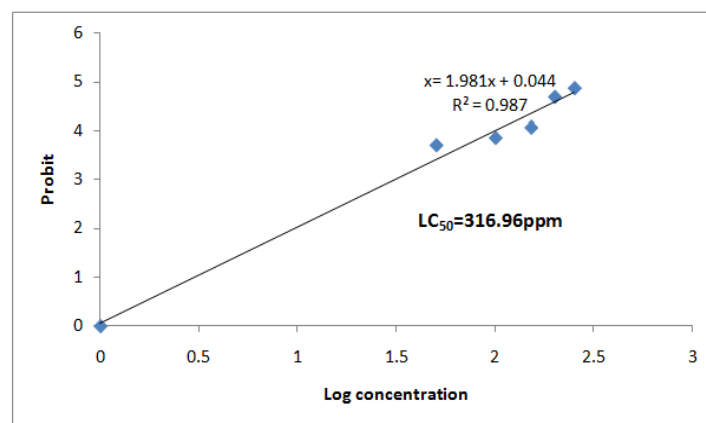


Figure 1. LC₅₀ value of *Anopheles gambiae* larva exposed to cold water extracts of ginger for 24 hours

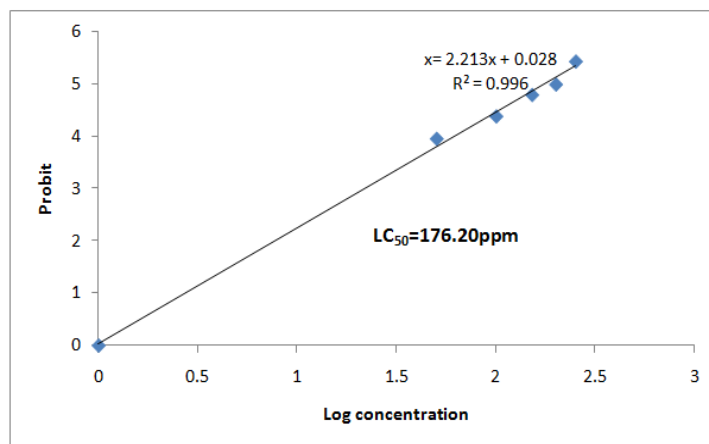


Figure 2. LC_{50} value of *Anopheles gambiae* larva exposed to hot water extracts of ginger for 24 hours

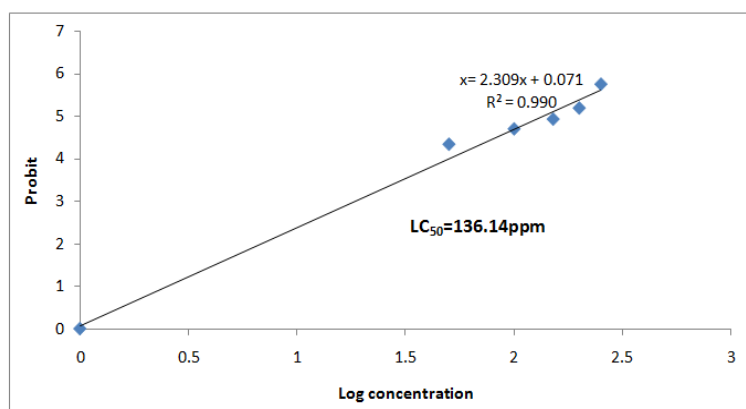


Figure 3. LC_{50} value of *Anopheles gambiae* larva exposed to boiled extracts of ginger for 24 hours

Conclusion

This study evaluated the larvicidal activities of aqueous extracts of ginger against *Anopheles gambiae*. The study found that ginger has larvicidal activity which varies in the order; cold water extract < hot water extracts < boiled extracts. Based on the findings of this study there is the need for study to focus on isolation and purification of exact bioactive compound that makes the plant confer larvicidal activity for possible development. Furthermore, there is the need to also try the extracts on the breeding ground of *Anopheles gambiae*.

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