BAOJ Biotechnology

Izah and Youkparigha, BAOJ Biotech 2019, 5: 1
5: 040

Research Article

Larvicidal Activity of Fresh Aqueous And Ethanolic Extracts of *Cymbopogon citratus* (DC) Stapf on Malaria Vector, *Anopheles gambiae*

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Abstract

Mosquitoes transmit several diseases including chikungunya, lymphatic filariasis, Japanese encephalitis, malaria and dengue fever. There is growing concerns on the use of chemical based insecticides on the environment, and resistance developed by some mosquito species. Hence research into search for suitable alternative has increased and plants have emerged as a potential substitute. Plants have shown to possess several metabolites and bioactive compounds such as alkaloids, saponins, tannins, phenol, flavonoids, glycoside, terpenes, etc. These compounds may be responsible for the diverse pharmacological potentials of *Cymbopogon citratus* (DC) Stapf. This study assessed the larvicidal activity of aqueous and ethanolic extracts of lemon grass, *C. citratus on malaria* vector, *Anopheles gambiae*. The bioassay was carried out following standard methods using larvae of *A. gambiae* obtained from the wild. The mortality rate at concentrations of 50, 100, 150, 200 and 250 ppm was 23.33%, 36.67%, 48.33%, 63.33% and 71.67%, respectively (aqueous extracts), and 23.33%, 50.00%, 55.00%, 76.67% and 85.00%, respectively (ethanolic extracts). Analysis of variance showed that there were significant variations at $p<0.05$ for each of the extracts under study. The mortality rate increased as the concentration of the extracts increased. The findings showed that ethanolic extracts had superior activity compared to the aqueous extract with LC50 value of 104.47 ppm and 161.06 ppm, respectively. The mortality rate showed a significant increase as the concentration of the extracts increased. The study showed that ethanolic extracts of *C. citratus* is more potent against larvae of *A. gambiae*. Based on the results of this study, there is the need for research to focus on isolation and purification of compounds that enable the plant confer larvicidal efficacy, and possibly field trial.

Keywords: *Cymbopogon citratus*; Malaria; Vector Borne Disease; Vector Control

Introduction

Mosquitoes, an important iniquitous dipteran flies, are vectors of etiologic agents of many dreaded diseases such as chikungunya, lymphatic filariasis, Japanese encephalitis, malaria and dengue fever [1,2,3,4,5,6]. There have been many attempts to eradicate and/or suppress the mosquito population in many parts of the world through the use of insecticides. Mosquitoes carry out some of their life cycles (oval, larval and pupal) in stagnant and/ or low flowing freshwater. As such, the need for improved sanitation cannot be overemphasized. But due to poor sanitation system in many developing countries and the cosmopolitan nature of the fly in many regions of the world(such as Nigeria, Uganda, Mozambique etc), its control through elimination of breeding areas is quite difficult.

Malaria exists in many regions of the world; but the incidence rates vary from one geographical location to another [5,2]. According to *World malaria report*, malaria cases have been on the increasing trend from 217 million cases in 2016 to 219 million cases in 2017 with about 435 000 deaths arising from malaria [7]. Most of the global

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Sub Date: March 23rd 2019, **Acc Date:** March 31st 2019, **Pub Date:** April 3rd 2019

Citation: Izah SC, Youkparigha FO (2019) Larvicidal Activity of Fresh Aqueous And Ethanolic Extracts of *Cymbopogon citratus (DC) Stapf on* Malaria Vector, *Anopheles gambiae*. BAOJ Biotech. 5: 040.

malaria cases occur in Africa [1, 2,4,6]. WHO [7] reported that 92% and 93% of malaria cases and deaths respectively occurred in Africa in 2017.Specifically, the authors also reported that approximately 50% of global cases of malaria in 2017 occurred in 5 nations including Nigeria (25%), Democratic Republic of the Congo (11%), Mozambique (5%), Uganda (4%) and India (4%) [7].This could be due to the presence of large populations of Anopheles mosquitoes in the areas.

Malaria affects adults, adolescents, children and infants. Malaria in children and infants is usually severe which requires urgent and careful attention. WHO [7] reported that children of 5 years and below are susceptible to infection, illness and death with 70% of malaria deaths occurring within this age grade (≤5 years). Though the incidence rate within this age grade (≤5 years) is on the declining trend from 440 000 deaths in 2016 to 285 000 in 2017.

Most *Anopheles* mosquitoes dwell in small, stagnant, shallow fresh water bodies often found in inappropriately discharged waste materials. There are several species of the *Anopheles* mosquito and approximately 30 of them are malaria vectors [5,7]. The distribution of these vectors also varies from one region to another. In Nigeria, the species that are mostly associated with the transmission of malaria include *A. gambiae* and *Anopheles arabiensis* [8,9,2] The intensity of malaria transmission by the vectors depends on the parasite, species of the vector, the human host, as well as the environmental conditions. WHO [7] have reported the long lifespan and strong human-biting habit of the African vectors of malaria to be the reasons why significant cases of malaria are found in the region.

With the discovery of the environmental contamination by chemical based insecticides and development of resistant mechanisms by the malaria vectors [5] research into the discovery of environment friendly substitutes have increased and to this effects several plants have been reported to have insecticidal potentials for the control of mosquitoes at various stages of development [2]. Consequently, some bio-products such as pyrethrum and neem are available [10].

Cymbopogon citratus (lemon grass) which belongs to the Poaceae family have been reported to possess several pharmacological properties including anti-amoebic, anti-microbial, antidiarrhoea, antifilarial, hypotensive, anticonvulsant, analgesic, antiemetic, antitussive, antirheumatic, antiseptic, anti-inflammatory, antimalarial, antimutagenicity, antimycobacterial, antioxidants, hypoglycemic and neurobehaviorial potentials [11]. Decoctions made from *C. citratus* are used to treat gastrointestinal disturbances, nervous disorders, hypertension, fever, elephantiasis, coughs, flu, headache, gingivitis, malaria, leprosy, ophthalmia, vascular disorders and pneumonia [12,13]

In addition, Kimutai et al. [14] reported the repellent activities of essential oils of *C. citratus* on the sandfly, *Phlebotomus duboscqi*. Rabha et al. [15] has reported that *C. citratus* has larvicidal activities against dengue and filariasis vectors, Goselle et al. [16] reported larvicidal activity of lemon grass on Culex mosquitoes, Ebe et al. [17] reported larvicidal effect of *C. citratus* root and leaf on larvae of *A. gambiae, Culex quinquefasciatus* and *Aedes aegypti, Araonu et al.* [18] reported larvicidal effects of crude methanolic and n-hexane extracts of *C. citratus* on, *A. gambiae* mosquito.

Typically, mosquito larviciding could be an effective approach for incorporating into integrated vector management strategies toward minimizing the transmission of malaria [19].Therefore, this study is aimed at assessing the larvicidal activities of crude and ethanolic extracts of *C. citratus* on malaria vector, *A. gambiae*.

Materials and Methods

Plant Collection and Extraction

The leaf of *C. citratus* used for the study was obtained from Ndemili in Ndokwa West Local Government Area of Delta state, Nigeria. The fresh *C. citratus* was cut into small sizes and grinded. 40g of the powder was weighed into 200ml of water and ethanol separately. The mixtures were filtered separately with double muslin cloth after 48 hours. The filtrate from the ethanolic extracts was placed in water bath to remove the ethanol, before being reconstituted with distilled water to different concentrations viz: 0, 50, 100, 150, 200 and 250 ppm.

Culture of Anopheles gambiae

The *A. gambiae* larvae used for the study were obtained from the wild following the approach previously applied by Izah [4]. Some of the larvae obtained were allowed to develop into adults and then identified following the characteristics presented by [20 21]. The larvae of *A. gambiae* were fed with biscuit and yeast at a ratio of 3:1 at room temperature (27 ± 3 °C).

Larvicidal Bioassay

The larvicidal activity of aqueous and ethanolic extracts of *C. citratus* against malaria vector was carried out following the protocol of WHO as cited by [22]. Different concentrations of both extracts were made Viz: 0, 50, 100, 150, 200 and 250 ppm with de-chlorinated water. In each of the concentrations, 20 larvae were used. This process was carried out in triplicates. At 24 hours, mortality was determined when the larvae did not respond to repeated prodding with a soft brush.

Statistical Analysis

The data obtained was calculated as percentage mortality. SPSS version 20 was used to calculate the mean, standard error (descriptive statistics) and analysis of variance and multiple comparison using Duncan statistics (inferential statistics). Test of significance and comparison between means was carried out at $p<0.05$. The LC_{50} value was calculated based on probit analysis using Finney's Table [23] and Microsoft excel regression analysis techniques.

Results and Discussion

The mortality rate of larvae of *A. gambiae* exposed to aqueous and ethanolic extracts of C. citratus for 24 hours is presented in Table 1. The results showed increased mortality as the concentration of the extracts increased. However, at concentrations of 50, 100, 150, 200 and 250 ppm the mortality was 23.33%, 36.67%, 48.33%, 63.33% and 71.67%, respectively (aqueous water extracts), and 23.33%, 50.00%, 55.00%, 76.67% and 85.00%, respectively (for ethanolic extracts). Analysis of variance showed that there were significant variations at $p<0.05$ for each of the extracts under study. Furthermore, Duncan multiple comparison showed that there is no significant variation between 200ppm and 250ppm for aqueous extracts, and between 100 and 150 ppm and 200 and 250 ppm for ethanolic extracts at $p<0.05$. The trend observed in this study is in accordance with previous works by [18,24] .The mortality also showed that ethanolic extract had superior effect on the larvicidal activities of *C. citratus* on malaria vector, *A. gambiae.* Previous studies have indicated that different solvents have varying activities on test organisms [25,26]. The superior effect of the ethanolic extracts could be due to its polarity which differs from that of water.

The larvicidal activities of *C. citratus* on malaria vector, *A. gambiae* are presented in Figures 1–2. The LC_{50} values for aqueous and ethanolic extracts were 161.01 ppm and 104.47 ppm, respectively. The findings of this study support previous studies indicating that *C. citratus* has insecticidal properties [14,15,16, 17, 18]. The LC_{50} values mosquitoes larvae exposed to varying concentration of *C. citratus* observed in this study differ from results 55 ppm for *A.arabiensis* [18], 74.2 ppm of methanol leaf extracts against *A.arabiensis* [24], 19.50mg/ml of ethanolic leave extracts against *Aedes aegypti* [27] and 109.65 ppm of ethanolic leaf extracts against *Culex quinquefasciatus* [28].

The variation in values could be associated to the age of the plants, geographical distribution of the plants, moisture content of the plant, species of mosquitoes, solvent used for extraction, and method of extraction [29] and [4].

The larvicidal activity of the malaria vector, *A. gambiae* could be associated to the many phytochemicals and bioactive compounds it contains. Previous works have reported the presence of flavonoids, phenolic compounds, terpenes, alcohols, ketones, aldehyde and esters, Citral α, Citral β, NerolGeraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone in *C. citratus* [11]. Ranitha [30] also reported that the essential oil of *C. citratus* contain Citral, Geranic Acid, Geranyl Acetate, Linalool, Neric acid, (Z) Citral, β-mycrene and β-Thujene. Allison et al. [31] reported the presence of flavonoids, glycosides and alkaloids and absence of tannins and saponins in *C. citratus*, Ekpenyong et al. [32] have also reported tannins, saponins, flavonoids, phenols, anthraquinones, alkaloids, deoxysugars, and various essential oil in *C. citratus*. The presence of alkaloids could account for its potentials to ward off pests including insects [33]. In addition, plant extracts have the capacity to alter the midgut epithelium in mosquito thereby leading to their death [34, 35]

Table 1: Larvicidal activity of ethanolic and aqueous extracts of *C. citratus* on *A. gambiae* at 24 hours

Concentration, ppm	Aqueous extract	Ethanolic extract
50.00	23.33±1.67a	23.33±1.67a
100.00	36.67±4.41b	50.00±2.89b
150.00	48.33±3.33c	55.00±2.89b
200.00	63.33±4.41d	76.67±4.41c
250.00	71.67±1.67d	85.00±2.89c

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

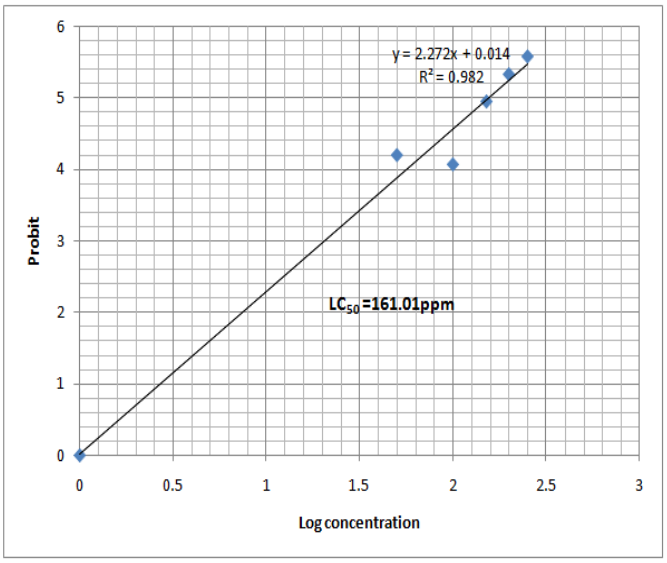

Figure 1: LC_{50} value of *Anopheles gambiae* larvae exposed to aqueous extracts of *Cymbopogon citratus* for 24 hours

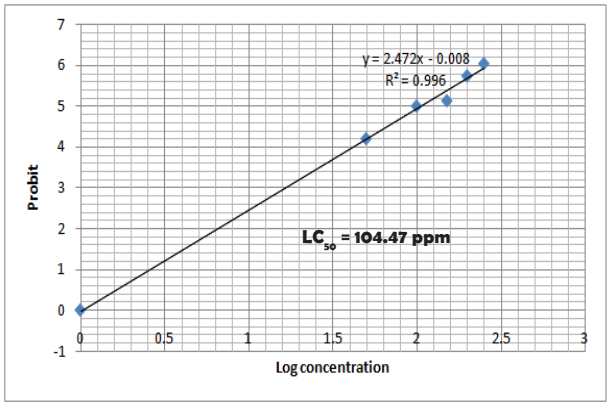

Figure 2: LC_{50} value of *Anopheles gambiae* larvae exposed to ethanolic extract of *Cymbopogon citratus* for 24 hours

Conclusion

The larvicidal activity of ethanolic and aqueous extracts of *C. citratus* on malaria vector, *A. gambiae* was evaluated. The results showed that larvae of *A. gambiae* can be controlled using *C. citratus*. In addition, the results showed that ethanolic extracts have superior effect on the mosquito compared to the aqueous extracts. Therefore, future studies need to be focused on the isolation and purification individual compounds that confer the larvicidal activity on this plant.

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