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Larvicidal activity of some selected medicinal plant extracts against the vector of filariasis

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ABSTRACT

The present study assessed the role of larvicidal activities of hexane, ethyl acetate and methanol dried leaf and bark extracts of *Ocimum gratissimum*, *Gleditsia triacanthos*, *Eucalyptus globulus* and *Azadirachta indica* against the fourth instar larvae of filariasis vector, *Culex quinquefasciatus* mosquito (Diptera: *Culicidae*). Larvicidal activities of four medicinal plant extracts were studied in the range of 4.69 to 1000 mg/l in the laboratory bioassays against early 4th instar larvae of *Culex quinquefasciatus*. The mortality data were subjected to probit analysis to determine the lethal concentrations (LC_{50} and LC_{90}) to kill 50 and 90 per cent of the treated larvae of the respective species. All plant extracts showed moderate effects after 24 h of exposure; however, the highest toxic effect of leaf methanol extract of *Ocimum gratissimum*, bark ethyl acetate extract of *Eucalyptus globulus*, methanol extract of *Azadirachta indica* and methanol bark extract of *Gleditsia triacanthos* against the larvae of *Culex quinquefasciatus* with LC_{50} =43.00, 41.02, 40.12 and 20.36 mg/l; LC_{90} =278.32, 218.72, 215.01 and 86.29 mg/l respectively. The result of findings shows that leaf and bark extract of *G. triacantha*, *A. indica*, *O. gratissimum* and *E. globulus* can be developed as ecofriendly larvicides.

KEYWORDS: *Culex quinquefasciatus*, medicinal plant, extracts, larvicide

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INTRODUCTION

Mosquitoes are the major vector of filariasis and play an important role in spreading of vector-borne diseases like malaria, dengue, chikungunya and Japanese encephalitis which leads to cause thousands of deaths per year [1]. *C. quinquefasciatus* is a vector of lymphatic filariasis, (tropical disease) which accounted 120 million people infected worldwide, and 44 million people having common chronic manifestation. According to World Health Organization (WHO) estimates, globally about 90 million people are infected with *W. bancrofti* and ten times more peoples are at the risk of being infected. So far, 25 million people harboring microfilaria and 19 million people suffering from filarial disease manifestations were recorded from India [2]. The controlling of mosquito populations using chemical insecticides had some advantages viz. their speedy action and easy application. With the continuous use of chemical insecticides, the mosquitoes are becoming resistant. Alternatively, natural pesticides (especially from plants), act as more potential agents for mosquito vectors. Aromatic plants and their essential oils are best sources of many active compounds for multipurpose uses. Plant based phytochemicals having mosquitocidal properties are now recognized as potent alternative insecticides to replace synthetic insecticides [3]. Previously, many naturally occurring chemicals have been reported to influence mosquito oviposition [4], act as

general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent [5] essential oils isolated from *Cinnamomum zeylanicum*, *Zingiber officinale* and *Rosmarinus officinalis* showed oviposition-deterrent and repellent activities against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* [6]. Another plant *Cipadessabaccifera* extract showed adulticidal activity against selected three mosquito vectors [7]. The present investigation will chose to study the mosquitocidal activities of *G. triacantha*, *A. indica*, *E. globulus* and *O. gratissimum* which are traditional medicinal plants. The essential oils obtained from leaves and stems of this plants were used as remedy for several health problems like fever, throat inflammations, ears or eyes, stomach pain, diarrhea, skin diseases[8]. Considering the above information's the present investigation deals with the use of *G. triacantha*, *A. indica*, *E. globulus* and *O. gratissimum* plant extracts for controlling *Culex quinquefasciatus* mosquito vector (Diptera: *Culicidae*).

MATERIALS AND METHODS

Plant Materials

The fresh and healthy leaves of *Ocimum gratissimum*, *Gleditsia triacanthos*, *Eucalyptus globulus* and *Azadirachta indica* were

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collected from Kano botanical garden (Latitude – 11.7794°N, Longitude – 78.2034°E), which is rich in floral biodiversity and indigenous plant populations.

Preparation of Plant Extracts

The leaf and bark were dried for 7-10 days in the shade at the environmental temperatures (27-37°C/day time). The dried bark (600 g) and leaf (700 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with hexane (2,200 ml), ethyl acetate (2,500 ml) and methanol (2,800 ml), in a soxhlet apparatus (boiling point range 60–80°C) for 6 h. The extracts were filtered through a Buchner funnel with Whatman No. 1 filter paper. The extracts were concentrated under reduced pressure 22 - 26 mm Hg at 45°C and the residue obtained was stored at 4°C. The residues were then made in to a 1 % stock solution with acetone (stock solution). From the stock solution, 1000-4.69 mg/l, dilutions were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05 per cent in the final test solution [9,10].

Mosquito Culture

C. quinquefasciatus larvae were collected from rice field and stagnant water areas of Kura local government area of Kano state Nigeria and identified in department of biological sciences Yusuf Maitama Sule University, Kano State. Larvae were kept in plastic and enamel trays containing well water. They were maintained and all the experiments were carried out at 27 ± 2°C and 75–85 % relative humidity under 14:10 h light and dark cycles. Larvae were fed a diet of Brewer's yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively as per the method of Kamaraj *et al.* [11].

Mosquito Identification

The filariasis vector larvae were identified by their simple morphological features as well as their behavior in water according to Linton *et al.* [12]. The larval head is short and stout, becoming darker toward the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, siphon, and saddle. Each segment has a unique setae pattern. The larvae of *Culex* mosquito tend to position themselves at an angle due to presence of siphon in which they use for respiration [13].

Larval Bioassay

During preliminary screening with the laboratory trial, the larvae of *C. quinquefasciatus* were collected from the insect-rearing cage and identified in Biological Department Yusuf Maitama Sule University, Kano. The larval bioassay was determined as per the procedure of WHO [14] and Rahuman *et al* [15]. Five batches of 20 larvae were used in each bioassay. Six different concentrations (15.63, 31.25, 62, 125, 250 and 500 ug/mL) were papered with 100 mL dechlorinated tap water, in a separate plastic container. Polysorbate 80 was used as an emulsifier at the concentration of 0.05% in the final test solution. The control was

set up with acetone, polysorbate 80 and dechlorinated tap water. The numbers of dead larvae were counted after 24 h of exposure and percentage of mortality was reported from the average of five replicates. The experimental media in which 100% mortality of larvae occurs alone were selected for dose-response bioassay [15].

Dose-Response Bioassay

The different concentrations ranging from 15.63 to 500 µg/mL were prepared. Based on the preliminary screening results, different solvents of leaf and bark extracts prepared from *G. tricantha*, *A. indica*, *E. globulus* and *O. gratissimum* were subjected to dose-response bioassay for larvicidal activity against the larvae of *C. quinquefasciatus*. The numbers of dead larvae were counted after 24 h of exposure, and the percentage of mortality was reported from the average of five replicates [15].

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC₅₀, LC₉₀, and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated by using the software developed by Reddy *et al.* [16]. P<0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal properties. In the present observation, hexane, ethyl acetate and methanol extract of leaf, and bark of *G. tricanthos*, *A. indica*, *O. gratissimum* and *E. globulus* extracts were tested against *C. quinquefasciatus*, mortality of four plant extracts were recorded, and the findings, related to the larvicidal activities of various plant extracts reported by the earlier authors corroborate the present study results[10]. 100 % larval mortality was observed in the bark methanol extract of *A. indica*, and *G. triacanthos*. Bark ethyl acetate and methanol extract of *E. globulus* and leaf ethyl acetate extract of *O. gratissimum* (Table 1). All plant extracts showed moderate toxic effect on *C. quinquefasciatus* after 24 h of exposure at 500 mg/l; however, the highest mortality was found in leaf methanol extract of *O. gratissimum*, bark ethyl acetate extract of *E. globulus*, methanol bark extract of *A. indica* and lastly bark methanol extract of *G. triacanthos* against the larvae culex with LC₅₀=43.00, 41.02,40.12 and 20.36 mg/l;

Table 1: Larvicidal activity of crude plant extracts against fourth instar larvae of *Cx. quinquefasciatus* at 500 ug/mL (Mean±SD) (%)

| Botanical name | Mortality | | |
|-----------------------|------------|--------------|-------------|
| | Hexane | Ethylacetale | Methanol |
| <i>O. gratissimum</i> | 83.00±2.15 | 75.00±2.00 | 100.00±0.00 |
| <i>E. globulus</i> | 72.00±2.32 | 100.00±0.00 | 100.00±0.00 |
| <i>A. indica</i> | 86.00±1.63 | 75.00±3.21 | 100.00±0.00 |
| <i>G. tricantha</i> | 81.00±2.02 | 89.00±2.01 | 100.00±0.00 |

Control - Nil mortality

Table 2: Larvicidal activity of different solvent crude extracts against fourth instar larvae of *C. Quinquefasciatus*

| Plant extract | Solvents | LD ₅₀ | | LD ₉₀ | |
|-----------------------|---------------|------------------|--------------|------------------|----------------|
| | | Mean±SD | ULC- LCL | Mean±SD | ULC – LCL |
| <i>O. gratissimum</i> | Methanol | 43.00±3.52 | 47.76 -33.12 | 278.32±19.11 | 298.73 -137.85 |
| <i>E. globulus</i> | Ethyl acetate | 41.02±2.82 | 58.01 -22.01 | 218.72±16.01 | 291.21 -102.05 |
| <i>A. indica</i> | Methanol | 40.12±1.33 | 58.00 -21.76 | 215.01±13.15 | 268.31 -192.10 |
| <i>G. tricantha</i> | Methanol | 20.36±1.42 | 20.15 -15.28 | 86.29±12.18 | 109.32 -81.16 |

Control - Nil mortality; LC₅₀ - Lethal concentration that kills 50 per cent of the exposed larvae; LC₉₀, Lethal concentration that kills 90 per cent of the exposed larvae; ULC, upper confidence limit; LCL, lower confidence Limit; comparing experimental and control group, with a significance level established at P<0.05 and were chi-square value was significant

LC₉₀=278.32, 218.72,215.01 and 86.29 mg/l respectively. The data obtained were analyzed using Chi-squared test, comparing experimental and control groups, with a significance level established at P<0.05 (Table 2).

Crude extract of leaves or bark of the plants have been tested earlier by several investigators and larvicidal activities have been shown against *C. quinquefasciatus* [17-23].The leaf of *O. gratissimum* show more effective repellents activity against the *C. quinquefasciatus* which is in accordance with the Rajkumar's findings [24]. The methanol extracts shows 100% larval mortality at concentration of 500 ug/mL against *C.quinquefasciatus* is also in accordance with Rajkumar's finding [20].

In conclusion, the findings showed that leaf and bark extract of *G. tricantha*, *A. indica*, *O. gratissimum* and *E. globulus* can be developed as ecofriendly larvicides. Also the results open the possibility for further investigations of the efficacy of larvicidal properties of natural product extracts.

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