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Lethal Effects of *Calotropis procera* Leaves Extract on Mosquito Larvae

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ABSTRACT

***Calotropis procera*, is a medicinal plant that is widely reported to have antibacterial, antiparasitic, larvicidal and insecticidal properties. This study is aimed at evaluating the larvicidal potentials of *C. procera* on mosquito larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The plant leaves were collected locally and extracted using petroleum ether as solvent. Four varying concentrations were made using dilution method to test larvicidal activity on 4th instar larva of the aforementioned species of mosquito. It was observed that the plant extract was lethal against both species of mosquito. The results also showed that mortality of the mosquito larvae increased as the concentration of the extract increases. Furthermore, probit analysis of the results shows that the median lethal concentration LC_{50} of *C. procera* extract on *Aedes aegypti* and *Culex quinquefasciatus* are 0.116mg/ml and 0.249 respectively. This means that *Culex quinquefasciatus* is more susceptible to the plant extracts. It is evident from this study that *C. procera* has larvicidal properties that can be considered in the production of an affordable plant based larvicide. Since *C. procera* is known to naturally grow in the wild; it will therefore require lesser resources to be cultivated domestically. We recommend that further studies should be conducted on the plant to explore its full potential.**

Keywords- larva, Mosquito, *Calotropis procera*.

I. INTRODUCTION

Mosquitoes are a group of insects of re-known public health significance. They act as vectors that harbor many diseases, they constitute nuisance by their bites and also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, which contribute significantly to poverty and social debility in tropical countries [1]. The World Health Organization [2], has declared mosquitoes as "Public Enemy Number One". *Aedes* species are known to transmit causative agents of yellow fever, dengue fever, encephalitis viruses and many other arboviruses. They are also vectors of *Wuchereria bancrofti* and *Brugia malayi*, in a few restricted areas [3]. Anopheles is a genus of mosquito famous for transmitting the deadly *Plasmodium* parasite that causes malaria. *Culex quinquefasciatus* (Say), commonly known as the southern house mosquito is a medium-sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions. *Culex quinquefasciatus* is the primary vector of St. Louis encephalitis virus and also transmits West Nile virus [4]. Despite progress in vaccine development, no effective and acceptable multivalent vaccines are available against mosquito-borne diseases [5]. Lack of improved drainage systems, opened reservoirs, fish ponds, irrigation ditches, rock pools and rice fields, make provision for abundant mosquito breeding spots.

According to [6], to reduce the incidence of different mosquito borne diseases, regulation of mosquito population is very important. The approach for control of mosquito-borne diseases predominantly relies on the interception of disease transmission cycle by preventing mosquitoes from biting humans, using insecticides and causing larval mortality in a large scale at breeding sites with larvicides [7]. Proper control of mosquitoes is based on personal protection and public awareness for eradicating breeding sites [8]. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. Larviciding largely depends on the use of synthetic chemical insecticides such as organophosphates and insect growth regulators. Although effective, their repeated use disrupts natural biological control systems and often resulting in undesirable effects on non-target organisms [1]. This also fostered environmental and human health concern that initiates a search for alternative control measures [9]. Many plants produce secondary components that have insect growth inhibitory activities. Besides the use of such compounds as agricultural insect pest control agents, their use in mosquito larval control is an interesting perspective [10]. Plant derived products are being investigated for their larvicidal prospects to the extent that more than 2000 plant species are already known to have insecticidal properties [11][12][13][14]. *Calotropis procera* is a common blossoming plant belonging to the family Apocynaceae. It is indigenous to North and Tropical Africa, Western and South Asia and China. The plant popularly known as Osher, Mudar, Apple of Sodom, Sodom apple or Stabragh, has been found to exhibit purgative, antipyretic, alexipharmic, anticonvulsant,

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antihelmintic, analgesic, anxiolytic, and sedative effects. It has also been used for the treatment of various diseases, including ulcers, leprosy, leucoderma, lumps, piles and splenic, hepatic and abdominal diseases [15]. They have been shown to contain a noxious bitter and opaque sap because of which they are also commonly known as milkweeds. Variety of components has been isolated from the milky sap, such as cardenolides, cardiac glycosides and flavonoids. The possible efficacy of the extracts prepared from the leaves of *Calotropis procera* has been explored against the larvae of dengue vector *Aedes aegypti*, malarial vector *Anopheles stephensi* and filarial mosquito *Culex quinque fasciatus*. However, a few existing gaps in literature suggests that the plant needs to be investigated more extensively as amosquito larvicide. The assessment of mosquito control efficiency of *Calotropis procera* may provide constructive research with an objective of the formulation of new anti-mosquito agent above and beyond the weed management.

The aim of the present study is to determine mosquito larvicidal potentials of extracts from leaves of *Calotropis procera.*

II. METHODOLOGY

Larval Sampling Area

Mosquito larvae was sampled at four different location of Federal Polytechnic Mubi Main campus comprising of Student hostels, Rectory, Biological garden and Barama outskirts of the university. Mosquito larvae were collected from different prospective larval breeding sites which include; stagnant ponds, domestic water containers, small water pools, broken soak away pipes, tins and used tyres, flower pots and wells. Collections were made with dipper, while the contents of small containers were directly transferred into collecting vessels [16].

Identification and Rearing of the Larvae

The collected larvae were sorted in the laboratory. They were further identified to species level using the keys of [17] and illustrations of [18]. The larvae for each species identified was sorted and counted. After counting, larvae of same species were each combined in the same labeled beakers containing 0.5 liters of breeding site water respectively.

Collection and Identification of Plant Materials

Leaves of *Calotropis procera* were collected from fruiting trees within the main campus of Federal Polytechnic Mubi, Adamawa, Nigeria. The collection was done during the months of April to June (raining season). The leaves were taken to the herbarium unit of the Department of Biological Sciences of the same Polytechnic for Identification and were assigned voucher numbers.

Preparation of Plant Extracts

The leave samples of the plant were dried using cabinet dryer set at 40^0C for one hour. The dried leaves were pulverized into fine powder using pestle and mortar and stored in separate labeled polythene bags prior to extraction.

Extraction of Plant Materials

Soxhlet extraction method using petroleum ether (60^0C-80^0C) as solvent was used to extract each of the powders already prepared.

Formulation of Concentration

Four (4) varying concentrations were prepared in accordance with [20] protocol using dilution formula to test the larvicidal activity.

Larvicidal bioassay

Larvicidal activity of the plant extracts was tested against 4th instar larvae of *Aedes aegyti* and *Culex quinquefasciatus* mosquito collected within the sample area.

Determination of Lethal Concentration (LC50) of Leaf Extract against Mosquito Larvae

The LC_{50} for each extract under laboratory and outdoor conditions was computed by transforming the various concentrations.

Statistical Analysis of Data

Data obtained from the study was analyzed using Percentage mortality of the larvae in the different doses of the leave extracts that was determined. Probit analysis of mortality data were done to determine the median lethal concentrations (LC_{50}) of the extracts against the treated mosquito species after 48 hours of treatment (Finney, 1971). Logarithms of the concentrations of extracts used was plotted against the empirical probit of the killed larvae to obtain regression equations and coefficients of determination (R2) for the extract type against each mosquito species.

Table 1: Lavicidal activity of *Calotropis Procera* Extract on *Aedes* larvae.

Concentration of Extract (mg/ml)	Percentage of Mortality (%)	Logarithm of Concentration	Empirical Probit of Mortality
3.0	75.1	0.48	5.67
2.0	69.7	0.30	5.52
1.0	64.8	0.00	5.39
0.5	62.0	-0.30	5.31

$Y=0.447x+5.418$, $R^2=0.947$, $LC_{50}=0.116$

Table 2: Lavicidal activity of *Calotropis Procera* Extract on *Culexquinquefasciatus* larvae

Concentration of Extract (mg/ml)	Percentage of Mortality (%)	Logarithm of Concentration	Empirical Probit of Mortality
3.0	90.7	0.48	6-34
2.0	86.9	0.30	6.13
1.0	74.7	0.00	5.67
0.5	65.9	-0.30	5.41

Y=1.227x+5.740, R^2=0.987, LC_{50}=0.249

III. RESULTS

The results for the larvicidal activity of *Calotropis Procera* on the larvae of *Aedes aegypti* and *Culex quinquefasciatus* of Mosquito are presented in table 1 and 2 respectively. It was observed that the plant extract was lethal against both species of mosquito. The results also showed that mortality of the mosquito larvae increased as the concentration of the extract is increased. Against *Aedes aegypti*, the highest mortality rate of 75.1 % was recorded at a concentration of 3mg/ml, followed by 69.7%, 64.8% and 62.0% at 2.0mg/ml, 1.0mg/ml and 0.5ml/ml respectively. *Culex quinquefasciatus* larva was comparably more susceptible to *C. procera* extract than *Aedes aegyti* larva. This is because at 3.0mg/ml, a higher mortality was recorded in *Culex quinquefasciatus* (90%) when compared to that of Aedes at the same concentration. Similar observations on percentage mortality (86.9%, 74.7% and 65.9%) were seen across other concentrations (2.0, 1.0 and 0.5mg/ml) with regards to species susceptibility to the plant extract respectively. However, probit analysis shows that the median lethal concentration LC_{50} of *C. procera* extract on *Aedes aegypti* and *Culex quinquefasciatus* is 0.116mg/ml and 0.249 respectively.

IV. DISCUSSION

Calotropis procera has been reported by many scholars to have Antibacterial, larvicidal and insecticidal properties. These findings have generated a lot of interest by researchers in fields of Biomedical and Pharmaceutical Sciences, most especially at a time when larvicide resistance is widely reported. Larvicide resistance is a major setback in vector-borne disease control efforts. The use of chemicals in pest control has always faced serious criticism from environmentalist because of its harmful effects on non-target species.In this present study, the larvicidal potentials of *C. procera* leave exract on *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae were evaluated. Findings from this study revealed that the plant extract was lethal against larva of both species, which is in agreement with the findings of [21], who reported that methanolic extracts of *C. procera* leaves have larvicidal properties against mosquito larva of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegyti*. Similarly, another study conducted by [22], aimed at investigating the larvicidal activity of aqueous leaves extract of *C. procera* against *Anopheles arabiensis* and *Culex quinquefaciatus*, reported that the extract caused high mortality against 3rd instar larvae of the aforementioned mosquito species. Investigation from this research also revealed that *Culex quinquefasciatus* are more susceptible to treatment with *C. procera* than *Aedes aegypti*, which is in partial agreement with the works of [21]. A study on *C. procera* latex and extract conducted by Shahi *et al.*, (2010), found out that the"latex" is more lethal than the "extract" on both *Anopheles* and *Culex* Species. Furthermore, results in this present study revealed that efficacy of *C. procera* increased with concentration which is in conformity with the findings of [22][21][23]. In a related study conducted by [24], 1ml of *C. procera* latex recorded an estimated mortality of100% against *Culex pipens* larva after 72hrs. In a related experiment conducted by [25], they found out that the aqueous extract of *C. procera* latex eliminated 50% of *Culex quinquefasciatus* larval population at 0.0062% (V/V) concentration. Additionally, they also found out in the same study that *Ficus benghalensis* extract produced about 25% mortality in a range of about 0.0195 and 10% (W/V) concentration.

V. CONCLUSION

C. procera has noticeable larvicidal effects against both *Aedes aegypti* and *Culex quinquefasciatus*. It is a good a candidate that can be explored in the quest for a more potent and affordable alternative larvicide. Interestingly, it is a plant that grows naturally in the tropics and sub-tropical regions where mosquito borne diseases are common. We recommend that further studies should be conducted at molecular levels in a bid to develop a more advanced larvicide.

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