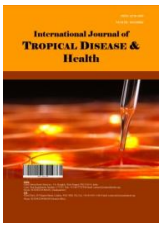

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Insecticidal Activities of Leaf Methanol Extract of *Clerodendrum polycephalum* Baker against *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In a view to determining the capacity of *Clerodendrum polycephalum* to control mosquitoes, the methanol extract of the leaf was investigated for insecticidal activities using three species of mosquitoes, *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus.*
Methodology: The leaves of *C. polycephalum* were collected, dried and extracted with methanol. Mosquito larvae were exposed to different concentrations for 24/48h. and sublethal concentrations ($L_{25,\ 50,\ 75}$) of the extract to determine larvicidal activity and monitor growth and development respectively. Twenty (20) blood-fed female *Aedes aegyti* mosquitoes were allowed to lay eggs on treated filter papers for antioviposition bioassay. The crude extract was separated into N-hexane, Dichloromethane, ethyl-acetate, ethanol fractions using Vacuum Liquid Chromatography to determine the active fraction.
Results: Results showed that larval mortalities were in the order *C. quiquefasciatus>A. gambiae>A. aegypti* with effective concentration ranging from 250 – 8000 ppm. Mortalities at 48hr were significantly different ($p<0.05$) from mortalities at 24h. Of the four fractions obtained, ethanol

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fraction gave the highest larval mortality of 100% at 2000 ppm. Larval duration for all the three species of mosquitoes exposed to the crude extract varied between 2.25±0.5 and 3.25±0.5 days. As Antioviposition Index increased from 46.4 - 89.9, percent hatching of mosquito eggs deceased from 87.25 – 67.5% with increasing concentration.

Conclusion: The extract of *C. polycephalum* was found to contain insecticidal compounds which are soluble in polar solvent. The plant could be exploited in mosquito control programme.

Keywords: Clerodendrum polycephalum extract; fractions; antimosquito.

1. INTRODUCTION

Mosquitoes are dipteran flies family, Culicidae, under nematocera suborder. They are responsible for the transmission of malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, *etc*. [1]. Mosquito control is still a major challenge in countries where the vector is supported by favourable environment especially in the temperate region. There is hardly any aquatic habitat anywhere in the world that does not permit mosquitoes to breed. They are therefore found in ground pools, gutter, domestic runoffs, tree holes and so on [2]. Many approaches have been employed to curb their growing population. The major tool in mosquito control is the use of synthetic insecticides such as organochlorine and organophosphate compounds [3]. This has not been very successful due to many factors. Some of the problems associated with the use of synthetic insecticides are lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non-biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale. An alternative to these synthetic compounds is to explore botanical insecticides which are generally considered safer, but more importantly, they are readily available. *Clerodendrum. polycephalum* Baker is a species of flowering plant of the family Verbenaceae. It is an erect or scandent shrub or lianas, or small tree of about 4 m or taller. Leaves are decussate (whorled), white flowers, sepals usually connate, lobes are unequal [4]. The phytochemical components [5] and antimalarial activities [6] of *C. polycephalum* have been reported. The plant is rich in bioactive components such as tannin, saponin, and alkaloids which can be useful as anti-infective agent. In this background, *C. polycephalum* plant was investigated for its insecticidal properties against mosquitoes.

2. METHODOLOGY

2.1 Plant Collection and Preparation

Fresh leaves of the plant were collected at an abandoned site around Sabo area of Ile-Ife, Nigeria in June, 2015. Voucher specimen was deposited at the herbarium of department of Pharmacognosy, Faculty of Pharmacy of the Obafemi Awolowo University. The leaves were oven-dried at 40^{o}C and powdered using a grinding machine, Daiki, 2002 model. Powdered leaves were soaked with 100% methanol and subjected to a shaker for 72 h. The preparation was filtered through a Buchner funnel with Whatman filter paper number 1 (24 cm in diameter). The extract was evaporated to dryness using a rotary evaporator and stored in a glass vial. Stock solutions were prepared using dimethyl sulfoxide (DMSO) dissolving agent.

2.2 Larvicidal Assay

The crude methanol extract was tested against three mosquito larvae, *Aedes aegypti* Linn., *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say. All mosquitoes were obtained from their natural habitats, identified and transferred into the laboratory. Five concentrations, 250, 500, 1000, 1500, 2000 ppm were prepared by diluting the stock with dechlorinated water. The larvicidal test was done according to standard method [7] with slight modifications. Plastic cups with capacity of 120 ml each were arranged, and 99 ml of dechlorinated water added to each. Ten mature larvae (late 3rd and 4th instars) were transferred into each of the cups. Larvae were first poured into a petri dish from which matured ones were picked using a cotton bud. Different quantities of extracts from the stock, equivalent to 250-2000 ppm, were transferred into cups and shaken gently to allow a good even dispersal of the extract. Each concentration was replicated thrice.

The leaf of a known potent natural insecticide, *Nicotiana tabacum* was collected, dried, powdered and extracted with methanol. The *N.*

tobacum leaf extract was used as the positive control.

Larvae mortalities were determined after 24 and 48hrs. The LC_{25}, LC_{50} and LC_{75} were calculated using Regression analysis.

2.3 Effects on Growth and Development

Insect growth regulator assay [3] was followed. Third instar larvae of each of *Aedes aegypti, Culex quinquefasciatus* and *Anopheles gambiae* mosquitoes were exposed to three sub-lethal concentrations (LC_{25}, LC_{50} and LC_{75}) obtained from their larvicidal activities results. Against *A. aegypti* mosquito, sub-lethal concentrations used were 560, 1120 and 1675 ppm; *C. quinquefasciatus* sub-lethal concentrations were 240, 4800 and 7200 ppm while *A. gambiae* sub-lethal concentrations included 1875, 3750 and 5625 ppm. Four replicates of 10 larvae each were used for each concentration. Larva and pupa mortality as well as adult emergence were recorded daily up to emergence of all the adults or death of the last larva or pupa. From the test results, percentages of both emerged and dead pupae and percentage of adult emergence, larval duration, and adult emergence inhibition (% IE) were determined.

Percent adult emergence inhibition is calculated thus:

$$\%IE = 100 - \frac{(T \times 100)}{C}$$

where T adult survival in treated medium and C = adult survival in control.

Oviposition preference

The method employed was that of [7]. Four 200 ml cups containing 100 ml of the crude extract in solution with sub lethal concentrations and controls were placed inside a one - cubed cage. Twenty (20) blood-fed female *Aedes aegyti* mosquitoes were exposed to the extract in solution. The mosquitoes were fed for at least three days before the commencement of the test. The cups were observed daily for eggs for 7 days.

Percent inhibition of oviposition is expressed as anti-oviposition index I and calculated as follows:

$$\text{Anti-oviposition Index (I)} = 100 \times \frac{(Ec - Ee)}{Ec}$$

Where Ec is the number of eggs laid in the control, Ee is the number of eggs laid in the treated dishes.

2.4 Chromatographic Technique

The crude extract was separated into four fractions namely N-hexane, Dichloromethane, Ethyl-acetate and ethanol with the aid of Vacuum Liquid Chromatography. Each fraction was evaporated to dryness, weighed, and tested for larvicidal activities using *A. aegypti* larvae.

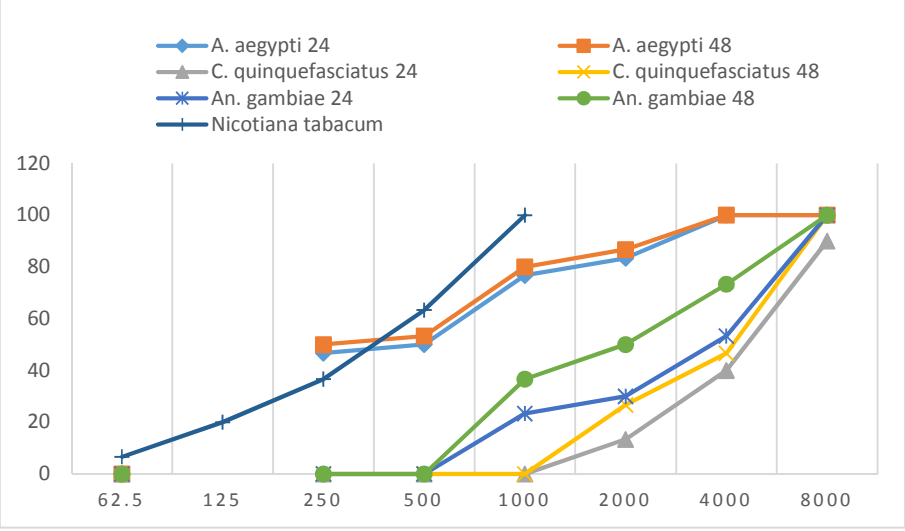

Fig. 1. Larvicidal activities of methanol extract of *C. polycephalum* against *Aedes aegypti, Anopheles gambiae,* and *Culex quinquefasciatus*

Table 1. Effects of methanol extract of *Clerodendrum polycephalum* leaf on oviposition of *Aedes aegypti*

Conc. (ppm)	Total no of eggs laid	% Hatching	Anti-oviposition index (I)
250	881	84.25	46.4
500	319	78.75	80.5
1000	190	68.75	88.4
2000	165	67.5	89.9
Control	1644	86.25	

Anti-oviposition Index (I) **=** 100 X [(Ec – Ee)/Ec]

Where Ec is eggs laid in the control; Ee is eggs laid in each of the concentration

Table 2. Effects of methanol extract of *Clerodendrum polycephalum* on growth and development of *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus* larvae

Mosquito	*Aedes aegypti*				*Anopheles gambiae*				*Culex quinquefasciatus*			
	Positive control	LC_{25}	LC_{50}	LC_{75}	Positive control	LC_{25}	LC_{50}	LC_{75}	Positive control	LC_{25}	LC_{50}	LC_{75}
Larval duration	2.75±0.5	2.75±0.5	2.5±0.5	2.25±0.5	3.25±0.5	3.25±0.5	3.0±0.5	3.25±0.5	3.25±0.5	3.25±0.5	2.75±0.5	2.5±0.57
Dead larvae	2	12	23	27	2	8	18	30	1	12	23	28
Dead pupae	0	0	7	7	0	4	10	10	0	1	6	8
Dead adults	0	0	0	0	0	0	4	0	0	0	2	2
Total emerged adult	37	28(24.33)*	10(72.98)	6(83.8)	38	28(26.3)	1(97.4)	0(100)	39	27(22.8)	8(77.2)	2(94.6)

**Figure in parenthesis = % IE (Emergence Inhibition)*

Table 3. Percentage yield and larval mortalities of *Aedes aegypti* (at 2000 ppm) of crude extract and VLC fractions

Crude/Fractions	Weight (g)	Percent (%) Yield	Percent Larval mortalities±SD
Crude methanolic extract	68	9.7	96.67±0.47
N-hexane extract	3.15	4.60	10.00±0.81
Dichloromethane (DCM)	0.65	0.96	0.00
Ethyl-acetate	2.35	3.46	0.00
Ethanol	13.75	20.22	100

3. RESULTS

3.1 Larvicidal

Fig. 1 shows the larvicidal activities of the crude extract on the species of mosquitoes used. Larval mortalities were in the order *C. quiquefasciatus>A. gambiae>A. aegypti* with effective concentration ranging from 250 – 8000 ppm. Mortalities at 48hr were significantly different ($p<0.05$) from mortalities at 24hr. The LC_{50} at 24/48hrs for the three mosquitoes were *A. aegypti,* 1122/1231ppm, *A. gambiae,* 3750/3760 ppm and *C. quinquefasciatus,* 4152.25/4794.4 ppm. The ethanol fraction gave the highest larval mortality of 100% at 2000ppm, followed by N-hexene with 10.00±0.81 while DCM and ethyl acetate were not active (0%) at all (Table 3). The positive control used, *N. tabacum* extract, produced 100% mortality of *A. aegypti* at 1000ppm within 24h while the extract under investigation produced 100% mortality at 2000ppm within 48h.

3.2 Effects on Growth and Development

Table 2 shows the effects of the crude methanol extract of *C. polycephalum* on growth and development of mosquito larvae. At the time of larva death, discoloration of larvae occurred. Some turned black from the living light pink color. Larval duration (in days) was the same with control at lowest concentrations for all the three mosquitoes. However, at sublethal concentrations, larval durations were slightly reduced which was significantly different from the control ($p<0.05$). At the same concentration levels, there were effective inhibitions of adult emergence in the three mosquitoes used. Emergence inhibition was low (22.8 – 26.3 %) at lowest concentrations for all the mosquitoes. The concentration that killed 50% larvae was sufficient enough to inhibit greater number from emerging. In all, *Anopheles gambiae* was the most affected, recording complete (100 %) adult emergence inhibition at 1675 ppm (LC_{75}).

3.3 Anti-oviposition

Table 1 shows the result of anti-oviposition against adult *Aedes aegypti.* The extract showed concentration-dependent anti-oviposition i.e. anti-oviposition index (I) increased (46.4 - 89.9) with concentration (250 – 2000 ppm). There was an effective prevention of egg- laying in higher concentrations (500 – 2000 ppm). Hatching of eggs was also affected as hatching reduced from 87.25 - 67.5%.

4. DISCUSSION

Several studies have been conducted on plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and more selective mosquito insecticides [8,9,10,11].

The 24/48 h bioassay was recommended by [3] as a tool for evaluating toxicity of phytotoxins however, a number of researchers have used either the 24 h bioassay [12] or 48 h [13]. The 48 h bioassay is actually necessary when the toxicity at 24 h is low [7]. Some researchers have found it necessary to extend the period to even 72 h [14,15]. Longer duration of exposure (48 h) allowed greater contact with the active ingredients and perhaps the case slow release of the compound to the experimental media at 48h also contributed to the increase in mortality at 48 hr. In addition to the duration, the concentrations used for the larvicidal was on the high side (1000 – 4000 ppm). The use of crude extract, in some cases, requires large amount to produce expected mortality [16,8] except in highly toxic plant [17]. Little amounts are required by the time the various components are separated and tested. Very little amount is also needed if the plant material is volatile oil [18,19,20] which is more or less an isolate.

Vacuum Liquid Chromatography was used to separate the components using non-polar to more polar solvents. This system of extraction has the advantage of bringing out the components faster than the regular column chromatography because it uses electric pumping machine. The differences in the extract yields from the crude could be linked with the different availability of extractable components resulting from the varied chemical composition of plants [21]. The use of vigor during extraction also contributed to the yields, [22] as it was subjected to a shaker for 72 hrs. The most active fraction, ethanol also gave the highest yield (20.22%). It has been discovered that phenolics are often extracted in higher amounts in more polar solvents such as ethanol [22].

The effects on the morphological changes on pupae and shortening of larvae duration in this study suggested that the mode of action of the extract could be by contact and that the plant extract contains Insect Growth Regulator (IGR)

components which could be exploited in mosquito control. In a similar study [23,24], a wide range of morphogenetic deformities in different categories including larval pupal mosaic, abnormal pupae and pupal adult mosaic was observed, according to stage of metamorphosis when death occurred due to abnormal growth and moulting. In a more recent study [25,26], microscopic observations revealed significant lesions on larval cuts treated with aqueous extract of *Ricinus communis*, it also induced histopathological changes at different levels of the body leading to disorganization of movements followed by immobilization and subsequent death.

The extract used in this study prevented successful egg-laying of mosquitoes (I=88.4 and 89.9) in higher concentrations (1,000 and 2,000 ppm respectively) of the extract used. This suggests that the extract produced some volatile compounds, which may act as repellents. Certain strong odor often repel mosquito from feeding and from breeding successfully. Incidentally, it has been revealed that the plant contains volatile compounds [27]. *Aedes* is a container breeder mosquito, which follows visual and olfactory cues to find appropriate oviposition sites and then both physical and chemical factors of water, to assess the suitability of potential larval habitats in order to maximize the fitness of such habitats for reproduction [28]. The extract also affected the hatching of the eggs laid.

5. CONCLUSION

It is concluded from the foregoing that the plant *C. polycephalum* contains substances which are lethal to all stages of mosquitoes. The substances are found soluble in polar solvent. Therefore, the plant deserves further work to explore this potential for mosquito control.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. India J. Med. Res. 2012;135(5):581-598.
2. Egwu O, Ohaeri CC, Amaechi EC, Ehisianya CN. Distribution and abundance of mosquito larvae in Ohafia, Abia State, Nigeria. Cuadernos de Investigación UNED. 2018;10(2):379-385.
3. World Health Organisation. Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHOPES/IC/96.1. Geneva: WHO. 1996;69.
4. Briquet JI. Clerodendrum. In: Verbenaceae In: Die Natürlichen Pflanzenfamilien volume IV, part 3a. Verlag von Wilhelm Engelmann: Leipzig, Germany. 1895;174-176.
5. Egharevba HO, Ibrahim JA, Ezeh G. Phytochemical, pharmacognostic and microscopic analyses of the leaves of *Clerodendrum polycephalum* Baker, J. App Pharmac Sc. 2015;5(07): 060-063.
6. Adewoyin FB, Omisore NO, Odaibo AB, Adewunmi CO, Iwalewa EO. *In vivo* antiplasmodial activity and haematological parameters of the methanolic extract of *Clerodendrum polycephalum* Baker leaves on *Plasmodium berghei berghei* in mice. European J. Medicinal Plant. 2016;12(1): 1–8.
7. W. H. O. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP/2005.13; 2005.
8. Siddiqui BS, Afshan AF, Gulzar AT, Sultana AR, Naqvi AS, Tariq RM. Tetracyclic Triterpenoids from the Leaves of Azadirachta indica and their insecticidal activities. Pharmaceutical Society of Japan. 2003;51:415-417.
9. Alouani AO, Ababsia T, Rahal I, Rehimi N, Boudjelida H. Activity evaluation of botanical essential oils against immature mosquitoes of *Culex pipiens* (Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2017; 5(4):829-834.
10. Amira K, Chouaib T, Djeghader NE, Boudjelida H. Laboratory study of the larvicidal efficacy of a local plant *Hertia cheirifolia* against the most abundant mosquito species, in Algeria. Journal of Entomology and Zoolological Studies. 2018;6(1):258-262.
11. Mohsen ZH, Jarvad ALM, Al-Chalabi BM, Al-Naib AA. Biological activity of

Callistemon lanceolatus against *Culex quinquefasciatus*. Fitoterapia. 1990;61: 270-274.

12. Remia KM, Lagaswamy S. Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae). Indian Journal of Natural Product and Resources. 2009; 1(20):208-212.
13. Candido LP, Cavalcanti MT, Beserra EB. Bioactivity of plant extracts on the larval and pupal stages of *Aedes aegypti* (Diptera, Culicidea). Revista da Sociedade Brasileira de Medicina Tropical. 2013;46(4): 420-425.
14. Granados-Echegoyen C, Pérez-Pacheco R, Soto-Hernández M, Ruiz Vega J, Lagunez-Rivera L, Alonso-Hernandez N, Gato-Armas R. Inhibition of the growth and development of mosquito larvae of *Culex quinquefasciatus* (Diptera: Culicidae) treated with extract from leaves of *Pseudocalymma alliaceum* (Bignonaceae). Asian Pacific Journal of Tropical Medicine 2014;7(8): 594–601.
15. Haldar KM, Ghosh P, Chandra G. Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine. 2011;1(6): 199-203.
16. Nour AH, Jessinta D, Nour AH. Larvicidal activity of extracts from different parts of Neem (*Azadirachta indica*) against Aedes aegypti mosquitoes' larvae. Scientific Research and Essays. 2012;7(31):2810-2815.
17. Perich MJ, Wells C, Bertsch W, Tredway KE. Toxicity of extracts from three Tagetes species against adults and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera: Culicidae). Journal of Medical Entomology. 1994;31:834-839.
18. Al Dakhil MA, Morsy TA. The larvicidal activities of the peel oils of three citrus fruits against *Culex pipiens*. Journal of Egypt Society of Parasitology. 1999;29: 347-351.
19. Deletre E, Martin I, Campagne P, Bourguet D, Cadin A, Menut C, Bonafos R, Chandre F. Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. PLOS ONE. 2013;8(12):e82103.
20. Ezeonu FC, Chidume GI, Udedi SC. Insecticidal properties of volatile extracts of orange peels. Bioresource Technology. 2001;76:273-279.
21. Hsu B, Coupar IM, Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chemistry. 2006;98:317-328.
22. Siddhuraju P, Becker K. Antioxidant antioxidant properties of various extracts of total phenolic constituents from three different Agro climatic origins of drumstick tree (*Moringa olefera* Lam.) leaves. Journal of Agriculture and Food Chemistry. 2003; 51:2144-2155.
23. Mehdi SH, Qamar A, Khan I, Jacob P. Larvicidal and IGR potential of *Ocimum tenuflorum* and *Datura alba* leaf extracts against malaria vector. European Journal of Experimental Biology. 2012;2(4):1370-1375.
24. Echegoyen CG, Pacheco RP, Hernández MS, Vega JR, Rivera LL, Hernandez NA, Armas RG. Inhibition of the growth and development of mosquito larvae of *Culex quinquefasciatus* (Diptera: Culicidae) treated with extract from leaves of *Pseudocalymma alliaceum* (Bignonaceae) Asian Pacific Journal of Tropical Medicine. 2014;594-601.
25. Aouinty B, Chennaoui M, Mahari S, Rihane A, Mellouki F. Larvicidal effects of aqueous extract from *Ricinus communis* L. leaves against mosquito *Culex pipiens*: mortality and histopathology of treated larvae. Journal of Materials and Environmental Sciences. 2018;9(2):619-623.
26. Sumba LA, Okoth K, Deng AL, Githure J, Knols BGJ, Beier JC, Hassanali A. Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. Journal of Circadian Rhythms. 2004;2:6-10.

27. Ogundajo AL, Owoeye OA, Ogunwande IA, Owolabi MS. Chemical composition of essential oil from the leaves of *Clerodendrum polycephalum* Baker growing in Nigeria. Journal of essential oil-bearing plant. 2016;19(1):119-124.

28. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. Journal of America Mosquito Control Association. 1991;7(2): 210-237.

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