



Larvicidal Activity of Calotropis procera Root Extract Against Aedes Mosquito Larva

Abdullahi M. A.^{1*}, Ferdinand C.², Adegbenro A. T.³, Odih C.⁴ and Usman, M. B.⁵

¹Department of Chemistry, Directorate of Science, Remedial and General Studies, Federal University of Health Sciences Azare, Bauchi State, Nigeria.

²Department of Chemistry, University of Abuja, Abuja, Nigeria.

³Department of Biochemistry and Molecular Biology, Obafemi Awolowo University Ile-Ife

Nigeria.

⁴Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

⁵Department of Chemistry, Faculty of Sciences, Gombe State University, Gombe State, Nigeria. Correspondence Author: abdullahi.muhammadjika@fuhsa.edu.ng

ABSTRACT

The environment and living things are significantly harmed by commercial pesticides. It is crucial to seek out new, eco-friendly alternatives to these synthetic pesticides that are more efficient and cost-effective. This study aimed to assess the effectiveness of ethyl acetate root extract from *Calotropis procera* in killing mosquito larvae. Cold maceration for 48 hours was used to extract the plant material (100 g) using (0.5L) of ethyl acetate. Superfluous filtrate were collected using a Winchester bottle, a glass funnel, and fluted Whatman No. 1 filter paper. The extracts (filtrate) were concentrated using a rotary evaporator. Concentrated extracts were air-dried in a fume hood, prior to the analysis. Various quantities of the root extract such as (400µg/ml, 300µg/ml, 200µg/ml, and 100µg/ml) were prepared from stock solution of the extract of *Calotropis procera*. This study showed that ethyl acetate extract of *Calotropis procera root* possessed larvicidal activity of 100% mortality at 400µg/mL, 300µg/mL, 200µg/mL, and 80% mortality at 100µg/mL against *Aedes* mosquito larva species at 35 °C and 91 % humidity. From the result there is a great increase in the mortality rate with increase in concentration of crude extract. Because of its larvicidal activities, the root portion of this plant can be utilized to control and manage *Aedes* mosquito larva.

Key words: Larvicidal, Calotropis Procera, Aedes, Larva, Mosquito

INTRODUCTION

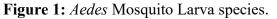
Calotropis procera, often called calotrope, is a tiny crown flower that is a member of the Asclepiadaceae family (Semimul and Hussain, 2019). According to Kareem *et al.* (2008), *Calotropis Procera* is native to the Arabian Peninsula (which includes Yemen, Saudi Arabia, and Oman), the Middle East, and southern Asia. *Calotropis procera* is also found in northern Africa (which includes Equatorial Guinea, Gambia, Ghana, Guinea-Bissau, Mauritania, Nigeria). This species was formerly grown as an ornamental garden plant, but it is no longer in vogue due to its deadly nature and frequent misconception as a weed (Semimul and Hussain, 2019).

Mosquitoes constitute a major hazard to public health because they can spread a number of diseases, some of which can be fatal in tropical and subtropical regions, such as dengue vellow fever. fever. malaria. chikungunya, and filariasis (Jang et al., 2002). Aedes aegypti mosquitoes transmit the dengue virus serotypes 1 through 4 (DENV-1 to DENV-4), which causes the disease dengue (Gould and Solomon, 2008). Simple fever is the first sign of a dengue infection, which progresses to severe and occasionally fatal



dengue hemorrhagic fever (DHF); dengue shock syndrome (DSS) is the other variety (Guzman *et al.*, 2016).





Fever often develops four to seven days after a mosquito bite and is the initial symptom of normal uncomplicated dengue. The bites of female Aedes aegypti mosquitoes (Figure 1) carrying the Dengue virus expose the susceptible human host to the virus (Parakash et al., 2011). When it comes to identifying and keeping an eye on Aedes populations at low densities, ovitrap surveys are seen to be an effective and sensitive method. They are affordable. secure, and environmentally beneficial (Upadhyay, 2013). Treating typical dengue fever is not possible. Source reduction, or getting rid of containers that are good places for oviposition and the development of aquatic stages, has been the main strategy used for control (Elimam et al., 2009). Use of chemical pesticides is one of the most widely used techniques for controlling the mosquito population. Mosquitoes have developed a resistance to commercial insecticides as a result of their use (Meier et al., 2022).

Commercial pesticides have a significant negative impact on both the environment and

living things. Finding innovative, environmentally acceptable alternatives to synthetic insecticides that are both more effective and more affordable is crucial. Many naturally occurring, physiologically powerful compounds found in plants can be employed as larvicidal agents. Plant-based bio-chemicals are thought to be safe for the environment and have a broad spectrum of activity against target species (Upadhyay, 2013).

It was discovered that Calotropis procera works well as a natural mosquito larvicidal agent against Culex quinquefasciatus and Anopheles arabiensis. The larvicidal activity was seen against the mosquito larvae 24 hours after treatment. The results showed that against the mosquito species, the Calotropis procera leaf extract shown remarkable larvicidal, adult emergence inhibitory, oviposition preventive repellent, and properties. Because of these characteristics, the extract may be effective as a natural biocide to reduce mosquito populations (Elimam et al., 2009). The purpose of this study was to determine the larvicidal activities of Calotropis procera roots extracts by subjecting Aedes larvae at various concentrations.

MATERIALS AND METHODS

Plant Collection

The *Calotropis procera* root was collected adjacent to Centre for African Medicinal Plants Research (CAMPRE) North-Eastern University, Gombe, along Gombe-Bauchi Express way, Lafiyawo Gombe, Nigeria at Latitude 10.2881^oN and Longitude 11.0537^o E, and was identified by Mr Muhammad Chindo of the Department of Botany, Faculty of Science, Gombe State University, Gombe State, Nigeria. Voucher specimen GSU/H/365 has been maintained at the herbarium (Abdullahi *et al.*, 2024b; Ferdinand *et al.*, 2023).





The plant material (100 g) was extracted using cold maceration for 48 hours with 0.5 L of ethyl acetate. Using a glass funnel, fluted Whatman No. 1 filter paper, and a Winchester bottle, superfluous filtrate was collected. A rotary evaporator was used to concentrate the extracts (filtrate). Before the analysis, superfluous concentrated extracts were allowed to air dry in a fume hood (Abdullahi *et al.*, 2024a).

Collection of Larvae

The larvae were obtained from Federal Medical Centre (FMC) Gombe State, Nigeria and kept in a mosquito cage.

Larvicidal Activity

400 µg/mL, 300 µg/mL, 200 µg/mL and 100 µg/mL concentrations were prepared from stock concentration of 3000 µg/mL obtained from ethyl acetate extract of *Calotropis procera* root and was tested against four test tubes containing 50ml distilled water and 10 larva successively, the whole component was allowed to stayed for 24 hours. The number of dead larva and percentage mortality was recorded

Percentage Mortality of Larvae

Mortality of the larvae was checked after 24 hours.

Mortality rate =	no of total tested larvae – no of dead larvae	× 100%
	no of total tested larvae	× 100%

RESULTS AND DISCUSSION

The results presented in Table 1. Suggested that the crude extract exhibited larvicidal activity against *Aedes* larval mosquitoes (SP) at all tested concentrations. In experiments 1, 2, and 3, all larvae were killed after exposure to the extract for 24 hours. In experiment 4, a slightly lower efficacy was observed, with 8 out of 10 larvae had died. The study had shown that the ethyl acetate extract of *Calotropis procera* root possess high larvicidal activity with a high percentage mortality rate of 100% at concentration of 400 μ g/mL, 300 μ g/mL, 200 μ g/mL than 80% of mortality at 100 μ g/mL concentration, there is a great increase in the mortality rate from 100 μ g/mL, 200 μ g/mL, 300 μ g/mL and 400 μ g/mL concentrations from this study.

 Table 1: Larvicidal Activities of Ethyl acetate Extract of Calotropis Procera Root against Aedes

 Mosquito Larva

S/N	Volume of the water(mL)	Concentration of extract (µg/mL)	Time (hrs)	Number of Larva	No of death Larva	% Mortality
1	50	400	24	10	10	100
2	50	300	24	10	10	100
3	50	200	24	10	10	100
4	50	100	24	10	8	80

Key: $\mu g/mL = Microgram per milliliter$

Calotropis procera was tested against *Anopheles labranchiae* mosquito larvae and exhibited high larvicidal activity with LC_{50} ranging from 28 to 325 ppm (Gaurav and Neelam, 2016). Larvicidal activity of *C. procera* leaf extract against *A. aegypti* showed the mortality rate of 13%, 20%, 33%, 66% and 100% against 20 mL, 40 mL, 60 mL, 80 mL and 100 mL extract (Aqsa *et al.*, 2016). In this study it was observed that the 400 μ g/mL, 300 μ g/mL and 200 μ g/mL concentrations of root extract was found to be the most effective



against larvae and showed 100% of death of larvae after 24 hours. Finding of this study showed that due to its larvicidal effect, all parts of the plant can be used for the prevention and control of Aedes mosquito larva. Different species of Calotropis had received great interest with respect to their effects including cytotoxicity, and antiinflammatory function (Arya and Kumar, 2005). Using the standard techniques advised by (WHO, 2009) the larvicidal activity of aqueous latex extracts of two medicinal plants, Calotropis procera and Ficus benghalensis. was evaluated against the fourth instar larvae of the lymphatic *filariasis* vector, *Culex* quinquefasciatus. Double transformation regression analysis was used to quantitatively determine the dose/response mortality connection and was found to be effective in agreement with the result obtained in this study (Ali and El-Rabaa, 2010).

The larvicidal and antioxidant capabilities of two common Calotropis species, Calotropis and Calotropis procera, were gigantea compared using their ethyl acetate extract. It was shown that, in comparison to C. gigantea, C. procera had a higher level of antioxidant activity in the reduction of ferric ions. The antioxidant potential of the leaves of the plant was also higher than that of the flower and root extract. The leaf methanol extract showed concentration-dependent larvicidal activity with a low LD₅₀ value of 387 mg/l in contrast to other extracts (Patel et al., 2014). Which is in agreement with the result obtained in this study. Calotropis procera plant is considered an effective and natural larvicide against Anopheles arabiensis and Culex quinquefasciatus (Elimam et al., 2009). This is consistent with the outcome of this investigation. The results concerning the larvicidal action of the designated plant are corroborated by these experiments. The results showed that the root extract of Calotropis

procera has remarkable larvicidal activity and might be used as a natural biocide to manage the mosquito population.

CONCLUSION

The larvicidal activity of the Calotropis procera was shown to be maximum at 400 μ g/mL, 300 μ g/mL and 200 μ g/mL and lowest at 100 µg/mL. As a result, it is recommended that a higher dosage of the ethyl acetate crude extract of Calotropis procera be used for optimal activity and efficiency. Isolating strong larvicidal substances that might be employed to control Aedes mosquito larvae could result from purifying these extremely active concentrations. To confirm Calotropis proceras larvicidal effect, additional studies on adult Aedes mosquitoes are advised. By directly burning the roots of Calotropis procera on Aedes mosquito larva species, the results of this study can be used domestically to stop the growth of Aedes mosquito larvae.

REFERENCES

- Abdullahi, M. A., John, V. A., Esther, M. T., and Isyaka, M. S. (2024a). Isolation and Characterization of Betulinic Acid, Stigmasterol and β-Sitosterol from *Calotropis Procera. Nigerian Research Journal of Chemical Sciences*, 12(1):387-401.
- Abdullahi, M. A., Olime, M. N., Usman, G., Muhammad, A. M., Muhammad, I. N., Muhammad, B. U., and Ibrahim, M. U., (2024b). Isolation of squalene from dichloromethane extract of *Croton nigritanus* leaf. *FUDMA Journal of Sciences (FJS)*, 8(2): 212-216.
- Ajaegbu, E.E., Onah, G.T., Ikuesan, A.J., Bello, A.M. (2023). Larvicidal Potency of Some Selected Nigerian Plants against Aedes aegypti . Chem. Proc., 14:35. https://doi.org/10.3390/ecsoc-27-16156
- Ali, N., and El-Rabaa, F. (2010). Larvicidal activity of some plant extracts to larvae of the



mosquito Culex quinquefasciatus (Say 1823). European review for medical and pharmacological sciences, 14:925-933.

- Aqsa, B., Beenish, Z. B., and Seemal, E. V. (2016). Larvicidal potential of *Calotropis* procera
- against Aedes aegypti. International Journal of Mosquito Research, 3(5): 47-51.
- Arya, S., and Kumar, V. L. (2005). Antiinflammatory efficacy of extracts of latex of *Calotropis* procera against different mediators of inflammation. *Mediators of inflammation*, 4:228- 232. doi: 10.1155/MI.2005.228.
- Elimam, A. M., Elmalik, K. H., Ali, F. S.(2009). Efficacy of leaves extract of *Calotropis* procera Ait. (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. Saudi journal of biological sciences,16(2): 95-100.
- Ferdinand, C., Isyaka, M. S., Tiwa A. A., Ibekwe, N. N., John, I., and Abdullahi, M. A. (2023).

Isolation of sitosterol and stigmasterol from the roots of *Croton penduliflorus*. *FUDMA Journal of Sciences (FJS)*, 7(6): 52-56.

- Guzman, M., Gubler, D., Izquierdo, A., (2016). Dengue infection. *Nat Rev Dis Primers* 2, 16055
- https://doi.org/10.1038/nrdp.2016.55
- Gould, E., Solomon, T. (2008). Pathogenic flaviviruses. *The Lancet*, 500-509.
- Jang, Y. S., Kim, M. K., Ahn, Y. S., Lee, H. S. (2002). Larvicidal activity of Brazilian plant
- against Aedes aegypti and Culex pipiens. Agri. Chem. Biotechnol., 4:131-134
- Kareem, S. O., Akpan, I., and Ojo O.P. (2008). Antimicrobial activities of *Calotropis Procera* on selected pathogenic microorganisms. *African Journal of Biomedical Research*,

11: 105- 110.

Meier, C. J., Rouhier, M. F., Hillyer, J. F. (2022). Chemical Control of Mosquitoes and the Pesticide Treadmill: A Case for Photosensitive Insecticides as Larvicides. *Insects*, 13(12):1093. doi: 10.3390/insects13121093. PMID: 36555003; PMCID: PMC9783766.

Patel, H. V., Patel, J. D., Patel, B. (2014). Comparative efficacy of phytochemical analysis and antioxidant activity of methanolic extract of *Calotropis gigantea* and *Calotropis Procera. International Journal of Biological & Pharmaceutical Research*, 5(2): 107-113.

- Parakash, P. K., Prasad, M.N., Kumar, M.V. and Rao, K. S. (2011). Evaluation of heptaprotective effect of *Calotropis* procera (Ait).R. Br. Root extract against paracetamol heptooxidative stress in albino rats. *Int. Res. J. Pharm.*, 2(1):285-291
- Semimul, M. A., and Hussain, S. (2019). An overview: identifications and activities of
- Calotropis procera leaves. World Journal of Pharmaceutical Research, 9(1):764-774.

Upadhyay R.K. (2013). Bio-efficacy of latex extracts from plant species *Thevetia nerifolia*, and *Artocarpus heterophyllus*, *Ficus* glomerata and *Calotropis procera* on survival, feeding, development and reproductive behavior of *Spodoptera litura* (F.) Noctuidae: Lepidoptera). *International Journal of Chemical and Biochemical Sciences*, 4:86-98.

Van, Q. E., Simon, G., André, A., Dewelle, J., Yazidi, M.E., Bruyneel, F. (2005). of a Novel Cardenolide Identification (2"-Oxovoruscharin) from *Calotropis* procera and the Hemisynthesis of Novel Derivatives Displaying Potent in Vitro Antitumor Activities and High in Vivo Tolerance: Structure-Activity Relationship Journal Analyses. of 48(3):849medicinal chemistry, 856. doi: 10.1021/jm049405a. PMID: 15689169.