

## Comparative Evaluation of the Larvicidal Activities of Crude Acetone Root Extract of *Allamanda cathartica* L (Apocynaceae) on the Larvae of *Aedes aegypti* and *Anopheles gambiae*.

Okwubie Lambert,<sup>1\*</sup> Chima Akunna precious.<sup>1</sup>

*1 Department of Pharmacognosy and Phytotherapy, university of Port Harcourt, Nigeria*

Submitted: 15-10-2022

Accepted: 31-10-2022

### ABSTRACT

In this study the evaluation of the larvicidal activities of Acetone root extract of *Allamanda cathartica* on *Aedes aegypti* compare with that of its activity on *Anopheles gambiae* was carried out. 1600g of the powdered root of the of the *Allamanda cathartica* was extracted by maceration using sufficient quantity of the acetone and with intermittent shaking for 72 hours. It was filtered and the filtrate was dried. The extract was then used for the larvicidal assay. The larvicidal assay was evaluated using the method by WHO guideline for larvicidal assay, 2005. Examination of the results showed that the acetone root extract of the *Allamanda cathartica* was very active against the two species of mosquito. However, activity on that of *Anopheles gambiae* is higher than that on the *Aedes aegypti*. The  $LC_{50}$  after 24 hours for *Aedes aegypti* was 0.671mg/ml, while that of *Anopheles gambiae* was 0.363mg/ml. After 48 and 72 hours the  $LC_{50}$  for *Aedes aegypti* was 0.1153 and 0.0255mg/ml respectively, while after 48 hours there were 100% mortality for all the concentrations used on the *Anopheles gambiae*. From the results we conclude that the *Allamanda cathartica* acetone root extract has high activities on both the two species of mosquitoes, though with higher activity on the *Anopheles gambiae* than on *Aedes aegypti* and can developed and used to fight these two dangerous mosquito species.

**KEYWORD:** *Aedes aegypti*, *Anopheles gambiae*, larvicidal.

### I. INTRODUCTION

One of the most dangerous animal as described by WHO is mosquito (WHO, 2015). Mosquito is the vector that transmit different types of disease parasites in man and animals. Because of this mosquito borne diseases are major challenges all over the world especially in third world countries, like those of Africa.

The two species of mosquitoes of interest in this study are the *Anopheles gambiae* and *Aedes aegypti*. *Anopheles gambiae* is of interest because it is the mosquito responsible for the transmission of falciparum, which is the protozoa that causes malaria in man. *Aedes aegypti* is mosquito species that transmit various virus that causes diseases in man. The diseases transmitted by *Aedes aegypti* include yellow fever, dengue fever, zika virus fever and chikungunya.

Although efforts were made to control these vector through different methods, some of these methods have certain disadvantages for instance the disadvantage in the use of DDT is well known. Thus there is the need to look for environmentally safe, degradable, affordable and target-specific compounds against these insect-vectors. The search for such compounds has been directed to the plant kingdom, (marthur, 2003). Before the discovery of synthetic insecticides, natural insecticides such as pyrethrum, rotenone, nicotine, sabadilla, ryania, among others, have been extensively used for insect control. (Balandrin, 1985)

The genus *Allamanda* belongs to the family Apocynaceae. It is warm plant that has yellowish trumpet-shaped flowers which is found in the whole plant all through the year. The plant can easily be planted it also grows very fast, the flowers are beautiful and can be used for decorative purposes. This genus is native to south and central America and then distributed commonly in the tropics (Min et al., 2006).

*Allamanda* species has been shown traditionally to have antifungal, antileukemic and anti-HIV activities (Tan et al., 1991), anticancer (Dobhal et al., 2004), cytotoxic activity against Madison lung carcinoma (Abdel-Kader et al., 1997) and also strong fungi toxicity against some dermatophytes which causes dermatomycosis in both humans and animals (Tiwari et al., 2002). The leaves and roots extracts of *allamanda cathartica*

are also used traditionally as herbal medicine for strong purgative, as antimalarial and in the management of jaundice and enlarged spleen (Liogier, 1995; Nayak et al., 2006). It has also been shown the extracts have good wound healing ability. It also has certain level of antibiotic action against *Staphylococcus* (Nayak et al., 2006). The acetone root extract has strong larvicidal activity against *Aedes aegypti*, (Okwubie and Onu, 2017)

The aim of this study is to compare the larvicidal activity of the acetone root extract of *Allamanda cathartica* against *Anopheles gambiae* and *Aedes aegypti*.

## II. MATERIALS AND METHOD

### Materials

Some of the materials used in this work includes rotary evaporator (Labsience, England), water bath (Techmel and Techmel, USA) analytical weighing balance, glass maceration jar, glass funnels, beakers, crucibles, measuring cylinder, desicator, etc.

Reagents includes acetone (LobaChemie, India), distilled water, Dragendoff's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, hydrochloric acid, chloroform, ethanol, etc.

### Method

#### Collection of plant materials and maceration

The root of the *Allamanda cathartica* was collected from choba park, University of Port Harcourt, Choba, Rivers state, Nigeria. It cut in pieces and cleaned to ensure the absence of impurities. It was then air-dried under shade at room temperature. After drying it was grounded into powder with grinder/blender. 1600g of the powdered root materials was macerated with

sufficient volume of acetone for 72 hours with intermittent shaking. It was then filtered and the filtrate was concentrated with rotary evaporator and finally dried in a water bath at 50°C. the dried extract was then used for larvicidal comparative evaluation.

#### Preparation of stock solution

The stock solution was prepared by dissolving 6g of the sample in 600mls of water. The sample was first dissolve with about 10ml of dimethylsulfoxide (DMSO) and then enough water was added to make up to 600ml.

#### Larvicidal bioassay

The bioassay was carried out according to the method by WHO Standard guidelines for larvicidal assay (WHO, 2005) with some modifications. From the stock solution various concentrations of the extract was prepared for the larvicidal assay. The concentrations used are 0.5, 1, 2, 3, and 4mg/ml. 100mls of each concentration were used. larvae between 3<sup>rd</sup> and 4<sup>th</sup> instar of development was use. The assay was carried out in triplicate with control for each concentration containing 1ml of acetone in 99ml of water. The assay was run simultaneously for the *Anopheles gambiae* and the *Aedes aegypti*. 20 healthy larvae were introduced into each of the concentration in 100ml container and larvicidal activities were monitored by checking for mortality after 24, 48 and 72 hours. The results were then recorded. The dead ones were identified if they do not respond to stimuli after being probed using needle.

Statistical analysis;

The result obtained from the bioassay was analyzed statistically using probits analytical method according Finney, 1971

## III. RESULTS

**Table No. 1: The result showing the percentage yield of the extraction of the root of *allamanda cathartica* using acetone as solvent.**

Parameter	Weight
Weight of plant material	1600g
Weight of extract	23.2g
Percentage yield	1.45%

From table one, the percentage yield after extraction is 1.45%. the weight of material used

was 1.6kg. the amount extracted was 23.2g which gave the above percentage.

**Table No. 2: result of the larvicidal activity of acetone root extract of Allamanda cathartica against Aedesegypti**

Conc. mg/ml	Number of Larvae	Mean Percentage mortality After 24 hours for extract		LC <sub>50</sub> mg/ml	Mean Percentage Mortality After 48 hours for extract		LC <sub>50</sub> mg/ml	Mean Percentage mortality After 72 hours for extract		LC <sub>50</sub> mg/ml
		Control	Extract		Control	Extract		Control	Extract	
0.5	20	0	56.5±15.46	0.671	0	86.5±15.46	0.115	0	95±7.07	0.026
1	20	0	78.5±10.28		0	93.5±9.43		0	96.7±4.7	
2	20	0	96.5±2.36		0	100±0		0	100±0	
3	20	0	100±0		0	100±0		0	100±0	
4	20	0	100±0		0	100±0		0	100±0	

Values are represented as mean of ± S.E.M (standard error of means)

From the result in table two above we see the activities of the extract on the Aedesegypti larvae. LC<sub>50</sub> after 24 hours was 0.671mg/ml, while

after 48 hours the LC<sub>50</sub> reduced to 0.115mg/ml. This further reduced to 0.026mg/ml after 72 hours.

**Table No. 3:Result of the larvicidal activity of acetone root extract of Allamanda cathartica against Anopheles gambiae.**

Conc. mg/ml	Number of Larvae	Mean Percentage mortality After 24 hours		LC <sub>50</sub> mg/ml	Mean Percentage mortality After 48 hours		LC <sub>50</sub> mg/ml	Mean Percentage mortality After 72 hours		LC <sub>50</sub> mg/ml
		Control	Extract		Control	Extract		Control	Extract	
0.5	20	15	86.7±10.7	0.0363	15	100±0		15	100±0	
1	20	5	81.67±8.5		5	100±0		5	100±0	
2	20	10	98.3±2.36		10	100±0		10	100±0	
3	20	0	100±0		0	100±0		0	100±0	
4	20	0	100±0		0	100±0		0	100±0	

Values are represented as mean of ± S.E.M (standard error of means)

The result in table 3 gives the activity of the extract against Anopheles gambiae. After 24 hours the LC<sub>50</sub> was 0.0363mg/ml, but after 48 hours the LC<sub>50</sub> could not be defined because there was 100% mortality signifying very strong activity.

#### IV. DISCUSSION

Aedesegypt and Anopheles gambiae are two species of mosquito that are of very concern to humanity because of their activities in transmitting various disease agents. The activity of the acetone root extract of Allamanda cathartica have been shown to exhibit strong larvicidal action against Aedesegypti. (Okwubie and Onu, 2017)

In this study activities of the acetone root extract on the larvae of two species of mosquitos was carried out and compared. From the results it is very clear that the root extract of the plant is active against the two species of the mosquito larvae, however the activity tends to be more active against the Anopheles gambiae than that of the Aedesegypti. After 24 hours the LC<sub>50</sub> of the Aedesegypt and Anopheles gambiae were 0.671 and 0.363mg/ml respectively. Showing that the activity of the extract is more on the

Anophelesgambiae than Aedesegypt. Also after 48 and 72 hours the LC<sub>50</sub> of the Aedesegypt were 0.115 and 0.026 respectively. However, the LC<sub>50</sub> of Anopheles gambiae after 48 and 72 hours were not defined because there was 100% mortality of the larvae after 48 hours for all the concentrations used, confirming that the extract is a little more active against Anopheles gambiae than Aedesegypt.

Examination of the larvicidal activities of this extract on both the Aedesegypti and Anopheles gambiae larvae showed that they both are time and concentration dependent. As the time and concentration increase, the activities increase. This is also similar to the activity of the root extract of Allamanda cathartica as reported by Okwubie and Onu, 2017.This is also similar the larvicidal activities of some plant extracts as reported by(Okwubie and John 2017), (Ubulom et al., 2012), (Nwabor, et al., 2014) etc. Examination of the results also showed that there were dead of some of the larvae in the control of concentrations 0.5, 1 and 2mg/ml of the Anopheles gambiae larvicidal assay, this may be as a result of the weakness of the dead larvae. This may have no effects on the outcome of the results as this death

occurred within the first 24 hours of the assay and no more death after this time for the remaining 48 hours. Moreover, other concentration both in the *Anopheles gambiae* and *Aedes aegypti* has no death of the control larvae

## V. CONCLUSION

The result of this work showed that *Allamanda cathartica* crude acetone root extract has strong larvicidal activities against both *Anopheles gambiae* and *Aedes aegypti* mosquito species. It can further be worked on to isolate the active agent for development and subsequently used in the control of mosquitoes and by extension the control of the diseases which they are the vectors of their causative agents.

## REFERENCES

- [1]. Abdel-kader, M.S., Wisse, J. Evans, R., Van Der Werff, H, Kingston, D.G.I. Bioactive Iridoids and New Lignin from *Allamanda cathartica* and *Himatanthus fallax* from the Suriname rainforest. *J. Nat. Prod.*(1997): 60: 1294-1297
- [2]. Balandrin: Natural plant chemicals, [http://www.researchgate.net/publication/19272122/public\\_natural\\_plant\\_chemicals](http://www.researchgate.net/publication/19272122/public_natural_plant_chemicals), 1985.
- [3]. Dobhal, M.P., G, Li, A. Gryshuk, A.G., Bhatnager A.K. Structural Modifications of Plumieride Isolated from *Plumeria bicolor* and the effect of these modifications on in vitro anticancer activity. *J. Org. Chem.*,(2004); 69: 6165-6172
- [4]. Finney, D.J. (1971). Probit analysis. Cambridge: Cambridge University Press.
- [5]. Liogier, H.A., Descriptive flora of Puerto Rico and Adjacent Islands. De La Universidad De Puerto Rico, San Juan, Puerto Rico House. *Trials Biological Control*,(1995); 37: 237-342.
- [6]. marthur, S.J.N, Prospects of using Herbal Products in the control of Mosquito vectors. *The Indian council of Medical Research*, New Delhi, 2003; 1 (33):1-10.
- [7]. Min BC., Omar-Hor K., Lin O. Y. C (2006); 1001 Garden Plants in Singapore. 2<sup>nd</sup> Edition, National Park Board, Singapore.
- [8]. Nayak, S., Nalabothu P., Sandiford, S., Bhogadi, V., Adogwa, Evaluation of wound healing activity of *Allamanda cathartica* L and *Lauria nobilis* L. extracts on Rat. *BMC Complement Altern. Med.*;(2006): 6 12
- [9]. Okwubie Lambert and Dan John., Evaluation of Larvicidal Activities of Stem Bark and Seed Extracts of *Picralima Nitida* (Stapf.) T. Durand and H. Durand (Apocynaceae) on *Aedes Aegypti* Larvae. *International Journal of Pharmacy and Biological Sciences*, 2017; 7(3): 65-70
- [10]. Okwubie, Lambert and Onu, Umunakwe. Evaluation of the Larvicidal Activity of the Root Extracts of *Allamanda Cathartica* L (Apocynaceae) On *Aedes Aegypti* Larvae. *Nigerian Journal of Pharmaceutical and Applied Science Research*,(2017): 6(1):61-64,
- [11]. Nwabor, Ozioma Forstinus Dibua, Uju Marie- Esther Nnamonu, Emmanuel Ikechukwu Odiachi,
- [12]. Osita Dickson, Dickson Okoro, Onyekwwe Joseph. Pulp Extracts of *Picralima nitida*: a
- [13]. Larvicidal Agent in Malaria Vector Control. *Journal of Biology, Agriculture and Healthcare*, (2014): 4(8): 69 –73
- [14]. Tan, G.T., Pezzuto J.M., A.D. Kingborn, Hughes, S.H., Evaluation of Natural Products as Inhibitors of Human Immunodeficiency Virus Type 1 (HIV-1) Reverse Transcriptase. *J. Nat. Prod.*, 1991: 54 143-154
- [15]. Tiwari, T.N., Pandey V.B., Dubey, N.K. Plumieride from *Allamanda cathartica* as an Antidermatophytic agent. *Phytother. Res.*,(2002): 16: 393-394.
- [16]. Ubulom, M. E., Imandeh, N. G., Udobi, C.E. and Ilya, I. Larvicidal and Antifungal Properties of *Picralima nitida* (Apocynaceae) Leaf Extracts. *European Journal of Medicinal Plants*, (2012):2(2): 132-139
- [17]. WHO,(2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO communicable disease control, prevention and eradication. WHO pesticide evaluation scheme WHO/CDS/WHOPES/GCDPP/2005.13.
- [18]. WHO,(2015). Mosquito borne diseases, [www.who.int/neglected\\_diseases/vector\\_ecology/mosquito-borne-diseases](http://www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases)