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Research Paper

Evaluation of the Larvicidal Activity of Crude Acetone Stem Extract of *Allamanda cathartica*L (Apocynaceae) on *AedesAegypti* Mosquito Larvae.

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ABSTRACT
Aedes aegypti is the mosquito species responsible for the spread of viruses that cause yellow fever, dengue fever zika, and, chikungunya diseases. The spread of these diseases could be control by controlling this vector responsible for their transmission. In this study the larvicidal activity of the crude acetone stem extract of Allamanda cathartica on Aedes aegypti larvae was evaluated. Sufficient quantity of the stem of the plant was collected and dried under the shield. It was then grinded and macerated with enough volume of the acetone solvent for about 48 hours. It was then filtered and concentrated with rotary evaporator and dried on the water bath at temperature of about 50^{o}C. A stock solution of 10mg/ml was prepared for the extract from which five different concentrations (1.0mg/ml, 2.0mg/ml, 3.0mg/ml, 4.0mg/ml and 5mg/ml in 100ml in triplicate for each of the concentration) were made and used for the test in accordance with World Health Organization (WHO) guideline with some modifications. A control was set up with only water for each concentration in 100ml of water. A total of 20 larvae were used for each of the concentration and control and mortality was recorded at 24, 48 and 72 hours.Examination of the result showed that crude acetone stem extract of the plant gave good larvicidal activity with LC_{50} of 2.17mg/ml after 24 hours. This reduced to 1.09 and 0.74mg/ml after 48 and 72 hours respectively. The highest concentration gave 100% mortality within 24 hours while the lowest concentration gave 68.35% mortality within 72 hours. The results of this study showed that the crude acetone stem extract of Allamanda cathartica gave strong larvicidal activity and could be developed into a strong mosquito larvicidal agent.

Keywords: *Allamanda Cathartica, stem extract, aedes aegypti.*

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I. INTRODUCTION

Mosquito borne diseases are major health challenges to human race. In fact, the WHO has described mosquito as one of the most dangerous animals in the world (WHO, 2015). Different species of mosquito spread different diseases. For instance, *Aedes aegypti* is the vector to the viruses causing yellow fever, dengue fever zika virus disease and chikungunya, while *Anopheles gambiae* is the vector to the spread of protozoa that cause malaria especially the falciparum protozoa.

Different method could be used to control mosquito multiplication. Targeting the adult mosquito through spraying chemical insecticides or killing the mosquito larvae before the emerge int adults through using synthetic larvicides or botanical extracts as an alternative larvicide are the method which could be use in controlling the mosquitoes (Mohini et al., 2007), these all depend on the growth stages of its life cycle of egg, larvae, pupae and adult.

The mosquito genus of interest in this study is the *Aedes aegypti*. The eggs of *aedes aegypti* and *Aedes albopictus* hatch within a few days into larvae when there are good conditions particularly at high temperature and flooding. Between 9 and 13 days the larvae undergo four molts. The male mosquitoes develop faster than the females and molt earlier into pupae. The pupae develop further into adult mosquitoes after 2 days (Olivia et al., 2020)thus the multiplication of this mosquitoes can be very rapid. The control of these mosquitoes is challenging because of the disperse and transient egg laying pattern of the female *Aedes aegypti* mosquitoes and its effective adaptation to the urban habitat. (Muktar et al., 2016)

The golden trumpet as *Allamanda cathartica* is commonly called belongs to the family apocynaceae. It has yellowish trumpet-shaped flowers which is found in the whole plant all through the year. It is a warm plant.

It grows very fast and can easily be planted. It has beautiful flowers 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).

The plant has been shown to have different medicinal properties.Many herbalists have claimed to use the leaves, roots, flowers or stem bark for the treatment of various fevers, jaundice, gastrointestinal disorders and malaria. (Iwu et al., 1993)(Etukudo et al., 2003).Allamanda species has also been shown traditionally to haveantifungal, 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).It also has certain level of antibiotic action against Staphylococcus. (Nayak et al 2006).(Okwubie and Onu, 2017) has shown that acetone root extract of the plant has strong larvicidal activity against *Aedesaegypti*. The aim of this study is to evaluate the larvicidal activity of the acetone stem extract of *Allamanda catharticaon Aedes aegypti.*

II. MATERIALS AND METHODS

Materials

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

Method

Collection of plant materials and maceration

The stem of the *Allamanda cathartica* was collected from Chobacampus, University of Port Harcourt, Choba, Rivers state, Nigeria. It was cut in pieces and air-dried under shade at room temperature. After drying it was grounded into powder with grinder/blender. A total of 2,170g of the powdered root materials was macerated with sufficient volume of acetone for48 hours after shaking. It was then filtered and the filtrate was concentrated with rotary evaporator and finally dried in a water bath at 50^{O}C. the dried extract was then used for the larvicidal evaluation.

Preparation of stock solution

The stock solution was prepared by dissolving 5g of the sample in 500mls of water. The sample was first dissolve with about 5ml of dimethysufoxide (DMSO) and then enough water was added to make up to 500ml. This gave stock solution of concentration10mg/ml.

Larvicidal bioassay.

The bioassay was carried out according to the method by WHO Standard guidelinesfor 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 1, 2, 3, 4 and5mg/ml. 100mls of each concentration were used. larvae between 3rd and 4th instar of development was use. The assay was carried out in triplicate with control for each concentration containing only water. 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 resultswere then recorded. The dead ones were identified if they do not respond to stimuli after being probed with shape material.

Statistical analysis.

The result obtained from the bioassay was analyzed statistically usingLdp line software forprobits analysis according to(Finney, 1971).

III. RESULTS

Table 1: The result showing the percentage yield of the extraction of the stem of *Allamanda cathartica* using acetone as solvent.

Parameter	Approximate Weight
Weight of plant material.	2,170g
Weight of plant extract.	13g
Percentage yield	0.6%

From the table 1 above, the percentage yield of the extraction is 0.6%. this may be regarded as low percentage

Table 2 Laboratory result for the larvicidal assay.

Conc. Mg/ml	Number of larvae use for each replicate is 20														
	Mortality after 24 hours					Mortality after 48 hours					Mortality after 72 hours				
	replicates			mean	control	replicates			Mean	control	replicates			mean	control
	1	2	3			1	2	3			1	2	3		
1	3	1	0	1.33	0	10	9	10	9.67	0	15	14	12	13.67	0
2	11	11	6	9.33	0	12	16	17	15	0	15	18	18	17	0
3	10	12	12	11.33	0	16	15	17	16	0	20	20	20	20	0
4	20	20	19	19.67	0	20	20	20	20	0	20	20	20	20	0
5	20	20	20	20	0	20	20	20	20	0	20	20	20	20	0

Table 2 showed the results for the larvicidal assay. Examination of the result showed that the extract is active against the *Aedes aegypti* larvae with complete mortality after 72 hours at concentration of 3mg/ml but at a concentration of 5mg/ml there was total mortality within 24 hours.

Table 3 Table showing the Percentage mortality and $LC_{50}$of the larvicidal assay.

Conc. mg/ml	After 24 hours			After 48 hours			After 72 hours		
	Mean mortality	% Mortality	LC_{50} mg/ml	Mean mortality	% Mortality	LC_{50} mg/ml	Mean mortality	% mortality	LC_{50} mg/ml
1	1.33	6.65±6		9.67	48.35±2.4		13.67	68.35±10.8	
2	9.33	46.65±11.1		15	75±10.8		17	85±7.1	0.74
3	11.33	56.65±4.7	2.17	16	80±4.1	1.09	20	100±0.0	
4	19.67	98.35±2.3		20	100±0.0		20	100±0.0	
5	20	100±0.0		20	100±0.0		20	100±0.0	

Table 3 showed the results for percentage mortality and LC_{50} of the larvicidal assay of the extract. Examination of the results showed 6.65 was the list percentage mortality observed with concentration of 1mg/ml after 24 hours while at a concentration of 3mg/ml we have 100% mortality of after 72 hours.

IV. DISCUSSION.

Mosquitoes borne diseases are major concerns to health providers in the world and one major way to control these diseases is to control the vector which is mosquitoes. The disadvantages of synthetic insecticides made it possible to turn our attention to plant biochemical that are insecticidal in general and larvicidal in particular. Many plants have been shown to have insecticides properties for instance, Roark describe about 1,200 plant species having potential insecticidal value (Roark, (1947)

Examination of these results showed acetone crude stem extract of *Allamanda cathartica* has very significant larvicidal activity at concentration of 1mg/ml which is the lowest concentration considered in this evaluation within 24 hours though with low percentage mortality. By 72 hours the percentage mortality has increased to 68.35% at this concentration. However, the percentage mortality increased to 100% within 24 hours at concentration of 5mg/ml. Thus, it can be seen that the larvicidal activity depend on the concentration and time of exposure.This is similar to the activity of some larvacidal plants as reported by (Ubulom et al., 2012) and (Nwabor et al., 2014), which also showed concentration and time dependent in their activities. Also, Comparative Evaluation of the Larvicidal Activities of Crude Acetone Root Extract of *Allamanda cathartica*L(Apocynaceae) on the Larvae of *Aedesaegypti* and *Anopheles gambiae* by (okwubie and Chima, 2022) gave the same time and concentration activities dependent on both the *Aedes aegypti* and *Anopheles gambiae* larvae.

The LC_{50} decreased with increase in time of exposure and concentration. After 24 hours the LC50 was 2.17mg/ml. this decreased to 1.09mg/ml after 48 hours it also further decreased to 0.74mg/ml after 72 hours. This was also observed by (Okwubie and Onu, 2017)in the larvicidal activity of the acetone root extract of this plant on the *Aedesaegypti* larvae. On the overall, the stem extract of this plant *Allamanda cathartica*gave a strong larvicidal activity *against Aedes aegypti.*

V. CONCLUSION

The result of this investigation on larvicidal activity of acetone crude stem extract of *Allamanda cathartica* against *Aedes aegypti* showed that the crude extract is very active with low LC_{50}. More investigation could be done to isolate and identify the active agent(s) responsible for the larvicidal activity and could further be developed and use for effective control of this mosquito which is a vector to many diseases causing organisms.

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