Evaluation of the Larvicidal Activity Of The Root Extracts of *Allamanda Cathartica* L (Apocynaceae) On *Aedes Aegypti* Larvae

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ABSTRACT

Aedes aegypti is a species of mosquito that transmits yellow fever, zika virus fever, dengue fever and chikungunya diseases. *Allamanda cathartica* L is an evergreen tropical plant that is grown as a climbing vine or pruned as a shrub, belonging to the family Apocynaceae. The aim of this research is to evaluate the larvicidal activity of *Allamanda cathartica* root on *Aedes aegypti* larva. The acetone and methanol extracts were obtained by exhaustive soxhlet extraction, while the aqueous extract was obtained through maceration. A stock solution of 10mg/ml was prepared for each extract from which six different concentrations (0.5mg/ml, 1.0mg/ml, 2.0mg/ml, 3.0mg/ml, 4.0mg/ml and 5mg/ml in 100ml) were made and used for the test in accordance with World Health Organization (WHO) guideline. A control was also prepared for each extract by adding 1ml of the solvent to 100ml of water. A total of 25 larvae were used for each of the concentration and mortality was recorded at 24, 48 and 72 hours. The three extracts showed larvicidal activity against *Aedes aegypti* larva with acetone extract having the highest activity followed by methanol and aqueous extract respectively. At 72 hours, the LC_{50} of acetone, methanol and aqueous extracts were 0.61mg/ml, 2.98mg/ml and 6.52mg/ml respectively. The result shows that acetone extract of the root of *Allamanda cathartica* could be a veritable source of Larvicides.

Keywords: *Allamanda cathartica, Aedes aegypti,* larvicide, larvicidal bioassay

1. INTRODUCTION

Mosquito borne diseases constitute a major health menace all over the world especially in Africa. According to WHO, 2015, Mosquitoes are one of the most deadly animals in the world. Their ability to carry and spread diseases to humans causes millions of deaths every year. Annually, millions of people and man-hours with attendant economic implications are lost to this pandemic. Different genera of mosquito are known to transmit different diseases. The genus of interest in this study is the Aedes. Diseases transmitted by *Aedes aegypt* include yellow fever, dengue fever, zika virus fever and chikungunya . Different strategies have been devised to curb disease transmission by these vectors but these have suffered certain limitations. These limitations have necessitated the search for environmentally safe, degradable, affordable and target-specific compounds against these insect-vectors. Searching for new control agents from natural products such as plant secondary metabolites have gained popularity among researchers in countries with a strong herbal tradition.

Large numbers of plants have been reported to possess insecticidal activity (Yang and Tang, 1988). Larvicides are insecticides that are specifically targeted against the larval life stage of an insect. Their most common use is against mosquitoes. Larviciding is a preferred option in mosquito control because the larvae occur in specific areas and can thus be more easily controlled.

Allamanda cathartica (Apocynacaea) is a medicinal plant, commonly called golden trumpet. 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, 1993 and Etukudo, 2003). Its larvicidal activities against *ancylostoma* species have also been reported by researchers such as (Santos et al, 2013). However, no scientific document has been encountered on the larvicidal effect of aqueous, acetone and methanolic root extracts of *Allamandac athartica* on the mosquito species used in this study, which is the main objective of this study.

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2. METHODOLOGY

2.1 Collection of Plant Materials

Plant sample (root) of *Allamanda cathartica* was collected from Choba campus, University of Port Harcourt, Rivers State, Nigeria. The plant was identified by Dr. Oladele of Department of Forestry, University of Port Harcourt. It was deposited at the Department of Pharmacognosy herbarium, University of Port Harcout with the voucher number UPH0290

2.2 Sample Preparation

The roots of the test plant were collected, cleaned and carefully sorted to ensure the absence of impurities such as sands, leaves, pulp and stone particles. They were then spread out on a clean surface and allowed enough time to air-dry under shade at room temperature. The dried plant sample was then pulverized using a mechanical blender. The pulverized sample was divided into four portions of 150 gram each.

2.3 Extraction

The first portion of the pulverized plant sample was subjected to soxhlet extraction using acetone. The second portion was also subjected to soxhlet extraction using methanol. The extracts obtained were respectively transferred to crucibles and dried by placing on water bath at temperature of about 50^0C. The third portion of the pulverized plant sample was macerated using water for 48 hours with intermittent shaking after which it was decanted and filtered. The filtrate (extract) was transferred to crucible and dried using water bath at temperature of about 50^0C. The extracts obtained were transferred to air tight containers and stored in desiccators until the time of use. The percentage yields of the extracts were determined.

2.4 Phytochemical Screening

Phytochemical tests were carried out on the pulverized sample using standard phytochemical screening method according to (Harborne, 1984; and Sofowora, 2006). The phytochemicals that were screened for includes; saponins, Tannins, phlobatannins, flavonoids, anthraquinones, cardiac glycosides, terpenes, carbohydrates and alkaloids.

2.5 Test organism

Fourth instar larvae of *Aedes aegypti* used in this investigation were provided by National Arbovirus and Vectors Research Centre (NAVRC), Enugu, Nigeria.

2.6 Larvicidal Bioassay

The larvicidal tests were carried out against 4^{th} instar larvae of *Aede* saegypti in accordance with WHO guidelines for larvicidal testing 2005 with slight modification. Stock solution of 10mg/ml was prepared. From the stock solution, different concentrations (0.5, 1.0, 2.0, 3.0, 4.0, & 5.0mg/ml in 100ml volume) were made for each extract. A control was also prepared for each extract by adding 1ml of the solvent used for extraction to 100ml of water. The tests were conducted in plastic containers of 100ml. Three replicates and a control were run simultaneously for each concentration, and a total of 25 healthy larvae were used for each container. The tests were carried out at room temperature (28 ± 2^0c). Mortality was observed at 24, 48 and 72hours. Larvicidal activity of each extract was determined by counting the number of dead larvae on daily basis (24hours interval). Dead larvae were recorded when they failed to move after probing with a needle. (WHO, 2005)

2.7 Statistical Analysis

The LC_{50} and LC_{90} of each of the extracts were calculated using standard method of probits, (finney 1971).

3. RESULTS

The yield value of the acetone, methanol and aqueous extracts of *Allamanda cathartrica* are 6.16%, 10.02% and 7.57% respectively. The phytochemicals detected by phytochemical screening of the crude root extract were terpenoids and steroids, carbohydrate, cardiac glycoside, flavonoids and tannins (Table 1).

From the table above the larvicidal activity of acetone extract after 24hours, 48hours and 72hour are shown. After 24hours, the LC_{50} and LC_{90} are 0.9298mg/ml and 1.4264mg/ml respectively, while after 48hours LC_{50} and LC_{90} are 0.885mg/ml and 1.3358mg/ml. After 72hour the LC_{50} and LC_{90} became 0.6131mg/ml and 0.9642mg/ml which gave us the highest activity (Table 2).

The result showed that the methanol extract has lesser activity than that of acetone. The LC_{50} and LC_{90} after 24hours are 7.8779mg/ml and 42.4598mg/ml respectively, while after 48hours the LC_{50} and LC_{90} became 3.6357mg/ml and 22.7181mg/ml respectively. On the other hand the LC_{50} and LC_{90} after 72hours became 2.9817mg/ml and 15.6893mg/ml respectively (Table 3).

The aqueous extracts showed the least lavicidal activity. After 24hours, the LC_{50} and LC_{90} were respectively 9.367mg/ml and 16.8241mg/ml, while after 48 hours they were respectively 7.107mg/ml and 11.4364mg/ml and after 72hours the LC_{50} and LC_{90} were respectively 6.517mg/ml and 9.8815mg/ml

Table 1: Larvicidal Activity of Acetone Root Extract Of *Allamanda Cathartica*

	24hour			48hour				72hour	
Conc (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml	% Mortality	LC_{50} (mg/ml	LC_{90} (mg/ml)
Control 0	0	0.9298	1.4264	0	0.885	1.3358	0	0.6131	0.9642
0.5	4			4			28		
1	56	Lower limit	Lower limit	64	Lower limit	Lower limit	92	Lower limit	Lower limit
2	100	0.7979	1.2031	100	0.7604	1.1309	100	0.5136	0.8072
3	100			100			100		
4	100	Upper limit	Upper limit	100	Upper limit	Upper limit	100	Upper limit	Upper limit
5	100	1.0818	1.9568	100	1.0266	1.8308	100	0.716	1.3879

Table 2: Larvicidal Activity of Methanol Root Extract Of *Allamanda Cathartica*

	24hour			48hour				72hour	
Conc (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml	% Mortality	LC_{50} (mg/ml	LC_{90} (mg/ml)
Control 0	0	7.8779	42.4598	0	3.6357	22.7181	0	2.9817	15.6893
0.5	4			12			12		
1	4	Lower limit	Lower limit	16	Lower limit	Lower limit	20	Lower limit	Lower limit
2	12	5.0159	15.8465	24	2.6538	10.8882	24	2.2516	8.6864
3	12			48			56		
4	24	Upper limit	Upper limit	56	Upper limit	Upper limit	56	Upper limit	Upper limit
5	48	28.5973	1033.0047	60	6.1078	136.208	72	4.3337	57.0454

Table 3: Larvicidal Activity of Aqueous Root Extract Of *Allamanda Cathartica*.

	24hour			48hour				72hour	
Conc (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml)	% Mortality	LC_{50} (mg/ml)	LC_{90} (mg/ml	% Mortality	LC_{50} (mg/ml	LC_{90} (mg/ml)
Control 0	0	9.367	16.8241	0	7.107	11.4364	0	6.517	9.8815
0.5	0			0			0		
1	0			0			0		
2	0			0			0		
3	0			0			0		
4	4			8			8		
5	8			16			20		

4.1 DISCUSSION

The plant kingdom has proved to be a reliable reservoir of potent phytochemicals which can serve as suitable, efficient, readily available and eco-friendly alternatives in the fight against insect pest. For instance Roark describe about 1,200 plant species having potential insecticidal value (Roark, 1947). In line with the search for compounds with excellent activity against insect pest, this work evaluates the larvicidal potentials of crude acetone, methanol and aqueous root extracts of *Allamanda cathartica* on the yellow fever vector, *Aedes aegypti.*

From the result, the percentage yields of the extracts are 6.16%, 10.02% and 7.57% for acetone, methanol and aqueous extracts respectively. Methanol had the highest percentage yield. This could be as a result of the polarity index of methanol which confers on it the ability to extract both the polar and non polar constituents of the root sample. Water, being a polar solvent, extracted the polar constituents of the root sample and gave a percentage yield of 7.57. Acetone is the least polar of the three solvents used and it gave the least percentage yield. This implies that acetone extracted more of the non polar constituents of the root sample.

Phytochemical screening of the root of *Allamanda cathartica* revealed the presence of carbohydrates, free reducing sugars, tannins, flavonoids, cardiac glycosides, terpenoids and steroids. The larvicidal activity of the plant can attributed to one or more of any of these phytochemical which can be ascertain after proper

isolation and characterization of the constituents of the extracts.

From the larvicidal bioassay result, acetone extract was found to show more larvicidal activity followed by methanol extract and aqueous extract had the least activity. The result obtained showed a time and concentration dependent increase in larvicidal activity. The acetone extract, at concentration of 0.5mg/ml was able to cause 28% mortality in 72 hour. On increasing the concentration to 1mg/ml, 92% mortality was recorded. Further increase in concentration (2, 3, 4, & 5mg/ml) gave 100% mortality in 24 hours. Methanol extract gave a reduced larvicidal activity when compared to the acetone extract. None of the concentrations tested was able to give 100% mortality although the result still showed a time and concentration dependent increase in larvicidal activity. The highest concentration (5mg/ml) was able to give 72% mortality in 72 hours. Aqueous extract had the least larvicidal activity. It was not able to show larvicidal effect even up to concentration of 3mg/ml. Its highest concentration (5mg/ml) was only able to give 20% mortality in 72 hours.The result showed a strong time – dependent correlation between the concentration of the extracts and the mortality rate of the larvae. This is evident in carefully examining the effect of time on the mortality rate. It can be observed that at a longer time of 48 and 72hrs of exposure, more larval mortality was recorded at same concentration of extracts. The same trend was also observed on the values of the LC_{50} and LC_{90} which are concentration and time dependent. 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.

4.2 CONCLUSION

Results obtained from this study showed that extract of the root of *Allamanda cathartica* may serve as an alternative to synthetic insecticides in the control of the deadly yellow fever, dengue fever and zika virus disease vector, *Aedes aegypti*. Also since the acetone extract gave the highest activity then we can conclude that the larvicidal activity of the root of *Allamanda cathartica* could be attributed to its non polar constituents.

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