



Larvicidal Activity of Aqueous Pod Extract of *Adansonia Digitata* (Linnaeus, 1753) on *Culex Quinquefasciatus* (Say, 1823)

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Abstract

The study evaluated the effect of Baobab, *Adansonia digitata* pod extract on larvae of *Culex quinquefasciatus*. The plant material was sun-dried for two weeks and pulverized using mortar and pestle and stored at room temperature for further processes. Powdered plant material was extracted using aqueous cold extraction method. The condensed extract was screened for phytochemicals. Indoor resting adult blood-fed female *Culex* mosquitoes were collected and introduced into Entomological Cages in the Laboratory and oviposited on water in the bowls. The third instar larvae were confirmed as *Cx. quinquefasciatus* using Hopkins keys for Culicinae. Triplicates of twenty-five late third instar larvae were tested in pod extract concentrations of 0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L and 2.50mg/L. Mortality was recorded after 24hr of exposure. The LC₅₀ was determined using probit analysis, while one-way ANOVA was used to establish significant differences in larval mean mortality. Phytochemical constituents such as carbohydrate, cardiac glycosides, steroids, triterpenes, tannins, flavonoids and alkaloids were present. The mean mortality of test concentrations differed significantly ($p \leq 0.05$) from control. However, the concentrations: 0.5mg/L and 8.0mg/L had 49% and 71% mortalities, respectively with LC₅₀ of 0.6mg/L. It can be concluded that, the aqueous pod extract of *Adansonia digitata* demonstrated good activity against *Cx. quinquefasciatus*. *Adansonia digitata* pod extracts has potential for controlling larvae of *Culex* mosquitoes and can be incorporated into integrated mosquito management programme.

Keywords: *Adansonia digitata*, Pod Extract, Larvae, *Culex quinquefasciatus*.

INTRODUCTION

Mosquitoes belonging to genera such as *Aedes*, *Anopheles* and *Culex* constitute the foremost vectors of several debilitating diseases in the tropical regions affecting humans and domestic animals (Reuda, 2008; Olayemi *et al.*, 2014). These diseases have

not only caused very high level of morbidity and mortality, but also loss of manpower, man-hours and economic loss (Olayemi *et al.*, 2014). These and other pitfalls have compelled Scientists to advocate for a refocus on the use of Botanicals, in

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Integrated Mosquito Management (IMM) protocols.

Sub-Saharan Africa carries a disproportionately high share of the global mosquito burden. Nigeria is suffering from an outbreak of yellow fever, accounting for 67.8% with male to female ratio 2:1 (NCDC, 2019). The re-emergence of Dengue virus infections in Africa and Zika virus in South America can be best tackled through control of vector.

This is predicated on the fact that insects have been feeding on plants and co-evolved with insects, and, overtime, have been equipped with a superfluity of chemical defenses. Alkaloids, terpenoids, essential oils, steroids, phenols, saponnins, glycosides and tannins, are constituents of some defense system used against predatory insects and herbivore by plants (Pratheeba *et al.*, 2015). These compounds are known to play vital roles in the physiology of plants (Katie and Thorington, 2006; Arivoli *et al.*, 2012). Furthermore, these bioactive compounds have been used in local medicine (Manner *et al.*, 2013). With these bioactive chemicals, significant reduction in chances of development of resistance by pests has been reported, as the substances strongly act on both behavioural and physiological processes of insects (Ghosh *et al.*, 2012). They are also relatively cheap, readily available and affordable (Kumar and Maneemegalai, 2008), non-toxic to mammals, biodegradable (Gomathi *et al.*, 2014) and more environmentally friendly (Kamaraj *et al.*, 2011). Baobab has diverse biological properties such as antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities (Kamatou *et al.*, 2011). Extracts from Baobab roots eliminate the motility in *Trypanosoma congolense* within 60min and drastically reduce the motility of *Trypanosoma brucei brucei* (Manfredini, 2002). Baobab pod has not been tested on

mosquitos and this informs its use in this study.

The menace caused by mosquitoes through biting and transmission of diseases is on the rise. Frequent uses of insecticides have led to the development of resistance by mosquitoes, making their control difficult. Alternative approach using plant based biocide will offer solution to the problems associated with synthetic insecticide. In light of the diseases associated with mosquitoes this study aimed at evaluating the effect of Baobab (*Adansonia digitata*; Linnaeus, 1753) pod extract on larvae of *Culex quinquefasciatus*.

MATERIALS AND METHODS

Study Laboratory

The research was conducted in the Entomology and Parasitology Laboratory of the Department of Zoology, Ahmadu Bello University (ABU), Zaria. The Baobab pod was collected from the fruiting tree in the surrounding of the Department of former Biological Sciences ABU, Zaria. The Baobab pod was sun-dried for two weeks and pulverized using mortar and pestle and stored the powder in labeled polythene bags at room temperature till ready for use.

Extraction and Phytochemical Screening of Baobab Pod

The pulverized pod was extracted using aqueous extraction method in the Department of Pharmacognosy and Drug Development, ABU, Zaria. One hundred grams (100g) of the pulverized baobab pod was soaked in 200ml of distilled water for 24hours following the method of Adebote *et al.* (2011). After 24 hours it was filtered using muslin clothe, while Rotary evaporator was used to remove excess solvents from the extracts until solidification. Extract was stored in labeled container at laboratory

conditions for further use. The solvent extract was subjected to preliminary phytochemical screening according to the procedures and methods described by Harborne (1973) and Trease and Evans (2002).

Collection and Maintenance of Mosquitoes

Indoor resting adult blood-fed female *Culex* mosquitoes were collected using aspirator into plastic containers from classrooms in the Departments of Biology, Botany and Chemistry, Ahmadu Bello University (ABU), Zaria, Nigeria. Trapped mosquitoes were transferred to the Entomology and Parasitology Research Laboratory in the Department of Zoology, ABU, Zaria and were then released into Entomological Cages containing bowls of water and 10% sugar solution soaked in cotton wool that was placed on the floor of the cage. Mosquitoes then oviposit on water in the bowls (Adebote *et al.*, 2011). The laid egg rafts hatched into first instar larvae and were fed with biscuit (Cream crackers) for 5-6 days until they developed into third instar larvae. The third instar larvae were confirmed to belong to *Cx. quinquefasciatus* using Hopkins (1952) keys for Culicinae.

Larvicidal Bioassay

After preliminary study, the following concentrations, 0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L, and 2.5mg/L were prepared from baobab pod extract. To obtain miscible solutions of the solidified extracts, the solid residue was first homogenized with 1 or 2 drops of tween20 before diluting to the desired concentrations with distilled water.

Twenty-five (25) third instar larvae of *Cx. quinquefasciatus* were isolated from the culture into a test container using a dropping pipette. Batches of 25 larvae were carefully transferred into 250mL capacity plastic bowls containing 100mL test solution for each concentration and labeled. The experiment was set up in triplicate for each concentration. Mosquito larvae were exposed in the test concentration and distilled water as control at laboratory conditions for 24 hours after which larval mortality in each bowl was determined. Larvae were counted and recorded as dead when it did not respond to the probing by needle.

Statistical Analyses

Data generated from larval mortality of *Cx. quinquefasciatus* was subjected to probit analysis to determine the median lethal concentration (LC_{50}) and measure of goodness fit (R^2). Descriptive statistics was used to determine the percentage mortality of *Cx. quinquefasciatus* in each concentration. One-way Analysis of Variance (ANOVA) was used to test for significant differences in the mean larval mortality in control and the various experimental concentrations. Duncan Multiple Range Test was used to separate the differences in means. All data were tested at $\alpha = 0.05$ level of significant.

RESULTS

Table 1 shows the phytochemical constituents in aqueous extract of *Adansonia digitata*. Carbohydrate, cardiac glucoside, steroids, triterpenes, tannins, flavonoid and alkaloid, were all present in the extract. However, anthraquinones and saponnins were not detected in the extract.

Table 1: Qualitative Phytochemical Constituents of the Aqueous Extract of *Adansonia digitata* Pod

S/No.	Phytochemicals	Test	Result
1.	Carbohydrates	Molisch test	+
2.	Anthraquinones	Bontragers test	-
3.	Cardiac glycosides	Kelle-killiani test	+
4.	Saponins	Frothigs test	-
5.	Steroids	Liebermam-burchard test	+
6.	Triterpenes	Liebermam burchard test	+
7.	Tannins	Ferric chloride test	+
8.	Flavonoids	Sodium hydroxide test	+
9.	Alkaloids	Dragendoff's test	+

Key: + = present; - = absent

The mortality of late third instar larvae of *Cx. quinquefasciatus* on exposure for 24 hours to various concentration of *A. digitata* aqueous extract is shown in Table 2. Mortality of *Cx. quinquefasciatus* larvae ranged from 0/75 (0%) in the control to 53/75 (71%) at concentration of 2.5mg. Larval mortality increased in a concentra-

tion-dependent fashion. Mean larval mortalities at the various experimental concentrations were significantly ($P < 0.05$) higher than the control; but increase in concentrations 0.5mg/L (49%), 1.0mg/L (56%), 1.5mg/L (64), 2.0mg/L (63%) and 2.5mg/L (71%) did not produce significant ($P > 0.05$) increase in mean larval mortality.

Table 2: Mortality of *Culex quinquefasciatus* Larvae (n=75) on Exposure for 24 hours to Various Concentration of Aqueous Extract of *Adansonia digitata*

Treatment (mg/L)	No. exposed (Mean±SE)	% Mortality	F-value	ANOVA probability (p-value)
Control	0 (0.00±0.00 ^b)	0	5.439	0.008
0.50	37 (12.33±3.38 ^a)	49		
1.00	42 (13.88±3.50 ^a)	56		
1.50	48 (16.12±2.00 ^a)	64		
2.00	47 (15.65±3.65 ^a)	63		
2.50	53 (17.78±2.00 ^a)	71		

Means with the same superscript letter per column is not significantly different ($p > 0.05$).

Table 3 shows the results of the probits analysis of mortalities of *Cx. quinquefasciatus* larvae on exposure to various concentrations of aqueous extract of *A. digitata*. On conversions of the extract concentrations to natural logarithms, and percentage mortalities to empirical probits of

kill, a plot of probits of kill against logarithm of concentrations (Figure 4.1) gave a regression equation of $y = 0.8503x + 5.1904$ and a coefficient of determination ($R^2 = 0.8577$). The median lethal concentration (LC_{50}) was 0.5971mg/L that was calculated from the regression equation.

Table 3: Median Lethal Concentration (LC₅₀) of Aqueous Extract of *Adansonia digitata*

Conc. (mg/L)	Log-conc.	% Mortality	Empirical probit of mortality	Regression equation (R ² -value)	LC ₅₀ (mg/L)
2.5	0.3980	71	5.6501	y = 0.8503x + 5.1904 (R ² = 0.8577)	0.5971
2.0	0.3010	63	5.3110		
1.5	0.1761	64	5.3609		
1.0	0	56	5.1502		
0.5	-0.3010	49	4.970.1		
Control	-	0	-		

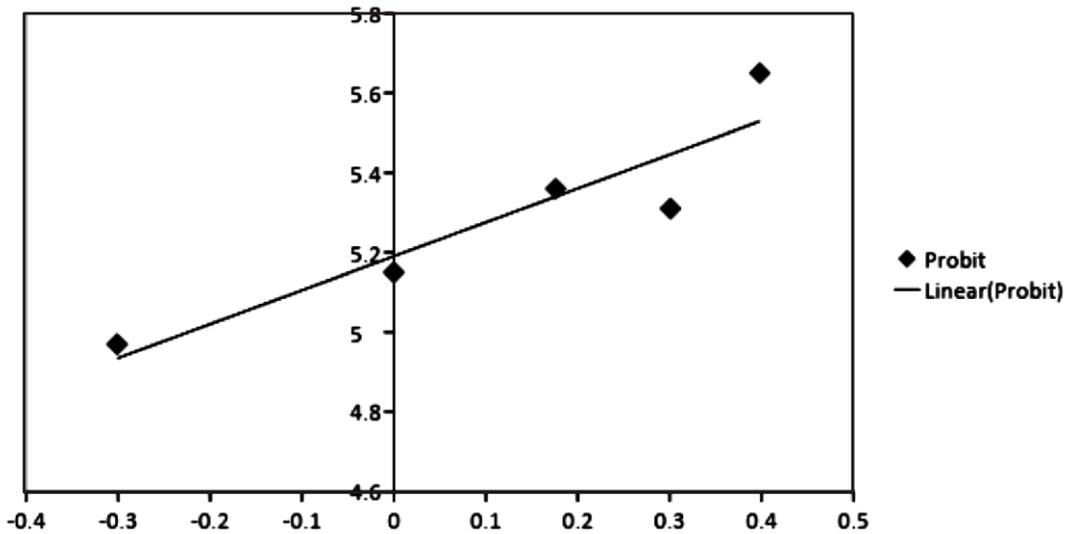


Figure 1: Empirical Probit of Mortality of *Culex quinquefasciatus* Larvae against the Logarithms of the Concentrations of Aqueous Pod Extract of *Adansonia digitata*

DISCUSSION

Adansonia digitata is rich in alkaloids, flavonoids, tannins, steroids/triterpenes, carbohydrate and cardiac glycosides (Kamatou *et al.*, 2011). These classes of phytochemicals could have accounted for their overt larvicidal efficacies against *Cx. quinquefasciatus*. Plants have been known to serve as huge storage of compounds that have biological action (Gutierrez *et al.*, 2014). Furthermore, Karou *et al.* (2011) had reported the universal occurrence of alkaloids in plants belonging to the Rubiaceae family; although Leal and

Elisabetsky (1996) demonstrated the absence of alkaloids in *Psychotria carthagenesis*. Compounds such as flavonoids, alkaloids, tannins and saponins in plants are known to be responsible for the insecticidal effects and toxicity to other animals (Gutierrez *et al.*, 2014). Although, the larvicidal prospect of *Adansonia digitata* against *Cx. quinquefasciatus* are not well documented, this study has however demonstrated the mortality of *Cx. quinquefasciatus* mosquito larvae to different dose concentrations of the aqueous pod extract of *A. digitata*. Thus, the extract of *A. digitata* could be employed, in

the population reduction management of *Cx. quinquefasciatus* vector mosquito and indirectly to control the diseases vectored by the species.

The results also indicated that there was a significant difference between some of the mean larval mortalities of the dose concentration of aqueous extract of *A. digitata*. This significant difference could be attributed to the heterogenous distribution, of the active compounds with larvicidal activity against *Cx. quinquefasciatus* larvae. Kamba et al. (2017) had a similar observation on the mortality of larvae of *Aedes* and *Culex* mosquito using n-hexane extracts of *Parkia biglobosa*.

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that: alkaloids, tannins, steroids, flavonoids, carbohydrate and cardiac glycosides were present, while anthraquinones and saponin were not detected in the aqueous extracts of *Adansonia digitata*. Aqueous extract of *A. digitata* produced significant larvicidal effect against *Cx. quinquefasciatus*.

There is need to evaluate pod extract of *A. digitata* in the field in mosquito control programmes to reduce the population of *Cx. quinquefasciatus*. Investigation of the effect of the aqueous extract on other life stages (eggs, pupae and adults) is recommended.

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