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Larvicidal Efficacy of *Cassia singueana* Methanol Leaf Extract Against *Aedes aegypti* and *Culex quinquefasciatus*

Jibrin, A.,[1] Ndams, I.S.,[1] Sow, G.J.,[1] Dangana, S.,[1] Sufi, D. A.,[2] Ahmed J.[1] and Mustapha M. B.[2]

[1]Department of Zoology,
Ahmadu Bello University, Zaria.
P.M.B 1044 Samaru. Zaria,
Kaduna,
Nigeria.

[2]Department of Animal and Environmental Biology,
Federal University Dutse,
P.M.B 7156, Dutse,
Jigawa Nigeria

Email: aliyujibrin2017@gmail.com

Abstract

Despite the persistent control measures, mosquitoes remain abundant and the disease they transmit persists globally posing significant health challenges. There is increasing enthusiasm for exploring botanical solutions as substitutes for conventional chemical insecticides, driven by their broad insecticidal attributes, eco-friendly nature and compatibility with various ecological settings. As such, the study was designed to assess the larvicidal properties of methanol leaf extract of Cassia singueana against Aedes aegypti and Culex quinquefasciatus. The plant was subjected to preliminary and GC-MS phytochemicals screening. Larvicidal bioassay was conducted using six different concentrations (0.10-3.20 mg/ml) and 31.25mg/l temphos and distilled water were used as positive and negative control respectively. The experimental set up was observed after 24 h and the lethal concentrations (LC_{50} and LC_{99}) were determined using Probit analysis. Preliminary phytochemical screening of C. singueana methanol leaf extract revealed the presence of alkaloids, cardiac glycosides saponins, phenolic compounds, tannins, steroid carbohydrates, flavonoids, and terpenoids. Eighteen (18) bioactive compounds were identified in the methanol leaf extract of C. singueana which includes 1,5-Heptadien-3-yne, 2-Hydroxy-3-methylbenzaldehyde, 2-Butenoic acid, Acetonitrile, 2,2'-iminobis-, 7,11-Hexadecadienal,2-Hexyne, among others. The extract had larvicidal activity against A. aegypti and C. quinquefasciatus with LD_{99} of 5.44 mg/ml and 15.85 mg/ml respectively at 24 h post-treatment. This study offers valuable perspectives on the possible application and advancement of C. singueana leaf extract as a potential bioinsecticide against A. aegypti and C. quinquefasciatus and consequently control the diseases transmitted by them.
Keywords: Aedes aegypti, Bioinsecticides, Cassia singueana, Culex quinquefasciatus, vector control.

INTRODUCTION

Vector-borne diseases transmitted by mosquitoes represent a major health concern in many countries, impacting the socioeconomic well-being of nations and posing a significant

Author for Correspondence

nuisance to humans due to their allergy-inducing properties like the local skin reaction at the bite site (Govindarajan *et al*., 2005). *Aedes aegypti* is an arboviral vector of dengue, zika and chikungunya that have persistently present substantial health risks particularly in the tropical and subtropical areas (WHO, 2020). Projections indicate that by 2050, nearly half of the global population may face the threat of arboviral infections with dengue leading the pack as the fastest-spreading virus on a global scale (Kraemer *et al*., 2019). In 2019, the World Health Organization reported the largest number of dengue globally with a high number of cases in Asia (WHO, 2019). In Africa, severe dengue is not very common with 176 cases being reported from 2011-2019 (Mwanyika *et al*., 2021). In Nigeria, dengue is considered a re-emerging disease with speculative prevalence reported across the country (Out *et al*.,2019; Mohammed *et al*., 2021). Dengue infections have not only compromised the health of individuals, resulting in hospitalizations and fatalities, but they have also contributed to economic stagnation. Given that many tropical regions consist predominantly of developing nations, the consequence of dengue outbreaks on the socio-economic landscape in these areas can disrupt already fragile societies, rendering them less equipped to handle the additional financial burden (Silver *et al*., 2020).

Culex quinquefasciatus is the principal vector of bancroftian filariasis and a potential vector of vector of West Nile virus (Vrzal *et al*., 2010) in the tropical and subtropical regions of the world. According to WHO (2023), bancroftian filariasis is responsible for 90% of reported lymphatic filariasis cases and over 880 million people in 44 countries are at risk of infection. Within the sub-Saharan Africa, a staggering 512 million individuals face the potential risk of being infected with lymphatic filariasis. Nigeria holds the highest ranking for endemicity worldwide and the North-West part of the country holds the highest disease burden (Hussaini *et al*., 2020). Although the mortality rate associated lymphatic filariasis is relatively low, the disease ranks as the fourth leading contributor to disability leading to social and financial loss which contributes to stigma and poverty (Cano *et al*., 2014; WHO, 2023).

Vector control strategies constitute the primary approach for controlling the majority of mosquito-borne diseases (Wilson *et al*., 2020; Jones *et al*., 2021; WHO 2023). Before the advent of chemical insecticides, these strategies primarily relied on environmental management involving the elimination of mosquito breeding sites, the use of mosquito bed nets, and screens on doors and windows to prevent mosquito entry into homes. Over time, the discovery and subsequent use of various chemical insecticides became the dominant method due to their swift action and high effectiveness (Wilson *et al*., 2020; Jones *et al*., 2021). However, prolonged use and misuse of chemical insecticides against mosquitoes have led to the development of resistance to available insecticides by mosquitoes (Mbatchou *et al.*, 2017, Demok *et al*., 2019; Kandel *et al*., 2019;; Sene *et al*., 2021; Omotayo *et al*., 2022). Furthermore, the persistent use of chemical insecticides has harmful effects on the environment, and beneficial organisms including humans (Liu *et al*., 2018). Hence, the central focus of the vector control program has shifted towards eco-friendly alternatives, emphasizing biological control methods over chemical insecticides. One highly effective strategy within the biological control program involves exploring floral biodiversity to identify potential botanical-based insecticides (Okoh *et al*., 2021; Musa *et al*., 2022 Lim *et al*., 2023). Plant-derived insecticides consist primarily of blends of bioactive compounds that work synergistically to affect the vector's behavioral and physiological processes thus reducing the likelihood of vectors developing resistance (Ghosh *et al*., 2012). Further, botanicals are favored alternatives due to their rapid degradation and low toxicity (Liu *et al*., 2018).

The plant *Cassia singueana* has numerous medicinal values across Africa (Asfaw *et al*., 2021; Kwamboka *et al*., 2021; Mamwa *et al*., 2021; Ripanda *et al*., 2023). The leaf juice is used to treat malaria, syphilis, ulcers, pneumonia, snake bite, and eye (Ghosh *et al*., 2012).
Over the past few years, many researchers have demonstrated the efficacy of many plant extracts against larvae of mosquitoes with many reporting up to 100% mortality 24 h post-exposure (Kumar *et al*., 2014; Okoh *et al*., 2021; Ojianwuna *et al*., 2023; Lim *et al*., 2023). Although, *Cassia singueana* has been studied for its medicinal properties (Asfaw *et al*., 2021; Ripanda*et al*., 2023), its larvicidal potential has not been explored. Therefore, this study was designed to evaluate the efficacy of *Cassia singueana* methanol leaf extracts against the third instars larvae of *Aedes aegypti* and *Culex quinquefasciatus*.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Healthy fresh leaves of *Cassia singueana*, were collected from their tree at Area A in the Main Campus of Ahmadu Bello University, Zaria with coordinates of 11.152223^0 latitude and 7.660125^0 longitude using cutlass after climbing the tree. The collected leaves were immediately carried to the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria for identification and the voucher specimens was numbered as *Cassia singueana*-- ABU01757.

Processing and Preparation of Plants Material

The collected leaves were cleaned and washed with tap water and allowed to air dry in a shady place for 7-14 days at an ambient environment temperature (25-37 °C). The dried leaves were separated and ground into fine powder mechanically using a laboratory grinder machine (FZ102) in Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, following the method used by Dass and Mariappan, (2014).

Extraction of Plant Materials

The powdered sample of *Cassia singueana* leaves were extracted using cold maceration with an extracting solvent (methanol). The pulverized leaves(400g) were soaked in 4 L of methanol in a conical flask and was thoroughly mixed using a shaker. The container was closed and kept for 72 hours. The extract was then filtered and dried at a temperature of 50°C in a water bath. The dried extract obtained was stored in a small container until used (Evans, 2009).

Preparation of Stock Solution

Cassia singueana methanol leaf extract (1g) was dissolved in 100 ml of distilled to form 10 mg/ml as a stock solution. Therefore, the concentrations of 0.10, 0.20, 0.40, 0.80, 1.60, and 3.20 mg/ml were prepared from this solution adopting the method used by Mughal *et al.* (2018).

Phytochemical Screening of Plant Extracts

Methanol leaf extract of *Cassia singueana* was screened for the following phytochemical constituents: alkaloids, cardiac glycosides, saponins, phenols compounds, tannins, steroids, flavonoids, terpenoids, and anthraquinones. These were carried out according to standard procedures described by Sofowora, (1993) and Trease and Evans, (2009).

Gas Chromatography and Mass Spectrophotometry of the *Cassia singueana* leaf extract

Gas chromatography of mass spectrophotometry (GC-MS) analysis of methanol leaf extract of *Cassia singueana* was done in the Multi-User Science Research Laboratory of Ahmadu Bello University, Zaria. Agilent technologies 7890B Gas Chromatography (GC) system fitted with a 30μm × 250 μm×0.25 μm capillary column coupled to Agilent 19091S-433UI Mass

Spectrometric (MS) was used at a temperature of 325 °C. The injector, transfer line, and ion source temperature were set at 290 °C. The ionizing energy was 70.00ev. The oven temperature was programmed from 50°C for 2 mins, then 10 °C /min to 110aqueous plant crude extract C/min, and then 280 °C at the rate of 5 °C/min. The samples were injected into an inlet port of the GC device. The GC instruments vaporized, separated, and then analyzed the various components within the samples. Each component produces a specific spectral peak that was recorded electronically. The size of the peaks is proportional to the quantity of substances present in the samples analyzed. Mass spectrometry identified the compound present in the three samples of methanol leaf extracts by charging each sample molecule, accelerating them into a magnetic field followed by breaking each molecule into charged fragments and detecting the different charges. The result was printed out from the computer system connected to the GC-MS machine (Ezekwe *et al*., 2020).

Collection, Identification, and Rearing of Mosquitoes
Mosquito larvae used for bioassay were collected from different sources ranging from discarded containers, pots, holes, etc. around Ahmadu Bello University Zaria, using the standard straining method with a strainer (Dares Salaam, 2005). Adult *Culex quinquefasciatus* were collected from a male ~~H~~ hostel: Oba Akenzua in ABU, Main Campus Samaru. All the collected samples were transported to the Entomology and Parasitology Research Laboratory, Department of Zoology Ahmadu Bello University, Zaria for identification and rearing. Collected larvae were identified as *Culex quinquefasciatus* and *Aedes aegypti* microscopically according to the keys of Hopkins (1952) and Rueda (2004). The larvae of *Aedes aegypti* and *Culex quinquefasciatus* were mass-reared in a small container placed inside an entomological cage. During the rearing process, larvae were fed with a mixture of finely ground fish feed and yeast powder in a ratio of 3:1 by weight. Care was taken to prevent the formation of any scum on the surface of the water. Larvae metamorphosed into second, third, fourth larval stage and transformed into pupae then emerged to adult stage. The larvae and the pupae were maintained at the temperature 28 ± 2℃ and relative humidity of 65±5% measured by handy hygrometer. The adults were fed with sugar solution and mated, female were then fed with blood of pigeon. Blood-fed females were transferred into oviposition cage where eggs were laid. Eggs were allowed to hatch into larvae and reared to reach third instars which were used for bioassay **(**Kumar *et al*., 2010).

Bioassays
The bioassay of *C. singueana* leaf extract was carried out in the Parasitology and Entomology Research Laboratory, Department of Zoology, Ahmadu Bello University, Zaria. The standard procedure of World Health Organization guideline for laboratory and field testing of mosquito larvicide with slight modifications was adopted (WHO, 2005). Six concentrations of the methanol leaf extracts of *Cassia singueana* were made up from the stock solution. Twenty-five (25) active third instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* were placed into each small plastic bowls of 160 ml capacity, containing 50 ml of distilled water using dropper and strainer. After then, the diluted methanol extract was added using syringe (5 ml size) and desired test concentration was obtained by modifying the WHO (2005) guidelines. Four replicates were set up for each concentration including controls (Negative and Positive). In negative control, larvae were put in 50 ml of distilled water devoid of any extracts and positive control in which 1ml of 31.25mg/l solution of temephos was added to 249 ml of water to obtain 0.125 mg/l concentration. During this experiment, temperature and humidity were also maintained at 28±2℃ and 65±5% using handy thermometer and humidity monitor. No food was provided to the test or control during the experiments. After 24hours exposure, observations were made and mortality was recorded. The percentage mortality was calculated by using the following formula (WHO, 2005)

$$larval\ mortality\ = \frac{\text{Number of dead larvae}}{\text{No. of larvae introduced}} \times 100$$

The larvae were counted as dead when they were not able to move and swim to the surface for respiration and probe insensitive.

Data Analyses

One-Way Analysis of Variance (ANOVA) was used to test significant differences in larval mortalities between controls and experimental groups. The lethal concentration (LC_{50} and LC_{99}) was computed using Probit analysis. All the mortality data were tested at $p<0.05$ level of significant.All analyses were carried out using the SPSS (Statistical Package Social Science) software version 26.

RESULTS

Phytochemical Constituents

Preliminary analysis of the leaf extract of *Cassia singueana* revealed that it contained alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, flavonoids, and terpenoids with anthraquinones being absent (Table 1). A total of 18 compounds were identified from methanol leaf extracts of *Cassia singueana* using GC-MS analysis. The chromatogram is presented in Figure 1. The phytoconstituents include1,5-heptadien-3-yne, 2-hydroxy-3-methylbenzaldehyde, 2-butenoic acid, acetonitrile, 2,2′-iminobis-, 7,11-hexadecadienal,2-hexyne, Z-1,9-tetradecadiene. (Table 2).

Table 1: Phytochemical constituents of *Cassia singueana* methanol leaf extract

S/NO.	Phytoconstituents	*Cassia singueana*
1	Alkaloids	+
2	Cardiac Glycosides	+
3	Saponins	+
4	Phenolic compounds	+
5	Tannins	+
6	Steroids	+
7	Carbohydrates	+
8	Flavonoids	+
9	Terpeniods	+
10	Anthraquinones	-

Keys + PRESENT -ABSENT

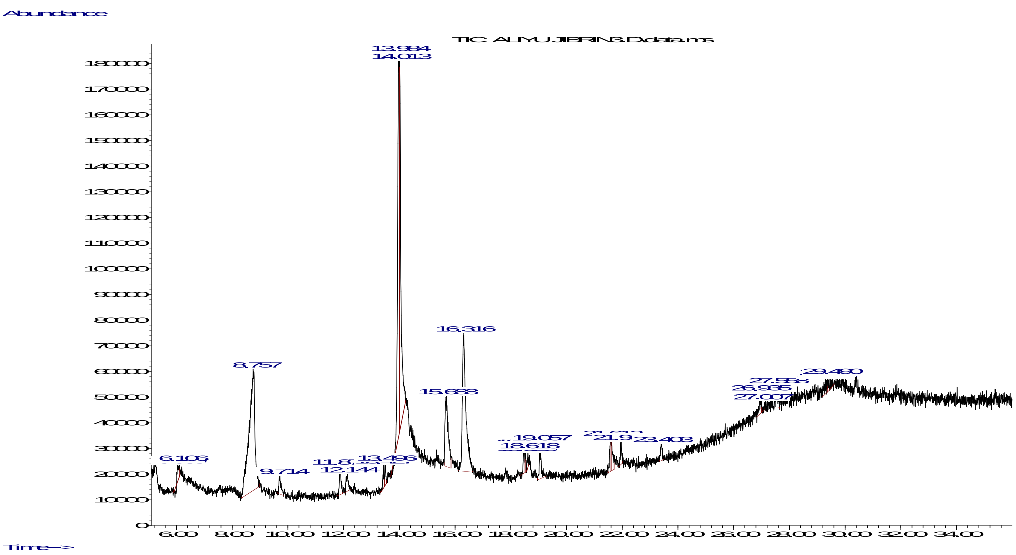

Figure 1: Gas chromatography-mass spectrophotometry chromatogram of Methanol

leaf extract of *Cassia singueana*

Table 2: Phytocomponents identified in the methanol leaf extract of *Cassia singueana* using GC-MS analysis

Peak No.	Retention time	Name of the compound	Molecular formular	Molecular weight	Peak area
1	6.039	1,5-Heptadien-3-yne	C_7H_8	92.14	0.43
2	6.106	2-Hydroxy-3-methylbenzaldehyde	$C_8H_8O_2$	136.15	0.22
3	8.757	2-Butenoic acid	$C_4H_6O_2$	86.09	22.25
4	9.714	Acetonitrile, 2,2'-iminobis-	$C_4H_5N_3$	95.1026	1.18
5	11.874	7,11-Hexadecadienal	$C_{16}H_{28}O$	236.393	1.43
6	12.144	2-Hexyne	C_6H_{10}	82.143	1.13
7	13.427	Z-1,9-Tetradecadiene	$C_{14}H_{26}$	194.36	0.65
8	13.496	17-Octadecynoic acid	$C_{16}H_{32}O$	280.4	0.73
9	13.984	Linoelaidic acid	$C_{18}H_{32}O_2$	280.44	18.77
10	14.013	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O$	280.45	17.73
11	18.528	4-Nonyne	C_9H_{16}	124.22	0.54
12	19.057	cis-11-Hexadecenal	$C_{16}H_{30}O$	238.408	2.14
13	21.583	8-Hexadecyne	$C_{16}H_{30}$	222.409	1.33
14	21.612	1,3,12-Nonadecatriene	C_9H_{34}	262.47	1.55
15	23.403	9,12-Octadecadienal	$C_{18}H_{32}O$	264.4	0.86
16	26.935	Cyclopentaneundecanoic acid	$C_{16}H_{30}O_2$	254.41	1.03
17	27.558	1,E-11,Z-13-Octadecatriene	$C_{18}H_{32}$	248.45	1.17
18	27.007	3-Decyne	$C_{10}H_{18}$	138.24	0.22

Larvicidal Efficacy of Methanol Leaf Extract of *Cassia singueana* AgainstA *edes aegypt*i and *Culex quinquefasciatus*

Methanol leaf extract of *Cassia singueana* caused varying degrees of mortalities in the larvae of *Aedes aegypti* and *Culex quinquefasciatus* at varying concentrations. Mortality was not observed in *Aedes aegypti* larvae exposed to the extract at 0.10, 0.20 and 0.40 mg/ml concentrations. However, mortality (16-90%) was observed in *Ae. aegypti*, exposed to 0.80, 1.6 and 3.20 mg/ml of the extract. *Culex quinquefasciatus* larvae were more susceptible to *C. singueana* leaf extract and the mortality ranges from 21-92%. The bioactivity of the extract was significantly dose-dependent ($P<0.05$) and mortality was not observed in the negative controls while the positive control caused 100% mortality (Table 3). The LC_{50} and LC_{99} of the extract against *Ae. aegypti* are 1.7923 mg/ml and 5.44 mg/ml respectively while the LC_{50} and LC_{99} against *Cx. quinquefasciatus* are 2.5021 and 15.85 mg/ml respectively.

Table 3: Larvicidal activity of the methanol leaf extract of *Cassia singueana* against *Aedes aegypti* and *Culex quinquefasciatus*

		Aedes aegypti		*Culex quinquefasciatus*	
Conc. (mg/ml)	No. of larvae exposed	Mean Mortality (±SE)	% Mortality	Mean Mortality (±SE)	% Mortality
Distilled H_2O -ve (Control)	100	$0.00±0.00^e$	0.00	$0.00±0.00^f$	0.00
Temephos +ve (Control)	100	$25.00±0.00^a$	100.00	$25.00±0.00^a$	100.00
0.10	100	$0.00±0.00^e$	0.00	$6.75±0.48^e$	27.00
0.20	100	$0.00±0.00^e$	0.00	$9.50±0.87^d$	38.00
0.40	100	$0.00±0.00^e$	0.00	$15.25±1.11^c$	61.00
0.80	100	$4.00±0.41^d$	16.00	$16.00±0.82^c$	64.00
1.60	100	$14.25±0.48^c$	57.00	$19.75±0.85^b$	79.00
3.20	100	$22.50±1.56^b$	90.00	$23.00±0.71^a$	92.00
P – value		0.000		0.000	

Values with the same superscript across column are not significantly different ($P≤0.05$)
Keys: SE = Standard Error of Mean

Table 4: Lethal Concentrations (LC_{50} and LC_{99}) of *Cassia singueana* Methanol Leaf Extract against *Aedes aegypti and Culex quinquefasciatus*

		Aedes aegypti		*Culex quinquefasciatus*	
Conc. (mg/ml)	No. of Larvae exposed	LC_{50} (mg/ml)	LC_{99} (mg/ml)	LC_{50} (mg/ml)	LC_{99} (mg/ml)
Distilled H_2O –ve (Control)	100	NIL	NIL	NIL	NIL
Temephos +ve (Control)	100	NIL	NIL	NIL	NIL
0.1	100	NIL	NIL	NIL	NIL
0.2	100	NIL	NIL	NIL	NIL
0.4	100	NIL	NIL	NIL	NIL
0.8	100	1.793	5.44	2.503	15.85
1.6	100	NIL	NIL	NIL	NIL
3.2	100	NIL	NIL	NIL	NIL

$LC_{50}$is the concentrations that kills 50% of the populations, $LC_{99}$is the concentrations that kills 99% of the populations

DISCUSSION

The preliminary phytochemical screening of the methanol leaf extract of C. *singueana* revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids. A recent study on the ethanol leaf extract of *C. singueana* reported the absence of tannins and flavonoids in the leaf (Kolawole *et al*., 2021) and this may be due to the difference in the solvent used for extraction (Truong *et al*., 2019). Similarly, a study on the phytochemical constituent of the ethanolic root extract of *C. singueana* (Abdul et.*al*., 2021) reported the absence of flavonoids and alkaloids and the differences may be attributed to the different plant parts used (Gunwantrao *et al*., 2016). Research findings indicatedthat some categories of phytochemicals reported in this study exhibit larvicidal activity against mosquitoes. For instance, alkaloids like pellitorine have been reported to have larvicidal activity against *Cx. pipens* and *Ae. aegypti* (Kim *et al*., 2017). Similarly, alkaloids like murrayanine, girinimbine, mohanimbine, and mahanimbine have also been reported to have larvicidal activity against the third instar larval stage of *Ae. Aegypti* (Hernández-Morales *et al*., 2015; Maziet *al*., 2017). Flavonoids like lanceolatin B and chrysoeriol isolated from *Tephrosia purpurea* and *Maerua siamensis* respectively have been reported to be active against *Ae. Aegypti* (Arriaga *et al*., 2014; Nobsathian*et al*., 2018). Other compounds like saponins and terpenoids have also been reported to have larvicidal activities against mosquitoes (Santos *et al*., 2017)). It can be suggested that the larvicidal activities observed in this study are likely due to bioactive compounds like alkaloids, flavonoids, saponins, and terpenoids.

The most effective method for characterizing the components of volatile substances, long - chain and branched-chain hydrocarbons, alcoholic acids and various other compounds is GC-MS (Kolawole *et al*., 2021). Peak area, retention times and molecular formulas are employed to validate the presence of phytochemical constituents. The GC-MS analysis of methanol leaf extract of *Cassia singueana*revealed 18 bioactive phytocontituentsthat includes 1,5-Heptadien-3-yne, 2-Hydroxy-3-methylbenzaldehyde, 2-Butenoic acid, Acetonitrile, 2,2'-iminobis-, 7,11-Hexadecadienal. All the identified phytoconstituents are in line with the compounds identified using preliminary phytochemical screening in this study. Eighteen phytoconstituents were also identified from the ethanolic leaf extract of *C. singueana* (Kolawole*et al*., 2021). However, different compounds were identified from this and their study and this may be because of the extraction solvent used (Gunwantrao *et al*., 2016).Similar findings have been reported from the methanol leaf extract of *Vernonia cinerea* (Abirami and Rajendran, 2012), *Kayea assamica* (Homen, *et al*., 2017), *Piper longum* (Dey *et al*., 2020) and *Hyptis suaveolens* (Dakum*et al*., 2021) all having larvicidal activities against mosquitoes.

There is a growing awareness and a consistent preference for utilizing natural, environmentally friendly compounds for larvicidal purpose globally. This is because they offer a handful of advantages over the use of chemical insecticides. In this study, a dose-dependent larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* was observed. *Cx. quinquefasciatus* were more susceptible exhibiting 27% mortality when exposed to 0.1 mg/ml of the extract. Mosquitoes generally vary in their level of susceptibility to plant extracts either because the toxicants in the extract are less specific to a particular mosquito or because the mosquito have a stronger detoxifying mechanism (Yahaya *et al*., 2021). Similar findings have been reported in a study where *Cx. quinquefasciatus* was observed to be more susceptible to benzoate and formate extract of *Catharanthus roseus* as compared to *Aedes aegypti* and *Anopheles stephensi* (Kamatchi *et al*., 2023). Furthermore, a study on the larvicidal property of seaweeds also reported more susceptibility to larvicides by *Cx. quinquefasciatus* as compared to *Ae. aegypti*.

However, *Aedes aegypti* larvae responded more at higher concentrations (1.60 mg/ml and 3.20 mg/ml) and that is the reason for $LC_{50}$and LC_{99}values of 1.793 and 5.44 mg/ml. Third instars larvae of *Culex quiquefasciatus* responded better in terms of mortality to various concentrations of *Cassia singueana* leaf extract than *Aedes aegypti*. Up to 79% and 92% larval mortalities were observed at highest concentrations of 1.60 mg/ml and 3.20 mg/ml. 27% mortality was obtained at least concentration (0.10 mg/ml) of *Cassia singueana* leaf extract. Mortality of *Culex quinquefasciatus* due to concentrations of *Cassia singueana* extract was dead at highest concentrations (1.60 mg/ml and 3.20 mg/ml) which was the responsible for LC_{50} and LC_{99}values of 2.503 and 15.85 mg/ml respectively.

Kanis *et al.* (2013) investigated the extract of *P. ovatum* roots on *Aedes aegypti* and exhibited LC_{50} 2.9 mg/ml and LC_{99} 6.1 mg/ml. Also Carla *et al.* (2020) the crude extract of *Piper corcovadensis* and *Piperrovatine* roots, reported LC_{50} of 4.86 mg/ml and LC_{99} of 15.50 mg/ml with *Piper corcovadensis* and LC_{50} value of 17.78 mg/ml and LC_{99} of 48.55 mg/ml with *Piperrovatine* on *Aedes aegypti* larvae. Sharowe r*et al*. (2018) in the study of larvicidal impact of some local medicinal plant extract against *Aedes aegypti* reported $LC_{50}$of 90.89 ppm and LC_{99} of 441.88 ppm with *Maesa indica* acetone extract which was consistent with values observed here. Humayun *et al*. (2016) with ethanol extract of *Azadirachta indica* reported the LC_{50} and LC_{99} values of 1.805 and 6.261 mg/ml against 4^{the} instar larvae of *Culex quinquefasciatus* which was also supported by the findings observed in this study. The lower LC_{50} and LC_{99}indicating the high potentials to serve as a larvicidal agents. The differences in LC_{50} and LC_{99} may be due to inherent physiological differences between two species of mosquitoes (Zia *et al*., 2018).

CONCLUSION

In conclusion, the methanol leaf extract of *Cassia singueana* investigated has substantial larvicidal potency against larvae of *Aedes aegypti* and *Culex quinquefasciatus.* The extract is rich in phytochemicals including alkaloids, flavonoids, tannins, and saponins which are known for their characteristic larvicidal prospects. Further research is warranted to explore the potential of these compounds for developing environmentally friendly insecticide formulations in the future.,

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REFERENCES

Abdul, A.O. Hajara, A, M. and Shafia, M.I. (2021).Evaluation of Phytochemicals and Antimicrobial Activities of Cassia singueana Root Extracts. *Dutse Journal of Pure and Applied Sciences*,**7**:2635−3490.

Abirami, P. and Rajendran, A. (2012). GC-MS analysis of methanolic extracts of *Vernonia cinerea*. *European Journal of Experimental Biology*, **2**(1): 9-- 12.

Alkali, Y.I., Abdulgafar, O.J., Yerima, M. and Abubakar, K. (2018).Antischizophrenic and central nervous system depressant effects of *Cassia singueana*. Australian *Journal of Science and Technology*, **7**:2−4.

Aline, C., Jesus, S., Wuillda, R., Carlos, C. and Fernanda, N.C.(2019). Larvicidal Activity of Secondary Plant Metabolites in *Aedes aegypti* Control: An Overview of the Previous 6 Years. *Natural Product Communications* 14:7.

Amusan, A.A.S. (2005). Distribution of mosquitoes (Diptera:Culicidae) and disease Transmission patterns in Ogun State. PhD Thesis University of Agriculture, Abekuta, Ogun State, Nigeria (unpublished) p336.

Arriaga, A.M.C., Oliveira, M., da, C.F. and da Silva, M.G.R. (2014). *Tephrosia purpurea*: a source of larvicidal compounds against *Aedes aegypti*. *Chemical Natural Compound*. **50**(6):1125-1127.

Asfaw, A., Lulekal, E. and Bekele, T. (2021). Ethnobotanical Investigation on Medicinal Plants Traditionally Used against Human Ailments in Ensaro District, North Shewa Zone, mhara Regional State, Ethiopia

Badaki, J.A. (2010). Parasitological and social aspects of Lymphatic filariasis in Taraba State. Ph.D Thesis in the Department of Zoology, University of Jos, Nigeria.

Braks, M.A., N.A., Honorio, R., Lourenco- De- oliviera, S.A. Juliano, and Lounibos, P. (2003).

Convergent habitat segregation of Aedesaegypti and Aedesalbopictus in southeastern Brazil and Forida.*Journal of Medical Entomology,* **40**:785-- 794.

Borkent, A. and Grimaldi, D. A. (2004). The earliest fossil mosquito (DipteraCulicidae), in mid-cretaceous Burmese amber *Annauls of the Entomological Society of America,* **97**: 882-- 888.

Buddhadev, S. G. and Buddhadev, S. S. (2016). Ayurvedic medicinal plant *Lawsoniainermis* L. A complete Review.*International Journal of Pharmaceutical Sciences*, **7**(2): 240-- 2.

Cano, J, Rebollo, M.P., Golding, N., Pullan, R.L., Crellen, T. and Soler, A. (2014). The global distribution and transmission limits of lymphatic filariasis: past and present. *Parasit Vectors.* 2014;**7**:466.

Collen, M., Elizabeth, B.A, Salis, M., Mandyiwa, K. and Charles, K. (2020). *Parkiabiglobosa* botany, uses, phytochemical properties and pharmacological potential. *Journal of pharmacy and Nutrition Science*, **10**:101-- 115.

Dakum, Y. D., Amajoh, C. N., Ombugadu, A., Istifanus, G., Agwom, F., Joseph, S. T., Jwanse, I. R., Lapang, P. M., Kopdorah, S. W. and Pam, D. D. (2021). Larvicidal Efficacy and GC-MS Analysis of Hyptissuaveolens Leaf Extracts against Anopheles Species. *International Journal of Biochemistry Research and Review*, **30**(1), 8–19.

Dares Salaam Urban Malaria Control Programmed (2005). Contact Urban Malaria Control Programme, City Medical Office of Health City Council, P.O. Box 63320,Dares Salaam.

Dass, K. and Mariappan M. (2014).Larvicidal activity of *Lawsoniainermis* and Murraya exotica leaves extract on filarial vector, *Culexquinquefasciatus. International Journal Mosquito Research,* **1**(2): 25— 27.

Dey, P., Goyary, D., Chattopadhyay, P., Kishor, S., Karmakar, S. and Verma, A. (2020). Evaluation of larvicidal activity of *Piper longum* leaf against the dengue vector, *Aedes aegypti*, malarial vector, *Anopheles stephensi* and filariasis vector, *Culex quinquefasciatus*. S *African Journal of Biology,* **132**:482–490

Dieu-Hien, T., Dinh, H., Nguyen,N., Thuy, A., Ta, A., Tuong, H.and Hoang C.N. **(2019)**. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-Inflammatory Activities of *Severiniabuxifolia. Journal of food quality*, **20**-19.

Ezekwe, A.S., Rizwan, A. A.,Karimah, M. R., and Ewa, O. (2020). Qualitative phytochemical and GC-MS analysis of some commonly consumed vegetables. *GSC Biological and Pharmaceutical Sciences*, **12**(03), 208-- 214.

Ghosh, A.; Chowdhury, N.; Chandra, G. Plant extracts as potential mosquito larvicides. *Indian . Medical. Research,***135**:581–598.

Govindarajan, M., Jebanesan, A. and Reetha, D. (2005). Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus*.

Gunay, F., Alten, B., Ozsoy, E.D., (2010). Estimating reaction norms for predictive population parameters, age specific mortality, and mean longevity in temperature-dependent cohorts of *Culexquinquefasciatus* Say (Diptera: Culicidae). *Journal of Vector Ecology*. **35**(2):354-362.

Hernández-Morales, A., Arvizu-Gómez, J.L. and Carranza-Álvarez, C. (2015). Larvicidal activity of affinin and its derived amides from Heliopsislongipes A. Gray Blake against Anopheles albimanus and Aedesaegypti. *J Asia Pac Entomol*. 2015;**18**(2):227-231.

Homen, P., Chitta, A., Bora, R., Pradip, J. and Mitra, K. (2017). Phytochemical screening and GC-MS analysis of methanolic leaf extract of an endemic plant Kayeaassamica. *Journal of Pharmacy and Biology Science*, Pp. 07-- 16.

Hopkins, G.H.E.(1952). *Mosquitoes of the Ethopian region l: larval bionomics of mosquitoes and taxonomy of culicinelarvae.*British Museum (National History), London.Pp. 1--355.

Humayun, R.K., Jinifath, I. R., Taiyeba, T. and Humayera, A. (2016). Effect of toxicity of Neem

And Mohaneem on the larvae of mosquito *Culexquinquefasciatus*. *Journal of Asia Science,* **42**(2): 209 -- 218.

Hussaini, A., Isaac, C., Rahimat, H., Inegbenosun, C., Obasuyi, C. and Solomon, E. (2020). The Burden of Bancroftian Filariasis in Nigeria: A Review. *Ethiopian Journal of Health Sciences*. **30**(2):301-310.

Isma'il, M., Muhammad, M. A., Salisu, A., Kim, I. Musa, I. and Ibrahim, I. D. (2013).Climate Change Perception in Samaru, Sabon–gari local government area of Kaduna state, Nigeria. *The Zaria Geographer,* **20**(1): 122-- 129.

Jayapriya, G. and Gricildasho, F. (2015). GC-MS analysis of bioactive compounds in ethanolic leaf extracts of Justicaadhatoda. *Journal of Pharmacognosy and Phytochemistry*, **4**(1):113-- 117.

Jones, R.T., Ant, T.H., Cameron, M.M. and Logan, J.G. (2021). Novel control strategies for mosquito-borne diseases. *Journal of Biological Sciences,* pp*376*.

Kanis, L.A., Rabelo, B.D., Moterle, D., Nogaretti, R.M., Nunes, T. Silva, O.S. and Prophiro, J.S. (2013). Standardized extract of Piper ovatum (Piperaceae) to control *Aedesaegypti* larvae (Diptera: Culicidae). *Journal of Crop Production*, **50**:816 -- 820.

Kim, S.I. and Ahn, Y.J. (2017). Larvicidal activity of lignans and alkaloid identified in Zanthoxylumpiperitum bark toward insecticide-susceptible and wild *Culex pipiens pallens* and *Aedes aegypti*. *Parasitology Vectors*. **4**;10(1):221.

Kraemer, M.U.G., Reiner, R.C., Brady, O.J., Messina, J.P., Gilbert, M., Pigott, D.M., Yi, D., Johnson, K., Earl, L. and Marczak, L.B. (2019). Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat. Microbiol.* **4**:854–863.

Kamatchi, P.A.C., Maheswaran, R. and Sivanandhan, S. (2023). Bioefficacy of ursolic acid and its derivatives isolated from *Catharanthus roseus* (L) G. Don leaf against *Aedes aegypti*, *Cule xquinquefasciatus*, and *Anopheles tephensi* larvae. *Environmental Sciences Pollutant Research,* **30**:69321–69329.

Kolawole, O.S., Isyaka, M.S., Dahiru, M. and Gani, A.M. (2021). Chemical constituents of leaves of Sennasingueana(Del.) lock. Journal of Pharmacognosy and Phytochemistry 2021; **10**(1): 131-136

Kumar, S. R. Warikoo, R. and Wahab, N. (2010).Larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different instars of an Indian strain of dengue fever mosquito, *Aedesaegypti* L. (Diptera: Culicidae), *Parasitology Research,* **107**: 901-- 907.

Kwamboka, S. (2021). Ethnobotanical Documentation, Phytochemistry, and Cytotoxicity of Anti-snakebite Envenomation Plants of Mwingi West Sub- County, Kenya

Lim, H., Lee, S.Y., Ho, L.Y. and Sit, N.W. (2023). Mosquito Larvicidal Activity and Cytotoxicity of the Extracts of Aromatic Plants from Malaysia. *Insects*

Liu, J., Zhang, M., Fu, W.J., Hu, J.F. and Dai, G.H. (2018). Efficacy of bioactive compounds from *Curcuma longa* against mosquito larvae. *Journal of Applied. Entomology,* **142**, 792–799.

Mahnaz, K., Hassan, V., Nafiseh, K., Dehaghi, L., Alireza S. Dehkordi, M., Mehdi, S., Abbas, H., and Farzaneh, H. (2013).Larvicidal Activities of Some Iranian Native Plantsagainst the Main Malaria Vector, *Anopheles stephensiActaMedicaIranica*, **51**(3): 141-- 147.

Mamwan., P.D., D, N.D., Sila, M.D., Dawang, S.N., Abok, C.J., Kwon-Ndung, E.H. and (2021). Ethnobotanical Survey of Plants in Bokkos District of Bokkos Local Government Area of Plateau State; pp. 13–21.

Mbatchou V.C., Tchouassi D.P., Dickson R.A., Annan K., Mensah A.Y., Amponsah I.K., Jacob J.W., Cheseto X., Habtemariam S and Torto B., (2017). Mosquito larvicidal activity of *Cassia tora*seed extract and its key anthraquinonesaurantio-obtusin and tusin.*Parasites and Vectors,***10**: (562).

Masi, M., Westhuyzen, A.E. and Tabanca, N. (2017). Sarniensine, a mesembrine-type alkaloid isolated from *Nerinesarniensis*, an indigenous South African Amaryllidaceae, with arvicidal and adulticidal activities against *Aedes aegypti*. *Fitoterapia*, **116**:34-38.

Missa, M., Saleh, A. A., Malik, A., Ahmed, E. and Saeed, M. (2015). Phytochemical screening, total phenolics content and antioxidants activity of *Cassia Singueana*. *Journal of Medical Plant*, **3**(5): 160-- 165.

Mohd, Y. (2016). Study on phytochemical analysis and anti-bacterial studies of *Lawsonia inermis* leaf extract. *Journal of Chemical and Pharmaceutical Research,* **8**(3):571-575.

Mohammed, A. S., Odegbemi, O. B., Igwe, C., Hussain, N. A., Abaye, B. and Adekanye, U.O. (2021). Prevalence and determinants of dengue virus immunoglobulin among febrile atients attending naval medical centre Victoria Island, Lagos State. *Global Biosecurity*, **3**(1) n/a-n/a. *Tropical Biomedicine*, pp1–3.

Mughal,Z., Ullah, H., Atif, L.Talha, K. and Khurram, Z.(2018).Larvicidal activity of medicinal plant extracts against *Culexquinquefasciatus*.(Culicidae, Diptera).*International Journal of Mosquito Research* 2018; 5(2): 47-- 51.

Musa, N., Banerjee, S., Maspalma, G. A., Usman, L. U., & Hussaini, B. (2022). Assessment of the phytochemical, antioxidant and larvicidal activity of essential oil extracted from Simpleleaf Chastetree [vitex trifolia] leaves obtained from Ganye Local Government, Adamawa State-Nigeria. *Materials Today: Proceedings*, ***49***, 3435-3438.

Mullen, L. Gary, D. and Lancet, L. (2009).*Medical and Veterinary Entomology London*Academic Press.Pp. 45.

Mwanyika, G.O., Mboera, L.E.G., Rugarabamu, S., Ngingo, B., Sindato, C., Lutwama, J.J. Paweska, J.T. and Misinzo, G.(2021). Dengue Virus Infection and Associated Risk Factors in Africa: A Systematic Review and Meta-Analysis. *Viruses,*13-536.

Nobsathian, S., Bullangpoti, V., Kumrungsee, N., Wongsa, N. and Ruttanakum, D. (2018). Larvicidal effect of compounds isolated from Maeruasiamensis (Capparidaceae) against *Aedes aegypti* (Diptera: Culicidae) larvae. *Chem Bio Technol Agric*. 2018;**5**(1):8-14.

Oduola, A.O., Awe, O.O. (2006). Behavioural biting preference of *Culexquinquefasciatus* in human host in Lagos metropolis Nigeria. *Journal Vector Borne Diseases*, **43**:16-20.

Okoh, H. I., Mogaji, H. O., Adekoya, M. A., Morikwe, U. C., Nwana, A. O., Ahmed, J., Makanjuola, W. A. and Otubanjo, O. A. (2021). Ethno-botanical survey of plant species used for mosquito control in Nigeria. *Nigerian Journal of Parasitology,* ***42***(11).

Omotayo, A.I., Dogara. M.M., Sufi. D., Shuaibu, T., Balogun, J.and Dawaki, S. (2022). High pyrethroid-resistance intensity in *Culexquinquefasciatus* (Say) (Diptera: Culicidae) populations from Jigawa, North-West, Nigeria. *Neglected Tropical Diseases*,**16**(6): e0010525.

Otu, A., Ebenso, B., Etokidem, A., and Chukwuekezie, O. (2019). Dengue fever - an update review and implications for Nigeria, and similar countries. *African health sciences*, **19**:(2) 20–27.

Remia, K.M. and Logaswamy, S. (2010). Larvicidal efficacy of leaf extract botanicals against mosquito vector *Aedesaegypti* (Diptera: Culicidae). Indian *Journal of Natural Products and Resources* **1**(2), 208-- 212.

Rueda, L.M. (2004). Pectorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with Dengue Virus Transmission. *Biology Zootaxa*. **589**: 1-- 11.

Sajal, B. and Probal, B. (2016). The Southern House Mosquito, Culexquinquefasciatus: profile of a smart vector. *Journal of Entomology and Zoological Studies,* **4**(2): 73-81

Sani I., Yusuf U and Umar KM (2017).Larvicidal Efficacy of Entomopathogenic Fungus, *Metarhiziu manisopliae* against *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Annals of Experimental Biology,* **4**(4):17-21.

Santos, A., Santos, F., Lima, H., Silva, G., Uzêda, R., Dias, E. and Batatinha, M. (2018). In vitro ovicidal and larvicidal activities of some saponins and flavonoids against parasitic nematodes of goats. *Parasitology*, **145**(14), 1884-1889.

Service, M.W. (2012). Medical entomology for students.Edn 5, Cambridge University Press, New York, Pp 303.

Sharowa, M.G. and Latif, M.A. (2018).Larvicidal impact of some local medicinal plants extracts Against *Aedesaegypti. Journal of Asia Sciences*, **44**(1): 61 -- 67.

Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria

Teklehaimanot, Z., (2004). Exploiting the potential of indigenous agroforestry trees.*Parkia biglobosa* and *Vitellariaparadoxa* in sub-Saharan Africa. Agroforestry Systems, **61**: 207-220.

Torrades, S. (2001). La Malaria La ConroversiaSobresuVaccina p 140-- 143.

Trease, G. E. and Evans, W. C. (2009). Phenols and phenolic glycosides, in harmacognosy, Textbook.Balliese, *Tindall and Co Publishers, London,* **12**: 343–383.

Vrzal, E.M., Allan, S.A., Hahn, D.A., (2010). Amino acids in nectar enhance longevity of female *Culexquinquefasciatus*mosquitoes.*Journal of Insect Physiology*, **56**(11):1659-1664.

Walker, K. and Lynch, L.(2007). Larval source management for malaria control in Africa. *Journal of Medical and Veterinary Entomology,* **21**:2 -- 21.

Wilson, A.L. Courtenay, O., Kelly-Hope, L.A., Scott, T.W., Takken, W., Torr, S.J., Lindsay, S.W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *Neglected Tropical Diseases.* pp20-79.

WHO, (2023). https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis

World Health Organization (2005). Global Malaria Programme.World malaria Report 20, AvenueAppia,Geneva 27.

Zia, U, Atif, I., Talha, K., Mughal J.andKhurram, Z. (2018). Larvicidal activity of medicinal plant extracts against *Culexquinquefasciatus*Say. (Culicidae, Diptera). *International Journal of Mosquito Research*5(2): 47-- 51.