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Phytochemical Constituents and Larvicidal Efficacy of Methanolic Extracts of *Cymbopogon citratus, Ocimum gratissimum* and *Vernonia amygdalina* against *Culex quinquefasciatus* Larvae

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ABSTRACT: This study assessed the larvicidal efficacy of the methanolic extract of *Cymbopogon citratus*, *Ocimum gratissimum* and *Vernonia amygdalina* against the third instar larvae of *Culex quinquefasciatus*. Qualitative analysis of the plants revealed that alkaloids, flavonoids, saponins, steroids, tannins, terpenoids and glycosides were present in all three plant extracts. Phlobatannins was present in trace amounts in *O. gratissimum* and absent in *C. citratus* and *V. amygdalina*. Larvicidal activities of the leaf extracts were studied on laboratory reared larvae of *Cx. quinquefasciatus* at a concentration range of 250 ppm to 1000 ppm. The percentage mortality was calculated and $LC_{50,}$ LC_{90} values were obtained from probit analysis using SPSS version 16.0 at 95% confidence limit (CL). Result of this study indicated that the percentage mortality of *O. gratissimum* extract was dose dependent with 250 and 1000 ppm having the percentage mortality of 18.33 and 43.3% respectively after 72 hrs. The percentage mortality in *C. citratus* extract after 72 hrs was 66.67% at 1000ppm concentration whereas at 750 ppm mortality was 8.33%. The percentage mortality for *V. amygdalina* increased from 250 to 750 ppm but decreased at 1000ppm with 750 ppm having a mortality of 63.33% and 1000ppm having a percentage mortality of 56.6% after 72 hrs. The LC_{50} and LC_{90} values of the methanolic leaf extract obtained after 72 hrs was 1008.19 and 1930.992 ppm for *C. citratus*, 1148.47 and 2210.727 ppm for *O. gratissimum* and 754.712 and 1548.499 ppm for *V. amygdalina* respectively. The methanolic extract of *V. amygdalina* exhibited a higher degree of potency when compared with the methanolic extract of *C. citratus* and *O. gratissimum* with a low LC_{50} value of 758.403ppm, 758.03 ppm and 754. 712 ppm at 24, 48 and 72 hrs respectively. In summary, this study reports the larvicidal effects of *C. citratus*, *O. gratissimum* and *V. amygdalina* against *Cx. quinquefasciatus* larvae which can serve as an alternative to synthetic pesticides in Nigeria.

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Culex quinquefasciatus Say 1823 is known globally as a nuisance and a vector of many diseases of humans. One of such diseases of public health concern is lymphatic filariasis which is ubiquitously known to be endemic in Africa. Nigeria is believed to bear the highest burden with over a hundred million people infected with the disease (WHO, 2011). This disease is caused by threadlike parasitic filarial worms; *Wuchereria bancrofti*, *Brugia malayi* and *B. timori. Wuchereria bancrofti* is the most widely spread responsible for over 90% of infections in Nigeria (WHO, 2011). Although, mosquito control measures in integrated pest management has been focused on disruption of disease transmission either by eliminating the mosquitoes, preventing human contact with the mosquitoes or killing the larvae at their breeding site, the most effective control measures are those targeted at the larval stages due to their confined habitats (Elimam *et al*., 2009). In recent times, natural products have been advocated as an economically productive and environmentally friendly substitutes for chemical pesticides. Although the use of conventional chemicals such as pyrethroids against mosquitoes showed promising results in reducing the spread of these vectors, there are undesirable side effects which occurred as a result of indiscriminate use. This resulted in loss of the environmental integrity, increased cases of secondary pest resurgence and non-target effects on native flora, fauna and humans (Koul *et al*., 2008), hence, it has become necessary to seek alternative control strategies. Unlike conventional insecticides which are based on a single active ingredient and plant-based insecticides which comprise of botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes in this insect. Thus, there is very little chance of pests developing resistance to such substances (Ferreira *et al.,* 2009; Ghosh *et al*., 2012). The insecticidal properties of plants have been the subject of intense research with an explosion of research over the past decade. One of such plants is *Cymbopogon citratus* (Poaceae) widely distributed in the tropical and subtropical regions of Africa, Asia and America. It a plant of great interest due to its commercially valuable essential oils used in food technology and traditional medicine (Mirghani *et al*., 2012) and is known to contain important phytochemicals that possess insecticidal properties

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(Asaolu *et al*., 2009; Desai and Parikh, 2012). Similarly, *Ocimum gratissimum* commonly called "scent leaf" plant is indigenous to tropical areas especially South-east Asia and West Africa. In Nigeria, it is found in both savannah and coastal areas (Effraim *et al*., 2003). It is commonly grown around houses and it repels mosquitoes and used in traditional medicine (Akinmoladun *et al*., 2007). Another plant from which natural product of pest management have been sourced is *Vernonia amygdalina* (Asteraceae). Apart from its importance in local meals (Egedigwe, 2010), traditional medicine (Iwu, 1986) and phytochemicals (e.g. Ghamba *et al*., 2014; Usunomena and Okolie, 2016; Lyumugabe *et al*., 2017), *V. amygdalina* is drought tolerant and thrives on a range of ecological zones and is used as a hedge plant in some communities (Bonsi *et al*., 1995).

Several accounts of larvicidal efficacy of *O. gratissimum* (Okigbo *et al*., 2010; Mgbemena, 2010; Pratheeba *et al*., 2015 and Tamilselvan *et al*., 2015), *C. citratus* (Mgbemena, 2010; Ebe *et al*., 2015; Goselle *et al*., 2017 and Ekesiobi *et al*., 2017) and *V. amygdalina* (Arivoli *et al*., 2011) have been well documented; however, the comparative efficacy of all three plants have never been reported. Therefore, the objective of this study is to determine the larvicidal efficacy of methanolic extracts of *O. gratissimum*, *C. citratus* and *V. amygdalina* on *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Plant Collection, Identification and preparation: Fresh leaves of scent leaf (*Ocimum gratissimum*), bitter leaf (*Vernonia amygdalina*) and lemon grass (*Cymbopogon citratus*) were collected within the University of Benin, Benin City, Nigeria. They were identified by a botanist in the department of plant biology and biotechnology. The freshly collected leaves were rinsed with tap water, shade dried (28 ± 2°C) for 20 days and pulverized using an electric blender. Methanolic extract of the leaves were made by weighing out 200g of the powdered leaves separately. Each of the weighed leaf powder was soaked in 5 litres plastic gallon containing 2500 ml of methanol and kept homogenous by constant shaking for 72 hours. It was sieved with muslin cloth and the filtrate was filtered again using Whatmans' filter paper No.1. The final filtrate of each leaf (inside different containers) was concentrated by evaporation in a water bath at controlled temperature (65°C). The yields were weighed and the percentage yield calculated. All extracts were collected and stored in the refrigerator at 4°C pending use.

Qualitative phytochemical screening: The phytochemical constituents of the plants were analysed according to reports from Keay *et al*. (1964) and Ejikeme *et al*. (2014).

Test for alkaloids: To 1 ml of methanolic extract in two different test tubes, 2-3 drops of Dragendroffs and Meyer's reagents were added into the different test tubes. The presence of orange red precipitate/turbidity with dragendroffs reagent or white precipitate with Meyer's reagent infers the presence of alkaloids.

Test for flavonoids: To 4 ml of the plant extract, a piece of magnesium ribbon was added followed by few drops of concentrated HCL. The presence of colour ranging from crimson to magenta indicated that flavonoids are present.

Test for glycosides: Keller Killianoi test was used to test for the presence of glycosides. 2 ml of plant extract, add 1 ml of glacial acetic acid with Iron (III) chloride and conc H_2SO_4. The appearance of blue colour indicates the presence of glycosides.

Test for saponins: 1ml of plant extract was measured in a test tube and 5ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes and on addition to few drops of olive oil formed an emulsion indicated that saponins are present.

Test for tannins: Measure 2ml of the extract diluted with distilled water in separate test tubes and 2-3 drops of 5% Iron (III) chloride ($FeCl_3$) solution added. A green - black or blue - black coloration indicated the presence of tannins.

Test for Terpenoids: A mixture of (2 ml) chloroform and 3 ml concentrated H_2SO_4 acid was added to 5 ml of each extract to form a layer. The presence of a reddish-brown colouration at the interface shows positive results for the presence of terpenoids (Ejikeme *et al*., 2004).

*Test for steroids***:** To 2.0 ml of the plant extract, 1ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. A red colour produced in the chloroform layer infers the presence of steroids.

Test for Phlobatannins: 10 ml of plant extract of each sample was boiled with 5 ml of 1 % aqueous hydrochloric acid. Deposits of red precipitates showed positive result.

Collection and maintenance of larval Culture in the laboratory: Egg rafts of *Cx. quinquefasciatus* were obtained from containers with polluted water found around the animal house within the university. One egg raft was placed in a plastic plate with 300 ml of rain water and covered with a net cover to prevent contamination. The eggs hatched into first instar larvae after 24 hrs and they were fed with powdered baked beans and dried liver. Feeding was done every other day until they developed into pupae. The pupae were transferred into bowls with tap water and placed in a cage until adults emerged. Adults were maintained in wooden cages and incessantly provided with 10%

sugar solution in a jar with a cotton wool. Five days after feeding with sugar solution, the adults were given a blood meal from a chicken placed in resting cages overnight for females to blood feed. Petri dishes containing 50ml of tap water lined with filter paper were kept inside the cage for oviposition. The eggs hatched and were fed until third instar larvae were obtained (WHO, 2005).

Preparation of Stock Solution: Standard WHO (2005) procedures were adopted for this study with slight modifications. Stock solution of each plant extract was prepared by measuring 1 g of solid extract and dissolved with 10 ml of acetone. 90 ml of distilled water was added to the mixture to make 1% stock solution.

Larvicidal bioassay: To perform the larvicidal bioassay, 2.5, 5.0, 7.5, and 10 ml from stock solution was added to each bowl and made up to 100ml by adding distilled water to make a concentration of 250, 500, 750 and1000 ppm respectively. A test solution without any plant extract was used as control (0ppm). Twenty (20) third instar larvae of *Cx. quinquefasciatus* were isolated from the culture using a dropping pipette and transferred to a bowl with 100 ml of test solution. Experimental plastic bowls were replicated thrice and maintained at a room temperature of 27 ± 2 (°C) and a relative humidity of 85 ± 5 %. The dead and moribund larvae were recorded after been observed for 24, 48 and 72 hrs respectively for all treatments. Larvae were confirmed dead when they failed to respond to probe with micropipette and mortality percentage was calculated.

Statistical Analysis: The percentage mortality was calculated and when the control mortality ranged from five to twenty per cent, the observed percentage mortality was corrected by Abbott's formula (Abbott, 1925).

$$\%M = \frac{Nd_L}{Nd_i} x100$$

Where % M = Percentage mortality; Nd_L = Number of dead larvae /pupae and Nd_i = Number of larvae introduced
(Abirami *et al*., 2011)

The Abbot's formula used is

$$M_{Cor} = \frac{M_T - M_C}{100 - M_C} x100$$

Where M_{cor} = corrected mortality; M_T = % test mortality; M_C = % control mortality

The mortality effect was analysed using Analysis of Variance (ANOVA) on SPSS Statistical software, version 16.0 (SPSS, Chicago, USA). Larval mortality data obtained was subjected to probit analysis on SPSS to determine the lethal concentration (LC_{50} and LC_{90}) of each plant extract against *Cx. quinquefasciatus* larvae. Fiducial limits of upper and lower confidence LC_{50} were determined. Regression equation and co-efficient of determination (R^2) were also obtained.

RESULTS AND DISCUSSION

Qualitative phytochemical constituents of the methanolic extracts of leaf extracts:

Table 1: Qualitative phytochemical constituents of the methanolic extracts of *O. gratissimum, C. citratus and V. amygdalina*

Plant Species	Phytochemicals							
	Alkaloids	Flavonoids	Glycosides	Phlobatannins	Saponins	Steroids	Tannins	Terpenoids
O. gratissimum	+++	+++	++	+	+++	+++	+++	+++
C. citratus	+++	+++	++	-	+++	+++	+++	+++
V. amygdalina	+++	+++	++	-	+++	+++	+++	+++

NB: +++ = Strongly Positive, ++ = moderately positive, + = Trace, - = Not Detected

This study investigated the phytochemical contents and larval efficacy of *O. gratissimum*, *C. citratus* and *V. amygdalina* against larvae of *Cx. quinquefasciatus*. The phytochemical results revealed presence of alkaloids, steroids, phlobatannins, saponins, tannins, flavonoids, glycosides in varying degrees ranging from highly present to moderate or even trace amount (Table 1).

The presence of flavonoids, alkaloids, saponins, phlobatannins, tannins, glycosides were revealed in *O. gratissimum* could have accounted for its larvicidal properties which agrees with the report of Gupta *et al*. (2011), Veniprasad *et al*. (2014) and Alexander (2014). This finding also agrees with Talabi *et al*. (2017) who reported alkaloids and saponins in appreciable amounts, moderate quantity of glycosides and traces of phlobatannins. Saponin's larvicidal potentials has also been reported. Meanwhile, saponins can react with larval cuticles by reconfiguring the cuticular membrane -a probable reason for larval death (Chowdhury *et al*., 2008). It should, however, be noted that most importantly, the phytochemicals can exhibit synergistic effects in their crude form (Mohammed *et al*., 2010). The presence of saponins in *O. gratissimum* does not agree with the report of Afolabi *et al*. (2007), who had previously reported absence of saponins. Unachukwu *et al*. (2015) reported the absence of glycoside in the methanolic extract of *O. gratissimum*, while Talabi *et al*. (2017) reported the absence of flavonoids whereas flavonoids and glycosides were detected in *O. gratissimum* in this report which may be due to the method of extraction used or plausibly differences in localities of the plant

itself. Alexander (2014) stated that the presence of saponins, alkaloids and flavonoids in *O. gratissimum* suggests its potential as a medicinal plant. Akinmalodun *et al*. (2007) observed that both aqueous and methanolic extracts of *O. gratissimum* contained flavonoids, glycosides, tannins and terpenoids. However, alkaloids detected were only present in the methanolic extract of *O. gratissimum* which agrees with our report whereas, saponins were absent in both extracts.

In *C. citratus*, flavonoids, alkaloids, saponins, glycosides and tannins were present in conformity with several reports (Asaolu *et al*., 2009; Ewanshiba *et al*., 2012; Christopher *et al*., 2014). The presence of glycoside in *C. citratus* did not conform with Mgbemma (2010), Unachukwu *et al*. (2016) and Umar *et al*. (2016) reports; and this could be due to the extraction method as most polar molecules are extracted by polar solvents (Ghosh *et al*., 2012). For instance, Geetha and Geetha (2014)'s report that methanolic extract of *C. citratus* had higher quantity of phytochemical constituents than the ethanolic extract; thus, suggesting that extraction method does play a role in detecting phytochemical constituents.

In *V. amygdalina*, there were alkaloids, flavonoids, steroids, saponins, phlobatannins, tannins, glycosides in conformity with different reports (Ghamba *et al*., 2014; Usunomena and Okolie, 2016). This agrees with a recent study by Lyumugabe *et al*. (2017) who reported high quantity of flavonoids and tannins in *V. amygdalina*. On the contrary, the findings here disagree with Unachukwu *et al*. (2015) as glycosides were not found in ethanolic extract of *V. amygdalina*; however, agrees with Anyi *et al*. (2016)'s report of terpenoids and glycosides in methanolic extract of *V. amygdalina*. Meanwhile it should be noted that the polarity of extraction solvents can influence the potency of extracted phytochemical constituents from plants (Ghosh *et al*., 2012; Tehri and Singh, 2015).

Larvicidal efficacy of leaf extracts against Cx quinquefasciatus larvae: The larval mortality of third instar larvae of *Cx. quinquefasciatus* after a period of exposure for 72 hrs and at various concentration of the plant extracts was observed. Varying percentage mortality were encountered while in some cases (control), no mortality was observed and as such the correction factor was not employed (Table 2).

Effects of methanolic extracts of C. citratus: The mortality of third instar larvae of *Cx. quinquefasciatus* at 24 hrs of exposure to various concentrations of *C. citratus* ranged from 3.3% in control to 55.22% in 1000 ppm, 3.30% to 62.05% at 48 hrs and 5.00% in control to 64.95% in 1000 ppm at 72 hrs of exposure. Increase in concentration from 250 ppm to 1000 ppm did not show any significant increase in mortality (p>0.05) whereas there was a significant difference (p <0.05) when comparing 0 ppm to 1000 ppm (Table 2). The result of the probit analysis on *Cx. quinquefasciatus* to different concentration of *C. citratus* after the period of exposure showed that the lethal concentration capable of killing 50% of the insect (LC_{50}) after 24, 48 and 78 hour were 1126.10, 1036.52 and 1008.19 ppm (Table 3).

Effects of methanolic extract of O. gratissimum: The mortality of third instar larvae of *Cx. quinquefasciatus* at 24 hrs of exposure to various concentrations of *O. gratissimum* ranged from 0% in control to 43.3% in 1000 ppm, 1.67% to 42.32% at 48 hrs and 6.7% in control to 39.23% in 1000 ppm at 72 hrs of exposure. Increase in concentration from 250 ppm to 1000 ppm did not show any significant increase in mortality (p>0.05) whereas there was a significant difference (p <0.05) when comparing 0 ppm to 750ppm and 1000 ppm (Table 2). The result of the probit analysis on *Cx. quinquefasciatus* to different concentration of *O. gratissimum* after the period of exposure showed that the lethal concentration capable of killing 50% of the insect (LC_{50}) after 24, 48 and 78 hour were 1059.58, 1077.29 and 1148.47 ppm (Table 4).

*Effects of methanolic extracts of V. amygdalina***:** The mortality of third instar larvae of *Culex quinquefasciatus* at 24 hrs of exposure to various concentrations of *V. amygdalina* ranged from 1.67% in control to 47.41% in 1000 ppm, 3.33% to 55.22% at 48 hrs and 6.67% in control to 53.59% in 1000 ppm at 72 hrs of exposure. Increase in concentration from 250 ppm to 1000 ppm did not show any significant (p > 0.05) whereas, there was a significant difference (p <0.05) when comparing 0 ppm with 750 and 1000 ppm (Table 2). The result of the probit analysis on *Cx. quinquefasciatus* to different concentration of *V. amygdalina* after the period of exposure showed that the lethal concentration capable of killing 50% of the insect (LC_{50}) after 24, 48 and 78 hour were 758.41, 758.03.29 and 754.72 ppm (Table 5).

Comparative efficacy of the three plant extracts against Culex quinquefasciatus: Table 6 compares the larvicidal efficacies of each plant extract at the different time of exposure against *Cx quinquefasciatus* larvae.

There was a significant difference (P<0.05) between the mean larval mortality of *Cx quinquefasciatus* due to exposure to methanol extract of *O. gratissimum*, *C. citratus* and *V. amygdalina*. The significant difference (p<0.05) was found in the *V. amygdalina* at a concentration of 750ppm.

Table 2: Mortality of *Cx. quinquefasciatus* to methanolic extracts of *C. citratus, O. gratissimum* and *V. amygdalina*

Plant type	conc (ppm)	n	Mean ± SE (Actual percentage mortality, corrected percentage mortality)		
			24 hours	48 hours	72 hours
Cymbopogon citratus	0	3	0.67 ± 0.33^{a} (3.3, 3.30)	0.67 ± 0.33^{a} (3.30, 3.30)	1.00 ± 0.00^{a} (5.00, 5.00)
	250	3	5.00 ± 1.73^{abc} (25.0, 24.05)	5.33 ± 2.03^{abc} (26.70, 24.05)	5.33 ± 2.03^{abc} (26.67, 22.84)
	500	3	5.00 ± 1.15^{abc} (25.00, 22.44)	5.00 ± 1.15^{abc} (25.00, 22.44)	5.00 ± 1.15^{abc} (25.00, 21.05)
	750	3	1.67 ± 1.20^{ab} (8.33, 5.17)	1.67 ± 1.20^{ab} (8.33, 5.17)	1.67 ± 1.20^{ab} (8.33, 3.47)
	1000	3	11.33 ± 0.88^{c} (56.67, 55.22)	12.67 ± 1.20^{c} (63.30, 62.05)	13.33 ± 1.45^{c} (66.67, 64.95)
	F value		8.30	8.36	8.17
	p value		0.01	0.01	0.01
Ocimum gratissimum	0	3	0.00 ± 0.00^{a} (0.00, 0.00)	0.33 ± 0.33^{a} (1.67, 1.67)	1.33 ± 0.67^{a} (6.67, 6.67)
	250	3	1.00 ± 0.58^{ab} (15.00, 0.00)	3.00 ± 1.53^{ab} (15.00, 13.28)	3.67 ± 2.18^{ab} (18.33, 12.43)
	500	3	3.67 ± 1.76^{ab} (18.00, 0.00)	3.67 ± 1.76^{ab} (15.00, 13.28)	3.67 ± 1.76^{ab} (18.33, 12.43)
	750	3	6.33 ± 0.67^{b} (31.70, 0.00)	6.33 ± 0.67^{b} (37.67, 30.52)	6.33 ± 0.67^{ab} (31.67, 26.80)
	1000	3	8.67 ± 3.28^{b} (43.00, 0.00)	8.67 ± 3.28^{b} (43.33, 42.32)	8.67 ± 3.28^{b} (43.33, 39.23)
	F value		22.07	15.06	9.54
	p value		0.00	0.00	0.00
Vernonia amygdalina	0	3	0.33 ± 0.33^{a} (1.67, 1.67)	0.67 ± 0.33^{a} (1.67, 3.33)	1.33 ± 0.88^{a} (6.67, 6.67)
	250	3	5.00 ± 1.53^{ab} (25.00, 23.70)	5.00 ± 1.53^{ab} (26.67, 22.41)	5.33 ± 1.45^{ab} (26.67, 21.44)
	500	3	5.66 ± 4.67^{ab} (28.33, 27.06)	5.67 ± 4.67^{ab} (28.33, 25.87)	5.67 ± 4.67^{ab} (28.33, 23.15)
	750	3	12.67 ± 1.47^{b} (63.33, 62.67)	12.67 ± 1.45^{b} (63.33, 62.05)	12.67 ± 1.47^{b} (63.33, 60.02)
	1000	3	9.67 ± 2.91^{b} (48.33, 47.41)	11.33 ± 2.33^{b} (56.67, 55.22)	11.33 ± 2.33^{b} (56.67, 53.59)
	F value		10.12	14.35	12.62
	p value		0.01	0.00	0.00

Note: Means followed by similar letters are not significantly ($p > 0.05$) different

Several accounts of larvicidal efficacy of *O. gratissimum*, *C. citratus* and *V. amygdalina* have been well documented; however, the comparative efficacy of all three plants have never been reported. This study further demonstrated the larvicidal efficacy of methanolic extracts of *O. gratissimum*, *C. citratus* and *V. amygdalina* on *Cx. quinquefasciatus* –an insect known for its vectorial capacity in the transmission of disease-causing agents. The result of the bioassay of *O. gratissimum* on *Cx. quinquefasciatus* revealed that the effect was dose dependent as mortality increases with increase in concentration. After 24 hrs of exposure, the highest mortality was reckoned in the highest dosage with 43.3% mortality, while 15% was recorded in 250ppm of *O. gratissimum*.

A much higher mortality of 96% than that recorded in this current study had earlier been reported on larvae of *Cx. quinquefasciatus* (Nzelibe and Chitem, 2013). Okigbo *et al*. (2010) reported 100% mortality of Culex mosquito after treatment with 50% petroleum ether *O. gratissimum* extract. Adefolalu *et al*. (2015) also reported 100% mortality in 2, 4, and 8% w/v concentration of methanolic extract of *O. gratissimum* after 24 hrs, while Rathy *et al*. (2015) stated that 8ml of aqueous extract of *O. gratissimum* inflicted 100% mortality on *Aedes albopictus* after 96hrs. The variations in mortality could be as a result of extraction method or plant's inherent variations that could have been influence by age (Sukumar *et al*., 1991).

The result of the larvicidal bioassay of the methanolic extract also indicated that there was a significant difference between the mean larval mortalities, which could be attributed to increase in the presence of active compounds as dose increases.

Table 3: Lethal concentration of *C. citratus* extract against *Cx. quinquefasciatus*

		Lethal conc	X^2	R^2
24HRS	LC_{50}	1126.10	28.17	0.46
	LC_{90}	2147.12		
48HRS	LC_{50}	1036.52	33.89	0.47
	LC_{90}	1965.53		
72HRS	LC_{50}	1008.19	34.74	0.44
	LC_{90}	1930.99		

Table 4. Lethal concentration of *O. gratissimum* extract against *Cx. quinquefasciatus*

		Lethal concentration	X^2	R^2
24 HRS	LC_{50}	1059.577	5.173	0.966
	LC_{90}	1842.501		
48 HRS	LC_{50}	1077.29	2.986	0.899
	LC_{90}	1911.359		
72 HRS	LC_{50}	1148.47	1.59	0.941
	LC_{90}	2210.727		

Table 5: Lethal concentration of *V. amygdalina* extract against *Cx. quinquefasciatus*

		Lethal conc	X^2	R^2
24HRS	LC_{50}	758.403	16.221	0.782
	LC_{90}	1426.209		
48HRS	LC_{50}	758.03	11.829	0.869
	LC_{90}	1477.87		
72HRS	LC_{50}	754.712	9.852	0.856
	LC_{90}	1547.499		

The mean lethal concentrations, LC_{50} and $LC_{90,}$ of the methanol extract of *O. gratissimum* against *Cx. quinquefasciatus* obtained here were 1148.47 and 2210.727ppm, respectively. On the contrary, much lower LC_{50} and LC_{90} of 2.57 and 2.52ppm had been reported (Nzelibe and Chintem, 2013) which again substantiates the view that extraction medium and methods may be culpable as Pratheeba *et al*. (2015) suggested that chloroform extract of *O. gratissimum* exhibited better mortality than other extracts. The findings here did not conform with Remia *et al*. (2017)'s report that methanolic extract of *O.*

gratissimum had mean lethal concentration (LC_{50}) of 65.15 and 74.49ppm, although, against egg and pupae of *Aedes aegypti*. Surprisingly, percentage mortality that was induced by *C. citratus* did not conform to the dose dependent pattern previously encountered. Although the highest mortality of 66.67% occurred in 1000ppm, a far lower rate was encountered in 750ppm compared to 500ppm, which contradicts Mgbemena (2010)'s position of dose-dependent mortality. The observed variation might have resulted from the interplay between biotic and abiotic factors which may have affected larval survival (Paaijmans, 2008). However, Aidaross *et al*. (2005) did not notice any mortality even at 250 and 500ppm of *C. citratus* extracts. Meanwhile, 16% and 62% mortality were observed in 1000 and 10000ppm after 24 hrs, which is similar to the findings here. Much higher mortality had been previously reported (Phasomkusolsil and Soonwera, 2010; Musa *et al*. 2015), though from petroleum ether extract of *C. citratus* against *Cx quinquefasciatus*. Variation in percentage mortality relative to this study could be due to extraction solvent and insect species (Ebe *et al*., 2015).

Table 6: Comparative larvicidal efficacies of *C. citratus*, *O. gratissimum* and *V. amygdalina*

		Concentration of plant extract (ppm)			
	Plant type	250	500	750	1000
24 hours	*Cymbopogon citratus*	5.00 ± 1.73^{a}	5.00 ± 1.15^{a}	1.67 ± 1.20^{a}	11.33 ± 0.88^{a}
	Ocimum gratissimum	1.00 ± 0.58^{a}	3.67 ± 1.76^{a}	6.33 ± 0.67^{ab}	8.67 ± 3.25^{a}
	Vernonia amygdalina	5.00 ± 1.53^{a}	5.67 ± 4.67^{a}	12.67 ± 1.45^{b}	9.67 ± 2.91^{a}
	F value	2.82	0.12	22.86	0.27
	p value	0.14	0.89	0.00156	0.77
48 hours	*Cymbopogon citratus*	5.33 ± 2.03^{a}	5.33 ± 2.03^{a}	1.67 ± 1.20^{a}	12.67 ± 1.45^{a}
	Ocimum gratissimum	3.00 ± 1.53^{a}	3.00 ± 1.53^{a}	6.33 ± 0.67^{ab}	8.67 ± 3.28^{a}
	Vernonia amygdalina	5.00 ± 1.53^{a}	5.00 ± 1.53^{a}	12.67 ± 1.45^{b}	11.33 ± 2.33^{a}
	F value	0.544	0.544	22.86	0.704
	p value	0.606	0.606	0.002	0.531
72 hours	*Cymbopogon citratus*	5.33 ± 2.03^{a}	5.00 ± 1.15^{a}	1.67 ± 1.20^{a}	13.33 ± 1.45^{a}
	Ocimum gratissimum	3.67 ± 2.19^{a}	3.67 ± 1.76^{a}	6.33 ± 0.67^{ab}	8.67 ± 3.28^{a}
	Vernonia amygdalina	5.33 ± 1.45^{a}	5.67 ± 4.67^{a}	12.67 ± 1.45^{b}	11.33 ± 2.33^{a}
	F value	0.25	0.12	22.86	0.897
	p value	0.79	0.89	0.002	0.456

Mean values with same superscript in the same column are not significantly different (P>0.05)

Mortality inflicted on the test insect from methanolic extract of *V. amygdalina* showed dose-dependent pattern up until 750ppm, after which a decline was registered at 1000ppm. Specifically, 63.3% mortality was obtained the highest obtained in the 750ppm concentration, but decline slightly to 56.57% even at a higher level of concentration than the former. It is suggestive of some factorial inconsistency in inflicting death on the larvae, but the actual reasons thereof remain unclear. The findings herein contradict Arivoli *et al*. (2011)'s report on how larval mortality is dose-dependent when treated with *V. cinerea* extract. This variation may be due to plant species used (Ghosh *et al*., 2012), or that the active components have high biodegradability that tends to deteriorate after a while, but these remain hypothetical until proven otherwise. Different plants have shown larvicidal effects against *Cx. quinquefasciatus*, for example, *Lantana camara*, *Ocimum sanctum* and *Adhatoda vasica* (Ghosh *et al*., 2016), *Azadirachta indica* (Subashini *et al*., 2016). *Leucas aspera* (Elumalai *et al*., 2017), *Hertia cheirifolia* (Amira *et al*., 2017) and much more in literature. Comparatively, the three plant extracts used herein indicate their larvicidal activities against the larvae of *Cx. quinquefasciatus* at various exposure times.

Conclusion: Conclusively, the result obtained here showed that leaf extracts of *O. gratissimum, C. citratus* and *V. amygdalina* can serve as alternatives to synthetic insecticides in control of *Cx. quinquefasciatus*. This is interesting because phytochemicals are ecofriendly, readily available and inexpensive, hence can be a favourable option in the management of mosquitoes and other insect vectors.

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