International Journal of Tropical Insect Science (2021) 41:999–1010
https://doi.org/10.1007/s42690-020-00281-x

ORIGINAL RESEARCH ARTICLE

Bioefficacy of two indigenous Nigerian botanicals on the developmental stages of malaria vector, *Anopheles gambiae* Giles [Diptera: Culicidae]

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Received: 23 January 2020 / Accepted: 18 September 2020 / Published online: 28 September 2020

Abstract

The use of botanical insecticides in place of synthetic ones is gradually gaining traction in mosquito control. This research examined the potency of *Clerodendrum volubile* and *Petivera alliacea* ethanolic leaf extracts against different life stages of *Anopheles gambiae*. Each plant extracts were formulated into concentrations of 50, 100, 200, 400 and 800 mg/l. Mosquito bioassays namely oviposition deterrent, larvicidal, pupicidal, adulticidal and repellency effect were investigated. The highest concentrations of *C. volubile* and *P. alliacea* had oviposition active index of −0.60 and − 0.76 respectively. Larval mortality of 58.33 and 100% were recorded for 800 mg/l concentration of *C. volubile* and *P. alliacea* after 24 h of exposure. However, the same concentration for both plant extracts recorded 100% after 48 and 72 h. The pupicidal activity of 800 mg/l of *C. volubile* were 51.67, 71.67 and 100%, and for *P. alliacea* 85, 100, 100% after 24, 48 and 72 h respectively. The adult mortality for both plant extracts were 16.67% at the highest used concentration after 30 min of exposure. Nevertheless, after 120 min, the same concentration of *C. volubile* and *P. alliacea* extracts recorded mortalities of 75 and 100% separately. *Clerodendrum volubile* extract provided protection of 100% against *An. gambiae* bites for 90 min while *P. alliacea* lasted for about 120 min. *Petivera alliacea* recorded the lowest LC_{50} and LC_{90} values for all the various life stages. The efficacies of these plant extracts imply that they can be incorporated into the integrated management of mosquitoes.

Keywords *Anopheles gambiae* · Oviposition deterrent · Larvicidal · Pupicidal · Adulticidal · Repellency

Introduction

Mosquitoes are seen as a menace in different climes as they are the vectors of several pathogens alongside with their annoying sounds and bites. These pathogens cause various public health diseases such as dengue fever, malaria, filariasis encephalitis, chikungunya fever, West Nile virus and Zika virus among other disease conditions (Anupam et al. 2012). *Anopheles gambiae* is responsible for the transmission of malaria fever and lymphatic filariasis (WHO 2007; CDC 2019).

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One of the efforts aimed at controlling mosquitoes is the use of synthetic insecticides which today is seen as an unpopular choice because they are perceived not to be eco-friendly. For instance, the use of Dichlorodiphenyltrichloroethane (DDT), organophosphates, organochlorines have shown to be non-biodegradable and toxic to humans based on their persistence in the ecosystem (Mahmood et al. 2016). Research has proven that the use of synthetic pesticides or insecticides as the case may be in terms of their effects, has a cognate effect on non-targeted organisms by altering their conditions through interference with natural processes leading to a system deficiency which may lead to their death (Aktar et al. 2009; Abdel-Tawab 2016). The World Health Organisation (WHO) estimates that over 200,000 people die globally through synthetic pesticide exposure annually due to synthetic pesticide overuse (Belmain et al. 2013). Aside from the environmental problem associated with the use of synthetic pesticides (insecticides) is the issue of resistance and pest resurgence (Ojo 2016). Pest resistance implies that a particular pest is resistant to a specific

pesticide rendering it ineffective in the control of the pest while pest resurgence emanates from scenario whereby pests become more virulent and difficult to control. Hence, the need to turn to the use of plants as insecticides which have been shown by researchers over the years to effectively control mosquitoes without causing the environment high cost in terms of preservation of the biodiversity and huge financial outlay.

Several researchers have demonstrated through their various works the pesticidal efficacy of plant materials and such plants could be referred to as botanicals. Their works have led to the development of numerous mosquito control agents such as ocimenone, rotenone, capllin, quassin, thymol, eugenol, neolignans, arborine and goniothalamin (Shaalan et al. 2005). Several species of plants have been shown to possess some mosquitocidal properties (Bekele 2018). A great number of these plant species were seriously investigated leading to the eventual characterization of their active constituents. The most notable of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (Shaalan et al. 2005; Alqasoumi et al. 2012; Adesina and Rajashekar 2018). Hence, plants containing them are said to have insecticidal properties. Common plants such as neem, tobacco, pawpaw inter alia have been used as insecticides (Habibullah et al. 2007).

Petiveria alliacea L. is a perennial shrub/herb from the family Phytolaccaceae (García-González et al. 2006). The plant is a straight herbaceous plant that can grow up to 1 m tall. It has small white flowers that are bisexual, zygomorphic, which are slightly imbricate to rather remote (Ferrer 2007). The sepals are white or greenish to pinkish, linear-lanceolate to linear-oblong, 3.5–6 mm long with superior ovary (Nienaber and Thieret 2003). Its fruit forms a cuneiform berry with narrow oblong achenes, 6–8 mm long with four hooks turned downwards and the seeds are solitary, erect and linear (Alegre and Clavo 2007). Phytochemicals present in *P. alliacea* include terpenoids (triterpenoids) saponins, phenols (polyphenols), coumarins, benzaldehyde, benzoic acid, flavonoids, fredelinol, pinitol and allantonin with varying concentrations in the root, stems and leaves (Kubec et al. 2002, 2003). The GC-MS analysis of the ethanolic extract of the leaves revealed Phenol,2,4-bis(1,1)-dimethylethyl, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Hexadecanoic acid, methyl ester, ethyl ester; 9,12,15-octadecatrienoic acid, ethyl ester; Stigmasterol; and Campesterol, n-Hexadecanoic acid, and Phytol as the major compound present (Abdul-Raheem et al. 2018). *Petivera alliacea* has been identified as a plant with medicinal, insecticidal and acaricidal properties (Nienaber and Thieret 2003; Gomes et al. 2005; Kim et al. 2006; Pérez-Leal et al. 2006; Williams et al. 2007; García-Mateos et al. 2007; Schmelzer and Gurib-Fakim 2008; Hernández et al. 2014; Hartmann et al. 2018).

Clerodendrum volubile P. Beauv is an indigenous plant that belongs to the family Lamiaceae (Verbenaceae). The plant is a green climbing shrub which can grow up to 3 m in height characterised with numerous flowers which are averagely 1.5 cm in length (Burkill 1985; Erukainure et al. 2011). *Clerodendrum volubile* is rich in several phytochemicals which include phenols, saponins, alkaloids and tannins (Erukainure et al. 2011). The gas chromatography-mass spectrometry (GC–MS) of its ethanolic crude extract revealed that oxirane, methyl 2-octylcyclopropene-1-heptanoate and Hexadecanoic acid, methyl ester were the top three major compounds as they accounted for 50.51%, 12.41% and 9.96% respectively (Molehin et al. 2017). Plants of the genus *Clerodendrum* which have been proven to be of high medicinal value (Erukainure et al. 2011, 2014). Research works on its insecticidal efficacies some members of the genus have been published (Patil et al. 2014; Muthu et al. 2015; Lurdu and Thampi 2017; Ileke and Adesina 2018). However, the insecticidal efficacy of *C. volubile* is yet to be reported. This study is aimed at determining the potency of *C. volubile* and *P. alliacea* ethanolic leaf extracts against different life stages of *An. gambiae* with oviposition deterrence, larvicidal, pupicidal, adulticidal and repellency potentials examined.

Methodology

Collection of plant materials

Defect-free fresh leaves of *P. alliacea* and *C. volubile* that were obtained from Ondo town (7.10° N, 4.84° E) in Ondo State, Nigeria. They were taken to the Crop, Soil and Pest Management Laboratory, Federal University of Technology, Akure, Ondo State for authentication. The leaves of the two plants were thoroughly cleaned with distilled water, air-dried in the laboratory for one month then pulverized into a fine powder using pestle and mortar. The resulting powder was stored inside dark bottles and refrigerated for preservation purpose until extraction procedure was carried out.

Plant active ingredient extraction

The cold maceration extraction method described by Udo (2011) was adapted in the study. Five hundred grams (500 g) of *P. alliacea* and *C. volubile* powders were soaked separately in an extraction bottle containing 1.5 l of absolute ethanol. The mixture was stirred occasionally with a glass rod and extraction was terminated after 3 days. The liquid filtrate was then concentrated with the aid of a rotary evaporator at a speed of 3 to 6 rpm, at temperatures of 30 to 40 °C for 8 h. The final extract is refrigerated at 4 °C until required.

Mosquito baits

The mosquito baiting method used for this study follows the ovitrap method described by Rawlins et al. (1998) with slight modification. Containers with 1 l of rainwater were sprinkled with 10 g of bakers yeast and then placed under a partially shaded area on an open field to serve as an attractant to wild mosquitoes to lay their eggs i.e. simulates the natural breeding ground of mosquitoes. After 2–5 days, the baits containing eggs/larva were then transferred to the laboratory. The eggs/larva were identified into species level using the morphological keys described by Gillies and De Meillon (1968) with the use of an Olympus stereo-dissecting microscope with model number VMT-2S-W. The *An. gambiae* larvae were then separated from the mixed culture and transferred into another plastic container containing distilled water, fed with bakers yeast and subsequently used or nurtured to the desired life stages (pupae and adults) as required by the different bioassays.

Concentrations preparation and experimental design

Two (2) grams of the crude extracts were dissolved in 1 l of solvent. From these stock solutions, different concentrations of 50 mg/l, 100 mg/l, 200 mg/l, 400 mg/l and 800 mg/l. The extract concentrations for oviposition deterrent, larvicidal and pupicidal bioassay were prepared using sterile distilled water as a solvent while 70% of ethanol was used for adulticidal and repellency bioassays. The experiment was carried out using a completely randomized design (CRD) under laboratory conditions.

Oviposition deterrent bioassay

The procedure for the oviposition deterrent of the plant extracts followed the ones described by Xue et al. (2001) and Fatima et al. (2011). An equal number (50) of a day-old male and female adults were placed inside a screen cage (45 X 45 X 30 cm) for mating purpose. They were provided with 10% sucrose solution soaked in cotton wool and placed in a plastic cup. After 4 days, an albino rat (*Rattus norvegicus*) with a shaved dorsal side (of about $20cm^2$ area) was used to feed the female mosquito. The rat with the help of wire mesh restrainer was placed on the cage with the shaved dorsal side towards it and left for about an hour in the absence of lightning. This was done to serve as blood source needed for oviposition by the female mosquito. About 50 ml of any given concentration of the plant extract was placed inside a plastic container that has filter paper serving as ovipositional surface. Three (3) cups were allotted to each concentration (50, 100, 200, 400, 800 mg/l) and control (water). The containers allotted to each concentration were arranged alternatively inside another screen cage of the same dimension to cancel out any effect on oviposition that may arise due to positioning. Ten gravid blood-fed female mosquitoes were then separated from the males and released inside the new cage containing the treatments where oviposition was observed. The test was replicated three times. The mosquitoes were sustained in conditions of 27° ± 2 °C and 75 ± 5% humidity. Egg deposition was observed and recorded after 24 h of egg-laying using a stereo-dissecting microscope.

The effective repellency (ER) was calculated using the formula below:

$$ER = \frac{NC-NT}{NC} \times 100 \quad (1)$$

Where ER is effective repellency, NC is the number of eggs in control and NT is the number of eggs in treatment.

The formula was used to calculate the oviposition active index (OAI) was described by Kramer and Mulla (1979) which is:

$$OAI = \frac{NC-NT}{NT+NC} \quad (2)$$

Oviposition active index of any substance can be categorised as follows:

- No oviposition deterrence or attractant substance when $OAI > 0$
- Moderated oviposition inhibit substance (moderate oviposition deterrence) - $0.5 < OAI < 0$
- Strong oviposition inhibit substance (strong oviposition deterrence) $-1 < OAI < -0.5$

Larvicidal bioassay

The method used for the larvicidal bioassay follows the procedure described by the WHO (2005) with little modifications. Twenty (20), 3rd/4th larval instar obtained from the culture were introduced separately into the various concentration of plant extracts along with a set of control containing sterile distilled water. All the tested concentrations were replicated three times. An aliquot of 50 ml of 50, 100, 200, 400, 800 mg/l and water (control) was used. Larvae that did not respond when pricked with a needle were recorded as been dead. The number of dead larvae was counted and recorded accordingly after 24, 48 and 72 h of exposure using the formula below.

$$\%\text{Larval mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (3)$$

Afterwards, the percentage of mortality was observed and corrected using Abbott (1925) formula shown below:

$$\%\text{corrected mortality} = \frac{\%\text{test mortality} - \%\text{control mortality}}{100 - \%\text{control mortality}} \times 100 \quad (4)$$

Pupicidal bioassay

A similar approach used for the larvicidal bioassay was used for the pupicidal bioassay. Twenty (20) two-day-old pupae were introduced separately into the various concentration of plant extracts along with a set of control containing sterile distilled water. All tested concentrations were replicated three times. An aliquot of 50 ml of 50, 100, 200, 400, 800 mg/l and water (control) was used. Pupae that didn't respond to any stimulus (needle pricks) were considered to be dead. The number of dead pupae was counted and recorded accordingly after 24, 48 and 72 h of exposure using the formula below:

$$\%\text{Pupal mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} \times 100 \quad (5)$$

Afterwards, the mortality is corrected using the Abbot formula given in eq. 4.

Adulticidal bioassay

The method described by WHO (2006) for mosquito adulticidal testing was slightly modified and used for this study. The Twenty (20) sugar-fed (10% sucrose solution) adults were introduced into a 1500 ml container perforated at the top and plunged with cotton wool. Cotton wool of 0.5 g was used to soak an aliquot of 0.5 ml of 50, 100, 200, 400, 800 mg/l and 70% ethanol (control) separately. These soaked cotton wools were left for a period of 2 h so that the ethanol used in the formulation of the concentrations can escape leaving behind the extract itself. The cotton wool with the extracts was then introduced into the containers containing the mosquitoes. Mortality was recorded when the mosquitoes failed to respond to an external stimulus such as tapping of the containers. All tested concentrations were replicated three times. The number of dead mosquitoes was recorded after 30, 60, 90 and 120 min. Percentage mortality was calculated using the formula below:

$$\%\text{Adult mortality} = \frac{\text{Number of dead adults}}{\text{Number of adults introduced}} \times 100 \quad (6)$$

The percentage mortality was then corrected using Abbot formula given in eq. 4.

Repellency test

The animal model procedures used by Oluyemi et al. (2018) was adapted for this study. Insect cages measuring 45 X 45 X 30 cm was used for this bioassay. The Albino rat was used as test animals. An area of 20cm^2 was shaved on the rat which was then washed with unscented soap. An aliquot of 0.5 ml of each concentration including the control (ethanol) was used to rub the shaved part of the rat separately. The rat with any given concentration was then placed inside the insect cage containing about disease-free 20 starved gravid female mosquitoes. The experiments were carried out during night time (19:00–05:00) when female *Anopheles* mosquitoes are considered to feed actively. When the mosquito lands on the surface and stays there for more than 2–3 s, the time of landing and the number of mosquitoes is recorded. The tests for each concentration were replicated three times and the percentage repellency was calculated with a formula described by Govindarajan and Sivakumar (2011) which is given as:

$$\%\text{Repellency} = \frac{\text{Ta} - \text{Tb}}{\text{Ta}} \times 100 \quad (7)$$

Where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

Data analysis

The results from the bioassays were subjected to one-way analysis of variance (ANOVA) and the means separated using Tukey's Honest Significance Test. Probit analysis was used to calculate lethal concentrations (LC) required to cause 50 and 90% (LC_{50} and LC_{90}) for bioassays that percentage mortalities were the values recorded (Finney 1971). Multivariate regression analysis was used to find the regression between concentration with oviposition (mean no. of eggs laid) and concentration with repellency time (minute). All level of significance was set at $p < 0.05$. The ANOVA and Probit analysis were done using Statistical Package for the Social Sciences (SPSS) version 20 while the multivariate regression analysis was done using Paleontological Statistics (PAST) version 4.01.

Results

Oviposition deterrent activities of *C. volubile* and *P. alliacea* against *An. gambiae*

All the different concentrations of both plant extracts tested yielded a negative oviposition activity index which ranged between −0.02 to −0.76 (Table 1). This goes to show that they all inhibited the oviposition of *An. gambiae*. When the concentration was high, the oviposition was also high which shows that there is a concentration-dependent relationship existing between the extracts and their oviposition deterrent ability. This also indicates that an increment in the concentration resulted in a corresponding increase in effective repellency percentage (Table 1).

Table 1 Oviposition deterrent activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Concentration (mg/l)	Mean No. of eggs ± S. E	ER%	OAI
C. volubile	Control	286.33 ± 8.27*	–	–
	50	272.78 ± 6.77^{g}	4.22	−0.02
	100	253.56 ± 8.14^{fg}	11.15	−0.06
	200	222.89 ± 4.85^{e}	21.70	−0.12
	400	123.00 ± 5.49^{c}	56.53	−0.40
	800	72.11 ± 4.34^{b}	74.53	−0.60
P. alliacea	Control	299.00 ± 5.12*	–	–
	50	240.78 ± 9.73^{ef}	19.46	−0.11
	100	220.78 ± 7.52^{e}	26.08	−0.15
	200	157.78 ± 4.92^{d}	47.17	−0.26
	400	100.89 ± 5.33^{bc}	66.01	−0.50
	800	40.44 ± 3.64^{a}	86.45	−0.76

Each value is Mean of 3 replicates; OAI = Oviposition Active Index; S.E: Standard Error; ER = effective repellency; * = Significance not calculated; Means followed by the same letter are not significantly different using Tukey's Honest Significance Test($p = 0.00$; df = 29)

The total number of eggs laid in the control plastic cup was very different from the number of eggs laid in treated plastic cups ($p < 0.05$) for all the extracts. Treated cups containing 50 mg/l of *C. volubile* extract had the highest number of eggs laid which was 272.78 ± 6.77. However, cups with 800 mg/l of *P. alliacea* presented the lowest eggs laid which were 40.44 ± 3.64. The effective repellency percentages for the extracts ranged from 4.22–86.45%. *C. volubile* extract at 50 mg/l had the lowest percentage while at 800 mg/l, *P. alliacea* recorded the strongest oviposition deterrence. Strong oviposition deterrence was observed for *C. volubile* at only 800 mg/l but at 400 mg/l and 800 mg/l for *P. alliacea*. This, therefore suggests that *P. alliacea* extract was the most active of the two extracts (Table 1). Table 2 shows the relationship that exists between the concentration and the mean number of eggs laid (oviposition). The result showed that the two plant extract significantly affected the number of eggs laid. This was so because the different concentrations of the two plant extracts reduced oviposition with respect to their various control.

Larvicidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Mortality of larvae of *An. gambiae* were recorded for all concentrations of the two plant extracts used. The percentage of mortality is presented in Table 3. Significant differences were observed between different concentrations of *C. volubile* and *P. alliacea* that had the same period of exposure at $p < 0.05$. Exposing the larvae to the lowest (50 mg/l) and highest (800 mg/l) concentrations for 24 h yielded 20.00 and 58.33% for *C. volubile*; 25.00 and 100.00% for *P. alliacea* extracts respectively. Forty-eight (48) hours of the exposure recorded 100% mortality for 800 mg/l of *C. volubile*, 400 and 800 mg/l of *P. alliacea* concentrations. At about 72 h of exposure, three concentrations of *P. alliacea* extracts namely 200, 400 and 800 mg/l had 100% mortality while 400 and 800 m/l of *C. volubile* extract had the same result. The overall result points to the fact that the extracts had increasing mortalities proportionate to increasing concentrations and time of exposure. This result suggests that *P. alliacea* extract was the most active of the two extracts tested.

Table 2 Relationship between the concentration and mean number of eggs laid (oviposition)

Variable	Slope ± error	Intercept ± error	r	p
C. volubile	−0.282 ± 0.036	277.950 ± 13.540	−0.969	**0.001**
P. alliacea	−0.299 ± 0.053	253.960 ± 20.107	−0.942	**0.005**

Bold numbers show significant value (Significance set at $p < 0.05$; df = 5)

Pupicidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Mortality of pupae of *An. gambiae* were recorded for all concentrations of the two plant extracts used. The percentage of mortality is presented in Table 4. Significant differences were observed between different concentrations of *C. volubile* and *P. alliacea* that had the same period of exposure at $p < 0.05$. When the pupae were exposed to the lowest (50 mg/l) and highest (800 mg/l) concentrations for 24 h yielded 6.67and 51.67% for *C. volubile*; 13.33 and 85.00% for *P. alliacea* extracts. After 48 h of exposure 800 mg/l concentrations of *C. volubile* and *P. alliacea* extracts, mortalities of 71.67% and 100.00% respectively. At about 72 h of exposure, 100% mortality was recorded for 800 mg/l of *C. volubile* and, 400 and 800 mg/l of *P. alliacea* extract. The overall result points to the fact that the extracts had increasing mortalities proportionate

Table 3 Larvicidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Concentration (mg/l)	Exposure Period (hours)		
		24	48	72
C. volubile	50	20.00 ± 0.00^{b}	31.67 ± 1.67^{b}	55.00 ± 2.89^{b}
	100	28.33 ± 1.67^{bcd}	43.33 ± 1.67^{c}	73.33 ± 4.4^{cd}
	200	35.00 ± 2.89^{cd}	60.00 ± 2.89^{de}	85.00 ± 2.89^{e}
	400	48.33 ± 1.67^{e}	75.00 ± 2.89^{f}	100.00 ± 0.00^{f}
	800	58.33 ± 4.41^{e}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}
P. alliacea	50	25.00 ± 2.89^{bc}	43.33 ± 1.67^{c}	65.00 ± 2.89^{b}
	100	36.67 ± 1.67^{d}	55.00 ± 2.89^{d}	81.67 ± 1.67^{de}
	200	48.33 ± 1.67^{e}	68.33 ± 1.67^{ef}	100.00 ± 0.00^{f}
	400	80.00 ± 2.89^{f}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}
	800	100.00 ± 0.00^{g}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}
	Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of 3 replicates; Means followed by the same letter in the same column are not significantly different using Tukey's Honest Significance Test ($p = 0.00$; df = 32)

to increasing concentrations and time of exposure. This result suggests that *P. alliacea* extract was the slightly more active of the two extracts tested as pupicides.

Adulticidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

The results for the adulticidal efficacies of *C. volubile* and *P. alliacea* are presented in Table 5. There was a significant difference ($p < 0.05$) in the mortalities recorded among the various concentrations used with respect to the period of exposure. After 30 min of exposure, the lowest concentration used (50 mg/l) did not produce any mortality but the highest concentration produced the same mortality of 16.67% for both plant extracts. At the maximum period of exposure (120 min) used for this study, 400 and 800 mg/l of *P. alliacea* recorded mortalities of 100% while 800 mg/l of *C. volubile* had a mortality of 75%. The percentage of mortality recorded seems to appear in a concentration and exposure period dependent manner.

Repellent activities of *C. volubile* and *P. alliacea* against *An. gambiae*

The results of the repellent activities of *C. volubile* and *P. alliacea* is presented in Table 6. At a period of 15 min, all the concentrations of the two plant extracts showed a 100% repellency. 800 mg/l of *C. volubile* provided 100% repellency until 90 min while *P. alliacea* still had 100% repellency until

Table 4 Pupicidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Concentration (mg/l)	Exposure Period (hours)		
		24	48	72
C. volubile	50	6.67 ± 1.67^{ab}	15.00 ± 2.89^{b}	33.33 ± 1.67^{b}
	100	11.67 ± 1.67^{bc}	28.33 ± 1.67^{c}	45.00 ± 2.89^{c}
	200	30.00 ± 2.89^{de}	55.00 ± 2.89^{d}	66.67 ± 1.67^{d}
	400	38.33 ± 4.41^{e}	63.33 ± 1.67^{de}	78.33 ± 1.67^{e}
	800	51.67 ± 1.67^{f}	71.67 ± 1.67^{ef}	100.00 ± 0.00^{f}
P. alliacea	50	13.33 ± 1.67^{bc}	18.33 ± 1.67^{bc}	51.67 ± 1.67^{c}
	100	21.67 ± 1.67^{cd}	28.33 ± 1.67^{c}	65.00 ± 2.89^{d}
	200	35.00 ± 0.00^{e}	60.00 ± 2.89^{d}	66.67 ± 1.67^{d}
	400	56.67 ± 1.67^{f}	80.00 ± 2.89^{f}	100.00 ± 0.00^{f}
	800	85.00 ± 2.89^{g}	100.00 ± 0.00^{g}	100.00 ± 0.00^{f}
	Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of 3 replicates; Means followed by the same letter in the same column are not significantly different using Tukey's Honest Significance Test($p = 0.00$; df = 32)

Table 5 Adulticidal activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Concentration (mg/l)	Time (minutes)			
		30	60	90	120
C. volubile	50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.67 ± 1.67^{a}	13.33 ± 1.67^{b}
	100	6.67 ± 1.67^{b}	15.00 ± 2.89^{b}	23.33 ± 1.67^{b}	41.67 ± 1.67^{d}
	200	6.67 ± 1.67^{b}	28.33 ± 1.67^{c}	40.00 ± 2.89^{c}	53.33 ± 1.67^{e}
	400	8.33 ± 1.67^{b}	35.00 ± 2.89^{cd}	48.33 ± 1.67^{cd}	63.33 ± 1.67^{f}
	800	16.67 ± 1.67^{c}	40.00 ± 1.67^{de}	58.33 ± 4.41^{def}	75.00 ± 2.89^{g}
P. alliacea	50	0.00 ± 0.00^{a}	8.33 ± 1.67^{ab}	20.00 ± 2.89^{b}	31.67 ± 1.67^{c}
	100	3.33 ± 1.67^{a}	16.67 ± 1.67^{b}	41.67 ± 6.01^{c}	60.00 ± 2.89^{ef}
	200	5.00 ± 0.00^{ab}	31.67 ± 4.41^{cd}	53.33 ± 3.33^{cde}	78.33 ± 1.67^{g}
	400	5.00 ± 0.00^{ab}	41.67 ± 1.67^{e}	65.00 ± 2.89^{ef}	100.00 ± 0.00^{h}
	800	16.67 ± 1.67^{c}	48.33 ± 1.67^{e}	71.67 ± 1.67^{f}	100.00 ± 0.00^{h}
	Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of 3 replicates; Means followed by the same letter in the same column are not significantly different using Tukey's Honest Significance Test($p = 0.00$; df = 32)

120 min of post-exposure. However, after 150 min of exposure, none of the plant extracts could boast of 100% with 800 mg/l of *C. volubile* and *P. alliacea* only managing a 64.44 and 93.75% respectively. Table 7 shows the relationship between the concentrations of the two plant extracts and repellency time. The result revealed that repellency by all concentration significantly reduces after 90 min for *C. volubile* and 120 min for *P. alliacea.*

Lethal concentrations (LC) of *C. volubile* and *P. alliacea* against *An. gambiae*

Lethal concentrations of *C. volubile* and *P. alliacea* against required to cause 50 and 90% mortality in different life stages of *An. gambiae* are shown in Table 8. The 50% lethal concentration (LC_{50}) of *C. volubile* and *P. alliacea* extracts after 24 h was 471.24 and 142.76 mg/l respectively for the larva stage. However, these values were significantly reduced to 48.20 and 40.00 mg/l for *C. volubile* and *P. alliacea* extract after 72 h. The 90% lethal concentration (LC_{90}) of *C. volubile* and *P. alliacea* was 13,572.54 and 612.94 mg/l for 24 h and after 72 h, 194.13 and 109.39 mg/l respectively. The pupicidal LC_{50} and values for *C. volubile* extract after 24 and 72 h were 677.00 and 105.73 mg/l, and LC_{90} of 6640.76 and 549.86 mg/l correspondingly. Nevertheless, the lethal concentrations values recorded for *P. alliacea* extract were lower compared to *C. volubile* extract. Its LC_{50} values were 274.56 and 57.37 mg/l after 24 and 72 h and LC_{90} values of 1485.35 and 241.22 mg/l respectively. Generally, the lethal concentrations tend to decrease as the period of post-exposure increases for the larva and pupa stages. The adulticidal LC_{50} for *C. volubile* and *P. alliacea* extracts after 120 min was

Table 6 Repellency activities of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Concentration (mg/l)	Time (Minutes)						
		15	30	60	90	120	150	180
C. volubile	50	100.00 ± 0.00*	75.00 ± 0.00*	50.00 ± 9.62^{a}	40.00 ± 5.77^{a}	22.22 ± 2.78^{a}	13.33 ± 3.85^{a}	8.33 ± 1.67^{a}
	100	100.00 ± 0.00*	75.00 ± 0.00*	61.11 ± 5.56^{ab}	46.67 ± 8.82^{a}	30.56 ± 2.78^{ab}	24.44 ± 2.22^{ab}	16.67 ± 1.67^{a}
	200	100.00 ± 0.00*	100.00 ± 0.00*	77.78 ± 5.56^{b}	56.67 ± 3.33^{ab}	44.44 ± 2.78^{b}	37.78 ± 2.22^{b}	30.00 ± 2.89^{b}
	400	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00^{c}	83.33 ± 3.33^{cd}	69.44 ± 2.78^{cd}	60.00 ± 0.00^{c}	48.33 ± 1.67^{c}
	800	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00^{c}	100.00 ± 0.00^{d}	80.56 ± 2.78^{de}	64.44 ± 2.22^{cd}	55.00 ± 2.89^{cd}
P. alliacea	50	100.00 ± 0.00*	100.00 ± 0.00*	76.67 ± 5.09^{b}	51.85 ± 6.06^{ab}	38.89 ± 5.56^{b}	29.17 ± 4.17^{b}	10.00 ± 2.89^{a}
	100	100.00 ± 0.00*	100.00 ± 0.00*	82.22 ± 1.11^{bc}	68.89 ± 1.11^{bc}	61.11 ± 2.78^{c}	54.17 ± 2.08^{c}	31.67 ± 1.67^{b}
	200	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00^{c}	79.26 ± 0.74^{cd}	66.67 ± 4.81^{cd}	54.17 ± 2.08^{c}	50.00 ± 2.89^{c}
	400	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00^{c}	100.00 ± 0.00^{d}	88.89 ± 2.78^{ef}	77.08 ± 2.08^{d}	65.00 ± 2.89^{d}
	800	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{f}	93.75 ± 3.61^{e}	86.67 ± 1.67^{e}

Each value is a mean ± standard error of 3 replicates. * = Significance not calculated; Means followed by the same letter are not significantly different using Tukey's Honest Significance Test($p = 0.00$; df = 29)

Table 7 Relationship between the concentrations of the plant extracts *C. volubile* and *P. alliacea* and repellency time

Botanicals	Variable Time (minutes)	Slope ± error	Intercept ± error	r	P
C. volubile	15	0.00 ± 0.00	100.00 ± 0.00	0.00	1.00
	30	0.03 ± 0.02	80.21 ± 7.61	0.70	0.19
	60	0.06 ± 0.02	58.10 ± 9.07	0.86	0.06
	90	0.08 ± 0.01	40.28 ± 4.96	0.97	**0.01**
	120	0.08 ± 0.02	25.58 ± 6.61	0.94	**0.02**
	150	0.07 ± 0.02	19.81 ± 7.61	0.90	**0.04**
	180	0.06 ± 0.02	13.06 ± 6.23	0.92	**0.03**
P. alliacea	15	0.00 ± 0.00	100.00 ± 0.00	0.00	1.00
	30	0.00 ± 0.00	100.00 ± 0.00	0.00	1.00
	60	0.03 ± 0.02	83.61 ± 6.35	0.70	0.19
	90	0.06 ± 0.02	62.22 ± 8.68	0.84	0.07
	120	0.07 ± 0.02	48.84 ± 7.71	0.91	**0.03**
	150	0.08 ± 0.02	38.37 ± 7.15	0.93	**0.02**
	180	0.09 ± 0.02	20.70 ± 8.45	0.93	**0.02**

Bold numbers show significant value ($p < 0.05$; df = 13)

208.45 and 80.65 mg/l respectively. The adulticidal LC_{90} of *P. alliacea* was lower than that of *C. volubile*.

Discussion

The usefulness and the prospect in the use of botanical based insecticide as a substitute for their synthetic contemporaries in the control of mosquito are gradually gaining traction (Ranganatha et al. 2013; Ileke et al. 2017; Ileke and Olabimi 2019). This study showed that ethanolic extracts of *P. alliacea* and *C. volubile* had mosquitocidal effects in terms of oviposition deterrence, larvicidal, pupicidal, adulticidal and repellent capabilities on *An. gambiae*.

The oviposition active index (OAI) recorded in this study revealed that the extracts were active in the deterring the oviposition of *An. gambiae* because of their negative values. These results corroborate the work of Wangrawa et al. (2016) on the oviposition-deterrence activities of ethanol, acetone and hexane extracts of *Lantana camara* L., *Hyptis suaveolens* Poit., *Ocimum canum* Sims. and *Hyptis spicigera* Lam. on sensitive and local strains of *An. gambiae* s. l. Also,

Table 8 Lethal Concentrations (LC) of *C. volubile* and *P. alliacea* against *An. gambiae*

Extract	Life stages	Slope ± S. E	Intercept ± S. E	χ^2	Exposure period	LC_{50} (LCL-UCL) (mg/l)	LC_{90} (LCL-UCL) (mg/l)
C. volubile	Larva	0.88 ± 0.14	−2.35 ± 0.33	0.27	24 h	471.24 (338.44–776.15)	13,572.54 (4894.35–92,399.01)
		1.75 ± 0.16	−3.61 ± 0.37	14.86	48 h	117.78 (38.21–219.19)	638.93(308.43–11,941.41)
		2.12 ± 0.25	−3.57 ± 0.50	7.28	72 h	48.20 (36.05–59.28)	194.13 (160.34–251.78)
	Pupa	1.29 ± 0.16	−3.66 ± 0.39	2.24	24 h	677.00 (513.42–1006.22)	6640.76 (3421.57–19,310.86)
		1.36 ± 0.15	−3.23 ± 0.35	5.34	48 h	237.77 (194.97–293.23)	2085.85 (1345.51–4004.44)
		1.79 ± 0.17	−3.62 ± 0.37	11.64	72 h	105.73 (87.23–124.76	549.86 (429.29–770.15)
	Adult	1.32 ± 0.15	−3.06 ± 0.34	7.44	120 min	208.45 (169.57–256.71)	1944.59 (1251.163–3770.86)
P. alliacea	Larva	2.03 ± 0.17	−4.36 ± 0.38	18.23	24 h	142.758 (58.27–278.48)	612.94 (304.24–9679.22)
		2.14 ± 0.21	−4.03 ± 0.44	23.72	48 h	76.48 (2.47–153.85)	303.66 (151.508–127,337.10)
		2.93 ± 0.43	−4.70 ± 0.81	6.42	72 h	40.00 (29.48–48.43)	109.39 (93.50–136.96)
	Pupa	1.75 ± 0.16	−4.26 ± 0.38	5.63	24 h	274.56 (233.77–327.14)	1485.35 (1074.89–2322.39)
		2.37 ± 0.19	−5.14 ± 0.42	9.92	48 h	149.95 (131.45–170.28)	521.85 (429.82–669.53)
		2.06 ± 0.22	−3.61 ± 0.46	13.92	72 h	57.37 (6.378–100.80)	241.22 (135.76–2721.01)
	Adult	2.68 ± 0.25	−5.12 ± 0.51	8.11	120 min	80.65 (69.45–91.75)	242.31 (204.91–302.05)

χ^2 = Chi-square value, S.E = Standard error, LCL = Lower confidence limit and UCL = Upper confidence limit

Prathibha et al. (2014) demonstrated the efficacies of petroleum ether and ethyl acetate extract of *Eugenia jambolana, Solidago canadensis, Euodia ridleyi* and *Spilanthes mauritiana* in inhibiting the egg-laying capabilities of *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi*. The submissions of Prathibha et al. (2014) and Wangrawa et al. (2016) showed that OAI and effective repellency percentage exhibited by the different plant extracts used in their studies presented themselves in a dose-dependent manner synonymous to results from this present study. In this study, only a concentration of *C. volubile* had very high OAI and effective repellency percentage while for *P. alliacea* was two. It then follows that they can be very useful when added into stagnant waters that could serve as oviposition sites of *An. gambiae* species to control them. Thus, effectively controlling the egg-laying habits of any species of mosquito will go a long way towards reducing the menace they constitute. This is so because oviposition is a vital part of their life cycle (Xue et al. 2001; Qiu et al. 2006; Prathibha et al. 2014).

The larvicidal activity of the ethanolic extract of *P. alliacea* showed the highest percentage mortality throughout the different period of exposure. The work of Hartmann et al. (2018) showed that different extract of *P. alliacea* showed that they were highly effective in controlling the larva of ***Ae. aegypti***. Unlike *P. alliacea*, *C. volubile* extract caused absolute mortality on *An. gambiae* larva only after the second day of exposure. The larvicidal activity of *C. volubile* in this study is similar to the ones produced by some other species of the genus *Clerodendrum* (Patil et al. 2014; Muthu et al. 2015; Lurdu and Thampi 2017). For instance, Patil et al. (2014) showed that extracts of *Clerodendrum inerme* were able to control the larva of *Ae. aegypti* and *Cx. quinquefasciatus*. Also,Muthu et al. (2015) demonstrated the efficacy of Pectolinaringenin, a compound isolated from *Clerodendrum phlomidis* Linn. F. against *An.* stephensi and *Earias vittella* larvae. The result gotten from this study conforms with several other works that have tested the efficacy of botanicals as potential mosquito larvicides (Ileke et al. 2017; Ileke and Olabimi 2019; Ileke and Adesina 2019). The high larvicidal efficacies of the plants used in this study may be because the larvae will swim around ingesting the extracts alongside phytochemicals present in them (Ileke et al. 2014).

The pupicidal bioassay in this study revealed that none of the two plants extracts caused maximum mortality on the test organism used until after the second day of exposure. Nevertheless, both plant extracts controlled the pupa of *An. gambiae* after the third day of exposure. The outcomes of this study agree with previous works carried out in this field. Ileke and Adesina (2018) reported the efficacy of *Clerodendrum capitatum* and *Bridelia machrantha* leaves extracts against *An. gambiae* pupae. Also, Panneerselvam et al. (2012) and Kashte et al. (2015) in their respective research showed the pupicidal activities of various plant leave extract against different malaria vectors. The mortality rate recorded in the pupicidal bioassay for the same exposure period was a bit lower compared to those recorded in the larvicidal bioassay. This might be due to the morphological differences that exist between the larvae and the pupae of mosquito thereby suggesting that the mode of action of these plant products is either by choking or contact and not necessarily ingestion as did pupal stage does not ingest whatsoever (Raveen et al. 2017).

Petiveria alliacea however, had more potent adulticidal effects after the maximum exposure period causing absolute mortality of the tested mosquito species. Although at lower concentrations and period of exposure, *C. volubile* induced very close percentage mortality as *P. alliacea* extract. The findings of this present study are in line with the works of other authors who have tested the efficacies of plant extract on different mosquitoes adults. For instance, difference extracts of *G. pentaphylla* leaves were effective in controlling the adults of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* (Ramkumar et al. 2016). Kovendan et al. (2013) in their work found the extract of *Acalypha alinifolia* leaves to be potent against adults of three different mosquito species. Mavundza et al. (2014) reported the adulticidal activities of ethanolic extract of about ten different plant species against *An. arabiensis* with the conclusion of them being effective. Also, Ileke and Ogungbite (2015) demonstrated the potency of *Alstonia boonei* in the management of adult *An. gambiae* mosquitoes.

The protectant ability of the plant extracts used in this study showed higher concentrations of the extract offered absolute protection from *An. gambiae* bites for a longer time. This increase of the plant extracts protection time at higher extract concentrations could be due to increased concentration of the active ingredient present in the extracts (Afolabi et al. 2018). *Petiveria alliacea* was observed to have better repellency efficacy as it provided absolute protection for a longer time compared to *C. volubile*. The result gotten from this study is in synchronisation with previous submissions of several authors in this area of study. Govindarajan and Sivakumar (2011) stated that different solvent extracts of *Eclipta alba* and *Andrographis paniculata* were efficacious in repelling mosquitos from the skin for a period of 150–120 min. *Tribulus terrestris* extracts have been proven to be able to repel *Ae. aegypti*, the dengue fever mosquito (El-Sheikh et al. 2016). Afolabi et al. (2018) proved that different solvent extracts of *Ocimum caninum, Ocimum gratissimum, Chromolaena odorata* and *Datura stramonium* were effective as repellents of *An. gambiae.*

The LC_{50} and LC_{90} values recorded in this study was dependent on the period of exposure. For instance, the LC_{50} and LC_{90} values for the larvicidal and pupicidal were observed to significantly decrease as the period of exposure increased.

This was in agreement with previous studies of whereby plant extracts were used to control different mosquito species (Dahchar et al. 2016; Asiry et al. 2017; Abok and Ombugadu 2018; Ullah et al. 2018). It, therefore, means that higher concentration of the tested plant extracts is required to effect desired levels of control within a short time and lower concentration for a longer period. It was also observed that the LC_{50} and LC_{90} values were lower for the larval stages as compared to pupa stages. This observation is in tandem with Ileke (2018) who reported that the LC_{50} and LC_{90} values of *Monodora myristica* and *Conyza sumatrensis* extracts against the larvae of two mosquito species to be lower than the pupae. Overall, *P. alliacea* recorded the lowest LC_{50} and LC_{90} for all the bioassays were mortality was examined in this study. In general terms, the mosquitocidal effects of the two plant extracts used in this study were concentration and time-dependent as higher mortalities were observed as the concentration and period of exposure increased. This was also the observations of Edwin et al. (2013) and Kashte et al. (2015) in similar researches.

The effectiveness exhibited by the two plants used in this study in controlling different life stages of *An. gambiae* might be linked to the phytochemicals present in them. This is so because plants that have been found out to be insecticidal are rich in phytochemicals such as alkaloids, flavonoids, steroids, tannins, terpenes and terpenoids (Alqasoumi et al. 2012; Adesina and Rajashekar 2018; Afolabi et al. 2018). The results from this current study sides with several previous studies that have highlighted the utilization of the plants with bioactive chemicals as potential, environment safe, alternatives to synthetic insecticides.

Conclusion

The results obtained from the use of ethanolic extracts of *C. volubile* and *P. alliacea* in the management and control of different life stages of *An. gambiae* proved that these plants were highly efficacious most especially *P. alliacea*. This was so because it had the lowest LC_{50} and LC_{90} values across all the various bioassays. All the life stages were susceptible to all the concentrations of the plant extracts used in this study but concentrations of 400 and 800 mg/l were more effective in controlling them. Hence, they can be used to replace chemical insecticides as a way of curbing and reducing the hazardous effects associated with their use.

Acknowledgements We appreciate the Plant Taxonomist, Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Ondo State for the authentication of the plants.The authors also appreciate Mr. Akeju Adebayo Victor and Mr. Akintan Michael Olanrewaju for their various contributions to the success of this research work.

Compliance with ethical standards

Ethical approval The ethical permit for the animal assay used in this study was granted by the animal ethical review committee, Environmental Biology and Public Health Unit, Department of Biology, Federal University of Technology, Akure, Ondo State of Nigeria.

Conflict of interest The authors declare that they have no conflict of interest.

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