Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 12(3): 1423-1436, June 2018

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Mosquito oviposition-deterrent and ovicidal property of fractions and essential oils from *Plectranthus glandulosus* and *Callistemon rigidus* against *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*

Simon Pierre Yinyang DANGA[1*], Elias Nchiwan NUKENINE[1], Aurore Christelle SENGUE BATTI[2], Lame YOUNOUSSA[1], Ezeike Amarachi KEZIAH[3] and Charles Okechukwu ESIMONE[3]

[1] *Department of Biological Sciences, Faculty of Science, University of Ngaoundere, PO Box 454, Ngaoundere, Cameroon.*

[2] *Department of Animal Biology and Physiology, Faculty of Science, P.O. Box 812, University of Yaounde 1, Cameroon.*

[3] *Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria.*

[*]*Corresponding author, E-mail: dangayisipi@yahoo.fr, Tel: +237 651 55 24 69*

ABSTRACT

Due to ever-growing insecticide resistance in mosquito vectors and environmental contamination by synthetic insecticides, plants may be a source of alternative agents for mosquito control. Therefore, the aim of this study was to investigate the mosquito oviposition-deterrent and ovicidal activities of different solvent fractions and essential oils from *Plectranthus glandulosus* and *Callistemon rigidus* against three mosquito species, *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*. For oviposition deterrence, 150 gravid females of each mosquito species were introduced in each cage. For ovicidal bioassay, 100 eggs and 1 raft of at least 100 eggs were exposed to 500 and 1000 ppm for fractions, 150 and 300 ppm for essential oils, 1000 ppm for WARRIOR®, and were assayed in the laboratory conditions. The same concentrations were used for oviposition deterrence. The oviposition deterrence was observed 72 h and the ovicidal activity 4 h post-treatment. *P. glandulosus* essential oil and hexane fraction utterly prevented gravid mosquitoes from laying eggs/rafts on oviposition traps as in WARRIOR®. There was relatively no mosquito eggs/rafts deposited in chloroform fraction of *C. rigidus* as observed in WARRIOR®. The hatching rates of *P. glandulosus* hexane fraction were reduced to 9.67, 15.33 and 28.33% against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively at 1000 ppm; 5 and 11.33%, respectively for the essential oil at 300 ppm with no egg hatched in *An. gambiae* treatments. Hexane fraction of *C. rigidus* recorded 14.33, 25.33 and 39.00% of hatching eggs against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively at 1000 ppm. These results revealed that *P. glandulosus* and *C. rigidus* served as potent oviposition deterrents and ovicides against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*.

© 2018 International Formulae Group. All rights reserved.

Keywords: *Plectranthus glandulosus*, *Callistemon rigidus*, Mosquito, Oviposition, Ovicides.

© 2018 International Formulae Group. All rights reserved.

DOI: https://dx.doi.org/10.4314/ijbcs.v12i3.28

INTRODUCTION

Mosquito vectors in Cameroon transmit serious human diseases such as malaria, yellow fever, dengue fever, chigunkunya, lymphatic filariasis or elephantiasis, causing many severe cases or deaths (WHO, 2012a, 2012b). Of the numerous mosquito species in Cameroon and other countries, *Anopheles gambiae* Giles 1902 is the major vector of malaria (WHO, 2012a). *Aedes aegypti* Linn. 1762 is the principal transmitter of yellow fever, dengue fever, dengue haemorrhagic fever, Chikungunya fever (WHO, 2012b). *Culex quinquefasciatus* Say 1823 is a vector of *Wuchereria bancrofti, which is* responsible for filariasis (WHO, 2013). Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito borne diseases (WHO, 2012a). Control of the mosquitoes is frequently dependent on continued applications of organophosphates (chlorpyrifos, temephos, and fenthion) (Yang et al., 2002). The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of mosquito species, environmental pollution andtoxic hazards to humans and other non-target organisms (Baluselvakumar et al., 2012). To alleviate these problems, major emphasis has been set on the use of natural plant-based products as oviposition deterrents, ovicides, larvicides, pupicides and adulticides which can provide an alternate to synthetic insecticides (Govindarajan et al., 2011).

Plectranthus glandulosus Hook f is an herb belonging to the Lamiaceae family. It is a plant whose leaves are commonly used to protect stored grains in Cameroon against insect infestation (Nukenine et al., 2013). Still in Cameroon, leaf essential oil extracted from the plant has been found to be effective against 4th instar larvae and early pupae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* (Danga et al., 2014a). The qualitative phytochemical analysis of the plant leaf revealed the presence of alkaloids, terpenoids, steroids, saponins, lipids, fats, fixed oils, tannins and phenolic compounds, and the fractions of the same plant leaf showed strong larvicidal activity against 4th instar larvae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* (Danga et al., 2014b).

Callistemon rigidus is a stiff and upright shrub characterized by red flower spikes that are shaped like bottlebrushes (Danga et al., 2014c). In Cameroon, *C. rigidus* has been found to be rich in phytochemicals such as alkaloids, steroids, saponins, lipids, fats, fixed oils, terpenoids, tannins and phenolic compounds (Danga et al., 2014c). The fractions of the plant leaf appeared to be toxic against 4th instar larvae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* (Danga et al., 2014c). Leaf essential oil extracted from the same plant showed strong mosquito larvicidal and pupicidal activities against 4th instar larvae and early pupae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* (Danga et al., 2014a).

One of the successful strategies for mosquito control is focused on targeting breeding sites of mosquitoes for regulation of their population density (Siriporn and Mayura, 2012). Oviposition is one of the most important events in the life cycle of mosquitoes. If oviposition is prevented, the mosquito's life cycle is disrupted and eggs are not laid. In the case of oviposition behaviour interrupted by oviposition repellents, or the immediate lack of a suitable aquatic site (medium) for egg laying, the gravid female would be forced to retain mature eggs (Xue et al., 2005). The fecundity and fertility of gravid female *Ae. albopictus* have been affected by the time duration of forced egg-retention which affected vitellogenesis (Xue et al., 2005). In addition, it has been proved that ovicides penetrate through the egg shell and act on the embryo inside (Elumalai et al., 2004). Therefore, the aim of this study was to investigate the mosquito oviposition-deterrent and ovicidal activities of different solvent leaf

extract fractions and essential oils of *P. glandulosus* and *C. rigidus* against three vector mosquitoes, namely *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*. The most effective extracts could be used in stagnant water bodies including containers which are known to be the breeding grounds for mosquitoes in Cameroon.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *Plectranthus glandulosus* and *Callistemon rigidus* were collected in October 2011 (8:00 am-12:00 pm Cameroon time, GMT + 1) at Ngaoundere (latitude 7° 22' North and longitude 13° 34' East, altitude of 1100 masl), located in the Adamawa region (plateau), Cameroon. The identities of the plants were confirmed at the National Herbarium of Cameroon, Yaounde, where specimens were deposited with the following voucher number: 18564/SRF/CAM and 41168HCN for *C. rigidus* and *P. glandulosus*, respectively. The leaves were dried in a room with temperature of 25±3 °C and relative humidity of 81±2%, and then ground to powdered form using a Mini Electric grinding mill, manufactured by Alvan Blanch, Chelworth Malmesbury Wiltshire (England) until the particles passed through a 0.4 mm-mesh sieve. The powder was stored in opaque containers inside a refrigerator at -4 °C and transported by road in February 2012 to the Faculty of Pharmaceutical Sciences (located in the city of Agulu), Nnamdi Azikiwe University, Awka; Anambra state, Nigeria, where they were stored in a refrigerator at -4 °C until needed for extraction.

Preparation of plant extracts and fractions

The extraction scheme was performed according to the method adopted by Okoye and Osadede (Okoye and Osadede, 2009). From the collection of plant material powder, 700 g were extracted for 3 days by cold maceration in methanol, stirring it thrice every day (morning, noon and afternoon) in the laboratory of Pharmaceutical and Medicinal Chemistry. The maceration process was then repeated thrice for maximum extraction. The methanol crude extract was then collected and concentrated almost to dryness under vacuum at 40 ^{o}C using rotary evaporator RE300 (ROTAFLO, England). The methanol crude extract was first absorbed on silica gel (60-200 mesh size) and sequentially fractionated using hexane, chloroform, ethyl acetate and methanol in increasing order of polarity. All the fractions so obtained were filtered many times adding fresh solvent until clear phase was obtained before passing to the next solvent using Whatman No. 1 filter paper (size: 24 cm, England). The same rotary evaporator was used to concentrate the fractions at 40 ± 5 ^{o}C. The yields of the fractions are summarized in Table 1. The fractions were stored in the refrigerator (-4 °C) until needed for bioassay.

Extraction of essential oils

The method adopted by Diksha et al. (2012) was used for extraction and isolation of the essential oils in the laboratory of Pharmaceutical and Medicinal Chemistry. The fresh dried powdered leaves were completely immersed in distilled water, then hydro-distilled in a full glass Clevenger-type apparatus (India) to give a reddish-yellow oil for *P. glandulosus* and a white-clear oil for *C. rigidus*. The oil was allowed to stand for 2 h, to be clear, and then it was collected carefully after draining out condensed water. The essential oil samples were tightly kept in bottles, rolled with aluminium foil to avoid light and stored in the refrigerator at -4 °C until need for bioassay. The yields are presented in Table 1.

Source and maintenance of larvae

The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were collected from laboratory cultures format the WHO/ National Arbovirus and Vector Research Centre Enugu, Enugu state, Nigeria. The larvae of *An. gambiae* were collected from Awka market,

Anambra State, Nigeria inside the gutter and identified at the WHO/National Arbovirus Research Centre, Enugu, Enugu State, Nigeria and reared in the laboratory. Water used for rearing was continually drawn from Agulu Lake for *An. gambiae* and tap water for *Ae. aegypti* and *Cx. quinquefasciatus*. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were fed with chicken feed (grower) mixed with fish feed in 3:1 ratio. Ground chicken feed (grower), yeast and fish feed in 3:1:2 ratios were floated on the water surface for *An. gambiae* feeding. On every alternate day, the water from the culture bowl was changed carefully until pupation.

Maintenance of pupae and adults

The pupae were picked up with a plastic dropper from culture bowls and were transferred to glass beakers containing 500 ml of water. The glass beakers containing pupae were then kept in mosquito cages of 40 cm long, 40 cm wide and 40 cm height for adult emergence. The freshly emerged adults were maintained at 29±3 °C, 82±4% r.h. and 12:12 h light:dark photoperiod cycles. The adults were fed on 10% sugar solution.

Blood feeding of adult female mosquitoes and egg laying

Four days after emergence, the female mosquitoes were offered a Guinea pig (*Cavia porcellus* Wagner, 1976) shaved on the dorsal side using scissors and the shaved part was placed on top of a cage at day time for *Ae. aegypti*. For *An. gambiae* and *Cx. quinquefasciatus*, a one month old Chicken was tied and kept inside cage after removing the feathers, from 6 pm to 6 am Nigerian time (GMT + 1), two times successively to make sure that the female mosquitoes have sucked enough blood to properly mature the eggs. This study was given an ethical approval from Anambra State University Teaching Hospital, Amaku, Awka; Anambra State, Nigeria Ethics Review Committee with the reference number of ANSUTH/AA/ECC/36. From day 2 after the blood meal, ovitraps were placed in the adult-holding cages for the collection of eggs. A plastic container of 15 cm diameter and 10 cm depth was used for oviposition. A layer of cotton balls was placed on the bottom, covered with a sheet of filter paper then flooded with water so that the water surface barely covered the filter paper for *An. gambiae* (Eugene, 1979). For *Cx. quinquefasciatus*, the plastic container almost filled with tap water was set inside the cage to lay their egg rafts (Eugene, 1979). For *Ae. aegypti*, the filter paper was lined in the interior of the plastic container and filled with tap water to cover approximately 1/3 of the paper (Eugene, 1979).

Mosquito oviposition deterrence

The oviposition deterrent test of leaf methanol crude extracts, fractions and essential oils of *P. glandulosus* and *C. rigidus* was performed using the method of Kumar et al. (2012) with slight modifications. One hundred and fifty (150) gravid female mosquitoes *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* (7-10 days old) were collected from stock cages and transferred to each mosquito cage (40 cm long, 40 cm wide and 40 cm high). Mosquitoes in each cage continued to be fed on 10% sugar solution and were held for two days after blood feeding. Two concentrations (1000 ppm and 500 ppm) for methanol crude extract and fractions, (300 ppm and 150 ppm) for essential oil of each plant, and 1000 ppm for WARRIOR® (100% DDVP: 2,2-dichlorovinyl dimethyl phosphate) were made using 1 ml of pure Tween 80 (Polyoxyethylene Sorbitan Monooleate) as emulsifier to facilitate the mixing of material in water. Four replicates for each concentration were set, with 2 cages placed side by side for each bioassay. Two replicates were performed in each cage [(4 treated + 1 control) × 2 extracts] as presented in Figure 1. Separate beakers (250 ml capacity) containing 100 ml of tap or lake waters and treated with the different products were placed in each cage; eight treated with the test material and two with Tween 80 (1 ml) (as negative control). Each beaker was fitted inside with a

white filter-paper to provide a support for oviposition. The paper was located in each beaker such that the lower half of the paper was submerged in water to moisten it by capillary action for *Ae. aegypti* (Xue et al., 2005). A layer of cotton balls was placed on the bottom of each beaker, covered with a sheet of filter paper, and then flooded with water so that the water surface barely covered the filter paper for *An. gambiae* and *Cx. quinquefasciatus* (Eugene, 1979). The oviposition beakers were introduced in the cage for oviposition at 6 pm Nigeria time. The placement of the beakers within the cages was randomized on day 1 and the position of each beaker was switched over each day for 3 days, to nullify the effect of position. The females were blood-fed again a week later, used in a second oviposition bioassay and then discarded. All experiments were run at ambient temperature of 29 ± 3 °C, relative humidity of 75-85% and photoperiod of 12:12 h light:dark cycles. After 72 h, the number of eggs laid for *Ae. aegypti* and *An. gambiae* and egg rafts for *Cx. quinquefasciatus* in treated and control beakers were counted under a light microscope, after removal of the oviposition paper. The eggs were dried at room temperature before egg-counting for *Ae. aegypti*. The oviposition experiments were expressed as mean number of eggs and the Oviposition Activity Index (OAI) was calculated using the following formula (Siriporn and Mayura, 2012):

$$OAI = \frac{NT - NC}{NT + NC}$$

where NT is the total number of eggs/rafts in the test solution, NC is the total number of eggs/rafts in the control solution and the OAI ranges from -1 to +1, with 0 indicating neutral response. The positive index values indicate that more eggs/rafts were deposited in the test beakers than in the control ones, and that the test solutions were attractive. Conversely, more eggs/rafts in the control beakers than in the test ones resulted in negative index values and the test solutions were deterrent. The percent effective repellency (ER%) for each extract, fraction and essential oil was calculated in the case of the test solution as a deterrent using the following formula (Siriporn and Mayura, 2012):

$$ER\% = \frac{NC\text{-}NT}{NC} \times 100$$

Mosquito ovicidal bioassay

The method of Siriporn and Mayura (2012) was performed for ovicidal activity. One hundred (100) eggs of *Ae. aegypti* (3-7 days), *An. gambiae* (24 h) and one egg raft containing a minimum of 100 eggs of *Cx. quinquefasciatus* (24 h) (Samidurai, 2012) were treated topically with methanol crude extract, fractions and essential oil of each plant material. The same concentrations were used as in oviposition-deterrent for each treatment. Tween 80 was used as emulsifier to facilitate the dissolving of material in water. After 4 h of treatment, the eggs/rafts were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with distilled water for hatching assessment after counting the eggs under the same microscope used in oviposition deterrence. In these assays, each test comprising of four replicates and negative controls (distilled water + 1 ml of Tween 80) were carried out in parallel for comparison. The hatched larvae were counted 120 h post treatment and the percentage of hatchability was calculated by the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs in treated water}} \times 100$$

Statistical analysis

Data on the number of eggs laid, oviposition activity index, percentage of effective repellency and percentage of egg hatchability were subjected to ANOVA procedure using Statistical Package for Social Sciences (SPSS 17.0). The Student-Newman-Keuls (SNK) test at $p \leq 0.05$ was used for mean separation.

RESULTS

Oviposition deterrence

The oviposition deterrence of *P. glandulosus* crude extract, fractions and

essential oil against gravid female *Ae. aegypti* showed that all the extracts were effective at different levels (Table 2). The oviposition activity indices (OAI) for all the extracts were negative and ranged from -0.21 to -1 which meant that these extracts had oviposition deterrent activity and the more the OAI value, the grater the oviposition deterrent activity. Hexane fraction and essential oil were the most deterrent extracts and methanol fraction was the least. At the higher concentration of 1000 ppm, there were only 20 eggs deposited in beakers treated with hexane fraction as compared with 227 eggs laid in the control beakers. The essential oil utterly deterred the gravid mosquitoes from laying their eggs, regardless of concentration. As for *An. gambiae*, the results showed that all the extracts drastically reduced oviposition when compared with the control (Table 3). All the OAI values were negative from -0.22 to -1. The magnitude of oviposition deterrence was highest in essential oil. There was no egg found in the treated beakers, regardless of the concentration. The hexane fraction had the same achievement at the higher concentration of 1000 ppm. The chloroform and ethyl acetate fractions also provided satisfactory results with only 4 and 9 eggs deposited, respectively at higher concentration as compared with 164 and 128 eggs deposited, respectively in control. Concerning *Cx. quinquefasciatus*, results showed that in general, gravid female mosquitoes deposited more egg rafts in beakers devoid of extract (control) as compared with the treated beakers (Table 4). The OAI for all extracts were negative and ranged from -0.09 to -1. Deterrence increased from one extract to the other. The essential oil, crude extract, hexane and chloroform fractions provided 100% of effective repellency (ER). The lowest oviposition deterrence was recorded in methanol fraction with 31.44% of ER at higher concentration.

The oviposition deterrence of *C. rigidus* leaf crude extract, fractions and essential oil against *Ae. aegypti* showed that only the higher concentration of the crude extract, hexane and chloroform fractions presented oviposition effect with 77.9, 78.87 and 96.63% of ER, respectively (Table 5). The OAI for these extracts were negative and ranged from -0.37 to -0.94. Against gravid female *An. gambiae*, the highest oviposition repellency activity of 83.31, 80.03 and 100% was recorded in crude extract, hexane and chloroform fractions, respectively at the lower concentration of 500 ppm (Table 6). Against gravid females of *Cx. quinquefasciatus*, the highest oviposition repellent activity of 100% was noted in hexane and chloroform fractions, regardless of the concentration (Table 7). The methanol crude extract also registered a significant deterrence of 81.94% at the higher concentration. For the three target mosquito species, no egg was deposited in the beakers treated with WARRIOR®.

Ovicidal activity

The percentage of egg hatchability from the three mosquito species treated with the methanol crude extract, fractions and essential oil of *P. glandulosus* varied from one mosquito species to the other and from an extract to the other (Table 8). The eggs of *An. gambiae*, which recorded the lowest hatching rate, tended to be more susceptible to all the plant products, than the two other mosquito species. The highest ovicidal activity was observed in hexane fraction and essential oil. The hatching rates at 1000 ppm of hexane fraction were reduced to 9.67, 15.33 and 28.33% against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. With the essential oil at 300 ppm, the eggs hatching rate was 5 and 11.33% against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively, and no egg hatched in *An. gambiae* treatments.

The ovicidal activity of *C. rigidus* showed that *An. gambiae* still recorded the lowest hatching rate compared to the two other mosquito species. The hexane and chloroform fractions were the most effective products (Table 9). At 1000 ppm, hexane fraction showed strong ovicidal activity with only 14.33, 25.33 and 39.00% of eggs hatching against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. No larva hatched in beakers treated with WARRIOR®, irrespective of the mosquito species.

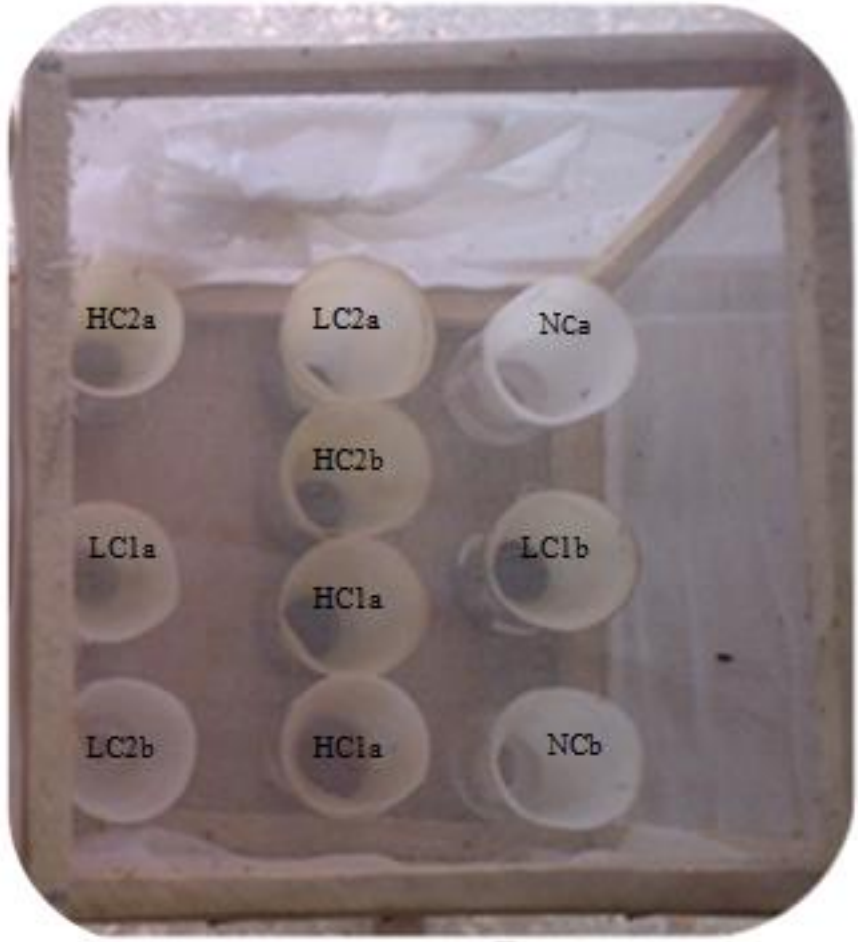

Figure 1: Oviposition deterrent bioassay with a: extract a, b: extract b, LC1: 1st low concentration (conc.), LC2: 2nd low conc., HC1: 1st high conc., HC2: 2nd high conc., NC: negative control.

Table 1: Yields of *Plectranthus glandulosus* and *Callistemon rigidus* leaf extracts.

Products	**Yields (%)**	
	P. glandulosus	***C. rigidus***
Methanol crude extract	9.96	27.28
Hexane fraction	10.94	8.18
Chloroform fraction	13.68	11.35
Ethyl acetate fraction	12.91	12.42
Methanol fraction	32.91	37.62
Essential oil	0.22 (w/w)	0.72 (w/w)

Table 2: Oviposition deterrence of *Plectranthus glandulosus* leaf methanol crude extract, fractions and essential oil against *Aedes aegypti* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of eggs in treated cups (Mean±SD)	Mean No. of eggs in control cups (Mean±SD)	OAI (Mean±S)	ER% (Mean±SD)
Methanol crude extract	500	111 ± 4^{de}	233 ± 12^{b}	-0.35 ± 0.04^{c}	52.28 ± 4.35^{e}
	1000	82 ± 7^{f}	237 ± 32^{b}	-0.48 ± 0.03^{d}	65.44 ± 2.23^{d}
Hexane fraction	500	33 ± 4^{g}	218 ± 7^{b}	-0.73 ± 0.04^{e}	84.65 ± 2.32^{c}
	1000	20 ± 2^{h}	227 ± 4^{b}	-0.84 ± 0.02^{f}	91.36 ± 0.93^{b}
Chloroform fraction	500	128 ± 9^{c}	206 ± 4^{b}	-0.23 ± 0.04^{b}	37.83 ± 4.79^{f}
	1000	102 ± 3^{e}	205 ± 6^{b}	-0.33 ± 0.03^{c}	50.02 ± 3.18^{e}
Ethyl acetate fraction	500	116 ± 8^{d}	226 ± 7^{b}	-0.32 ± 0.05^{c}	48.64 ± 5.41^{e}
	1000	104 ± 5^{e}	227 ± 10^{b}	-0.37 ± 0.03^{c}	54.19 ± 3.47^{e}
Methanol fraction	500	226 ± 8^{a}	239 ± 9^{b}	-0.03 ± 0.02^{a}	5.27 ± 2.37^{g}
	1000	209 ± 9^{b}	321 ± 10^{a}	-0.21 ± 0.01^{b}	34.80 ± 0.80^{f}
Essential oil	150	0 ± 0^{i}	207 ± 7^{b}	-1.00 ± 0.00^{g}	100.00 ± 0.00^{a}
	300	0 ± 0^{i}	224 ± 12^{b}	-1.00 ± 0.00^{g}	100.00 ± 0.00^{a}
WARRIOR®	1000	0 ± 0^{i}	221 ± 6^{b}	-1.00 ± 0.00^{g}	100.00 ± 0.00^{a}
F value		479.57***	17.86***	489.09***	314.15***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 3: Oviposition deterrence of *Plectranthus glandulosus* leaf methanol crude extract, fractions and essential oil against *Anopheles gambiae* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of eggs in treated cups (Mean±SD)	Mean No. of eggs in control cups (Mean±SD)	OAI (Mean±SD)	ER% (Mean±SD)
Methanol crude extract	500	77 ± 4^{b}	187 ± 6^{c}	-0.42 ± 0.04^{b}	58.57 ± 3.49^{e}
	1000	27 ± 4^{e}	193 ± 6^{c}	-0.75 ± 0.04^{d}	85.83 ± 2.40^{cd}
Hexane fraction	500	4 ± 1^{gh}	261 ± 3^{a}	-0.97 ± 0.01^{f}	98.34 ± 0.59^{a}
	1000	0 ± 0^{d}	267 ± 17^{a}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
Chloroform fraction	500	19 ± 2^{f}	166 ± 5^{d}	-0.80 ± 0.02^{d}	88.52 ± 1.31^{c}
	1000	4 ± 1^{gh}	164 ± 24^{d}	-0.96 ± 0.02^{f}	97.59 ± 0.81^{a}
Ethyl acetate fraction	500	51 ± 5^{c}	114 ± 4^{ef}	-0.38 ± 0.05^{b}	54.83 ± 4.58^{e}
	1000	9 ± 2^{g}	128 ± 5^{e}	-0.87 ± 0.03^{e}	92.95 ± 1.77^{b}
Methanol fraction	500	103 ± 5^{a}	162 ± 4^{d}	-0.22 ± 0.02^{a}	36.37 ± 2.19^{f}
	1000	33 ± 6^{d}	192 ± 8^{c}	-0.71 ± 0.06^{c}	82.70 ± 3.91^{d}
Essential oil	150	0 ± 0^{h}	118 ± 2^{ef}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
	300	0 ± 0^{h}	105 ± 6^{f}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
WARRIOR®	1000	0 ± 0^{h}	217 ± 2^{b}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
F value		292.65***	77.23***	290.71***	264.75***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 4: Oviposition deterrence of *Plectranthus glandulosus* leaf methanol crude extract, fractions and essential oil against *Culex quinquefasciatus* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of rafts in treated cups (Mean±SD)	Mean No. of rafts in control cups (Mean±SD)	OAI (Mean±SD)	ER% (Mean±SD)
Methanol crude extract	500	2 ± 1^{cd}	12 ± 2^{a}	-0.67 ± 0.18^{b}	79.29 ± 12.02^{ab}
	1000	0 ± 0^{d}	11 ± 2^{a}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
Hexane fraction	500	0 ± 0^{d}	7 ± 1^{b}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
	1000	0 ± 0^{d}	9 ± 2^{ab}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
Chloroform fraction	500	0 ± 0^{d}	9 ± 1^{ab}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
	1000	0 ± 0^{d}	10 ± 3^{ab}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
Ethyl acetate fraction	500	8 ± 2^{ab}	9 ± 1^{ab}	-0.08 ± 0.10^{a}	14.14 ± 17.23^{c}
	1000	3 ± 0^{c}	11 ± 3^{a}	-0.59 ± 0.05^{b}	74.24 ± 3.80^{b}
Methanol fraction	500	9 ± 1^{a}	10 ± 0^{ab}	-0.09 ± 0.08^{a}	16.36 ± 11.82^{c}
	1000	7 ± 1^{b}	10 ± 1^{ab}	-0.20 ± 0.16^{a}	31.44 ± 21.33^{c}
Essential oil	150	0 ± 0^{d}	9 ± 1^{ab}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
	300	0 ± 0^{d}	6 ± 1^{b}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
WARRIOR®	1000	0 ± 0^{d}	10 ± 2^{ab}	-1.00 ± 0.00^{c}	100.00 ± 0.00^{a}
F value		42.25***	540.42***	73.63***	43.10***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 5: Oviposition deterrence of *Callistemon rigidus* leaf methanol crude extract, fractions and essential oil against *Aedes aegypti* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of eggs in treated cups (Mean±SD)	Mean No. of eggs in control cups (Mean±SD)	OAI (Mean±SD)	ER% (Mean±SD)
Methanol crude extract	500	71 ± 2^{gh}	208 ± 4^{de}	-0.49 ± 0.02^{e}	66.06 ± 1.66^{c}
	1000	44 ± 5^{i}	200 ± 12^{e}	-0.64 ± 0.05^{f}	77.90 ± 3.47^{b}
Hexane fraction	500	94 ± 7^{g}	204 ± 6^{de}	-0.37 ± 0.03^{d}	54.04 ± 2.39^{d}
	1000	70 ± 1^{h}	334 ± 40^{a}	-0.65 ± 0.04^{f}	78.87 ± 3.12^{b}
Chloroform fraction	500	20 ± 2^{j}	212 ± 9^{de}	-0.83 ± 0.02^{g}	90.70 ± 1.18^{a}
	1000	10 ± 3^{jk}	299 ± 11^{b}	-0.94 ± 0.03^{h}	96.63 ± 1.13^{a}
Ethyl acetate fraction	500	296 ± 15^{c}	146 ± 4^{f}	0.34 ± 0.02^{a}	-92.48 ± 7.86^{g}
	1000	194 ± 7^{f}	185 ± 7^{e}	0.02 ± 0.01^{c}	-4.89 ± 1.01^{e}
Methanol fraction	500	254 ± 7^{d}	238 ± 4^{d}	0.03 ± 0.01^{c}	-6.85 ± 1.22^{e}
	1000	223 ± 11^{e}	207 ± 4^{de}	0.04 ± 0.04^{c}	-8.19 ± 8.16^{e}
Essential oil	150	392 ± 16^{a}	308 ± 8^{b}	0.12 ± 0.03^{b}	-27.24 ± 6.70^{f}
	300	332 ± 12^{b}	255 ± 15^{c}	0.13 ± 0.04^{b}	-30.48 ± 11.30^{f}
WARRIOR®	1000	0 ± 0^{k}	221 ± 6^{de}	-1.00 ± 0.00^{i}	100.00 ± 0.00^{a}
F value		719.56***	41.97***	802.09***	464.70***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 6: Oviposition deterrence of *Callistemon rigidus* leaf methanol crude extract, fractions and essential oil against *Anopheles gambiae* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of eggs in treated cups (Mean±SD)	Mean No. of eggs in control cups (Mean±SD)	OAI (Mean±SD)	ER% (Mean±SD)
Methanol crude extract	500	19 ± 2^{a}	114 ± 4^{h}	-0.71 ± 0.02^{f}	83.31 ± 1.18^{b}
	1000	3 ± 0^{ef}	144 ± 8^{f}	-0.96 ± 0.01^{gh}	98.13 ± 0.48^{a}
Hexane fraction	500	26 ± 3^{d}	132 ± 6^{gh}	-0.67 ± 0.05^{e}	80.03 ± 3.14^{b}
	1000	7 ± 2^{e}	172 ± 10^{c}	-0.92 ± 0.02^{g}	95.96 ± 1.02^{a}
Chloroform fraction	500	0 ± 0^{f}	136 ± 4^{g}	-1.00 ± 0.00^{h}	100.00 ± 0.00^{a}
	1000	0 ± 0^{f}	166 ± 11^{d}	-1.00 ± 0.00^{h}	100.00 ± 0.00^{a}
Ethyl acetate fraction	500	216 ± 13^{a}	121 ± 4^{gh}	0.28 ± 0.04^{a}	-78.20 ± 13.14^{f}
	1000	154 ± 7^{c}	117 ± 3^{h}	0.14 ± 0.02^{b}	-32.28 ± 5.05^{e}
Methanol fraction	500	218 ± 16^{a}	181 ± 2^{bc}	0.09 ± 0.04^{b}	-20.40 ± 8.20^{d}
	1000	152 ± 11^{c}	136 ± 2^{gh}	0.05 ± 0.04^{c}	-11.29 ± 8.61^{cd}
Essential oil	150	206 ± 5^{ab}	155 ± 7^{e}	0.14 ± 0.03^{b}	-33.23 ± 9.77^{e}
	300	194 ± 5^{b}	188 ± 6^{b}	0.02 ± 0.01^{d}	-3.39 ± 0.93^{c}
WARRIOR®	1000	0 ± 0^{f}	217 ± 2^{a}	-1.00 ± 0.00^{h}	100.00 ± 0.00^{a}
F value		514.76***	53.47***	370.93***	387.00***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 7: Oviposition deterrence of *Callistemon rigidus* leaf methanol crude extract, fractions and essential oil against *Culex quinquefasciatus* 72 h post treatment.

Products	Conc. (ppm)	Mean No. of rafts in treated cups (Mean±SD)	Mean No. of rafts in control cups (Mean±SD)	OAI (Mean±SD)	ER% (Mean±SD)
Methanol crude extract	500	4 ± 1^{e}	12 ± 1^{a}	-0.46 ± 0.17^{d}	62.00 ± 15.80^{b}
	1000	3 ± 0^{e}	15 ± 2^{a}	-0.70 ± 0.09^{e}	81.94 ± 6.22^{ab}
Hexane fraction	500	0 ± 0^{f}	8 ± 1^{ab}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
	1000	0 ± 0^{f}	11 ± 2^{a}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
Chloroform fraction	500	0 ± 0^{f}	10 ± 1^{a}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
	1000	0 ± 0^{f}	10 ± 3^{a}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
Ethyl acetate fraction	500	16 ± 1^{a}	11 ± 1^{a}	0.16 ± 0.10^{bc}	-40.58 ± 27.55^{cd}
	1000	12 ± 1^{c}	10 ± 1^{a}	0.12 ± 0.04^{bc}	-28.56 ± 9.74^{c}
Methanol fraction	500	16 ± 1^{a}	12 ± 1^{a}	0.16 ± 0.07^{bc}	-41.03 ± 20.68^{cd}
	1000	14 ± 1^{b}	12 ± 1^{a}	0.06 ± 0.02^{c}	-13.96 ± 5.08^{c}
Essential oil	150	11 ± 2^{cd}	6 ± 1^{b}	0.32 ± 0.05^{a}	-95.24 ± 21.63^{e}
	300	10 ± 0^{d}	6 ± 0^{b}	0.23 ± 0.04^{b}	-58.73 ± 13.75^{d}
WARRIOR®	1000	0 ± 0^{f}	10 ± 2^{a}	-1.00 ± 0.00^{f}	100.00 ± 0.00^{a}
F value		132.26***	563.92***	223.61***	96.83***

Means within a column followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); ***: p<0.001; OAI: Oviposition Activity Index; (+): more eggs were deposited in the test beakers than in the control beakers (attractive); (-): more eggs in the control beakers than in the test beakers (deterrent); ER%: Percent Effective Repellency; SD: Standard Deviation; Number of replicates: 4.

Table 8: Ovicidal activity of *Plectranthus glandulosus* leaf methanol crude extract, fractions and essential oil against *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* 4 h post-treatment.

Products	Conc. (ppm)	% mean ± Std. Dev. of mosquito eggs hatchability			
		Ae. aegypti	*An. gambiae*	*Cx. quinquefasciatus*	F value
Methanol crude extract	500	59.67 ± 3.06^{dA}	50.67 ± 1.53^{eB}	56.67 ± 1.53^{fA}	13.50**
	1000	28.00 ± 2.00^{gB}	23.33 ± 2.52^{hC}	38.67 ± 1.53^{iA}	43.89***
Hexane fraction	500	36.33 ± 2.52^{fB}	27.33 ± 0.58^{gC}	42.67 ± 1.53^{hA}	59.37***
	1000	15.33 ± 2.08^{hB}	9.67 ± 1.15^{iC}	28.33 ± 1.53^{jA}	103.04***
Chloroform fraction	500	51.00 ± 2.00^{eB}	44.33 ± 1.53^{fC}	56.67 ± 1.53^{fA}	39.57***
	1000	38.00 ± 1.73^{fB}	27.33 ± 1.53^{gC}	48.00 ± 1.00^{gA}	151.78***
Ethyl acetate fraction	500	88.00 ± 1.73^{bA}	74.67 ± 1.53^{cB}	89.67 ± 1.15^{cA}	91.25***
	1000	57.00 ± 2.00^{dB}	45.67 ± 2.52^{fC}	66.00 ± 2.65^{eA}	53.90***
Methanol fraction	500	90.33 ± 1.15^{bB}	83.00 ± 1.00^{bC}	95.33 ± 1.53^{bA}	74.21***
	1000	77.67 ± 2.08^{cA}	69.67 ± 1.53^{dB}	79.67 ± 1.53^{dA}	28.00**
Essential oil	150	9.00 ± 1.73^{iB}	6.00 ± 1.00^{jC}	26.33 ± 1.53^{jA}	171.21***
	300	5.00 ± 1.00^{jB}	0.00 ± 0.00^{kC}	11.33 ± 1.53^{kA}	87.10
Negative control	0	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	-
WARRIOR®	1000	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{l}	-
F value		784.96***	926.88***	977.39***	

Means within a column (comparing the different products using small lettres) and within a line (comparing the three mosquito species using capital lettres) followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); **: p<0.01; ***: p<0.001, Number of replicates: 4.

Table 9: Ovicidal activity of *Callistemon rigidus* leaf methanol crude extract, fractions and essential oil against *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* 4 h post-treatment.

Products	**Conc. (ppm)**	**% mean± Std. Dev. of mosquito eggs hatchability**			
		Ae. aegypti	***An. gambiae***	***Cx. quinquefasciatus***	**F value**
Methanol crude extract	500	86.67 ± 1.53^{cB}	75.67 ± 1.15^{cC}	90.67 ± 1.53^{cA}	90.50***
	1000	61.67 ± 1.15^{fB}	56.00 ± 1.73^{eC}	70.33 ± 1.53^{fA}	70.35***
Hexane fraction	500	39.00 ± 2.00^{iB}	19.33 ± 1.53^{iC}	50.33 ± 1.53^{iA}	255.50***
	1000	25.33 ± 2.08^{jB}	14.33 ± 1.53^{jC}	39.00 ± 1.00^{jA}	179.26***
Chloroform fraction	500	38.67 ± 1.53^{iB}	23.67 ± 1.53^{hC}	48.33 ± 1.53^{iA}	198.61***
	1000	22.00 ± 2.00^{kB}	15.33 ± 0.58^{jC}	31.33 ± 2.08^{kA}	67.02***
Ethyl acetate fraction	500	85.67 ± 1.15^{cA}	74.33 ± 1.53^{cB}	87.67 ± 1.53^{dA}	77.55***
	1000	58.67 ± 2.08^{gA}	46.67 ± 1.53^{fB}	61.33 ± 1.53^{hA}	61.03***
Methanol fraction	500	91.33 ± 1.53^{bA}	88.67 ± 1.15^{bA}	95.00 ± 2.00^{bB}	11.87**
	1000	81.00 ± 2.00^{dA}	68.67 ± 1.53^{dB}	64.33 ± 1.53^{gC}	77.65**
Essential oil	150	70.00 ± 1.00^{eB}	38.67 ± 1.15^{gC}	75.00 ± 2.00^{eA}	551.10***
	300	43.33 ± 1.53^{hB}	18.67 ± 2.51^{iC}	48.67 ± 1.53^{iA}	209.57***
Negative control	0	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	-
WARRIOR®	1000	0.00 ± 0.00^{l}	0.00 ± 0.00^{k}	0.00 ± 0.00^{l}	-
F value		952.29***	966.33***	861.15***	

Means within a column (comparing the different products using small lettres) and within a line (comparing the three mosquito species using capital lettres) followed by the same letter do not differ significantly (P≥0.05) (Student-Newman-Keuls's test); **: p<0.01; ***: p<0.001; Number of replicates: 4.

DISCUSSION

This study has revealed that the leaves of *P. glandulosus* and *C. rigidus* showed both oviposition-deterrent and ovicidal activity towards the three target mosquito species. In the search of what might be responsible for the oviposition-deterrent and ovicidal activity, the following nine chemical classes: alkaloids, terpenoids, phenolic compounds (tannins), steroids, saponins and fixed oils were found in the leaves of *P. glandulosus* and those of *C. rigidus* in a previous study. All the above-mentioned nine chemical classes were found in the methanol crude extracts of the two plant species. For the two plant species, the chemical classes were similar for hexane (terpenoids, steroids and fixed oils), ethyl acetate (tannins) and methanol (alkaloids, tannins and saponins) fractions. Chloroform fraction of *P. glandulosus* contained only steroids and that of *C. rigidus*, terpenoids (Danga et al., 2014c, 2014c). The above activities of both plants may be due to the action of those phytochemicals which, not only prevented the three mosquito species from laying their eggs, but also the laid eggs from hatching to the larval stage. The results of the present study corroborate the findings obtained by Siriporn and Mayura (2012), where the essential oils of six plants showed significant oviposition-deterrent and ovicidal activity against *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*, respectively. The results of our study are also consistent with the findings of Reegan et al. (2015) who demonstrated the oviposition deterrent and ovicidal activities of five medicinal plant extracts namely *Aegle marmelos* (Linn.), *Limonia acidissima* (Linn.), *Sphaeranthus indicus* (Linn.), *Sphaeranthus amaranthoides* (burm.f), and *Chromolaena odorata* (Linn.) against *Cx. quinquefasciatus* and *Ae. aegypti*. Among the different extracts of the five plants screened, the hexane extract of *L. acidissima* recorded the highest ovicidal activity of 79.2% and 60% at 500 ppm concentration against the eggs of *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. Similarly, the same hexane extract of *L. acidissima* showed 100% oviposition deterrent activity at all the tested concentrations against *Cx. quinquefasciatus* and *Ae. aegypti* adult females. In addition, the water-soluble *Moringa oleifera* lectin has been found very effective against oviposition and egg hatching of *A. aegypti* (Santos et al., 2012). Moreover, the ovicidal and oviposition response of ten plant volatile oils were evaluated against *Cx. quinquefasciatus*. Among the ten oils, clove, aniseed and cinnamon oils registered the highest (100%) ovicidal activity at 200 ppm. Lemon (97.5%) and tulsi (91.2%) oils were also highly effective against *C. quinquefasciatus* eggs at 200 ppm. Maximum oviposition response activity (100%) was obtained in clove oil (Ramar et al., 2014). Furthermore, Mario et al. (2009) stated that the selection of sites for oviposition is a critical factor for the survival and population dynamics of the species. It is initiated with the reception of environmental (visual, tactile and olfactory) stimuli, which may either attract or repel the insect, limiting the possibilities of finding oviposition sites. This suggests that one or more phytochemicals present in both plants may have repelled the vectors not to lay eggs. Elumalai et al. (2004) revealed that the efficacy to act on the embryo inside the egg shell depends on an efficient penetration of the insecticide. Xue et al. (2005) and Santos et al. (2012) reported more entry of the chemical inside the egg shell, when eggs were directly exposed to higher concentrations of the compounds, which affect the embryogenesis. In this light, one or more phytochemicals, individually or synergistically, may have penetrated the egg shell of the mosquitoes in the present study, killing the embryo in the egg.

Conclusion

In conclusion, *P. glandulosus* and *C. rigidus* leaf extracts showed high oviposition-deterrent and ovicidal activity against three mosquito species, *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*. These results could

encourage the search for new active natural compounds offering an alternative to synthetic ovicidal and oviposition-deterrent, from these two plant species. The findings of this study could also be useful in the integrated approach to mosquito control programmes against container-inhabiting mosquitoes.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

COE and ENN conceived the idea and designed the experiments. SPYD, EAK and LY conducted the experiments. SPYD analysed the data and wrote the draft manuscript. All the authors read and corrected the manuscript.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the equipment facilities provided by the Faculty of Pharmaceutical Sciences of the Nnamdi Azikiwe University, Awka; Anambra State, Nigeria. Sincere thanks are due to the staff members of the same institution for their cooperation. We are also thankful to the WHO/National Arbovirus and Vector Research Centre Enugu, Enugu state, Nigeria for the provision of mosquito species used in this project.

REFERENCES

Baluselvakumar, Gokulakrishnan J, Elumalai K, Dhanasekaran S, Anandan A, Krishnappa K. 2012. Mosquito ovicidal and repellent activity of *Melothria maderaspatana* plant leaf extracts against *Aedes aegypti* (Diptera: Culicidae). *Inter. J. Recent Sci. Res*., **3**(5): 325-328.

Danga YSP, Esimone CO, Nukenine EN. 2014c. Larvicidal and phytochemical properties of *Callistemon rigidus* R. Br. (Myrtaceae) leaf solvent extracts against three vector mosquitoes. *J. Vector Borne Dis*., **51**: 216-223.

Danga YSP, Esimone CO, Younoussa L, Nukenine EN. 2014a. Larvicidal and pupicidal activities of *Plectranthus glandulosus* and *Callistemon rigidus* leaf essential oils against three mosquito species. *J. Mosq. Res*., **4**(2): 5-14. DOI: 10.5376/jmr.2014.04.0002

Danga YSP, Nukenine EN, Younoussa L, Esimone CO. 2014b. Phytochemicals and larvicidal activity of *Plectranthus glandulosus* (Lamiaceae) leaf extracts against *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *J. Pure Appl. Zool*., **2**(2): 160-171.

Diksha, Navdeep KG, Rajesh K, Dhar KL. 2012. Study on contents and chemical constituents of the essential oil of the leaves of *Eucalyptus globules* Labill. Inter. *J. Universal Pharm. Life Sci*., **2**(4): 264-269.

Elumalai K, Jeyasankar A, Raja N, Ignacimuthu S. 2004. Ovicidal and larvicidal activity of certain plant extracts against the tobacco armyworm, *Spodoptera litura* (Fab.). *J. Curr. Sci*., **5**(1): 291-294.

Eugene JG. 1979. Manual for mosquito rearing and experimental techniques. In: Am Mosquito Control Association, Inc. Bulletin No. 5; 27-78.

Govindarajan, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. 2011. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed*., **1**: 43-48. DOI: 10.1016/S2221-1691(11)60066-X

Kumar SV, Mani P, Bastin TMMJ, Ravikumar G. 2012. Mosquito larvicidal, oviposition deterrent and repellent properties of *Acalypha indica* L. extracts against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. *Inter. J. Med. Biosci*., **1**(3): 33-41.

Mario AN-S, Marques FA, Duque EJL. 2009. Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae. *Rev. Bras. Entomol*., **53**(1): 1-6.

Nukenine EN, Chouka FP, Vabi MB, Reichmuth C, Adler C. 2013. Comparative toxicity of four local botanical powders to *Sitophilus zeamais* and influence of drying regime and particle size on insecticidal efficacy. *Int. J. Biol. Chem. Sci*., **7**(3): 1313-1325. DOI: http://dx.doi.org/10.4314/ijbcs.v7i3.36

Okoye BCF, Osadede OP. 2009. Study on the mechanism of anti-inflammatory activity of the extracts and fractions of *Alchornea floribunda* leaves. *Asian Pac. J. Trop. Med*., **2**(3): 7-14.

Ramar M, Ignacimuthu S, Gabriel Paulraj M. 2014. Ovicidal and oviposition response activities of plant volatile oils against *Culex quinquefasciatus* say. *J. Entomol. Zool. Studies.*, **2**(4): 82-86.

Reegan AD, Gandhi MR, Paulraj MG, Ignacimuthu S. 2015. Ovicidal and oviposition deterrent activities of medicinal plant extracts against *Aedes aegypti* L. and *Culex quinquefasciatus* Say Mosquitoes (Diptera: Culicidae). *Osong. Public. Health. Res. Perspect*., **6**(1): 64-69. DOI: http://dx.doi.org/10.1016/j.phrp.2014.08.009

Samidurai K. 2012. Mosquito larvicidal and ovicidal properties of *Pemphis acidula* Frost. (Lythraceae) against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed*., **2**(3): S1862-S1866. DOI: 10.1016/S2221-1691(12)60509-7

Santos NDdL, de Moura KS, Napoleão TH, Santos GKN, Coelho LCBB, Navarro DMdAF, Paiva PMG. 2012. Oviposition-stimulant and ovicidal activities of *Moringa oleifera* Lectin on *Aedes aegypti*. *PLoS ONE*, **7**(9): e44840. DOI:10.1371/journal.pone.0044840

Siriporn P, Mayura S. 2012. The effects of herbal essential oils on the oviposition deterrent and ovicidal activities of *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say). *Trop. Biomed*., **29**(1): 138-150.

WHO. 2012a. World malaria report. World Health Organization global malaria program, p. 276.

WHO. 2012b. World health statistics 2012. World Health Organization Library Cataloguing-in-Publication Data, p. 180.

WHO. 2013. Lymphatic filariasis: A handbook for national elimination programmes. WHO/HTM/NTD/PCT/2013.10, p. 107.

Xue R-D, Arshad A, Barnard DR. 2005. Effects of forced egg-retention in *Aedes albopictus* on adult survival and reproduction following application of DEET as an oviposition deterrent. *J. Vector Ecol*., **30**(1): 45-48.

Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS. 2002. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agr. Food. Chem*., **50**: 3765-3767. DOI: 10.1021/jf011708f