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Research Article

PHYTOCHEMICALS AND LARVICIDAL ACTIVITY OF *PLECTRANTHUS GLANDULOSUS* (LAMIACEAE) LEAF EXTRACTS AGAINST *ANOPHELES GAMBIAE*, *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquitoes transmit diseases, causing millions of human deaths every year. Due to the huge consequences of synthetic insecticides, plants may be alternative sources of mosquito control agents. The present study assessed the phytochemicals and larvicidal activity of different solvent leaf extracts of *Plectranthus glandulosus* against three major vector mosquitoes, *viz*. *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*. Twenty five early fourth instar larvae of each mosquito species were exposed to various concentrations ranging from 125-1000 ppm of methanol crude extract (MCE), hexane (HF), chloroform (CF), ethyl acetate (EAF) and methanol fractions (MF), from 250-2000 ppm of water extract (AE) and 2000 ppm of DDVP. The WHO standard protocol was followed. The larval mortality was observed 24 h post-exposure. The LC_{50} and LC_{90} values were determined by probit analysis. The qualitative phytochemical analysis revealed the presence of Alkaloids, Terpenoids, Steroids, Saponins, Tannins and Phenolic Compounds, Lipids, Fats and Fixed Oils. Among all solvent extracts and fractions tested, the maximum efficacy was observed in HF against all target mosquito species with LC_{50} values of 17.11, 89.08 and 610.40 ppm against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. MCE, CF and EAF against *An. gambiae* were also effective with LC_{50} values of 167.85, 201.50 and 76.21 ppm, respectively. The results of the leaf extracts and fractions of *P. glandulosus* are promising as good mosquito larvicides against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*. This is a new eco-friendly approach that may replace the chemical DDVP for vector control programs.

Keywords: *Plectranthus glandulosus*, Mosquito Larvicides, Fractions, Phytochemicals.

INTRODUCTION

Mosquitoes are the important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of death every year (Jeyasankar *et al*., 2012). These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as Democratic Republic of Congo, Nigeria, Cameroon etc. (WHO, 2012a). About half of the world's population is at risk of malaria, and an estimated 216 million cases in 2010 led to approximately 655 000 deaths; 86% of these were children under the age of five (WHO, 2012b). Cameroon reported a high (>70%) percentage of the population potentially covered by ITNs delivered in 2011 but did not report a decrease in malaria admissions and deaths (WHO, 2012b). In 2013, nearly 800 people died in a malaria outbreak in northern Cameroon, one described by public health officials as "a severe and sudden epidemic". More than 12,000 cases of the disease admitted in hospital in three weeks were mostly young children and pregnant women (CNN, 2013). The only *Plasmodium* species was *P. falciparum* (100%) and the major *Anopheles* species was *An. gambiae* (WHO, 2012b).

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For epidemiological and historical reasons, Cameroon is among the twelve most highly endemic countries for Yellow Fever (YF) in Africa (WHO, 2008). During the last two decades, two important YF outbreaks were reported in the Cameroon: in 1990 (180 cases, 125 deaths) and 1994 (10 cases, 5 deaths) in the Far-North and the Adamaoua Regions, respectively. In 2009, a preventive mass campaign vaccination was conducted targeting 7 469 615 people in 62 health districts. Despite this preventive campaign, isolated cases of YF were reported in certain districts which were not considered at risk of YF (Demanou *et al*., 2012). According to WHO (2012a), Cameroon occupied the sixth rank all over the world in YF cases. An outbreak of YF has been reported in the North Region of Cameroon. The first case occurred on 8 October 2011 in Baboudji (Bibemi Health District) and the last case was registered on 9 January 2012 in Guider. A total of 24 cases with 7 deaths were reported in Guider (3 cases), Bibemi (1), Ngong (1), Mayo Oulo (1) and Golombe (1) districts. 18 of these cases were identified as part of the surveillance system, with fever and jaundice within 14 days of onset (Demanou *et al*., 2012). Moreover, cases of Chikungunya Virus were detected among the populations of Douala and Yaoundé in Cameroon in 2006 (Peyrefitte *et al*., 2006).

Over and injudicious use of synthetic insecticides in vector control to prevent these diseases has resulted in environmental hazards through persistence and accumulation of non biodegradable toxic components in the ecosystem, development of insecticide resistance among mosquito species, biological magnification in the food chain and toxic effects on human health and non-target organisms (Francisco *et al*., 2002; Devine and Furlong, 2007).

With these problems in focus, it becomes increasingly necessary to search for an alternative in the development of environmentally safe, biodegradable, low cost, target specific insecticide for mosquito control which can be used with minimum care by individuals and communities (Dua *et al*., 2010). Plants may be a source of alternative agents to replace the synthetic insecticides for mosquito control (Bashir and Javid, 2013).

Roark (1947) described approximately 1 200 plant species having potential insecticidal value, while Sukumar *et al*. (1991) listed and discussed 344 plant species that only exhibited mosquitocidal activity. Essam *et al*. (2005) summarized the mosquitocidal activities of various herbal products from edible crops, ornamental plants, trees, shrubs, herbs, grasses and marine plants according to the extraction procedure developed in eleven different solvent systems and the nature of mosquitocidal activities against different life stages of different vector species as a ready reference for further studies.

Plectranthus glandulosus Hook f is one of 18 species of *Plectranthus* genus which are indexed in the world (Abdel-Mogib *et al.*, 2002). It belongs to the Lamiaceae family and whose species are adapted to nearly all types of areas and altitudes (Abdel-Mogib *et al.*, 2002). It is sometimes considered as synonym of *Coleus laxiflorus* (Benth.) Roberty (Ngassoum *et al*., 2001). This herb is found in West African flora (Ngassoum *et al*., 2001) and also in Cameroonian flora (Nukenine *et al.*, 2003). Still in Cameroon, it is known as medicinal plant used against infuenza, cough and chest complaints (Oliver-Bever, 1982). It is a plant whose leaves are commonly used to protect stored grains and flour (Nukenine *et al*., 2011, 2013; Goudoum *et al*., 2013), as mosquito repellent and anthelminthic (Nukenine *et al*., 2003).

Mosquitoes in the larval stage are striking targets for pesticides because they rear in water and therefore very easy to handle in this atmosphere (Nandita *et al*., 2008). The present study was carried out to report on the phytochemical composition and to determine the larvicidal activity of *P. glandulosus* leaf extracts against three medically important mosquito species, namely *Anopheles gambiae* (*An. gambiae*), the ugliest mosquito species, *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). Throughout our online search, this was the first time this investigation was undertaken for toxicity of different solvent extracts from *P. glandulosus* leaves against these vector mosquitoes.

MATERIALS AND METHODS

Plant collection

The fresh leaves of *P. glandulosus* were collected in October 2011 (6:00 am-11:00 am GMT) in Ngaoundere (latitude 7° 22' North and longitude 13° 34' East, altitude of 1100 masl),

located in the Adamawa region (plateau), Cameroon. Ngaoundere is located in the Sudano-Guinean agro-ecological zone. The Sudano-Guinean agro-ecological zone is characterized by two seasons: a dry season from November to March and a rainy season spanning April to October. Average annual temperature is 22°C with a maximum of 34°C in March and a minimum of 12°C in December or January. Annual precipitation is 1595 mm (Nukenine *et al*., 2013). The plants were less than one-year old and only the green leaves were harvested. The identity of the plant was confirmed at the National Herbarium of Cameroon in Yaounde, where voucher specimens were deposited with the following voucher number: 41168HCN. The leaves were dried at ambient temperature of 25±3 °C and 81±2 % of relative humidity in a room, and then ground to powder using an electric grinder until the powder 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 Faculty of Pharmaceutical Sciences, Agulu; Nnamdi Azikiwe University, Awka; Anambra state, Nigeria where the experiments were carried out and then stored in a freezer at -14 °C until needed.

Preparation of the plant extracts and fractions

The extraction procedure was performed based on Okoye and Osadede (2009) method. From the collection of plant material powder, 700g were extracted for 3 days by cold maceration in methanol shaking it thrice per day (morning, noon and afternoon) in the laboratory of Pharmaceutical and Medicinal Chemistry. The maceration process was then repeated thrice for maximal extraction. The suspension was filtered through Whatman® No 1 filter paper (size: 24 cm, England) using a Buchner funnel. The methanol crude extract (9.96%) was concentrated to dryness in a rotary vacuum evaporator RE300 (ROTAFLO, England). The MCE was adsorbed in silica gel (60-200 mesh size) and eluted in succession with hexane, chloroform, ethyl acetate and methanol fractions to obtain HF (10.94%), CF (13.68%), EAF (12.91%) and MF (32.91%) following the solvent polarities. The same rotary evaporator was used to concentrate the fractions at 40±5^{o}C. For the water extraction, 200g of plant material powder was soaked in 1 l distilled water for 6 h with occasional shaking to dissolve the active components in the laboratory of Pharmaceutics and Pharmaceutical Technology. The suspension was latter filtered using a fine muslin cloth. The filtrate was then lyophilized (freeze-dried) to remove the water solvent using the Yorco Freeze-drying machine (Lyophlizer); manufactured by York Scientific Industries PVT Ltd (India). The water extract (10.73%) was obtained. All the extracts and fractions were stored in the refrigerator (4°C) until needed.

Test organisms

The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were collected from 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. Water used for the rearing process was continually drawn from Agulu lake. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were fed on 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 replaced carefully with fresh lake water and the feeding was continued until the 4th instar larvae were obtained. The larvae were maintained at ambient laboratory condition (temperature of 29±3°C, relative humidity of 82±4% and photoperiod cycles of 12:12 (light:day)).

Larvicidal bioassay

The WHO standard procedure (WHO, 2005) was followed to determine the efficacy of the plant extracts and fractions against fourth instar larvae of *Ae. Aegypti, Cx. quinquefasciatus* and *An. gambiae*. Stock solutions of the extracts or fractions were performed using Tween 80 (Polyoxyethylene sorbitan monooleate) as emulsifier to facilitate the dissolution of the material in water. The stock solutions were further diluted up to 100 ml by adding tap water. From these stocks, various concentrations ranging from 125 to 1000 ppm were made. For comparison, a commercial formulation of WARRIOR® 1000 EC (100 % DDVP: 2,2-dichlorovinyl dimethyl phosphate) larvicide (2000 ppm, recommended concentration), bought in a shop from Awka market (Anambra State, Nigeria), was used as positive control. 1 ml of Tween 80 in 99 ml of tap water was used as

negative control. These controls were set up for each replicate, mosquito species and extract or fraction. For water extract, 2 ml of distilled water was used for dissolution and made up to 100 ml as stock solution by adding tap water. From the stock solution, concentrations ranging from 250 to 2000 ppm were made. 1 ml of distilled water in 99 ml of tap water was used as negative control. All the concentrations were chosen after a preliminary test for all extracts and fractions. Twenty-five fourth instar larvae of each mosquito species were released into each 250 ml beaker containing 100 ml of tap water and mortality was recorded after 24 h of exposure. No food was provided to the larvae either to the tests or controls during the experiments. The dead larvae were expressed as percentage mortality at each concentration. In cases where bioassay tests showed more than 20% (negative) control mortality, these were discarded and repeated. However when (negative) control mortality ranged from 5-20%, the observed percentage mortality was corrected by Abbott's formula. The larvae were considered as dead, if they were not responsive to a gentle prodding with a fine needle. All bioassays were carried out at room (temperature of 27±3°C and 78±4% of relative humidity. Experiments were set in four replicates along with control.

Percentage test mortality (%) = (number of larvae dead / total number of larvae used) × 100

Corrected Mortality (%) = [(% test mortality - % control mortality) / (100 - control mortality)] × 100 (Abbott, 1925).

Phytochemical tests

The phytochemical screening of the leaf extracts and fractions of *P. glandulosus* was carried out by using standard protocols (Harborne, 1973 and Prashant *et al*., 2011). The plant was screened for Tannins and Phenolic Compounds, Flavonoids, Saponins, Alkaloids, Terpenoids, Steroids and Lipids, Fats and Fixed Oils.

Statistical analysis

The corrected mortality was determined using Abbott's (1925) formula whenever required. The percentage of mortality data were subjected to the ANOVA procedure using the Statistical Package for the Social Sciences (SPSS 17.0). The Student-Newman-Keuls (SNK) test at P= 0.05 was used for mean separation. Probit analysis (Finney, 1971) was applied to determine lethal dosages causing 50 % (LC_{50}) and 90 % (LC_{90}) mortality of larvae, 24 h post exposure, and other statistics at 95% confidence limits (upper confidence limit (UCL) and lower confidence limit (LCL)), slope and Chi-square.

RESULTS

The larvicidal activity of the organic extract and fractions of *P. glandulosus* was found to be mosquito species dependent and extract or fraction dependent.

The results of the phytochemical composition of *P. glandulosus* presented in Table 1 showed the presence of Alkaloids, Tannins and Phenolic Compounds, Steroids, Saponins, Lipids, Fats and Fixed Oils and Terpenoids in MCE; Steroids, Lipids, Fats and Fixed Oils and Terpenoids in HF; Steroids in CF; Tannins and Phenolic Compounds in EAF, Alkaloids, Saponins, Tannins and Phenolic Compounds in MF.

Results from extracts and fractions against fourth instar larvae of *Ae. aegypti* 24 h post exposure showed that both extracts and fractions exhibited good larvicidal activities, varying from one fraction to another. At the lowest concentration of 125 ppm, 6.67%, 69.33%, 52.00, 4.33% of dead larvae were recorded in MCE, HF, CF and EAF, respectively (Table 2). At the highest concentration of 1000 ppm, only HF killed all exposed larvae, and even at 500 ppm. MF registered the lowest percentage of mortality (13.33%) followed by EAF (30.67%), MCE (90.67%) and CF (93.33%). The different extracts and fractions recorded LC_{50} values of 89.08, 209.38, 371.62, 1987.10 and 2533.66 ppm for HF, CF, MCE, EAF and MF, respectively (Table 2). The water extract registered 33.33, 57.33 and 62.67% of mortality at 500, 1000 and 2000 ppm, respectively with LC_{50} value of 1138.88 ppm (Table 5).

As for the results of the extract and fractions against fourth instar larvae of *An. gambiae* after 24 h of exposure, HF showed excellent larvicidal activity in killing approximately all the exposed larvae at all concentrations with LC_{50} value as low as 17.11 ppm. Statistically, there was no significant difference ($F=3$; $p>0.05$) among all the concentrations (Table 3). MCE, CF and EAF exhibited 100% mortality each at 500 ppm and 36, 20, 20.00 and 74.66% mortality, respectively at the lowest concentration of 125 ppm. Their LC_{50} values were 76.21, 167.85 and 201.50 ppm for EAF, MCE and CF, respectively. MF

registered the lowest larvicidal effect with 6.33 and 14.67% mortality, respectively in 500 and 1000 ppm to get the highest LC_{50} value of 2536.17 ppm. The water extract registered no mortality at all concentrations (Table 5).

Concerning the activity of the extract and fractions of *P. glandulosus* against fourth instar larvae of *Cx. quinquefaciatus* 24 h post exposure presented in Table 4, all the extracts and fractions did not kill the larvae at the two lowest concentrations of 125 and 250 ppm except HF (6%) at 250 ppm. MF recorded no dead larvae at all concentrations. The highest concentration of 1000 ppm registered low mortality of 22.67, 21.33 and 20% in MCE, EAF and CF, respectively. However HF recorded 97.33% mortality at 1000 ppm. The LC_{50} values were 610.40, 1702.51, 1718.37 and 1752.20 ppm for HF, MCE, EAF and CF, respectively (Table 4). The water extract registered no mortality at all concentrations (Table 5). No larvae survived in DDVP, the synthetic mosquito larvicide.

Table 1. Phytochemical screening test of *Plectranthus glandulosus* leaf extract and fractions.

Phytochemical Compounds	Extract and fractions				
	Methanol crude extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
Alkaloids	+	-	-	-	+
Tannins & Phen. Compounds	+++	-	-	+	+
Steroids	++	+	+	-	-
Saponins	++	-	-	-	+
Lipids, Fats and Fixed Oils	++	++	-	-	-
Terpenoids	++	++	-	-	-

+: present at low concentration; ++: present at moderate concentration; +++: present at high concentration; -: Not present.

Table 2. Larvicidal activity of *Plectranthus glandulosus* extract and fractions against fourth instar larvae of *Aedes aegypti* 24 h post exposure.

Extract and Fractions	Conc. (ppm)	Mean± Std. Deviation (%)	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol crude extract	125	6.67±2.30[a]	371.62 (295.47-469.25)	963.77 (708.48-1656.06)	0.02
	250	30.67±4.61[b]			
	500	65.33±2.30[c]			
	1000	90.67±4.61[d]			
	F value	309.70***			
Hexane fraction	125	69.33±6.11[a]	89.08 (23.49-129.63)	248.96 (183.40-509.93)	1.16
	250	85.33±6.11[b]			
	500	100.00±0.00[c]			
	1000	100.00±0.00[c]			
	F value	34.38***			
Chloroform fraction	125	52.00±13.85[a]	209.38 (119.17-295.26)	1076.49 (656.16-3676.35)	1.28
	250	38.67±6.11[a]			
	500	69.33±4.61[b]			
	1000	93.33±6.11[c]			
	F value	23.23***			
Ethyl acetate fraction	125	4.33±0.57[a]	1987.10 (*)	19151.04 (*)	2.07
	250	6.67±2.30[a]			
	500	30.67±6.11[b]			
	1000	30.67±4.61[b]			
	F value	39.54***			
	125	0.00±0.00[a]	2533.66	7634.79	2.65

Methanol fraction	250	0.00±0.00[a]	(-)	(-)
	500	5.67±2.08[b]		
	1000	13.33±2.30[c]		
	F value	49.50***		

Mean of mortality ± standard deviation (%) within a column followed by the same letter do not differ significantly at P = 0.05 (Student-Newman-Keuls's test); ***: p<0.001; LC_{50} and LC_{90}: Lethal Concentrations able to kill 50% and 90% of larvae, respectively; ppm: parts per million; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; (-): no Confidence Limit estimated; (*): very large values of Confidence Limit; χ2: Chi-square; Number of replicates: 4.

Table 3. Larvicidal activity of *Plectranthus glandulosus* extract and fractions against fourth instar larvae of *Anopheles gambiae* 24 h post exposure.

Extract and Fractions	Conc. (ppm)	Mean± Std. Deviation (%)	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol crude extract	125	36.00±4.00[a]	167.85 (127.43-205.52)	358.37 (280.31-583.23)	2.41
	250	65.33±6.11[b]			
	500	100.00±0.00[c]			
	1000	100.00±0.00[c]			
	F value	214.80***			
Hexane fraction	125	96.00±4.00[a]	17.11 (-)	82.71 (-)	0.55
	250	100.00±0.00[a]			
	500	100.00±0.00[a]			
	1000	100.00±0.00[a]			
	F value	3.00ns			
Chloroform fraction	125	20.00±6.92[a]	201.50 (166.41-241.17)	373.09 (299.94-559.51)	1.91
	250	53.33±15.14[b]			
	500	100.00±0.00[c]			
	1000	100.00±0.00[c]			
	F value	65.86***			
Ethyl acetate fraction	125	74.66±4.61[a]	76.21 (10.79-122.46)	260.51 (181.80-579.58)	2.64
	250	81.33±6.11[a]			
	500	100.00±0.00[b]			
	1000	100.00±0.00[b]			
	F value	34.51***			
Methanol fraction	125	0.00±0.00[a]	2536.17 (-)	7646.86 (-)	0.19
	250	0.00±0.00[a]			
	500	6.33±1.52[b]			
	1000	14.67±2.30[c]			
	F value	75.63***			

Mean of mortality ± standard deviation (%) within a column followed by the same letter do not differ significantly at P = 0.05 (Student-Newman-Keuls's test); ns: p>0.05; ***: p<0.001; ns: not significant; LC_{50} and LC_{90}: Lethal Concentrations able to kill 50% and 90% of larvae, respectively; ppm: parts per million; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; (-): no Confidence Limit estimated; χ2: Chi-square; Number of replicates: 4.

Table 4. Larvicidal activity of *Plectranthus glandulosus* extract and fractions against fourth instar larvae of *Culex quinquefaciatus* 24 h post exposure.

Extract and Fractions	Conc. (ppm)	Mean± Std. Deviation (%)	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Methanol crude extract	125	0.00±0.00[a]	1702.51 (*)	3773.81 (*)	0.69
	250	0.00±0.00[a]			
	500	6.67±2.30[b]			
	1000	22.67±4.61[c]			
	F value	51.46***			
Hexane fraction	125	0.00±0.00[a]	610.40 (-)	951.24 (-)	8.94
	250	6.00±2.00[b]			
	500	16.00±4.00[c]			
	1000	97.33±2.30[d]			
	F value	979.84***			
Chloroform fraction	125	0.00±0.00[a]	1752.20 (-)	3894.51 (-)	0.63
	250	0.00±0.00[a]			
	500	0.00±0.00[a]			
	1000	20.00±4.00[b]			
	F value	75.00***			
Ethyl acetate fraction	125	0.00±0.00[a]	1718.37 (*)	4519.87 (*)	0.27
	250	0.00±0.00[a]			
	500	0.00±0.00[a]			
	1000	21.33±4.61[b]			
	F value	64.00***			
Methanol fraction	/	/	/	/	/

Mean of mortality ± standard deviation (%) within a column followed by the same letter do not differ significantly at P = 0.05 (Student-Newman-Keuls's test); ***: p<0.001; LC50 and LC90: Lethal Concentrations able to kill 50% and 90% of larvae, respectively; ppm: parts per million; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; (-): no Confidence Limit estimated; (*): very large values of Confidence Limit; /: no mortality registered; χ2: Chi-square; Number of replicates: 4.

Table 5. Larvicidal activity of *Plectranthus glandulosus* water extract against fourth instar larvae of *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* 24 h post exposure.

Target mosquito species	Conc. (ppm)	Mean± Std. Deviation (%)	LC_{50} (LCL-UCL) (ppm)	LC_{90} (LCL-UCL) (ppm)	χ^2
Aedes aegypti	250	0.00±0.00[a]	1138.88 (-)	2801.98 (-)	4.91
	500	33.33±6.11[b]			
	1000	57.33±4.61[c]			
	2000	62.67±4.61[c]			
	F value	122.37***			
Anopheles gambiae	250-2000	/	/	/	/
Culex quinquefasciatus	250-2000	/	/	/	/

Mean of mortality ± standard deviation (%) within a column followed by the same letter do not differ significantly at P = 0.05 (Student-Newman-Keuls's test); ***: p<0.001; LC_{50} and LC_{90}: Lethal Concentrations able to kill 50% and 90% of larvae, respectively; ppm: parts per million; LCL: Lower Confidence Limit; UCL: Upper Confidence Limit; (-): no Confidence Limit estimated; /: no mortality registered; χ2: Chi-square; Number of replicates: 4.

DISCUSSION

Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites although the precise boundaries between the two groups can in some instances be somewhat blurred (Crozier *et al*., 2006). Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, and growth and development. These include phytosterols, acyl lipids, nucleotides, amino acids and organic acids. Other phytochemicals, many of which accumulate in surprisingly high concentrations in some species, are referred to as secondary metabolites. They are Alkaloids, Flavonoids, Steroids, Tannins, Saponins, Essential Oil, Phenolics, etc. (Crozier *et al*., 2006). Although ignored for long, their function in plants is now attracting attention as some appear to have a key role in protecting plants from herbivores and microbial infection, as attractants for pollinators and seed-dispersing animals, as allelopathic agents, UV protectants and signal molecules in the formation of nitrogen-fixing root nodules in legumes. Secondary metabolites are also of interest because of their use as dyes, fibres, glues, oils, waxes, flavouring agents, drugs and perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides (Crozier *et al*., 2006). In addition, insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction (Anupam *et al*., 2012).

Plectranthus glandulosus leaves in this study were screened for the presence of major phytochemical groups. The preliminary phytochemical screening revealed the presence of Tannins and Phenolic Compounds, Alkaloids, Saponins, Steroids, Terpenoids, Lipids, Fats and Fixed Oils. These phytochemical compounds could be obviously responsible for the mosquito larval phytotoxicity of this plant. In previous studies, Nigerian *P. glandulosus* was also reported to possess Flavonoids, Terpenoids, Steroids, Saponins, Alkaloids (Egwaikhide and Gimba, 2007).

The larvicidal activity of extracts and fractions from *P. glandulosus* in the present study corroborated the findings of other researchers. The larvicidal activity of extracts from *Aloe ngongensis* against the common malaria vector, *An. gambiae*, was determined. Among the tested fractions, hexane fraction was the most active with the LC_{50} value of 0.84 mg/ml followed by chloroform, acetone and ethyl acetate fractions with LC_{50} values of 0.98, 1.08 and 1.14 mg/ml, respectively. The methanol fraction was the least effective fraction with an LC_{50} value of 2 mg/ml (Matasyoh *et al*., 2008). In the present study, HF was also the most toxic fraction for all the mosquito species. This should be due to the presence of Fixed Oil, Terpenoids and Steroids revealed within the fraction. Earlier, it was proven that as typical lipophiles, the essential oil passed through the cell wall and cytoplasmic membrane, disrupted the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilized them. Cytotoxicity appears to include such membrane damage and dead (Mann and Kaufman, 2012). Also, Terpenoids are known to possess insecticidal properties (acute toxicity) (Mann and Kaufman, 2012). Phenolic Compounds including Tannins and Flavonoids are known to possess insecticidal properties and act as mitochondrial poisons for insect vectors (Mann and Kaufman, 2012). Alkaloids are nitrogenous compounds that show insecticidal properties at low concentration and are often toxic to Vertebrates. Nicotine and Anabasine are common Alkaloids used as pesticides. The mode of action of Alkaloids on insect vectors varies with the structure of heir molecules, but many are reported to affect acetylcholinestrase or sodium channels (Rameshwar, 2010). Earlier, water extract of *Calotropis gigantea* leaves containing Phenoic Compounds, Flavonoids, Alkaloids, Tannins, Saponins Glycosides and Phytosterols was found effective against fourth instar larvae of *Cx. gelidus* (LC_{50} = 137.90 ppm) and *Cx. tritaeniorhynchus* (LC_{50} = 110.05 ppm) (Kumar *et al*., 2012). Similar observations were obtained with other plant extracts against different mosquito species in earlier studies. Devan *et al*. (2013) observed that Carbohydrates, Tannins, Saponins, Flavonoids, Alkaloids, Quinones, Terpenoids, Triterpenoids, Phenols, Coumarins, Proteins, Cyanin, Cardiac Glycosides screened from *Tridax procumbens* extracts exhibited larvicidal activity in fourth instar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*.

The bioassay-guided fractionation of *Achyranthes aspera* led to the separation and identification of a Saponin as a potential mosquito larvicidal compound with LC_{50} value of 18.20 and 27.24 ppm against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively (Bagavan *et al*., 2008). Plant metabolites detected in the leaves of *P. glandulosus* may have exerted single, additive or synergistic effect on the larvae. Further investigation is needed to substantiate this.

In addition, *Cx. quinquefasciatus* was the most resistant mosquito species against both extracts and fractions in the present study. This result is also comparable to earlier reports of Govindarajan *et al*. (2013) who observed the larvicidal activity of crude benzene extract of *Caesalpinia pulcherrima* against vector mosquitoes with LC_{50} values of 119.27, 135.24 and 150.47 (higher) ppm for *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus*, respectively. In the same vein, the LC_{50} from crude ethyl acetate extract of the same plant were 127.80, 142.43 and 158.17 (higher) ppm for *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus*, respectively. Moreover, the larvicidal efficacy of *Cleistanthus collinus* leaf hexane, diethyl ether, dichloromethane and ethyl acetate extracts were evaluated against vector mosquitoes. *Cx. quinquefasciatus* was more resistant than *An. stephensi* and *Ae. aegypti* against all the extracts (Arivoli and Samuel, 2011). Larvicidal activity of the methanol extracts of *Calotropis procera* were tested against fresh third instar larvae of selected mosquito species. *Cx. quinquefasciatus* (LC_{50}=94.08 ppm) was still more resistant than *An. stephensi* (LC_{50}=81.99 ppm) and *Ae. aegypti* (LC_{50}=63.24 ppm) (Gokulakrishnan *et al*., 2013). This may be due to the ability of *Cx. quinquefasciatus* larvae to develop inner resistance, for which the mechanism is unknown.

The reduction in the activity of water extract against the three mosquito species observed in this study corroborates the findings of many authors. The water fraction of *Viola betonicifolia* at 600 ppm registered as low as 8% mortality while hexane, methanol, butanol, chloroform and ethyl acetate fractions of the same plant registered 14.34, 60.28, 26.56, 87.98 and 86.12% mortality, respectively (Naveed and Muhammad, 2011). Also, the water extract did not show meaningful mortality against late third and fourth instar larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* compared to methanol, acetone and petroleum ether (Bansal *et al*., 2012).

All the chi-square values were not significant ($p>0.05$) meaning that the observed results were closer to the expected ones.

CONCLUSION

From the results, it can be concluded from the present study that the larvicidal activity is due to the phytoconstituents present in the extracts or fractions of *P. glandulosus* leaves. As it is known that anything that holds water will breed mosquitoes, the HF can be moved out of the lab to small scale field trial targeting mosquito breeding tools such as old washing machines, tyres, boats, horse troughs, steel drums, cisterns, plastic containers, glass bottles and aluminum cans. Moreover, further studies are needed as bio-guided fractionation of HF for the isolation and identification of the principal constituents responsible for the larvicidal activity and their mode of action by different trails to recommend its use as a useful anti-mosquito agent. Also, carrying out comparative study between *P. glandulosus* from Cameroon and other countries like Nigeria would be interesting.

CONFLICT OF INTEREST STATEMENT

We declare that this is a team work and that we have no conflict of interest.

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