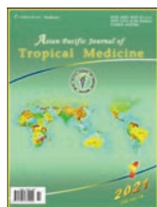

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi:10.4103/1995-7645.304298

Impact Factor: 1.94

Anopheles gambiae larvicidal and adulticidal potential of *Phyllanthus amarus* (Schumach and Thonn, 1827) obtained from different localities of Nigeria

Kingsley Uchenna Ozioko✉, Chris Ikem Okoye, Fabian Chukwuemenam Okafor, Rose Nduka Obiezue

Parasitology and Public Health Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka 400241, Nigeria

ABSTRACT

Objective: To screen phytochemicals in ethanolic leaf extracts of *Phyllanthus amarus* collected from three different geographical zones in Nigeria and evaluate their effects on larva and adult of *Anopheles gambiae*.

Methods: The sample extracts of *Phyllanthus amarus* prepared with ethanol solvent were tested against *Anopheles gambiae* at two important developmental stages of its life cycle using slightly modified WHO protocols.

Results: Alkaloids, saponins, tannins, flavonoids, glycosides, phenols, and terpenes were detected in each extract. Among these samples, the extract from northwest exhibited the highest larvicidal activity (LC_{50}=263.02 ppm), followed by southeast and southwest extracts (LC_{50}=288.40 and 295.12 ppm, respectively after 48 h), while the extract from southwest exhibited the highest adulticidal activity (LC_{50}=275.42 ppm), followed by northwest and southeast extract (LC_{50}=301.99 and 316.22 ppm, respectively after 24 h). A 50% larva mortality was almost attained at 600 ppm after 48 h duration of exposure to the northwest extract.

Conclusions: The tested samples possess strong larvicidal and adulticidal property against *Anopheles gambiae* which depends on their chemical composition and localities of collection. Further studies are needed to explore the insecticidal activity against a wider range of mosquito species, and to identify active ingredient(s) of the extract responsible for such activity.

KEYWORDS: Adulticidal; *Anopheles gambiae*; Different localities; Larvicidal; *Phyllanthus amarus*

1. Introduction

Anopheles (*An.*) *gambiae* is a pest of significant public health importance, responsible for the transmission of malaria in humans. Malaria, which is an infectious disease caused by single-celled protozoan parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes. In humans, the parasites multiply in the liver to infect red blood cells. Currently, there is no effective vaccine available for the prevention of malaria. One of the methods for reducing and interrupting malaria transmission is by the use of synthetic insecticides[1]. Insecticides are used to reduce the population density of hematophagous insects' vectors[2]. Despite the effectiveness and benefit of synthetic insecticide against several mosquito vectors, its use has resulted in several issues such as the rising occurrences of insecticide resistance and adverse effects on the ecosystem. The insecticides resistance of mosquito has increased the complexity of malaria elimination and its vectors control programme[3]. *An. gambiae* mosquitoes are the commonly known vectors of seasonal malaria transmission in Nigeria. Several cases of insecticide resistance of *An. gambiae* have been reported in Nigeria[4,5]. Indiscriminate and improper use of insecticides has brought insecticide selection pressure in killing the *Anopheles* mosquitos, as evidenced by the increased frequency of phenotypic and knockdown resistance[4]. Insecticide from plant origin has been known to be biodegradable, target-specific, and cost-effective[6]. Plants and their products have been used to control mosquito vectors before the synthetic organic insecticide was discovered[7,8]. Abirami *et al.*[9] reported that the insecticidal activity of plant extract depends upon the plant species, insect species, geographical varieties, plant parts used, extraction method adopted, and the polarity of solvents used during extraction. *Phyllanthus* (*P.*) *amarus* (Euphorbiaceae), also known as stone-breaker, is an herbaceous shrub distributed widely in Nigeria. The plant has been used to treat human-related

✉To whom correspondence may be addressed. E-mail: ukingsley82@yahoo.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2021 *Asian Pacific Journal of Tropical Medicine* Produced by Wolters Kluwer-Medknow. All rights reserved.

How to cite this article: Ozioko KU, Okoye CI, Okafor FC, Obiezue RN. *Anopheles gambiae* larvicidal and adulticidal potential of *Phyllanthus amarus* (Schumach and Thonn, 1827) obtained from different localities of Nigeria. Asian Pac J Trop Med 2021; 14(1): 27-33.

Article history: Received 19 November 2019 Revision 25 November 2020 Accepted 28 November 2020 Available online 5 January 2021

diseases over the years. It contains bioactive compounds that have behavioral and physiological effects on insects[10]. Euphorbiaceae family's medicinal nature has been attributed to their complex habitat with varying stress factors, as evidenced by the varied assemblage of secondary substances in different species[11]. Traditionally, it is called Geeron-tsuntsaayee (Hausa) Eyinolobe (Yoruba), and Enyikwonwa (Igbo) in Nigerian languages[12]. To the best of our knowledge, the insecticidal activity of *P. amarus* has not been demonstrated convincingly, therefore, this study aims to determine the insecticidal effect of extracts of *P. amarus* from different geographical zones in Nigeria by testing larvicidal and adulticidal activities against *An. gambiae*.

2. Materials and methods

2.1. Location and sample collection

The selection of the plant for the study was based on previous scientific literature such as widespread distribution and medicinal usage in Nigeria[13-15]. Fresh mature shoots and seeds of *P. amarus* were randomly collected (between April and November, 2018) from each of the three major ethnic districts in Nigeria, namely Yoruba metropolis in the southwest (SW), Hausa metropolis in the northwest (NW), and Igbo land in the southeast (SE) of Nigeria. The samples were collected between latitude 4.57˚ E and longitude 7.77˚N for SW; latitudes 7.43˚E and longitudes 10.52˚N for NW; latitudes 7.50˚E and longitude 7.77˚N for SE. Each of the three locations was selected as the representative of a zone with a similar climatic tendency in Nigeria. Southeast receives more than 120 inches (3 000 mm) of rain a year; southwest receives about 70 inches (1 800 mm); while Northwest receives no more than 20 inches (500 mm) a year[16]. The ranking order for monthly mean evapotranspiration (mm/day) in the various zones during the peak landscape activity from March to June is SW<SE<NW[17].

The collected *P. amarus* plants were identified in the field by an ethnobotanist and their shoots were transported to BioResources Development and Conservation Centre Nsukka, Enugu State Nigeria for further identification and authentication. A voucher specimen of the dried plant was deposited in the herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, with a voucher number designated as UNH No. 96. The seeds were discarded and the leaves were chopped up into small pieces and dried on the laboratory tables at room temperature. Each sample was further ground into fine powders with a grinding machine and sieved to give a particle size of 50-150 mm. The powdered samples were stored at room temperature in three airtight locked polythene bags and labeled as A, B, and C (SW, NW, and SE, respectively). They were later transported to the University of Nigeria, Nsukka, where extraction and analysis were carried out.

2.2. Extraction

The method earlier described with slight modifications was used for preparation of the extracts[18]. Powdered samples of each extract were extracted separately with 20 mL/g of ethanol and filtered with filter paper (Whatman No. 1) into three fresh conical flasks. The filtrates were subjected to solvent recovery using a rotary evaporator, leaving behind semi-solid extracts, kept in labeled bottles, and preserved in the refrigerator (4 ℃) before use. Standard stock solutions were prepared (at 1%) by dissolving 1 g of the residue in 100 mL of water containing 0.1% ethanol. From the stock solution, different concentrations (200-600 ppm) were prepared with water by serially diluting the stock solution according to WHO protocol[19]. The standard Rambo® insecticide powder (0.6% permethrin) at a concentration of 200-600 ppm was prepared separately from 0.5 g of the powder in 100 mL of water. They were conducted simultaneously for larvicidal and adulticidal bioassays.

2.3. Phytochemical analysis of the ethanolic plant extract

The presence of some important phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenes, and phenols was detected by the Sofowora, Trease GE and Evans standard methods described previously[20,21].

2.4. Test organisms

The larvae of mosquitoes were collected from stagnant water within the Nsukka area and identified into different species in the laboratory following the method as described in an earlier publication[22]. The *An. gambiae* larvae were separated from the mixed culture and transferred into mosquito rearing device, fed with yeast, and stabilized to produce adult. The adults were fed with 10% w/v sugar solution and blood-fed with albino rats; the rearing environment was under 12 h light/12 h dark condition at (27±5) ℃. The F2 generations were used for the assay.

2.5. Larvicidal activity

The larvicidal activity of the plant crude extract was conducted according to WHO standard procedure as described by Rohani *et al.*[19] with little modifications. Each of the treatments was tested in triplicate at various concentrations (200-600 ppm), along with the control experiment dosed up with an equivalent amount of each solution free of the tested treatment. Both treatment and control groups were kept under the same conditions. A total of 480 fourth-instar larvae were used per treatment. The larvae were randomly distributed into 4 groups (1, 2, 3, 4) each of 120 instars larvae and further randomized into 6 replicate experiments (A, B, C, D, E, F) of 20 instars larvae into plastic cups, each containing appropriate extract concentration in 200 mL of water mixed with a small amount of yeast powder. Mortality was observed after 16, 32, and 48 h post-treatment. There were three trials for each treatment from which data were pooled together for the analysis. Dead larvae were

identified when they failed to move after tapping their siphons or cervical regions with a needle. Dead larvae were removed as soon as possible to prevent decomposition, which may cause rapid death of the remaining larvae. If more than 2 larvae in each test pupate in the course of the experiment, that particular test is repeated. The mortality of the treated group was corrected using Abbott's formula as stated by Ashwini *et al*.[23].

2.6. Adulticidal activity

An adulticidal bioassay was performed according to a previous study[24] with slight modifications. The adulticidal activities of the treatments were evaluated at various concentrations and control groups. Each treatment was tested in triplicate at various concentrations (200-600 ppm), along with the control experiment dosed up with the equivalent amount of each solution free of the tested treatment. Both treatment and control groups were kept under the same conditions. The bioassay was conducted in experimental kits consisting of transparent plastic tubes. Each tube was sealed on top with a cover quaze. Some tubes were used to expose the mosquitoes to treatments, while some were used to hold them after the treatments. A total of 360 *An. gambiae* adults were used per treatment. The adults were randomly distributed into 4 groups (1, 2, 3, 4) each of 90 *An. gambiae* adults and further randomized into 6 replicate experiments (A, B, C, D, E, F) of 15 adults into each of the plastic holding tubes and allowed to acclimatize for 1 h. Subsequently, filter papers impregnated with various concentrations of the treatments were placed on top of the cover quaze and knockdown rate was recorded from the average of three replicates after 1 h post-treatment period. At the end of the exposure, the mosquitoes were transferred back to the holding tubes where pads of cotton wool soaked with 10 percent glucose solution were introduced. The holding tubes were then placed inside the mosquitoes rearing chamber and mortality was observed after 24 hours post-treatment recovery period. There were three trials for each treatment from which data were pooled together for the analysis. The adulticidal activity was calculated by counting dead mosquito from the introduced mosquito. Mosquito was considered dead if it was lying on its back or side and was unable to maintain flight after a gentle tap with a soft brush. The control mortalities were corrected using Abbott's formula as shown below:

$$\text{Percent mortality of test organism} = \frac{\text{No. of dead organisms}}{\text{No. of introduced organisms}} \times 100$$

$$\text{Corrected mortality (\%)} = \frac{\text{Mortality in control (\%)-mortality in treatment (\%)}}{\text{100-mortality in control (\%)}} \times 100$$

$$\text{Knocked down (\%)} = \frac{\text{No. of adults knocked-down (per unit time)}}{\text{No. of adults released}} \times 100$$

2.7. Statistical analysis

The data were subjected to log-probit analysis to calculate LC_{50} and LC_{90} with a 95% confidence limit. χ^2 goodness of fit tests, and regression co-efficient, were recorded. Whenever χ^2 value was found significant (P<0.05). A heterogeneity correction factor was used in the calculation of confidence limits. The effect of treatments at various concentrations on mosquito mortality was evaluated by one-way analysis of variance followed by Tukey test. Mortality data were tested for normality before analysis and log-transformed due to violations of homogeneity of variance. The collected data were analyzed statistically using MS Excel® 07 and SPSS software version 20.0. P<0.05 were considered to be statistically significant.

3. Results

3.1. Phytochemicals screening

Each of the plant samples revealed the presence of alkaloids, flavonoids, saponins glycosides, terpenes, phenols, and tannins (Table 1).

Table 1. Phytochemicals present in *Phyllanthus amarus* from three different geographical zones.

Phytochemicals	SW extract	NW extract	SE extract
Alkaloids	(++)	(+)	(+)
Flavonoids	(++)	(+++)	(++)
Saponins	(++)	(+)	(++)
Glycosides	(+)	(++)	(++)
Terpenes	(++)	(+)	(+)
Phenols	(+)	(+)	(++)
Tannins	(+)	(++)	(+)

SW: southwest; NW: northwest; SE: southeast. (-) denotes negative; (+) denotes positive in low amount; (++) denotes moderately; (+++) denotes highly positive.

3.2. Mortality of 4th instar of An. gambiae larvae at 48 h post-treatment with various extracts in comparison with a commercial insecticide

Table 2 shows the mean mortality of *An. gambiae* larvae after 48 h exposure to different concentrations of *P. amarus* plant extracts and standard insecticide. The mosquito mortality occurred in a dose-dependent manner. The highest mortality was observed in NW extract (LC_{50}=263.02 ppm; LC_{90}=512.86 ppm), followed by SE extract (LC_{50}=288.40 ppm; LC_{90}=562.34 ppm), and SW extract (LC_{50}=295.12 ppm; LC_{90}=602.55 ppm). No mosquito mortality was observed in the concentration control group. A significant difference in mortality (P<0.05) existed among SW extract, SE extract, NW extract, and standard insecticide at various concentrations, apart from 300 ppm concentration of SE extract that was not significantly different from that of SW extract.

3.3. Larvicidal-effect of ethanolic extracts of P. amarus against the 4th instar of An. gambiae larvae

The effect of the various extracts of *P. amarus* on the fourth instar larvae of *An. gambiae* is shown in Table 3. Mortality of the larvae was

Table 2. Mortality of 4th instar of *Anopheles gambiae* larvae after 48 h post-treatment at different concentrations of various extracts of *Phyllanthus amarus* in comparison with a commercial insecticide.

Treatment	Mortality of *Anopheles gambiae* larvae at different concentrations (%)$^{\#}$					Control	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2 (df=4)	R^2	P value
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm						
SW extract	31.45±0.19^{a}	42.97±2.92^{a}	58.79±0.72^{a}	81.44±0.20^{a}	90.21±0.18^{a}	0*	295.12 (215.70-379.96)	602.5 (514.26-1 675.28)	8.445	0.983	0.733
NW extract	39.06±0.58^{b}	50.47±0.23^{b}	70.60±0.35^{b}	89.25±0.14^{b}	97.37±.0.70^{b}	0*	263.02 (152.85-332.61)	512.86 (419.71-1 317.56)	11.821	0.983	0.874
SE extract	29.66±0.30^{c}	44.88±0.67^{a}	65.40±0.38^{c}	88.55±0.34^{c}	93.43±0.29^{c}	0*	288.40 (225.53-351.76)	562.34 (469.47-985.07)	8.168	0.972	0.594
Rambo®	96.00±1.00^{d}	99.66±0.57^{c}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	0*	50.118 (0.00-85.98)	131.82 (0.00-189.923)	2.563	0.563	0.464

$^{\#}$Data was expressed as mean±SD of 5 independent experiments performed in triplicates along with control. Means followed by different letters within the same column are significantly different (P<0.05); Treatment control: rambo® powdered insecticide (0.6% permethrin); SW: southwest; NW: northwest; SE: southeast; Concentration control, water (0.1% ethanol); *=No mortality; LCL: lower confidence limits; UCL: upper confidence limits; χ^2: *Chi*-square value; *df*: degrees of freedom; R^2=regression co-efficient.

Table 3. Effect of the extracts against the 4th instar of *Anopheles gambiae* larvae at three time interval of 16 h (0-16 h, 16-32 h and 32-48 h).

Treatment	Mortality of *Anopheles gambiae* larvae at different concentrations (%)$^{\#}$						Control	LC_{50} (LCL-UCL)	Slope±SEM	χ^2 (df=4)	R^2	P value
	Time	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm						
SW extract	0-16 h	3.06±0.31	7.53±1.04	7.85±0.38	13.14±2.06	20.65±6.03	0*	1 819.70 (1 013.40-5 926.80)	2.00±0.34	1.333	0.918	0.721
	16-32 h	14.15±0.22	15.19±0.22	17.42±0.82	33.17±1.15	34.09±4.46	0*	1 202.26 (778.73-2 967.90)	1.55±0.47	4.138	0.844	0.223
	32-48 h	14.03±0.19	20.03±1.05	33.17±1.19	35.07±4.99	36.05±15.67	0*	912.01 (664.25-1 814.29)	1.64±0.25	1.707	0.873	0.635
NW extract	0-16 h	9.08±0.15	9.83±1.02	13.15±3.00	16.19±0.94	21.07±1.86	0*	3 715.35 (1 317.06-8 852.87)	1.13±0.25	0.751	0.949	0.861
	16-32 h	12.00±0.07	19.49±1.63	22.06±1.94	27.19±3.03	26.52±5.89	0*	1 862.08 (967.38-4 076.65)	1.18±0.16	0.615	0.887	0.893
	32-48 h	18.09±0.19	22.08±2.17	35.13±2.45	46.08±3.54	50.09±0.21	0*	588.84 (571.32-948.83)	2.09±0.26	1.632	0.967	0.652
SE extract	0-16 h	6.14±0.24	9.50±0.91	10.55±3.92	20.14±0.21	22.07±0.16	0*	1 778.27 (1 016.15-7 979.95)	1.69±0.33	1.736	0.920	0.711
	16-32 h	6.95±1.99	16.19±0.24	19.10±2.18	21.08±1.11	23.80±2.49	0*	1 548.81 (995.76-14 036.37)	1.61±0.34	1.250	0.886	0.741
	32-48 h	16.17±0.17	19.02±3.53	36.88±1.86	47.18±3.02	47.44±2.18	0*	616.59 (516.346-7 650.18)	2.20±0.38	3.420	0.911	0.331

$^{\#}$Data was expressed as mean±SD of 5 independent experiments performed in triplicates along with control. SW: southwest, NW northwest, SE: southeast; LC_{50}: lethal concentration 50%; LCL: lower confidence limit, UCL=upper confidence limit; χ^2: *chi*-square value; *df*: degrees of freedom; Slope±SEM are the slope of regression lines where (SEM)=standard error; R^2=regression co-efficient; Concentration control, water (0.1% ethanol); *=No mortality.

Table 4. Mortality of *Anopheles gambiae* adults after 24 h post-treatment at different concentrations of various extracts of *Phyllanthus amarus* in comparison with a commercial insecticide.

Treatment	Mortality of *Anopheles gambiae* adults at different concentrations (%)$^{\#}$					Control	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2 (df=4)	R^2	P value
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm						
SW extract	37.15±1.02^{a}	41.21±1.16^{a}	73.14±1.11^{a}	86.20±1.03^{a}	92.25±1.10^{a}	0*	275.42 (163.60-353.16)	588.84 (450.16-1 665.95)	11.894	0.928	0.206
NW extract	25.30±1.31^{b}	40.26±1.02^{a}	70.26±1.03^{bc}	85.28±1.00^{ac}	89.19±1.08^{b}	0*	301.99 (284.38-328.56)	602.55 (550.77-669.13)	4.447	0.942	0.310
SE extract	23.87±1.02^{b}	38.21±0.96^{b}	68.21±1.06^{bc}	80.27±0.86^{b}	85.19±1.08^{c}	0*	316.22 (297.48-345.05)	660.69 (597.79-786.62)	3.564	0.939	0.264
Rambo®	37.85±0.95^{ac}	46.67±0.61^{c}	80.27±1.10^{d}	83.41±1.12^{c}	94.62±0.74^{a}	0*	263.02 (168.00-332.27)	549.54 (441.44-1 229.76)	10.101	0.918	0.071

$^{\#}$Data was expressed as mean±SD of 5 independent experiments performed in triplicates along with control. Means followed by different letters within the same column are significantly different (P<0.05). Treatment control: rambo® powdered insecticide (0.6% permethrin); SW: southwest; NW: northwest; SE: southeast; Concentration control, water (0.1% ethanol); *=No mortality; LCL: lower confidence limits; UCL: upper confidence limits; χ^2: *Chi*-square value; *df*: degrees of freedom ; R^2=regression co-efficient.

observed at different concentrations of each sampled extract. The larvae mortality of various extracts was found to increase with concentration and duration of exposure. All the extracts treated groups showed good larval mortality against *An. gambiae*, while concentration control groups showed no mortality after 48 h post-treatment. The LC_{50} values at 16, 32, and 48 h were 1 819.70 ppm, 1 202.26 ppm, and 912.01 ppm for SW extract, 3 715.35 ppm, 1 862.08 ppm, and 588.84 ppm for NW extract, 1 778.27 ppm, 1 548.81 ppm, and 616.59 ppm for SE extract, respectively. Apart from NW extract at 600 ppm that exhibited the highest mortality at 500 ppm after 32 h (16 h time interval), all other extracts caused the highest mortality against *An. gambiae* larvae at 600 ppm. Both the NW and SE extracts at 600 ppm exhibited around 50% mortality after 48 h (at 16 h time interval) post-treatment.

3.4. Mortality of An. gambiae adults after 24 h post-treatment with various extracts in comparison with a commercial insecticide

Table 4 indicated the number of adult death was proportional to the level of concentration in each plant extract. Generally, as the concentration increases, the rate of adult mosquito mortality increases. The LC_{50} and LC_{90} values of SW, NW, and SE extracts of *P. amarus* against *An. gambiae* were 275.42 & 588.84 ppm, 301.99 & 602.55 ppm, and 316.22 & 660.69 ppm, respectively. The exposure of ethanol extract of *P. amarus* at the concentration of 200 ppm for 24 h to *An. gambiae* caused no significant decrease (P>0.05) in the mosquito mortality as compared to the treatment control. The SW extract showed maximum adulticidal effect against *An. gambiae* with LC_{50} and LC_{90} values (275.42 ppm and 588.84 ppm, after 24 h). No mortality was detected in any of the concentration

Table 5. Median lethal concentration and knockdown rate of *Anopheles gambiae* adults to ethanolic leaf extract of *Phyllanthus amarus* and a commercial insecticide within 1 h exposure period.

Treatment	Knockdown rates within 1 h at different concentrations (%)#					Control	LC_{50} (LCL-UCL)	Slope±SEM	χ^2 (*df*=4)	R^2	*P* value
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm						
SW extract	9.37±0.70	17.40±0.95	27.43±0.68	53.20±1.00	54.20±1.03	0*	562.34 (418.59-1 984.30)	3.61±0.98	14.61	0.875	0.153
NW extract	7.23±0.83	8.36±0.86	25.40±0.81	44.20±1.00	51.23±0.85	0*	645.65 (465.68-2 797.35)	3.42±0.92	12.56	0.881	0.248
SE extract	8.16±1.00	11.43±0.75	25.34±1.11	45.43±0.95	47.21±1.10	0*	630.95 (487.61-1 319.20)	3.08±0.57	6.68	0.904	0.198
Rambo®	21.40±0.98	33.30±0.72	43.50±0.91	61.26±1.15	77.20±1.10	0*	389.04 (366.18-436.53)	3.12±0.45	4.88	0.989	0.659

All tests were run in triplicates. #Data was expressed as mean±SD; Slope±SEM are the slope of regression lines, where (SEM): standard error; R^2=regression co-efficient; Concentration control, water (0.1% ethanol); *=No knockdown.

control groups. The mortality of the SW extract was almost equal to that of the Rambo® insect powder at 600 ppm, and there was no statistically significant difference between the two groups. *An. gambiae* adults initially flew slowly and then ceased to fly when disturbed (knockdown) before death during the exposure period.

3.5. Median lethal concentration and knockdown rate of An. gambiae adults to ethanolic leaf extract of P. amarus after 1 h post-treatment

The knockdown rate of *An. gambiae* adults to various extracts of *P. amarus* using impregnated papers was shown in Table 5. The knockdown rate was recorded at various concentrations ranging from 200 ppm to 600 ppm. All extracts showed knockdown increases with concentrations from 200-600 ppm except for SE and SW extracts at 500 ppm where it slightly decreased. No knockdown was detected in any of the concentration control groups. The statistical data LC_{50}, 95% confidence limits and slope of regression lines were also calculated. The LC_{50} values were expressed as follows: LC_{50} value of SW extract was 562.34 ppm, NW extract was 645.65 ppm, SE extract was 630.95 ppm, and Rambo® insecticide was 389.04 ppm.

4. Discussion

Nigeria has various geographical distribution and climate types with unique biodiversity that provide supports to millions of people for centuries. The country also has numerous mountains, plains, and a desert that result in distinct weather and climate ranging from equatorial in south, tropical in center, and arid in north. Each of the geographical location is covered with plants of various species; many have been published in ethnobotanical data, and culturally characterized in literature, including traditional herbal insecticides for controlling insect pest. The present study has demonstrated that *P. amarus* plant extracts possess insecticidal properties against *An. gambiae* that transmit malaria parasites. Similarly, Okonkwo and Onyeji[27] have reported a considerable insecticidal effect of *P. amarus* on *An. gambiae* adults, where it caused 100% mortalities in 12 h exposure period. Several studies have also demonstrated the insecticidal effect of *P. amarus* plant extract on several mosquitoes species, including *Aedes aegypti* and *Culex tritaeniorhynchus*[25], and other disease causing organisms such as fungi and bacteria[26]. Insecticidal activities of other plant extracts against *An. gambiae* mosquitoes have also been previously reported. For instance, a study performed[28] with each of the *Ocimum gratissimum*, *Chromoleana odorata*, *Terminalia catappa*, *Carica papaya*, *Vernonia amygdalina*, and *Cymbopogon citrates* showed exposure dependent LC_{50} and lethal concentration values, which are consistent with our results on the mortality of *An. gambiae* at different concentrations and varying levels of LC_{50} values. Similar active compounds such as flavonoids, phenols, alkaloids, tannins, saponins, terpenes, and glycosides were identified from each sampled extract which is in line with the study reported previously[12,29]. Therefore, confirming the presence of similar active compounds in each plant extract probably indicates that they are basic compounds which are common to all the samples independent of the localities of collection. Secondly, the tested samples possess important bioactive compounds with insecticidal potential, which can act as natural candidates for the discovery of new products to combat *Anopheles*. The insecticidal activities observed can be attributed to the secondary metabolites presented in *P. amarus* that are readily extracted in ethanol[30]. Although the active compounds were common in all the sampled extracts, their chemical compositions vary from location to location, which is in line with the report of Boko-Haya *et al.*[31] that *P. amarus* extracts contain large families of chemical groups whose number and nature vary according to ecological zones. Variation in activities may be due to differences in the phytochemical composition of the various extracts. The sampled extracts were from three different geo-climatic zones of Nigeria. Each sampled extract contains flavonoids, saponins, and tannins that play a role in plant defense against insects[32] and might have been responsible for larval and adult deaths. Saponins possess insecticidal properties that affect insect physiology in many different ways[33], likewise flavonoids. Glycosides can cause physiological disruption and inhibition of insect vital respiratory enzymes[34]. Plant secondary metabolites can penetrate mosquito integument to disrupt its important metabolic reactions[35], hence deprive adult mosquitoes of oxygen and lead to suffocation[36]. The present study has indecated that the highest content of flavonoids in NW extract showed the best larvicidal activity, while the lowest content of glycosides in SW extract demonstrated the least larvicidal activities. LC_{50} greater than 200 ppm was recorded in the ethanol extract of *P. amarus* against 4th instars larvae of *An. gambiae*, likewise previously reported in petroleum ether extract of the same plant species against 4th instars larvae of mosquito vectors[37]. The insecticidal activities expressed by each of the extracts reflect their differences in chemical composition and climatic condition[38]. The concentration-dependent

mortality results of *P. amarus* plant against *An. gambiae* have shown that the concentration may be a factor in the method for toxicity tests on mosquitoes[30] as well as the climatic condition, which is in line with the work of Hada *et al.*[39]. The sequential mortality in correlation with an increase in exposure of plant extracts did not occur in the study as most of the test organisms could resist the effect of the treatments at various concentrations. However, non significant larva percent mortality effects (P<0.05), observed after 48 h at a higher concentration between 500 and 600 ppm might be to resist the effect of the extract by the larva. NW extract exhibited lesser saponins content than other extracts but still had the highest larvicidal activity. These might be due to the wrong proportion of bioactive compounds or different active compounds with a synergic effect[40]. The studied extracts demonstrated remarkable knockdown effects on *An. gambiae*, hence they can rapidly disrupt the mosquito life cycle at adult stages and effectively reduce its population. Additionally, the efficacy of each sampled extracts on 4th instar larvae of *An. gambiae* is directly comparable to that in the positive control.

In conclusion, our results indicated that extracts of *P. amarus* obtained from different geo-climatic zones in Nigeria would be effective against *An. gambiae* at larva and adult stages of its life cycle. The analysis of the mosquito mortality showed considerable variability in the activities of NW, SW, and SE plant extracts. The variability in insecticidal activities depends on the time of exposure and concentration of the extracts[41,42] as well as ecologic conditions (geographical regions, climate conditions and altitude). Although all extracts showed remarkable insecticidal effects on both *An. gambiae* larvae and adults, the intensity of these activities remained below that of the standard insecticide. Thus this plant extract provides the bases to act as an alternative to synthetic insecticide in the control programme of mosquito, especially in high prepared stock solutions. It constitutes a potential source of bioactive ingredients, cheaper than synthetic insecticides, and may pose no harmful effects to the ecosystem. Bioactive compounds analysis of extracts in different locations can help in selecting areas for mass production of this plant species to enhance its pharmaceutical and marketing values. The extract can as well be used directly as an insecticidal agent in small volume aquatic habitats or breeding sites of limited size around human dwellings, thereby controlling malaria transmission.

Conflict of interest statement

The authors report no declarations of interest.

Acknowledgements

The authors are grateful to Department of Zoology and Environmental Biology University of Nigeria, Nsukka Postgraduate Board for their lecturers specialist input to the discussion during the proposal presentation and progress of this research and BioResources Development and Conservation Centre, Nsukka for all the help, guidance and facilities provided to accomplish this work and also to the field workers that participated in the sample collection.

Authors' contributions

KUO, CIO, and FCO conceived of the presented idea. KUO, RNO and CIO wrote the protocol, KUO prepared the first draft of the manuscript and conducted the laboratory experiments while CIO supervised the work. KUO and FCO performed the statistical analysis. All the authors read, interpret, and approved the final manuscript.

References

[1] Ramkumar G, Karthi S, Muthusamy R, Suganya P, Natarajan D, Kweka EJ, et al. Mosquitocidal effect of *Glycosmis pentaphylla* leaf extracts against three mosquito species (Diptera: Culicidae). *PLoS One* 2016; **11**(7): e0158088.

[2] Pavela R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects-a review. *Plant Protect Sci* 2016; **4**: 229-241.

[3] Swargiary A, Daimari M, Roy M, Haloi D, Ramchiary B. Evaluation of phytochemical properties and larvicidal activities of *Cynodon dactylon*, *Clerodendrum viscosum*, *Spilanthes acmella* and *Terminalia chebula* against *Aedes aegypti*. *Asian Pac J Trop Med* 2019; **12**(5): 224-231.

[4] Awolola TS, Adeogun A, Olakiigbe AK, Oyeniyi T, Olukosi YA, Okoh H, et al. Pyrethroids resistance intensity and resistance mechanisms in *Anopheles gambiae* from malaria vector surveillance sites in Nigeria. *PLoS One* 2018; **13**(12): e0205230.

[5] Kristan M, Fleischmann H, Della Torre A, Stich A, Curtis CF. Pyrethroid resistance/susceptibility and differential urban/rural distribution of *Anopheles arabiensis* and *Anopheles gambiae* s.s. malaria vectors in Nigeria and Ghana. *Med Vet Entomol* 2002; **17**: 326-332.

[6] Soonwera M, Phasomkusolsil S. Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culexquinque fasciatus* (Say). *Asian Pac J Trop Biomed* 2017; **7**(11): 967-978.

[7] Bekele D, Petros B, Tekie H, Asfaw Z. Larvicidal and adulticidal effects of extracts from some indigenous plants against the malaria vector, *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia. *J Biofertil Biopestici* 2014; **5**: 144.

[8] Butt A, Butt BZ, Vehra SE. Larvicidal potential of *Calotropis procera* against *Aedes aegypti*. *Int J Mosq Res* 2016; **3**(5): 47-51.

[9] Abirami D, Kovendan K, Chandramohan B. Ovicidal and larvicidal properties of *Pergularia extensa* and *Spermacoce hispida* ethanol root extracts on *Anopheles stephensi* liston. *Southeast Asian J Trop Med Public Health* 2017; **48**(6): 1188-1199.

[10] Kosar H, Srivastava M. Management of sucking pests, by integration of organic sources of amendments and foliar application of entomopathogenic fungai on chilli. *J Biopest* 2016; **9**(1): 80-90.

[11]Julius TM, Patrick VD. Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. *J Med Plants Res* 2011; **5**(5): 652-662.

[12]Oluboyo BO, Oluboyo AO, Kalu SO. Inhibitory effects of *Phyllanthus amarus* extracts on the growth of some pathogenic microorganisms. *Afr J Cln Exper Microbiol* 2016; **17**(3): 166-172.

[13]Neeraj B, Lal S. A survey of some medicinally important plants of the Euphorbiaceae family used by the Santhal tribes of Santhal Pargana. *Indian J Tradit Know* 2019; **18**(3): 610-614.

[14]Mao X ,Wu L, Guo H, Chen W, Cui Y, Qi Q, et al. The genus *Phyllanthus*: An ethnopharmacological, phytochemical, and pharmacological review. *Evid–Based Complementary Altern Med* 2016; **2016**: 1-36.

[15]Meena J, Sharma RA, Rolania R. A review on phytochemical pharmacological properties of *Phyllanthus amarus* schum. and thonn. *Int J Pharm Sci* 2018; **9**(4): 1377-1386.

[16]National Bureau of Statistics, Federal Republic of Nigeria. *Annual abstract of statistics. 2010.* [Online]. Available from: www.nigerianstat.gov.ng. [Accessed on 25 December 2019].

[17]Chineke TC, Idinoba ME, Ajayi OC. Seasonal evapotranspiration signature under a changing landscape and ecosysterm management in Nigeria: Implication for agriculture and food security. *Am J Sci Ind Res* 2011; **2**(2): 191-204.

[18]Mavundza EJ, Maharaj R, Chukwujekwu JC, Finnie JF, Staden JV. Screening for adulticidal activity against *Anopheles arabiensis* in ten plants used as mosquito repellent in South Africa. *Malar J* 2014; **13**: 173.

[19]Rohani A, Azahary AR, Yu K, Zurainee MN. Insecticide susceptibility status and resistance mechanism of *Anopheles cracens* Sallum and *Peyton* and *Anopheles maculatus* Theobald (Family: Culicidae) from knowlesi malaria endemic areas in Peninsular Malaysia. *Asian Pac J Trop Med* 2019; **12**(2): 79-86.

[20]Raipuria N, Kori D, Saxena HO, Pawar G, Choubey SK. Qualitative and quantitative evaluation of secondary metabolites in leaves, roots, and stem of *Cleome viscosa* L. *Int J Green Pharm* 2018 ; **12**(4): 285-289.

[21]Ekwueme FN, Nwodo OFC, Joshua, PE, Nkwocha C, Eluka PE. Qualitative and quantitative phytochemical screening of the aqueous leaf extract of *Senna mimosoides*: Its effect in *in vivo* leukocyte mobilization induced by inflammatory stimulus. *Int J Curr Microbiol Appl Sci* 2015; **4**(5): 1176-1188.

[22]Gillies MT, De Meillon B. The anophelinae of Africa South of the Sahara. *Sou Afr Inst Med Res* 1968; **54**: 1-343.

[23]Ashwini U, Taju G, Thirunavukkarasu P, Asha S. Pupal emergence inhibition activity of *Acalypha indica* leaf extract against dengue vector, *Aedes albopictus* mosquito. *Int J Pharm Pharm Sci* 2017; **9**(8): 114-118.

[24]World Health Organization. *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd ed; 2016.* [Online] Available from: http://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575-eng.pdf?sequence=1. [Accessed on 16 January 2019].

[25]Khader SZ, Ahmed SZ, Sathyan J, Mahboob MR, Venkatesh KP, Ramesh KA. Comparative study on larvicidal potential of selected medicinal plants over green synthesized silver nano particles. *Egypt J Basic Appli Sci* 2018; **5**: 54-62.

[26]Senjobi CT, Ettu AO, Otujo CO. Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus* Schum and Thonn. *J Pharmacogn Phytother* 2017; **9**(1): 6-10.

[27]Okonkwo CO, Onyeji CM. Insecticidal potentials and chemical composition of essential oils from the leaves of *Phyllanthus amarus* and *Stachytarpheta cayennensis* in Nigeria. *Int J Biochem Res Rev* 2018; **22**(3): 1-16.

[28]Opoggen L, Rotimi J, Aigbodion FI, Omoregie AO. Larvicidal efficacy of six medicinal plants on *Anopheles gambiae* s.l mosquito. *Merit Res J Med Med Sci* 2019; **7**(1): 18-23.

[29] Etta HE, Ekaluo U, Okon E, Eneobong EE. Phytochemical evaluation and initial tlc profiling of two potential indigenous male contraceptive plants. *Eur J Pharm Med Res* 2015; **2**(5): 47-56.

[30]Oyedokun AV, Anikwe JC, Okelana FA, Mokwunye IU, Azeez OM. Pesticidal efficacy of three tropical herbal plants' leaf extracts against *Macrotermes bellicosus*, an emerging pest of cocoa, *Theobroma cacao* L. *J Biopest*icides 2011; **4**(2): 131-137.

[31]Boko-Haya YY, Ouinsavi AIN. Christine O, Houngbeme A, Gbaguidi AF. Ethnic differences in use, phytochemical screening and non-poisonous leaves of *Phyllanthus amarus* (Schum & Thonn.) in North of Benin. *J Ethnobiol Tradit Med* 2016; **126**: 1185-1196.

[32]Bilal H, Hassan SA. Plants secondary metabolites for mosquito control. *Asian Paci J Trop Dis* 2012; **2**: 168-178.

[33]Pineda-Cortela MR, Cabantoga RY, Caasia PM, Chinga CA, Pereza JB, Godisana PG, et al. Larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against *Aedes aegypti*. *Pharm Biol* 2019; **57**(1): 120-124.

[34]Yu S. *Classification of insecticides: The toxicology and biochemistry of insecticides.* New York: Taylor and Francis Group; 2015, p. 184-185.

[35]Pani M, Nahak G, Sahu RK. Review on larvicidal activity of medicinal plants for malaria vector control. *Int J Curr Pharm Rev Res* 2015; **6**(2): 94-114.

[36]Rattan R. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protec* 2010; **29**(9): 913-920.

[37]Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes *viz*. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. *Appl Entomol Zool* 2008; **43**(1): 57-63.

[38]Kumar S, Yadav M, Yadav A, Yadav JP. Comparative analysis of antimicrobial activity of methanolic extracts of *Aloe vera* and quantification of aloe-emodin collected from different climatic zones of India. *Arch Clin Microbiol* 2015; **6**(2): 1-10.

[39]Hada D, Sharma K. Effect of different physical factors on *Cassia fistula* fruit pulp extract and their herbal formulation efficacy. *Glob J Pharmaceu Sci* 2017; **4**(2): 555632. doi: 10.19080/GJPPS.2017.04.555632.

[40]Medhi SM, Reza S, Mahnaz K, Reza A, Abbas H, Faemeh M. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*. *Asian Pac J Trop Med* 2010; **3**(11): 841-845.

[41]Francine TN, Cabral BNP, Anatole PC, Bruno MM, Pauline N, Jeanne NY. Larvicidal activities of hydro-ethanolic extracts of three Cameroonian medicinal plants against *Aedes albopictus*. *Asian Pac J Trop Biomed* 2016; **6**(11): 931-936.

[42]Salah EM, Ibrahim RR, Hussien A, Salem K, Gonaid MH, Soliman HSM. Phytochemical screening and insecticidal activity of different extracts of *Acacia modesta* Wall. on adult *Culex pipiens* mosquito. *Egypt J Hosp* 2018; **73**(11): 8022-8030.