https://dx.doi.org/10.4314/jpb.v21i1.5
Vol. 21 no. 1, pp. 32-41 (January 2024)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Bio-efficacy of essential oils of *Eryngium foetidum* L. and *Plectranthus amboinicus* (Lour.) Spreng against *Anopheles gambiae* Giles

Peace M. E. UBULOM[1*], Paul S. THOMAS[2], Akaninyene U. AKPAN[1], Edidiong J. UDOFA[3]

[1]*Department of Animal and Environmental Biology, Faculty of Biological Sciences, University of Uyo, Uyo, Nigeria.*
[2] *Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.*
[3] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.*

Received 12th January 2024; Accepted 26th January 2024

Abstract
Malaria and lymphatic filariasis transmitted by female *Anopheles* mosquitoes remain health challenges in tropical Africa. Resistance by vectors to synthetic insecticides limits control efforts. This study evaluated efficacy of essential oils of *Eryngium foetidum* and *Plectranthus amboinicus* against *Anopheles gambiae*. Test oils were obtained by hydro-distillation using Clevenger apparatus**.** From 1 mL stock solution, 31.25, 62.50, 125 and 250 and 500 µL/mL concentrations of *E. foetidum* and *P. amboinicus* oils were prepared using serial-dilution with tween 80 as diluent. Each concentration had eighty adult mosquitoes exposed. Results of knockdown and insecticidal tests after 30 and 60 minutes respectively revealed higher susceptibility of *An. gambiae* to *E. foetidum. Eryngium foetidum* (250µL/mL) elicited 100% knockdown after 20 minutes. In the insecticidal test, the same concentration resulted in 100% mortality at 40 minutes. Same effect was observed for 500 µL/mL of *P. amboinicus* oil at 25 and 60 minutes. From Probit analysis, KD_{50} values were 66.793 and 251.350 µL/mL for *E. foetidum* and *P. amboinicus* respectively. Values for LC_{50} were 85.818 and 428.663 µL/mL for *E. foetidum* and *P. amboinicus* respectively. Results demonstrate that *E. foetidum* oil holds promise as control agent against *An. gambiae*. *Plectranthus amboinicus* oil did not demonstrate appreciable efficacy**.**

Keywords: Essential oils; insecticidal; *Anopheles gambiae; Eryngium foetidum*; *Plectranthus amboinicus*

INTRODUCTION

Disease epidemic and endemicity are challenges of many regions of the world especially Africa, south of the Sahara. Some factors account for this such as anthropogenic activities that result in the creation of suitable breeding habitats for vector species, including insect vectors. For instance, dumping of wastes in gutters has resulted in clogged gutters that in turn serve as breeding sites for mosquitoes and other vector species. Improper waste disposal practices increase the number of discarded water-holding containers in the environment and also enhance the breeding of mosquitoes.

*Correspondence. *E-mail*: upema84@yahoo.com *Tel*: +234-8169046483. ISSN 0189-8442

According to the World Health Assembly (WHA) update, serious outbreaks of mosquito-borne diseases have affected populations with resultant mortalities in many countries around the globe [1]. Besides disease transmission is the biting nuisance caused by some insect vectors. Females of exophagic species of mosquitoes could make life outdoors including recreational activities intolerable. Also, some mosquito species are noisy and the sounds produced by these species at night, together with their bites could disturb rest and sleep, resulting in negative physiological effect.

Vector control remains a viable option in the control of insect-borne diseases and hence should be strengthened as a strategy for disease prevention and response to disease outbreaks. Over the years, synthetic/chemical insecticides have been relied upon in the control of insect vectors. However, their use has the demerit of not being eco-friendly. Another problem linked to them, is the development over time of physiological resistance to these chemical insecticides by some vector species [2, 3, 4, 5]. These demerits among others have created the need for continuous search for effective as well as safe methods of vector control. Some degree of success has been reported with the use of natural products of plant origin that are eco-friendly, biodegradable and relatively less toxic [6, 7, 8].

The scourge of insect-borne diseases calls for complementary efforts by governments, the health sector and researchers, towards effective and safe control strategies. Secondary metabolites and aromatic compounds in some plants have been shown in scientific studies to elicit insecticidal effects in insect vectors. In a study, it was reported that extracts of *Senna alata* have larvicidal efficacy against *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti* [6]. The use of *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides has been documented [7]. The efficacy of fixed oil obtained from the seed of *Annona muricata* was evaluated and it was reported that the oil holds potential as repellent against *An. gambiae* [8].

Eryngium foetidum L. (Apiaceae) is a herb indigenous to Central America and West Indies, but has been introduced into countries of the West Africa subregion, including Nigeria. It is an aromatic herb used in ethno-medicine and as a traditional spice for foods [9]. *Plectranthus amboinicus* (Lamiaceae) is a perennial herb, found throughout the tropics including Africa and also occurs in Asia and Australia. It is sometimes grown as an ornamental plant [10]; but it also has nutritional therapeutic and biological properties [11]. However, there is a paucity of scientific report on the efficacy of the oils of *E. foetidum* and *P. amboinicus* on adult *An. gambaie*.

The objective of this research was to evaluate the bio-efficacy of essential oils of the aerial parts of *E. foetidum* and the leaves of *P. amboinicus* against the biological vector, *An. gambiae.* This was determined by the knockdown and insecticidal effects of the test oil concentrations on the experimental vector species.

EXPERIMENTAL METHODS

Collection, identification and authentication of *E. foetidum* and *P. amboinicus.* The plants were obtained from the medicinal plant garden of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. Identification and authentication with voucher numbers UUH40026 and UUH40019 for *E. foetidum* and *P. amboinicus* respectively were done by a plant taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Nigeria.

Oil distillation. Aerial parts of *E. foetidum* and the leaves of *P. amboinicus* used for this research were processed by separately washing

and shade drying on laboratory bench for two hours for water to drain. This was followed by shredding, after which each plant sample was separately weighed using a triple beam balance and stored in sterile, labelled sample bags. Thereafter, extraction of oil from 1000 g of each plant sample was separately carried out by hydrodistillation using Clevenger type apparatus (5 L capacity), in accordance with the British Pharmacopoeia [12]. In each case, 2.5 litres of water was added to the 1000 g of plant sample in the flask of the Clevenger apparatus and heated over a heating mantle maintained at 70^{o}C for four hours. Oils were collected in labelled glass sample bottles. The oil obtained from each sample was separately dried over sodium sulphate and stored in a deep freezer (-4^{o}C) prior to use.

Test mosquito species. *Anopheles gambiae* mosquitoes used for this research were obtained from larval stock in the Insectary of the Malaria Vector Laboratory of the Department of Animal and Environmental Biology, Faculty of Science, University of Uyo, Nigeria. They were reared using standard procedures [13], until they attained adulthood before commencement of the experiments. Rearing involved the selection of fourth instar larvae of *An. gambiae* from the larval stock using pipettes and their transference into cups containing water (100 mL each). These cups were placed in screened metal cages 30 x 50 x 20 cm^3 (length, width and depth, respectively). Larvae were fed with nutrients consisting of a tiny pinch of fine Quaker oats dissolved in water [8, 14, 15]. Emerged pupae were removed and placed in separate cups containing 100 mL water. No nutrients were added. They were also kept in the cages for adult emergence. Adults were identified by their possession of a distinct head, three thoracic segments and abdominal segments. They also had three pairs of jointed limbs and a pair of wings amongst other adult features. Adult *An. gambiae* mosquitoes that emerged were fed on 10% sucrose solution soaked in cotton pads [16].

Preparation of test oil concentrations. The oil concentrations used for this study were separately prepared for each plant oil from the stock (1 mL of pure oil), by a two-fold serial dilution, using 10% tween 80 as the diluent. From the dilutions, 31.25, 62.50, 125, 250 and 500 µL/mL of essential oils of *E. foetidum* and *P. amboinicus* were obtained for the experiments. Each control set-up consisted of 1 mL of 10% tween 80.

Knockdown tests. The knockdown potential of *E. foetidum* and *P. amboinicus* oils were separately tested against adult *An. gambiae*, in the laboratory of the Department of Animal and Environmental Biology, Faculty of Science, University of Uyo, Nigeria.

Each test oil had five (5) concentrations and each concentration was replicated four (4) times. The control which consisted of 1 mL of 10 % tween 80 was also replicated four times. Adult *An. gambiae* mosquitoes collected from the rearing cage, using an aspirator were exposed to each experimental set-up and the controls in WHO Insecticide Susceptibility Test tubes. For each replicate, twenty (20) adult mosquitoes were exposed. In other words, for each test concentration, a total of eighty (80) adult *An. gambiae* mosquitoes were exposed. The same was done for the control set-up and the replicates. The knockdown efficacy of each oil was separately determined by impregnating 12 x 15 cm of Whatman filter paper No.1 with 1 mL of the test oil, using a micropipette. Each impregnated filter paper was air-dried for 5 minutes before insertion into the WHO susceptibility test tubes. This was done according to standard procedures [17]. Observation of the knockdown effect of each oil on the test mosquitoes was done over a total exposure period of 30 minutes at intervals of 5 minutes. This was done by visual observation.

Insecticidal tests. For the determination of the insecticidal efficacy of the oils, five (5) oil concentrations (31.25, 62.50, 125, 250 and 500 µL/mL) were prepared for each oil. Each concentration had four (4) replicates. The control and the replicates consisted of 1 mL 10% tween 80 each. Adult mosquitoes were collected from the cages as was done in the knockdown tests and exposed to the test oil concentrations and their controls in transparent WHO Insecticide Susceptibility Test tubes. In each experimental set-up, twenty (20) adult *An. gambiae* mosquitoes were exposed, totaling eighty (80) mosquitoes per oil concentration and control. Insecticidal efficacy of each oil concentration and their control was determined for 60 minutes at intervals of 10 minutes each.

Statistical analysis. Data generated from this research were analyzed using one-way analysis of variance (ANOVA) to determine if the observed difference in the potency of the oils was significant. Probit analysis involving the determination of median lethal concentrations (LC_{50}) of the oils and their confidence intervals was also carried out using data from this study. Log-time Probit model was also used for the estimation of knockdown doses (KD). All data were analyzed using SPSS version 21.

RESULTS

Percentage oil yield. Hydro-distillation of the experimental plants yielded 0.05 % and 0.20% of essential oils from *E. foetidum* and *P. amboinicus* respectively.

Knockdown effect of the oils of *E. foetidum* and *P. amboinicus.* On exposure of the test mosquitoes to the lowest concentration (31.25 µL/mL) of the oil of *E. foetidum*, a total of 10 test mosquitoes representing 12.5% were knocked down at 15 minutes exposure period. At the end of 30 minutes the same concentration elicited 40% knockdown effect on the mosquitoes. *Eryngium foetidum* oil (500 µL/mL) knocked down 100% of the mosquitoes at 10 minutes exposure period. Results showing the knockdown effect of other concentrations of the oil are presented in Table 1 (a & b). Another observation made was that all knocked down mosquitoes exposed to 500 µL/mL of the oil of *E. foetidum* were moribund. Similarly, the mosquitoes that were not knocked down on exposure to the same concentration (500 µL/mL) for 5 minutes were no longer agile.

In this study 50% of the test mosquitoes exposed to 62.50 µL/mL of the oil of *P. amboinicus* were knocked down at exposure period of 30 minutes. When the mosquitoes were exposed to 250 µL/mL of the oil, 95% of the mosquitoes were knocked down at the end of the exposure period of 30 minutes. *P. amboinicus* oil at a concentration of 500 µL/mL elicited 100% knockdown of the mosquitoes at exposure period of 25 minutes. Other results on the knockdown effect on mosquitoes exposed to the other concentrations of the oil of *P. amboinicus* are presented in Table 2 (a & b). Mosquitoes exposed to the control (10% tween-80) and replicates were not knocked down throughout the duration of the experiment (30 minutes).

Insecticidal effects of oils of *E. foetidum* and *P. amboinicus.* Observations made on the insecticidal effect of 31.25 µL/mL of the oil of *E. foetidum* revealed 55% mortality of the test mosquitoes at the end of the exposure period of 60 minutes, whereas exposure of the test mosquitoes (*An. gambiae*) to 250 µL/mL of the same oil resulted in 100% mortality of the insects at exposure period of 40 minutes (Table 3a &b). Total mortality (100%) was elicited by the highest concentration of the oil (500 µL/mL) after an exposure period of 10 minutes. Other results on the insecticidal effect of the other concentrations of essential oil of *E. foetidum* are as presented (Table 3a &b).

Table 1a: Knockdown Effect of Different Concentrations of *E. foetidum* oil on *An. gambiae*

Concentration (µL/mL)	Total Number Knocked Down (%)/Time of Exposure					
	5 min	10 min	15 min	20 min	25 min	30 min
31.25	0(0)	0(0)	10(12.5)	20(25)	27(33.75)	32(40)
62.5	14(17.5)	28(35)	40(50)	52(65)	65(81.25)	80(100)
125	22(27.5)	44(55)	56(70)	68(85)	80(100)	80(100)
250	28(35)	56(70)	70(87.5)	80(100)	80(100)	80(100)
500	50(62.5)	80(100)	80(100)	80(100)	80(100)	80(100)
Control (1mL of 10% Tween 80)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

No. of test mosquitoes per group (n)=80; Values in bracket are percentage knockdown values

Table 1b: Mean Knockdown values of *An. gambiae* exposed to different concentrations of *E. foetidum* oil

Concentration (µl/mL)	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes
31.25	0.00±0.00^{d}	0.00±0.00^{e}	2.50±0.29^{e}	5.00±0.58^{d}	7.00±0.58^{c}	8.00±0.00^{b}
62.5	3.50±0.29^{c}	7.00±0.58^{d}	10.00±0.58^{d}	13.00±0.58^{c}	16.50±0.29^{b}	20.00±0.00^{a}
125	5.50±0.29^{b}	11.00±0.58^{c}	14.00±0.00^{c}	17.00±0.58^{b}	20.00±0.00^{a}	20.00±0.00^{a}
250	7.00±0.58^{b}	14.00±1.15^{b}	17.50±0.29^{b}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}
500	12.50±1.44^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}
Control (1mL of 10% Tween 80)	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
Total	**4.75±0.93**	**8.67±1.53**	**10.67±1.54**	**12.50±1.59**	**13.92±1.62**	**14.67±1.65**
p Value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

No. of test mosquitoes per group (n)=80; Values of knockdown are Mean ± SE Means along same column with same superscript = not significantly different. Means along same column with different superscripts = significant

Table 2a: Knockdown effect of different concentrations of *P. amboinicus* oil on *An. gambiae*

Concentration (µL/mL)	Total Number Knocked Down (%)/ Time Exposure					
	5 min	10 min	15 min	20 min	25 min	30 min
31.25	0(0)	0(0)	0(0)	9(11.25)	10(12.5)	20(25)
62.5	0(0)	0(0)	9(11.25)	18(22.5)	30(37.5)	40(50)
125	0(0)	6(7.5)	21(26.25)	34(42.5)	45(56.25)	58(72.5)
250	0(0)	16(20)	31(38.75)	54(67.5)	67(83.75)	76(95)
500	15(18.75)	30(37.5)	35(42.5)	70(87.5)	80(100)	80(100)
Control (1mL of 10% Tween 80)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

No. of test mosquitoes per group (n)=80; Values in bracket are percentage knockdown values

Table 2b: Mean Knockdown values of *An. gambiae* exposed to different concentrations of *P. amboinicus* oil

Concentration (µl/ml)	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes
31.25	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{e}	2.50±0.29^{e}	2.50±0.29^{e}	5.00±0.58^{d}
62.5	0.00±0.00	0.00±0.00^{d}	2.00±0.00^{d}	4.50±0.29^{d}	7.50±0.29^{d}	10.00±0.58^{c}
125	0.00±0.00	1.50±0.29^{c}	5.50±0.29^{c}	8.50±0.29^{c}	11.00±0.58^{c}	14.50±0.87^{b}
250	2.00±0.00	4.00±0.58^{a}	7.00±0.58^{b}	13.50±0.87^{b}	16.50±0.87^{b}	19.00±0.58^{a}
500	4.00±0.00	7.50±0.29^{a}	8.50±0.29^{a}	17.50±0.29^{a}	20.00±0.00^{a}	20.00±0.00^{a}
Control (1mL of 10% Tween 80)	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{f}	0.00±0.00^{f}	0.00±0.00^{e}
Total	**1.00±0.32**	**2.17±0.59**	**3.83±0.71**	**7.75±1.29**	**9.58±1.50**	**11.42±1.52**
p Value	NA	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

No. of test mosquitoes per group (n)=80; Values of knockdown are Mean ± SE Means along same column with same superscript = not significantly different. Means along same column with different superscripts = significant

Table 3a: Insecticidal effect of different concentrations of *E. foetidum* oil on *An. gambiae*

Concentration (µL/mL)	Total Mortality (%)/Time of Exposure					
	10 min	20 min	30 min	40 min	50 min	60 min
31.25	0(0)	0(0)	4(5)	16(20)	36(45)	44(55)
62.5	0(0)	0(0)	32(40)	52(65)	64(80)	68(85)
125	0(0)	16(20)	52(65)	68(85)	76(95)	76(95)
250	0(0)	36(45)	72(90)	80(100)	80(100)	80(100)
500	80(100)	80(100)	80(100)	80(100)	80(100)	80(100)
Control (1mL of 10% Tween 80)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

No. of test mosquitoes per group (n)=80; Values in bracket are percentage mortality values

Table 3b: Mean mortality values of *An. gambiae* exposed to different concentrations of *E. foetidum* oil

Concentration (µL/mL)	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
31.25	0.00±0.00	0.00±0.00^{d}	1.00±0.58^{e}	4.00±0.58^{d}	9.00±1.15^{c}	11.00±0.58^{c}
62.5	0.00±0.00	0.00±0.00^{d}	8.00±1.15^{d}	13.00±0.58^{c}	16.00±1.15^{b}	17.00±0.58^{b}
125	0.00±0.00	4.00±0.58^{c}	13.00±0.58^{c}	17.00±0.58^{b}	19.00±0.58^{a}	19.00±0.58^{a}
250	0.00±0.00	9.00±0.58^{b}	18.00±0.00^{b}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}
500	20.00±0.00	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}	20.00±0.00^{a}
Control (1 mL of 10% Tween 80)	0.00±0.00	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{d}
Total	**3.33±1.55**	**5.50±1.52**	**10.00±1.62**	**12.33±1.62**	**14.00±1.55**	**14.50±1.51**
p Value	NA	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

No. of test mosquitoes per group (n)=80; Values of mortality are Mean ± SE Means along same column with same superscript = not significantly different. Means along same column with different superscripts = significant

Table 4a: Insecticidal effect of different concentrations of *P. amboinicus* oil on *An. gambiae*

Concentration (µL/mL)	Total Mortality (%) / Time of Exposure					
	10 min	20 min	30 min	40 min	50 min	60 min
31.25	0(0)	0(0)	0(0)	2(2.5)	12(15)	22(27.5)
62.5	0(0)	0(0)	0(0)	6(7.5)	24(30)	44(55)
125	0(0)	0(0)	0(0)	22(27.5)	44(55)	60(75)
250	0(0)	0(0)	14(17.5)	44(55)	62(77.5)	78(97.5)
500	0(0)	0(0)	30(37.5)	60(75)	76(95)	80(100)
Control (1mL of 10% Tween 80)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

No. of test mosquitoes per group (n)=80; Values in bracket are percentage mortality values

Table 4b: Mean mortality values of *An. gambiae* exposed to different concentrations of *P. ambionocus* oil

Concentration (µL/mL)	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes	60 minutes
31.25	0.00±0.00	0.00±0.00	0.00±0.00^{c}	0.50±0.29^{d}	3.50±0.29^{e}	5.50±0.29^{d}
62.5	0.00±0.00	0.00±0.00	0.00±0.00^{c}	1.50±0.29^{d}	6.00±0.58^{d}	11.00±0.58^{c}
125	0.00±0.00	0.00±0.00	0.00±0.00^{c}	5.50±0.29^{c}	11.00±0.00^{c}	15.00±0.58^{b}
250	0.00±0.00	0.00±0.00	3.50±0.29^{b}	11.00±0.58^{b}	15.50±0.29^{b}	19.50±0.29^{a}
500	0.00±0.00	0.00±0.00	7.50±0.87^{a}	15.00±1.15^{a}	19.00±0.58^{a}	20.00±0.00^{a}
Control (1 mL of 10% Tween 80)	0.00±0.00	0.00±0.00	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{f}	0.00±0.00^{e}
Total	**0.00±0.00**	**0.00±0.00**	**1.83±0.61**	**5.58±1.20**	**9.17±1.40**	**11.83±1.52**
p Value	NA	NA	<0.001*	<0.001*	<0.001*	<0.001*

No. of test mosquitoes per group (n)=80; Values of mortality are Mean ± SE Means along same column with same superscript = not significantly different. Means along same column with different superscripts = significant

Table 5: 30 Minute KD_{50} values of *E. foetidum* and *P. amboinicus* oils tested on *An. gambiae*

Oils	KD_{50} Values (µL/mL)	Confidence Interval (µL/mL)
E. foetidum	66.793	52.544 - 81.512
P. amboinicus	251.350	199.191 - 338.182

Table 6: 60 Minute LC_{50} values of *E. foetidum* and *P. amboinicus* oils tested on *An. gambiae*

Oils	LC_{50} Values (µL/mL)	Confidence Interval (µL/mL)
E. foetidum	85.818	64.338 - 110.086
P. amboinicus	428.663	289.758 - 843.477

When adult *An. gambiae* mosquitoes were exposed to 250 µL/mL of essential oil of *P. amboinicus*, mortality of 97.50% was observed at the end of the exposure period (60 minutes). However, exposure to a higher concentration (500 µL/mL) of the oil resulted in 100% mortality of the mosquitoes at the end of the exposure period of 60 minutes. Other results are presented in Table 4 (a & b). No mortality was observed in the control (10% tween- 80) and replicates throughout the exposure period of 60 minutes.

Median 30 minutes KD_{50} and 60 minutes LC_{50} values of essential oils of *E. foetidum* and *P. amboinicus.* The 30-minute KD_{50} and 60-minute LC_{50} values of essential oils of *E. foetidum* and *P. amboinicus* tested against *An. gambiae* and their confidence intervals are presented in Tables 5 and 6, respectively.

DISCUSSION

This research was carried out to evaluate the bio-efficacy of essential oils of *E. foetidum* and *P. amboinicus*, against adult mosquitoes of the species *An. gambiae*. From the yield values obtained for both oils, *P. amboinicus* was richer in essential oil content (0.2%) than *E. foetidum* (0.05%). The knockdown and insecticidal effects elicited by the test oils on *An. gambiae* demonstrate the potency of the oil of *E. foetidum* against the mosquito species. Although, the oil of *P. amboinicus* elicited some knockdown and mortality effects, its efficacy was not appreciable when compared with the oil of *E. foetidum*. These results revealed that the effect of the oils were concentration and time dependent. Studies on the chemical composition and antioxidant capacity of *E. foetidum* [18] and on the insecticidal and phytotoxic activity of essential oil of *E. foetidum* [19] revealed that the oil consists of aliphatic and aromatic compounds that possess biological activities. It was reported that the essential oil of *E. foetidum* which is used extensively as a medicinal herb in most tropical regions was active among others against *Hyalomma lusitanicum* and could therefore be used to develop a natural biocide/acaricide for the control of *H. lusitanicum* [19]. They attributed this to the rich aldehyde and benzene derivatives composition of essential oil of *E. foetidum.* The essential oil of *E. foetidum* has been reported to possess no adverse phytotoxic effects [19]. Also, the repellency and contact toxicity of essential oil of *E. foetidum* to adults of the red flower beetle *Tribolium castaneum* were documented [20]. Findings from this study also reveal that the oil of *E. foetidum* was potent against *An. gambiae* mosquitoes.

This research revealed a concentration and time dependent effect of oil from the fresh leaves of *P. amboinicus* against *An. gambiae* adults too. Studies on the biological activity of oil from *P. amboinicus* against the seaside mosquito *Ochlerotatus caspius* [21] and *Aedes aegypti* [22] have been documented. Their results revealed that the oil was potent against the test mosquito species. In another study, it was reported that oil from the leaves of *P. amboinicus* is particularly rich in phenolic monoterpenes such as thymol and carvacrol

and these are speculated to exert various pharmacological and biological properties [11].

The GC-MS analysis of the chemical constituents of the oils used in this study which is another segment of this research is on-going and yet to be completed. However, evaluation of the effect of the oil of *P. amboinicus* on *Anopheles stephensi* was carried out and the reported efficacy of the oil was attributed to the presence of carvacrol and thymol [23]. In this study, the observed knockdown and insecticidal effects of the oil of *P. amboinicus* on the test mosquito species (*An. gambiae*) though not very significant is attributable to the compounds present in the test oil. The chemical composition of essential oils is known to vary due to factors such as geographical region, plant variety, age, time of harvesting and method of processing. This may account for the observed difference in the activity of the two oils, though both oils possess aromatic and other compounds. These compounds may have elicited their effects singly or in synergy. Review comments [24] buttress this assertion.

Toxicity of essential oil from *P. amboinicus* (Indian borage) on the larvae of *An. gambiae* was tested using 3.125, 6.25, 12.5, 25, 50 and 100 ppm oil concentrations [25]. Observations were made after 12, 24 and 48 hours. Mortality of 100% was recorded for larvae of the laboratory colony after 48 hours. Larvae of the wild population showed relatively poor response to the essential oil. Thus, their results revealed differential susceptibility of larvae of *An. gambiae* obtained from laboratory colony and the wild population [25].Observation made as well as results obtained from this study revealed differential susceptibility of the test mosquitoes to the two oils. Although the oil of *P. amboinicus* has been reported to be effective against other vector species [21, 22, 23], in this investigation, it did not prove to be very effective against *An. gambiae.* Different pest species are known to react differently to different classes of pesticides and this may have accounted for the observed difference in the susceptibility of *An. gambiae* to the two oils.

The higher efficacy of essential oil of *E. foetidum* than the oil of *P. amboinicus* against *An. gambiae* was substantiated by the fact that exposure of the test mosquitoes to 250 µL/mL of *E. foetidum* oil resulted in 100% knockdown of the mosquitoes at an exposure period of 20 min; whereas the same concentration of *P. amboinicus* oil at the same exposure period (20 min) elicited 67.50% knockdown effect on the mosquitoes. Also, 250 µL/mL of the oil of *E. foetidum* resulted in 100% mortality at the 40^{th} minute of exposure, while 55% mortality was observed at the same exposure period (40 min.) with 250 µL/mL of the oil of *P. amboinicus.* Only exposure to 500 µL/mL of the oil of *P. amboinicus* resulted in 100% mortality of the test mosquitoes. To further buttress the higher potency of the oil of *E. foetidum* to the mosquitoes than the oil of *P. amboinicus* were the 30 minutes KD_{50} and the 60 minutes LC_{50} values obtained from probit analysis which were 66.793µl/ml and 85.818µl/ml for *E. foetidum* and 251.350µL/mL and 428. 663µL/mL for *P. amboinicus* respectively. The KD_{50} and LC_{50} values represent the dose/concentration of a substance that knocks down and kills 50% of the test population respectively, during the observation period. The smaller the value the more toxic the test substance. The larger the KD_{50} or LC_{50} value, the lower the toxicity of the substance. That the test mosquitoes were not knocked down and no mortality of mosquitoes was observed on exposure to 10% tween-80 (control and replicates) in the knockdown and insecticidal tests implies that tween-80 did not exert deleterious effect on adult *An. gambiae.*

There are limitations associated with the use of synthetic insecticides which include but not limited to the development of

resistance to these synthetic insecticides by vector species. Natural products of plant origin with insecticidal potential could be used to circumvent these limitations. Plants possess biologically active compounds known as phytochemicals, some of which function as feeding deterrents, growth regulators, repellent and toxins against insect pest/vector species. Since these are known to be less toxic, biodegradable and induce less resistance [26], they could be explored in the search for potent insecticidal agents to combat mosquito borne diseases including malaria and lymphatic filariasis, transmitted by females of the species *An. gambiae.*

Acknowledgements. Authors appreciate laboratory staff of the Departments of Pharmacognosy and Natural Medicine and Animal and Environmental Biology, University of Uyo, Nigeria, for their technical assistance. Authors also owe a debt of gratitude to Mr. Idongesit A. Umohata and Dr. Clement A. Yaro for their technical assistance.
This research was funded by TETFUND through Award Number: TETF/DR&D/CE/UNI/UYO/IBR/2020/VOL 1).

REFERENCES

1. World Health Assembly (WHA). The Global Vector Control Response (GVCR) 2017 – 2030 7th World Health Assembly Update 30:5:2017.

2. Silva APB, Santos JMM, Martins AJ. Mutations in the voltage-gated sodium channel gene of anophelines and their association with resistance to pyrethroids – A review. Parasit. & Vect. 2014; 7(1):1371-1387.

3. Aguirre-Obando OA, Bona ACD, Duque L, Jonny E, Navaro-Silva MA. Insecticide resistance and genetic variability in natural populations of *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicdae) from Colombia. Zoologia (Curitiba) 2015; 32(1):14-22.

4. Aguirre-Obando OA, Pietrobon AJ, Dalla AC, Bona ACD, Navaro-Silva MA. Contrasting patterns of inseticde resistance and knockdown resistance in *Aedes aegeypti* populations from jacarezinho (Brazil) after a Dengue outbreak. Revista Brasileriria de entomologia 2016; 60(1):94-100

5. Seixas G, Grigoraki L, Weetman D, Vincente J L, Silva AC, Pinto J, Vontas J. and Sousa, CA. Insecticide resistance is mediated by multiple mechanism in recently introduced *Aedes aegypti* from Madeira Island (Portugal). Plos Neg. Trop. Dis. 2017; 11(7):1371-1387.

6. Ubulom PME, Imandeh GN, Akpabio EE, Opara K N, Ekanem M S. Larvicidal Effect of aqueous and ethanol extracts of *Senna alata* on *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti* Pak. J. Pharm. Sci. 2013; 26(3):561-566.

7. Chaudhary S, Kanwar R, Sehgal A., Cahill D, Barrow C, Sehgal R, Kanwar, J. Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. Front. Plant Sci. 2017; 8:600-610.

8. Ubulom PME, Umohata IA, Thomas PS, Ekpo ND, Jamabo RT. Efficacy of the Seed Oil, Leaf Extract and Fractions of *Annona muricata* as Repellent and Larvicide against *Anopheles gambiae*. Ann. Res. Rev. Bio. 2019; 34(1):1-13.

9. Jaramillo BE, Duarte E, Martelo I (2011). Volatile chemical composition of the essential oil from Colombian *Eryngium foetidium* L. and determination of its antioxidant activity. Rev. Cuba Plant. Med. 2011; 16:140-150

10. Lukhoba CW, Simmonds MSJ, Paton AJ. Plecthranthus: A review of ethnobotanical uses. J. of Ethnopharm. 2006; 103:1-24.

11. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinius* (Lour.) Spreng: Botanical, Phytochemical Pharmacological, and Nutritional Significance. Molecules 2016; 21:369.

12. Medicines and Health Care Products Regulatory Agency (MHRA) British Pharmacopoeia. The Book Depository Ltd. YK/TSO. 2018.

13. Ejeta D, Asme A, Asefa A. Insecticidal Effect of Ethnobotanical Plant extracts against *Anopheles arabiensis* under laboratory conditions. Malar. J. 2021; 20:466.

14. Umohata IA, Ubulom PME, Udofa EJ, Bala D N, James IV. Potentials of Ethanol Extracts of Aerial parts of *Diplazium esculentum* (Retz) RW. as Larvicide against *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say. Int. J. Trop. Dis. and Hlth. 2020; 41(3):40-47.

15. Umohata IA, Ubulom PME, Thomas PS, Nwangwu UC. Potency of Ethanol Extracts and

Fractions of the Leaf of *Annona muricata* against *Aedes aeygpti* Larvae. Nig. J. Para. 2020; 41(2):198-204.

16. Pappathi T, Dharani P, Packiam M, Martin P, Elumalai K. Mosquitocidal properties of *Plectranthus amboinicus* oil tested against the important three Human vector mosquitoes (Diptera: Culicidae). J. Emer. Tech. Inno. Res. (JETIR) 2021; 8(10): 255-270.

17. World Health Organisation (WHO). Test Procedure for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. 2nd Edition. WHO Document Production Services. Geneva, Switzerland. 2016.

18. Thomas PS, Essien EE, Ntuk SJ, Choudhary MI. *Eryngium foetidum* L. Essential Oils: Chemical Composition and Antioxidant Capacity. Medicines (Basel, Switzerland) 2017; 4(2):24. https://doi.org/10.3390/medicines4020024

19. Rodriguez-Orozio V, Jaramillo-Colorado BE, Olmeda AS. Insecticidal and phytotoxic activity of essential oil from Colombian *Eryngium foetidum* L. *Revista Colombiana de Ciencias horticolasi* 2023; 17(3): https:// doi.org /10.17584 /rcch. 2023 v17i 3.15925.

20. Ruchuon W, Mongkol W. Toxicity and Bioactivity of Essential oil of Cilantro (*E. foetidum* L.) against red flower beetle (*Tribolium castaneum* Herbst). Austr. J. of Crp. Sci. 2022; 16(02):259-265. doi: 10:214751ajcs.22.16.02.3414.

21. Knio KM, Usta J, Dagher S, Zournajiam H, Kreydiyyeh S. Larcividal activity of Essential oil extracted from commonly used herbs in Lebanon against the seaside mosquito, Ochlerotatus caspius Bioresour. Technol. 2008; 99.763-768 doi:10: 1016/j.biortech.2007.01.026.

22. Paramasivam D, Balasubramanian B, Park S, Alagappan P, Kaul T, Liu, W, Pachiappan P. Phytochemical profiling and biological activity of *Plectranthus amboinicus* (Lour.) mediated by various solvent extracts against *Aedes aegypti* larvae and toxicity evaluation: Asian Pac. J. of Trop. Med. 2020; 13(11):494-502.

23. Annaduri S, Venugoplan V. Chemical composition and larvicidal activity of Essential oil of *Pletranthus amboinicus* (Lour.) Spreng against *Anopheles stephensi*: a malaria vector mosquito. Para. Res. 2010; 107:1275-1278

24. Meryem SD, Emel C. Plant Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects*, 2022; 13(2):162. doi 10.3390insects/13020162.

25. Kweka EJ, Senthilkumar A, Venkatesalu V. Toxicity of Essential Oil from Indian borage on the larvae of the African malaria vector mosquito, *Anopheles gambiae*. Para. & Vec. 2012; 5:277 http://www.parasitesandvectors.com/content/5/1/227

26. Ohia CMD, Ana GREE. Bio-insecticides: The one-health response to mosquito borne diseases of public health importance. J. of Bio. Agric. & Hlthcare. 2015; 5:22-26.