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Adulticidal efficacy of *Annona senegalensis* and *Boswellia dalzielii* Leaf fraction extracts and essential oils against *Anopheles gambiae* Gilles, *Aedes aegypti* Linn and *Culex quinquefasciatus* say using CDC bottles

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Abstract
The repeated and misuse of synthetic insecticides for mosquito control has raised the problem of human toxicity, environmental pollution and insect resistance, and the search for alternative mosquito control methods using botanicals is nowadays warmly encouraged. For that, the methanolic crude extracts, fractions (n-hexane, chloroform, ethylacetate and methanol) and essential oils of the leaves of *Annona senegalensis* and *Boswellia dalzielii* were essayed for their toxic properties against the adults of *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* in the laboratory using CDC bottles method. The plant extracts and fractions were applied at doses of 312.5, 625, 1250 and 2500 mg/bottle while essential oils were tested at the concentrations ranging from 25 to 200 mg/bottle on adults of the three mosquito species. DDVP (2.2-dichlorovinyl dimethyl phosphate, 2000 mg/bottle) and acetone (1mL) were used as positive control and negative controls, respectively. Mosquito mortality was monitored after 24 h post-exposure plant extracts/fractions and 1, 6, 12, 18 and 24 h post-treatment for essential oils. In results, extracts and fractions of the two plant species caused a moderate mortality of the three mosquito species adults and the significant mortality (≥ 60%) was obtained with n-hexane fraction of the two plants against *An. gambiae* adults. The n-hexane fractions of *A. senegalensis* (1636.39 mg/bottle) and *B. dalzielii* (1836.7 mg/bottle) were the most potent against *An. gambiae* compared to other fractions and the two other mosquito species. Essential oils of the two plant species tested at 200 mg/bottle as well as DDVP (2000 mg/bottle) achieved complete mortality (100%) of the three mosquito species. LC50 values recorded after 24h post-treatment were 2.15, 12.23 and 13.44 mg/bottle for *A. senegalensis* and 7.33, 11.34 and 8.95 mg/bottle for *B. dalzielii*, respectively against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquito adults. Thus, n-hexane fraction and essential oils of *A. senegalensis* and *B. dalzielii* could be a source of safe and very potent insecticidal products for the preparation of traditional or refined insecticide formulations for mosquito control.

Keywords: Adulticidal, plant species, extracts fractions, essential oils, mosquito species

1. Introduction
Mosquitoes are considered nowadays as the most medically significant vectors, since they are continue to harm human, disrupt societies over the millennia and continue to have socio-economic and devastating impacts on human beings [1, 2]. Worldwide, those insects belonging to *Anopheles*, *Aedes* and *Culex* genera are mostly involved in the transmission of parasitic and viral diseases including malaria, yellow fever, dengue fever, diverse encephalitis forms and lymphatic filariasis, etc. [3]. In sub-Saharan Africa, *Anopheles gambiae* is the most important widely distributed and the most efficient vector of malaria [4]. According to WHO [5], 91% of world deaths occur in Africa and in 2016, 216 million malaria cases with 445,000 people died was reported. Dengue, yellow fever and chikungunya are arbovirus diseases transmitted through the bites *Aedes aegypti* and their incidence is also growing drastically around the world in recent decades [6]. Worldwide, 1.3 billion people in 72 countries are at risk of contracting the lymphatic filariasis transmitted by *Culex quinquefasciatus*, with 120 million people reported being infected, 65% of them living in Southeast Asia and 30% in Africa [7].
In the mosquito vector control programs, the application of synthetic insecticides is largely used and remains one of the most important strategies in the prevention and control of

mosquito-borne diseases. Like that, the most of the important methods commonly used for mosquito adults control include the long lasting insecticidal treated nets (LLINs) and the indoor residual spraying (IRS). However, synthetic insecticides used caused problems such as environmental pollution, the high operational cost, toxicity to humans and harmful effect on non-target organisms, as well as the development of insect resistance to those synthetic products, and these created the need for developing alternative approaches to control mosquito pest [8-10]. The characteristic of that alternative insecticide implied its adequate efficacy toward the target pest, rapid degradability, and safe to humans and other non-target species [11]. Botanical products (extracts, essential oils, etc.) with their richness in potent insecticide phytochemical constituents could be that alternative solution in the insect pest control programs. Moreover, there is a low chance for insect to develop resistance to plant products since these derived plant products are diversified in phytochemical components having different mode of actions compared to the synthetic insecticides with a single active ingredient [12, 13]. For these reasons, much research effort has been focused on plant extracts and essential oils or their constituents as potential sources of insect control agents. In this context, *Annona senegalensis* and *Boswellia dalzielii* belonging to the families of Annonaceae and Burseraceae respectively may rank among the most important insecticide plants. Several studies on the insecticidal properties of essential oils from *A. senegalensis* and *B. dalzielii* have been documented. Previous works reported the effectiveness of *A. senegalensis* products against stored products pests including *Sitophilus zeamais*, *Caryedon serratus*, *Tribolium castenum* and *Callosobruchus maculatus* [14-20]. The plant products were reported to be toxic against the immature stage development of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* [21-25] and also against fleas and lice in poultry [26].

Similarly, *Boswellia dalzielii* gum bark protected wood from termite attacks; its fumigation repels flies and mosquitoes [27, 28] and protected cereals insect attacks in the storage [17]. The mosquitocidal efficacy of the plant was reported against larvae of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* [24]. The present study aimed to evaluate the lethal (adulticidal) activity of extracts, fractions and essential oils from *A. senegalensis* and *B. dalzielii* plants against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* adults using CDC bottles method.

2. Materials and Methods

2.1 Collection and processing of plant materials

The leaves of *A. senegalensis* and *B. dalzielii* were harvested respectively at Dang in the Adamaoua region of Cameroon in November of 2011 and at Midjivin in the Far North region of Cameroon in December of 2011. The two plant species were identified and confirmed under the registration number of 7783/SRF-CAM for *A. senegalensis* and 20532/SRF-CAM for *B. dalzielii* at the National Herbarium of Cameroon. The leaves were dried in a room under ambient condition, then pulverised with an electric grinder and passed through 0.4 mm mesh size sieve. Then, powders were stored at -18ºC in a deep freezer until their utilization for extraction.

2.2 Extraction and fractionation of plants

The fractionation of each plant methanolic crude extract was done according to the method of Gueye *et al*. [19]. Indeed, 200 g of *A. senegalensis* and *B. dalzielii* crude extract each was split successively following the method of differential solubility in four solvents of different polarity including n-hexane, chloroform, ethyl acetate and methanol solvents according to the diagram below (Figure 1). Each fraction recovered was concentrated using Rotary evaporator and the yield of each the solid fraction gotten was stored at -4°C in refrigerator until needed bioassays.

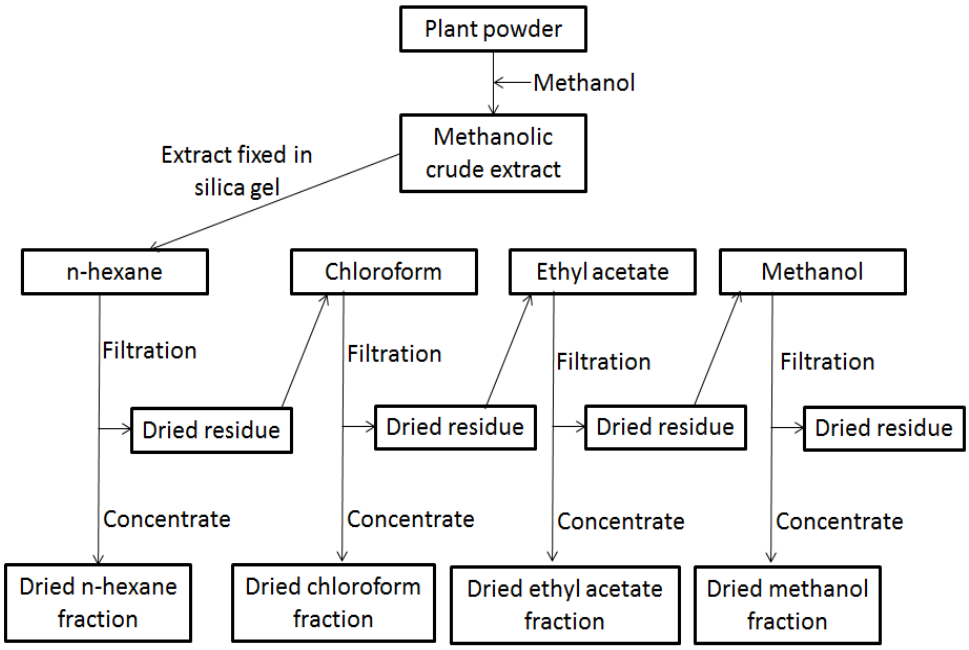

Fig 1: Diagram of extraction and fractionation of the plants

2.3 Extraction of plant essential oils

The finely grounded plant materials were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus (Garg Process Glass India Private Ltd, India). Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C in refrigerator until needed for bioassay.

2.4 Collection and rearing of mosquito species

The larvae of *An. gambiae* were collected from water in gutters in February 2013 at Awka market, Anambra State, Nigeria with the assistance of experts from the National Arbovirus Research Center (NAVRC), Enugu, Nigeria; kept in plastic trays containing tap water and were fed with a diet containing crayfish and biscuit (in ratio 3:1). *Ae. aegypti* larvae were obtained from laboratory cultures in the Department of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria and "chicken feed" food was provided to them. The larvae of *Cx. quinquefasciatus* were collected from National Arbovirus Research Center (NAVRC), Enugu, Nigeria in January 2013 and were fed with "chicken feed". The three mosquito species were reared in the insectarium of the Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria under fluctuating temperature and relative humidity (25±2ºC; 80-90% RH) under a photoperiod of 12L:12D and 12L:12D.

2.5 Adulticidal test using CDC bottles.

The solutions were prepared and the bottles coated according to the CDC [29] protocol while bioassay procedure was performed following Aizoun *et al*. [30] method.

2.5.1 Preparation of stock solutions

The bottles used for the bioassay need to be coated inside with the diagnostic dose of the insecticide under evaluation. The diagnostic dose is a determined amount of insecticide per bottle after preliminary screening test. For that issue, the extracts/fractions and essential oils were dissolved in acetone (Sigma-Aldrich, USA). The choice of this chemical was about the ability of this solvent to evaporate very fast. To obtain the concentrations of 12.5, 25, 50, 100 and 200 mg/bottle of essential oils; 312.5, 625, 1250 and 2500 mg/bottle of the extracts/fractions, products were dissolved in adequate quantity of acetone to make 10 mL of total solution. Each 1 mL of this solution would contain 12.5, 25, 50, 100 and 200 mg of the essential oils and 312.5, 625, 1250 and 2500 mg of extracts/fractions insecticides.

2.5.2 Procedure of cleaning, drying and coating the bottles

Bottles (250 mL) were cleaned with soap and rinsed with tap water. After bottles completely dried in the Oven set at 60°C, 1 mL of each stock solution concentration of plant extracts/fractions or essential oils were transferred into the bottles and swirled to coat the entire inner part of each bottle until the acetone solvent completely evaporated leaving the bottle dry. The bottle coated with 1 mL of acetone constituted the negative control while 1 mL Warrior™ (DDVP) diluted in 1 mL was used as positive control.

2.5.3 Bioassay procedure

The bioassay was performed with the cleaned, dried and coated bottles 24 h after their preparation as above (Section 2.5.2), in a lying position. Using a mouth aspirator, 10-25 mosquitoes were introduced into each test bottle including the control bottle. At start time (Time 0), the bottles were examined to count the number of dead and alive mosquitoes. The number of mosquitoes dead or alive was subsequently recorded every 15 minutes up to 24 h or in less time if all the insects died. However, data were grouped such that mortality counts were reported for 1, 6, 12, 18 and 24 h post-treatment. The mortality in the control bottle was taken into consideration at 2 hours (end of the bioassay) when reporting the results of the bioassay. The bioassay results were discarded, if mortality in the control bottle at the end of the test was >10%. Mosquitoes were considered dead if they can no longer stand.

2.6 Statistical analyses

The percentage of mosquito adult mortality data were subjected to the ANOVA procedure using the Statistical Package for the Social Science (SPSS 16.0).Turkey's test at P = 0.05 was applied for mean comparisons. Probit analysis [31] was applied to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality adults 1, 6, 12, 18 and 24 h after treatment application. Abbott's formula [32] was used to correct for control mortality when mortality in the control are comprised between 3% and 10% before probit analysis and ANOVA.

3. Results

3.1 Toxicity of plant extracts/fractions

Results from the present assessment showed a significant adulticidal activity of extracts/fractions and essential oils of *A. senegalensis* and *B. dalzielii* against the three mosquito species adults targeted. The efficacy of the leaf extract and fractions of *A. senegalensis* against *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* adults is presented in figure 2 and concentration-dependent mortality of the three mosquito adults was registered. At the highest tested concentration (2500 mg/bottle) of the methanolic crude extract of *A. senegalensis*, 21.33, 36.00 and 18.67% mortality of adults of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*, respectively were recorded. After fractionation, a moderate mortality was recorded with n-hexane fraction on *Ae. aegypti* (57.33%), *An. gambiae* (64.67%) and *Cx. quinquefasciatus* (46.67%) compared to the positive control (DDVP at 2000 mg/bottle) which achieved 100% mortality with the three mosquito species. No mortality was registered with the ethyl acetate and methanol fractions for all three mosquito species. Comparing the efficacy of the active fractions of *A. senegalensis*, the n-hexane fraction with 24 h LC_{50} of 1636.39, 2220.80 and 2730.91 mg/bottle was more potent to the adult mosquitoes than chloroform fraction with 24 h LC_{50} of 2403.37, 3753.10 and 3955.60 mg/bottle for *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively (Table 1).

The methanolic crude extract of *B. dalzielli* caused low mortality to adult *An. gambiae*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Figure 3). At the highest tested concentration (2500 mg/bottle), the extract achieved 31.33, 18.67 and 14.00% mortality of *An. gambiae*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. After fractionation, concentration-dependent significant mortality of the adult mosquitoes, ranging from 2.67 to 30.00% ($F=183.50$; df=4, 10; $P < 0.001$) for *Ae. aegypti*, 3.33 to 58.67% ($F=369.02$; df=4, 10; $P < 0.001$) for *An. gambiae* and 1.33 to 27.33% ($F=205.21$; df=4, 10; $P < 0.001$) for *Cx. quinquefasciatus* were registered with the n-hexane fraction. The chloroform fraction at 2500 mg/bottle caused 40.0, 22.67 and 20.67% adult mortality to *An. gambiae*, *Ae. aegypti*, and *Cx. quinquefasciatus*. The positive control (DDVP at 2000 mg/bottle) achieved complete adult mortality of the three mosquito species, while no mortality was recorded with ethyl acetate and methanol fractions as well as the negative control. Among the fractions of *B. dalzielii*, the toxic effect of n-hexane fraction was superior to the others, and recorded 24 h LC_{50} of 1836.7 and 5544.5 mg/bottle, respectively against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* (Table 1).

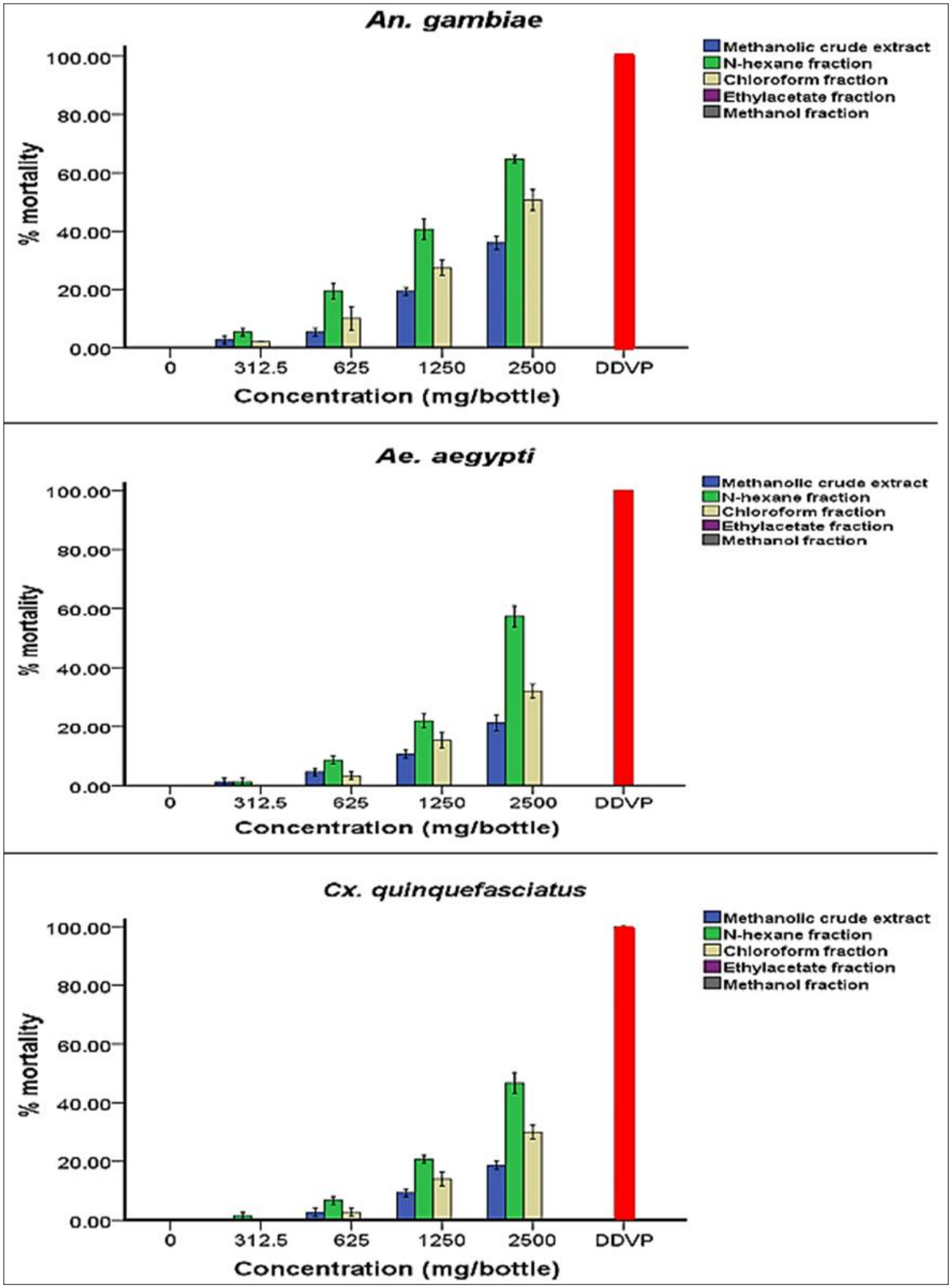

Fig 2: Percentage mortality of *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* adults treated with different concentrations of *Annona senegalensis* leaf extract/fractions in the laboratory (25±2˚C, 72±5% r.h.).

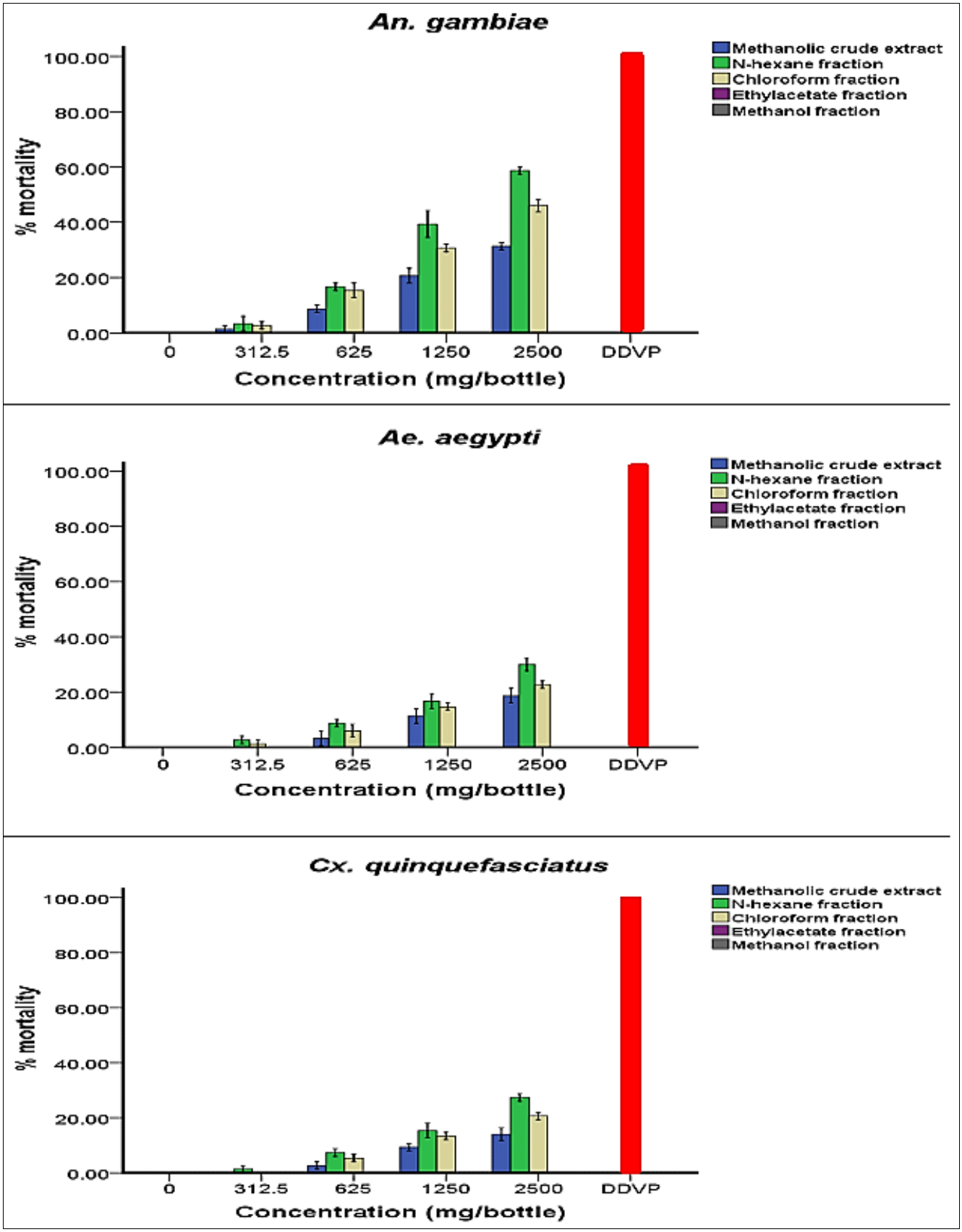

Fig 3: Percentage mortality of *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* adults treated with different concentrations of *Boswellia dalzielii* leaf extract/fractions in the laboratory (25±2°C, 72±5% r.h.).

Table 1: LC_{50} and LC_{90} values (mg/bottle) at 24 h post-treatment of *Annona senegalensis* and *Boswellia dalzielii* leaf extracts/fractions against adults of *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* in the laboratory (25±2°C, 72±5% r.h.).

Mosquito species	Plants/Extracts/Fractions		Slope±SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
Anopheles gambiae	*A. senegalensis*	MCE	1.87±0.16	0.98	3853.42(3182.71-4181.40)	18663.63(12467.63-22814.07)	4.40^{ns}
		NHF	4.54±0.38	0.97	1636.39(1488.99-1820.77)	6424.97(5205.27-8374.73)	2.66^{ns}
		CHF	4.37±0.27	0.98	2404.37(2140.98-2767.68)	9050.31(7030.45-12567.30)	4.56^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-
	B. dalzielli	MCE	1.68±0.16	0.95	4419.5(3515.5-6080.7)	25506.1(15681.9-51518.1)	7.25^{ns}
		NHF	2.16±0.14	0.96	1836.7(1662.3-2061.1)	7184.5(5747.3-9540.3)	8.35^{ns}
		CHF	1.82±0.14	0.96	2602.7(2249.8-3127.8)	13148.4(9403.7-20631.3)	8.10^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-
Aedes aegypti	*A. senegalensis*	MCE	1.52±0.18	0.97	8224.8(5645.65-14859.7)	56904.3(27378.7-184366.9)	3.47^{ns}
		NHF	2.63±0.17	0.98	2220.8(2019.5-2483.0)	6813.3(5586.7-8768.6)	6.96^{ns}
		CHF	2.36±0.21	0.98	3753.1(3186.6-4679.9)	13082.0(9370.6-20927.2)	5.71^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-
	B. dalzielli	MCE	1.77±0.21	0.93	7233.6(5186.9-12175.3)	38317.4(20271.3-106251.2)	11.18^{ns}
		NHF	1.49±0.16	0.97	5544.5(4156.6-8438.5)	40070.4(21847.8-99656.1)	2.88^{ns}
		CHF	1.48±0.17	0.98	7357.6(5170.9-12667.4)	53687.1(26502.4-163489.3)	5.68^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-
Culex quinquefasciatus	*A. senegalensis*	MCE	1.90±0.23	0.97	6945.2(5059.5-11410.3)	32822.6(18003.1-86040.7)	4.64^{ns}
		NHF	2.36±0.17	0.98	2730.9(2417.4-3176.2)	9524.8(7358.3-13379.9)	14.01^{ns}
		CHF	2.38±0.22	0.96	3955.1(3390.9-5005.6)	13638.7(9641.2-22381.2)	16.05^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-
	B. dalzielli	MCE	1.61±0.23	0.92	10413.1(6638.4-22488.9)	41551.7(28343.4-277263.9)	7.88^{ns}
		NHF	1.60±0.17	0.97	5684.8(4284.0-8598.5)	35803.2(20039.9-85698.5)	4.64^{ns}
		CHF	1.65±0.19	0.98	6978.0(5022.7-11568.3)	41551.9(21809.6-114653.9)	8.94^{ns}
		EAF	-	-	-	-	-
		MTF	-	-	-	-	-

ns= P>0.05, FL= Fiducial Limit, LC= lethal concentration, --: the values have not been determined because of the low or no mortality.
MCE=methanolic crude extract, NHF= n-hexane fraction, CHF= chloroform fraction, EAF= ethyl acetate fraction, MTF= methanol fraction.

3.2 Efficacies of plant essential oils

The essential oil of *A. senegalensis* caused significant mortality to adults of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* which increased with concentration and time post-exposure (Figure 3). One hour post-exposure, mortality varied significantly from 82.00 to 100.00% (F=793.99; df=5, 18; P < 0.001) for *An. gambiae*, 37.00 to 100.00% (F=399.12; df=5, 18; P < 0.001) for *Ae. aegypti* and 23.00 to 99.00% (F=716.33; df=5, 18; P < 0.001) for *Cx. quinquefasciatus*. Within 24 h of exposure, the essential oil of *A. senegalensis* as well as the positive control (DDVP at 2000 mg/bottle) generally achieved 100% mortality of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* adults, irrespective of concentration. Among the mosquito species assessed, the essential oil of *A. senegalensis* was more effective against the adults of *An. gambiae* (LC_{50}=10.23 and LC_{90}=37.33 mg/bottle) than *Ae. aegypti* (LC_{50}=32.82 and LC_{90}=95.27 mg/bottle) and *Cx. quinquefasciatus* (LC_{50}=41.11 and LC_{90}=98.63 mg/bottle) (Table 2).

The essential oil of *B. dalzielii* caused a significant mortality of the adults of the three mosquito species, which increased with doses and time post-exposure (Figure 4). The mortality varied significantly from 38.00 to 97.00% (F=578.30; df=5, 18; P < 0.001) for *An. gambiae*, 13.00 to 83.00% (F=157.27; df=5, 18; P < 0.001) for *Ae. aegypti* and from 7.00 to 90.00% (F=360.08; df=5, 18; P < 0.001) for *Cx. quinquefasciatus*, 1 h post-exposure. After 24 h post-exposure, all the tested concentrations achieved practically 100% mortality of *An. gambiae*. For the same time-point, mosquito adults mortality significantly ranged from 94.00 to 100.00% (F=4150; df=5, 18; P < 0.001) with *Ae. aegypti* and 89.00 to 100.00% (F=3087; df=5, 18; P < 0.001) with *Cx. quinquefasciatus*, across the tested concentration range of 25 to 200 mg/bottle. The positive control (DDVP at 2000 mg/bottle) caused 100% mortality to all the three mosquito species. The essential oil was more effective against the adults of *An. gambiae* (LC_{50}=32.36, LC_{90}=95.67 mg/bottle) than *Ae. aegypti* (LC_{50}=77.98, LC_{90}=278.54 mg/bottle) and *Cx. quinquefasciatus* (LC_{50}=88.55, LC_{90}=228.41 mg/bottle) (Table 2).

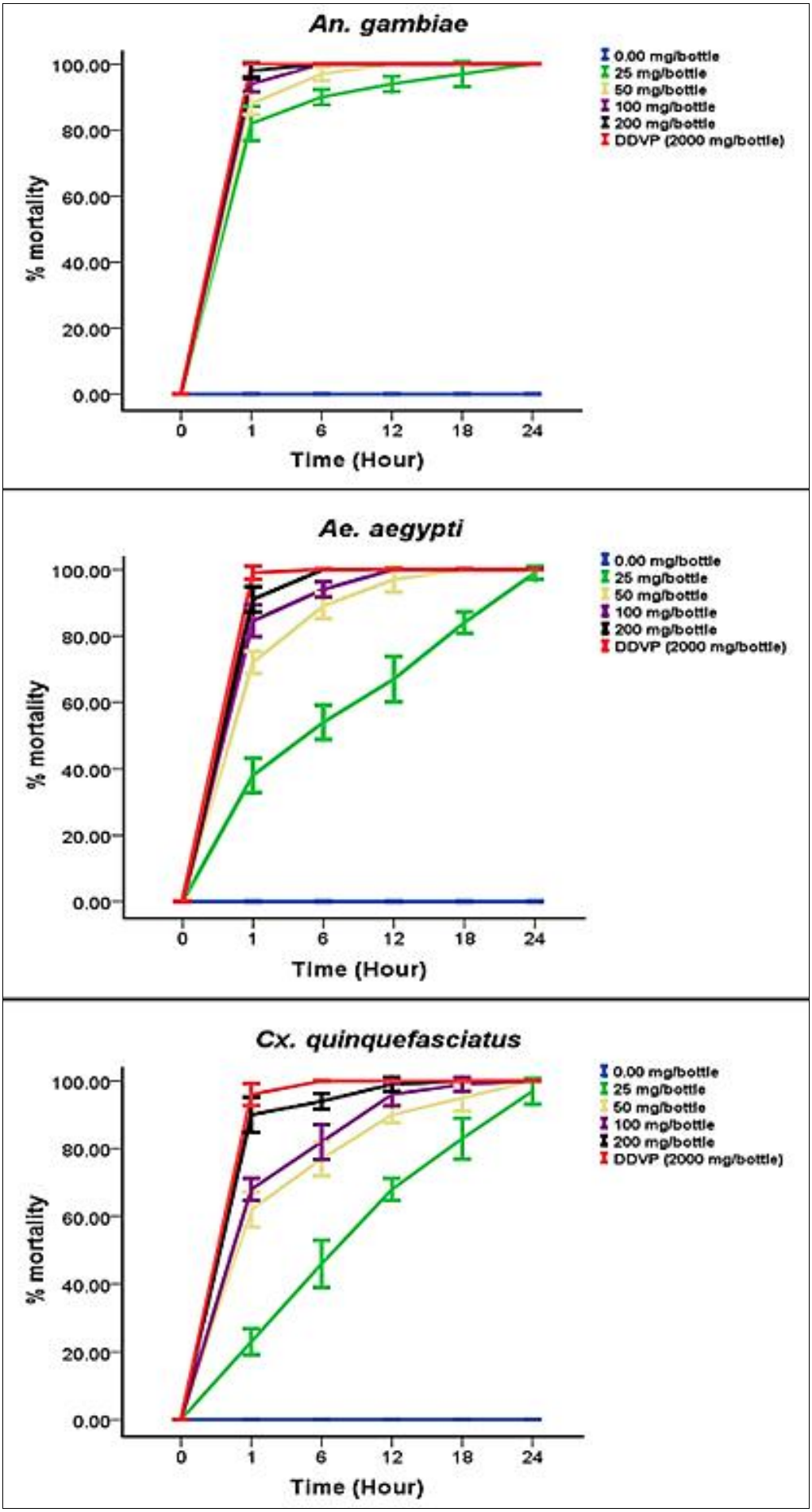

Fig 3: Percentage mortality of *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* adults treated with *Annona senegalensis* leaf essential oil after 1, 6, 12, 18 and 24h post-treatment in the laboratory (25±2°C, 72±5% r.h.).

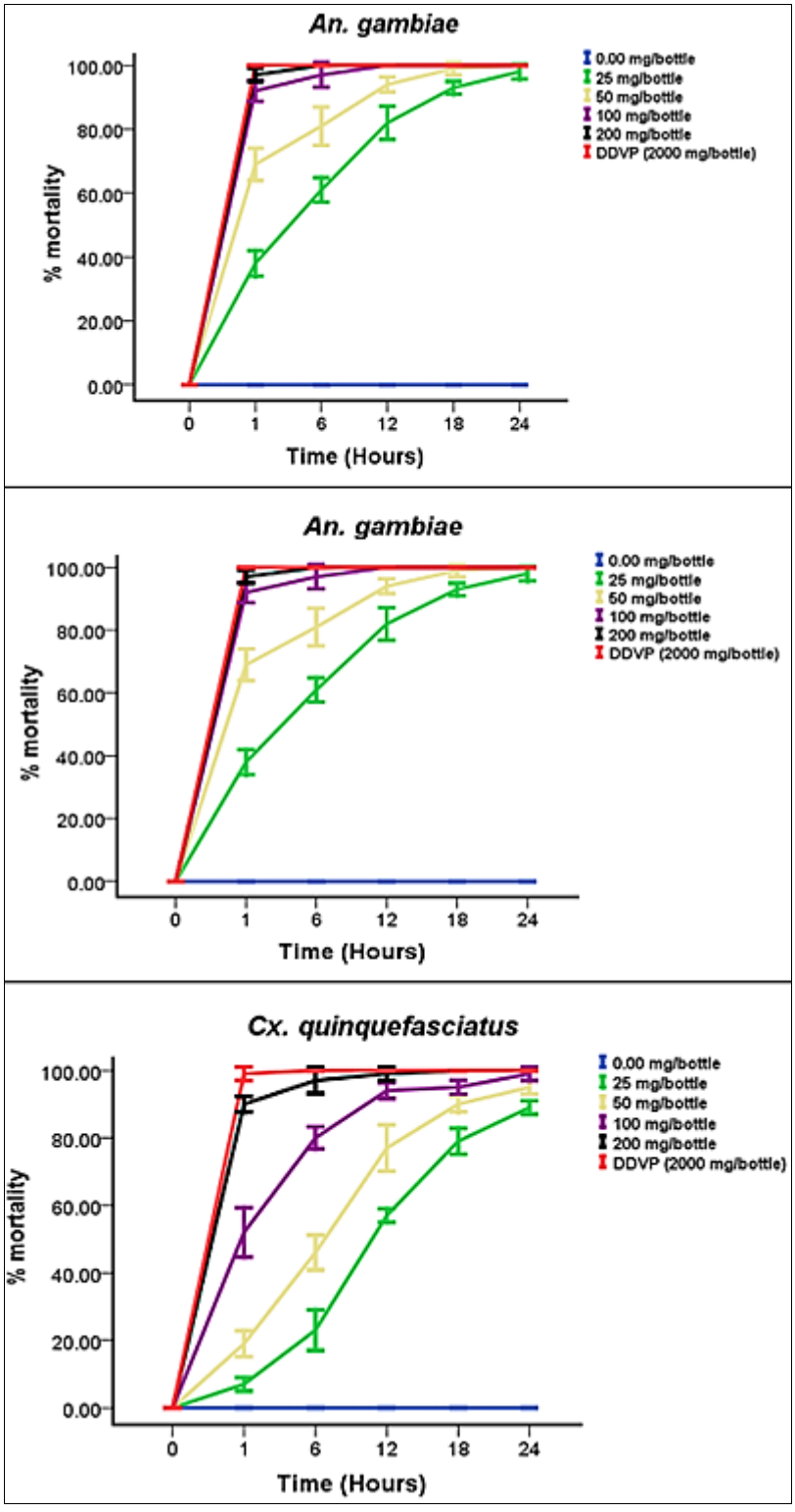

Fig 4: Percentage mortality of *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* adults treated with *Boswellia dalzielii* leaf essential oil after 1, 6, 12, 18 and 24h post-treatment in the laboratory (25±2˚C, 72±5% r.h.).

Table 2: LC_{50} and LC_{90}values (mg/bottle) of *Annona senegalensis* and *Boswellia dalzielii* essential oils against adults of *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* in the laboratory (25±2˚C, 72±5% r.h.).

Plant species	*Mosquito species*	Time (H)	Slope±SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
A. senegalensis	*An. gambiae*	1	2.28±0.25	0.40	10.23(5.56-14.31)	37.33(31.56-44.27)	25.40*
		6	2.86±0.46	0.34	9.03(5.06-12.310	25.34(21.60-28.50)	12.32^{ns}
		12	2.19±0.50	0.31	4.23(0.88-8.27)	17.01(9.77-21.75)	9.20^{ns}
		18	1.91±0.67	0.30	2.15(0.00-8.43)	10.12(0.00-19.13)	24.88*
		24	-	-	-	-	-
	Ae. aegypti	1	2.77±0.15	0.73	32.82(28.60-36.83)	95.27(82.73-114.3)	31.86**
		6	3.09±0.21	0.55	22.30(18.40-25.66)	57.92(50.99-68.93)	32.03**
		12	4.34±0.40	0.46	19.65(18.86-21.83)	38.79(35.62-43.48)	19.45^{ns}

		18	3.39±0.49	0.36	12.23(8.57-15.05)	29.21(26.57-32.10)	11.35^{ns}
		24	2.16±1.41	0.29	1.84 (---)	7.20(---)	17.75^{ns}
	Cx. quinquefasciatus	1	3.37±0.16	0.78	41.11(38.11-44.13)	98.63(89.37-110.98)	19.65^{ns}
		6	3.26±0.19	0.62	26.55(22.85-29.85)	65.58(57.77-77.54)	33.46**
		12	3.46±0.30	0.47	18.04(13.53-21.48)	42.35(37.29-50.43)	36.07**
		18	3.71±0.52	0.39	13.44(9.91-16.09)	29.75(27.37-32.54)	15.68^{ns}
		24	3.91±0.67	0.30	2.15(0.00-8.43)	10.13(0.00-19.13)	24.88*
B. dalzielli	*An. gambiae*	1	2.72±0.14	0.72	32.36(28.92-35.65)	95.67(85.04-110.57)	21.06^{ns}
		6	2.28±0.19	0.57	21.01(16.41-24.91)	60.85(52.62-74.14)	36.35**
		12	2.49±0.28	0.40	10.92(7.72-13.73)	35.74(32.17-39.80)	13.27^{ns}
		18	2.76±0.55	0.32	7.33(2.95-11.05)	21.33(16.20-24.88)	13.63^{ns}
		24	2.50±0.29	0.29	3.57(0.00-9.36)	11.59(0.95-18.17)	10.57^{ns}
	Ae. aegypti	1	2.32±0.11	0.92	77.98(70.83-86.04)	278.54(232.25-350.76)	23.43^{ns}
		6	2.70±0.14	0.75	34.61(31.11-38.00)	103.41(91.79-119.68)	20.75^{ns}
		12	2.97±0.22	0.53	19.87(17.45-22.05)	53.60(49.37-59.09)	16.01^{ns}
		18	2.39±0.25	0.42	11.34(7.13-14.97)	38.93(33.88-45.19)	21.07^{ns}
		24	2.19±0.46	0.32	4.76(1.23-8.46)	18.28(11.63-22.86)	13.73^{ns}
	Cx. quinquefasciatus	1	3.11±0.13	0.98	88.55(81.14-96.98)	228.41(197.13-274.63)	29.67^{ns}
		6	2.82±0.13	0.87	49.53(44.77-54.45)	140.92(122.26-168.45)	29.64**
		12	2.30±0.15	0.61	21.87(17.82-25.54)	78.81(66.78-93.73)	23.37^{ns}
		18	1.74±0.18	0.44	8.95(5.90-11.91)	48.92(43.20-55.87)	15.07^{ns}
		24	1.86±0.26	0.36	5.76(2.80-8.80)	28.08(22.93-32.76)	17.90^{ns}

^{ns}P>0.05, *P<0.05 and **P<0.01, FL= Fiducial Limit, LC= Lethal concentration. --: the values have not been determined because of the low or no mortality.

4. Discussion

The extracts, fractions and essential oils of the two plant assessed significantly demonstrated their efficacy against adults of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*. Numerous previous studies reported the mosquitocidal activity of the various plant extracts/fractions, compounds against major medical importance mosquito vectors. Previous studies reported the adulticidal activity of various plant extracts such as *Dysoxylum malabaricum* leaf methanol extract against *An. stephensi* [33]; *Apium graveolens* seed ethanol extract against *Ae. aegypti* [34] and *Acalypha alinifolia* leaf methanol extract against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* [35]. Adulticidal activity of *Curcuma aromatic*, *Zingiber zerumbet* and *Melia azedarach* extracts were also reported against *Ae. aegypti*; *Culex gelidus*, *Cx. quinquefasciatus* and *An. stephensi* mosquito species [36][37][38]. Moreover, the blend ethanolic with aqueous extract of seven plants including *Justicia gendarussa, Eucalyptus globulus, Artemisia annua, Cymbopogan citratus, Centella asiatica, Annona squamosal* and *Myristica fragrans* tested against *An. stephensi* adults showed a significant activity between 80 and 100% mosquito adult mortality in all extracts [39].

Extracts and fractions of *A. senegalensis* and *B. dalzielii* caused a moderate adulticidal activity against three mosquito species assessed in the present study and *Anopheles* mosquito species was the most affected. Similar observation was reported by Murugan *et al*. [40] in which methanol extract of orange peel was found to be more toxic against *An. stephensi* adults compared to *Ae. aegypti* and *Cx. quiquefasciatus* mosquito species. This finding also coincide with the results of Kovendan *et al*. [35] when among three vectors tested with crude hexane, benzene, ethylacetate, acetone and methanol leaf extracts of *Acalypha alnifolia*, the highest adulticidal activity was observed in high mortality of *An. stephensi*, followed by *Ae. aegypti* and *Cx. quinquefasciatus*. Difference in sensibility of some mosquito species to plant insecticide products may be link to the morphology and physiology of the insect in which during their development may inherit genes that might enable them to tolerate or develop minor resistance to the plant insecticides.

From this present finding, the non-polar solvent n-hexane and chloroform fractions as well as the methanolic crude extract were the most effective. Similarly, the hexane extracts of *Ocimum gratissimum, O. tenuiflorum, P. granatum* and *Moringa oleifera* were revealed to be more effective on *Cx. quinquefasciatus* adults than ethyl acetate and methanol extracts of these plant species [41]. In the same way, petroleum ether and methanol leaf extracts of *Rhinacanthus nasutus* were found as the most potent against *Aedes aegypti* and *Culex quinquefasciatus* adults compared to chloroform and ethyl acetate of the plant [42]. This result is also comparable to earlier reports of Govindarajan *et al*. [43] in which, the adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol leaf extract of *Pithecellobium dulce* against *Cx. quinquefasciatus* and a significant adult mortality of mosquito adults was observed in hexane and methanol. Similarly, Govindarajan and Sivakumar [44] reported the highest adulticidal activity of crude hexane, ethylacetate, benzene, chloroform, and methanol extracts of leaf of *Andrographis paniculata* tested against two important vector mosquitoes, viz., *Cx. quinquefasciatus* and *Ae. aegypti*, in which methanol extract was found the most effective with LC_{50} values of 149.81 and 172.37 mg/L, respectively. Dichloromethane extract of *Aloe ferox* exhibited a high insecticidal activity against *Anopheles arabiensis* mosquito adults compared to the ethanol extract of this plant [45]. In contrary, Govindarajan and Sivakumar [46] tested benzene, hexane, ethyl acetate, methanol and chloroform leaf extracts of *Eclipta alba* and *Andrographis paniculata* against malarial vector, *Anopheles stephensi* and they observed a maximum efficacy in the methanol extract of the two plants. The variation in the solvent extraction could be linked to the ability of each solvent to extract qualitatively and quantitatively, the phytochemicals like alkaloids, flavonoids, saponins, tannins, phenolic compounds, etc, which possess insecticidal activities. Indeed, as pyrethroid insecticides, plants are rich in phytochemical compounds able to induce neurotoxic symptoms in insect via the deactivation of acetylcholinesterase enzyme [47, 48].

In this present study, essential oils of the two plants exhibited also a significant adulticidal activity against the three major vectors mosquito species. Similarly, the adulticidal activity of

the essential oil of *Lantana camara* evaluated against *Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluvialitis*, and *An. stephensi* on 0.208 mg/cm^2 impregnated papers exhibited percent mortality of 93.3%, 95.2%, 100%, 100%, and 100%, respectively [49]. Jaffa (*Citrus sinensis*) oil was reported to be most lethal against *Aedes albopictus* adults through exposure tube method with LC_{50} values of 53.61, 11.07 and 3.41% at 6, 12 and 24 h, respectively [50]. Five essential oils including camphor, calamus, clove, citronella and eucalyptus tested in vaporizer, filter paper and aerosol forms exhibited a significant Knock-down and adulticidal activity against the filarial mosquito vector, *Culex quinquefasciatus* [51].

In this present work, the efficacy of the both plant essential oils was mosquito species dependent and these plant products were more effective against *An. gambiae* than *Ae. aegypti* and *Cx. quinquefasciatus* mosquito species. This response could be explained by the fact that the mosquito species (*Ae. aegypti* and *Cx. quinquefasciatus*) may develop physiological resistance and some tolerance to insecticides with which it has active defense before any insecticide action.

The toxic effect of these plant essential oils could be attributed to the oxygenated monoterpenes, diterpènes and sesquiterpenes reported on the two plant evaluated in the previous works [52-57]. Indeed, the toxic effect of essential oils are attributed to the numerous constituents including aliphatic aldehydes, ketones, esters, acids, terpenes, phenols and alcohols, which have strong impact on behavior and the mortality of insects [58]. Previous works reported pure insecticide compounds, essential oils or pyrethroid insecticides applied on insects may lead to neurotoxic symptoms in insects such as hyperactivity, seizures and tremors accompanying by knock down effect [59, 60, 61, 47]. The different compounds contained in essential oils may interfere with acetylcholinesterase enzyme acting as potent of the central nervous system where all cholinergic synapses are virtually located [62], and is responsible of the inhibition action of that enzyme leading to neurotoxic effect followed by the death of insect [63].

5. Conclusion

Globally, methanolic crude extract, n-hexane, chloroform, ethylacetate and methanol fractions as well as essential oils of the leaves *Annona senegalensis* and *Boswellia dalzielii* exerted a significant dose-dependent adulticidal activity against *An. gambiae, Ae. aegypti* and *Cx. quinquefasciatus*. The most active fraction, n-hexane (2500 mg/bottle) of the two plant species caused roughly ≤ 60% mortality to the three adult mosquito species assessed. For the same time-point, DDVP (2000 mg/bottle) achieved complete mortality of the three mosquito species, and the lowest tested concentration (25 mg/bottle) of the essential oil from both plants caused 100% mortality to the mosquito species. Thus, n-hexane fractions and essential oils of the two plants exhibited high activity on adults of *An. gambiae Ae. aegypti* and *Cx. quinquefasciatus*, and may constitute a potential alternative candidates for insecticides formulation for the control of the mosquito species assessed.

6. Competing Interest

The authors declare that they have no competing interests.

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