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Toxicity of *Boswellia dalzielii* (Burseraceae) Leaf Fractions Against Immature Stages of *Anopheles gambiae* (Giles) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae)

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ABSTRACT: Mosquitoes are vectors of several human pathogens, and great attention has recently been placed on insecticides from plant-derived products, in search for mosquito control agents. This study, thus, investigated the potency of *Boswellia dalzielii* methanol leaf extract and its four fractions as mosquito ovicide, larvicide, and pupicide against *Anopheles gambiae* and *Culex quinquefasciatus*. The plant products were tested at the following concentrations: 125, 250, 500, 1000, and 2000 ppm on eggs and 312.5, 625, 1250, and 2500 ppm on the larvae and pupae of the mosquitoes. For results, hatchability of *A. gambiae* eggs was reduced to 5% with *n*-hexane fraction at 2000 ppm. Among the plant products tested, *n*-hexane fraction was most toxic against *A. gambiae* (LC_{50} = 385.9 ppm) and *C. quinquefasciatus* (LC_{50} = 3394.9 ppm). The *n*-hexane fraction of *B. dalzielii* might be used as a mosquitocidal agent in the breeding sites of *A. gambiae* and *C. quinquefasciatus*.

KEYWORDS: mosquitocidal, extract, fraction, *Boswellia dalzielii*, *Anopheles gambiae, Culex quiquefasciatus*

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Introduction

Mosquitoes are insects renowned as a major health problem since they are important vectors of several diseases, including malaria, dengue fever, yellow fever, chikungunya, and filariasis, which cause millions of deaths among pregnant women and children under five years every year in Africa.[1] The mosquito *Anopheles gambiae* is a major vector of malaria in the sub-Saharian Africa. Observations made in different regions of Cameroon revealed that *A. gambiae* is found throughout the country.[2] In the savannah of northern and urban areas of Cameroon, *A. gambiae* is a major vector of malaria.[3,4] Malaria parasite remains a major public health problem in tropical countries. Over two billion people are exposed worldwide, and about 300–500 million people are infected each year.[5] In Cameroon, malaria causes 25.32 deaths per 100,000 people,[1] and *A. gambiae* is the principal vector of the disease in both rural and urban areas.[3,6]

Lymphatic filariasis (LF) also known as elephantiasis represents a major vector-borne public health problem worldwide. *Culex quinquefasciatus* is the most significant vector for the transmission of human LF worm, *Wuchereria bancrofti*.[7] Currently, 1.3 billion people in 72 countries worldwide are at risk of contracting the disease, with 120 million people reported being infected, 65% of them living in Southeast Asia and 30% of them living in Africa.[8] In the rural north-western Cameroon, the overall prevalence was 14.5% in the year 2000.[9] The disease itself is not fatal but inflicts a considerable socioeconomic impact due to human disability and ostracization.[7]

Vector control has been used for over a century to prevent disease because it is a more viable option than to have populations who regularly take preventative drug treatments for malaria, filariasis, dengue fever, yellow fever, West Nile virus, etc., and such preventative drug treatments are not available for some of these diseases. Therefore, eliminating mosquito vectors at immature stages in their breeding sites should be the best and most commonly recommended methods for preventing these diseases.[10]

The current mosquito vector control measures are mainly focused on the use of synthetic residual chemicals.[11] Synthetic pesticides have been extensively used for mosquito-borne disease control by killing, preventing adult mosquitoes from biting humans, or killing mosquito larvae at the breeding sites of the vectors.[12] The continuous use of these chemicals has disrupted natural enemies,[13] led to the development of insect resistance to synthetic pesticides, such as malathion, dichlorodiphényltrichloroéthane (DDT), and deltamethrin, and even biopesticides, such as *Bacillus thuringiensis*.[14] Other problems

posed by chemical insecticides include high operational cost, environmental pollution, and deleterious effects on nontarget organisms.[15] These potentially negative effects of synthetic chemicals have created the need for developing safer alternative approaches to control mosquito vectors.

Plants may be a source of alternative agent to replace synthetic insecticides for mosquito control because botanicals are environmentally safer, more biodegradable, and more target specific.[16] More than 1500 plant species belonging to 235 families having potential insecticidal value were described by Saxena.[17] Sukumar et al[18] discussed 344 plant species that exhibited mosquitocidal activity. The most promising botanical groups are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Aristolochiaceae, and Malvaceae, which provided numerous beneficial principles ranging from pharmaceuticals to insecticides.[19,20]

Boswellia dalzielii (Burseraceae) is a tree that grows up to 13 m in height, found in savannah regions, locally abundant in Northern Ivory Coast, Northern Nigeria, and Cameroon.[21] The plant is used to treat tuberculosis,[22] gingivitis,[23] skin disorders, digestive disorders, musculoskeletal disorders, nervous disorders, breathing problems, and other infections.[24] From early times, gum from the bark of *B. dalzielii* was thought to protect against termites when rubbed on wood.[25] In the northern part of Cameroon, the leaves of *B. dalzielii* are used locally to protect maize, millet, and sorghum against weevil attacks.[26] The stem bark secretes a fragrant white gum that is burnt to fumigate cloth and to drive out flies, mosquitoes, etc., from rooms.[27] From Cameroon, Lame et al[28] reported the toxic effect of this plant against *Aedes aegypti*. Nonetheless, no study has reported on the insecticidal efficacy of the extracts or fractions of this plant against mosquitoes. The goal of this study was to evaluate the bioactivity of fractions of *B. dalzielii* on immature developmental stages of *A. gambiae* and *C. quinquefasciatus*.

Materials and Methods

Collection and processing of plant materials. The green leaves of *B. dalzielii* were collected around 07:00 hours Greenwich Mean Time (GMT) + 1 from Midjivin (latitude 10°10.800′N, longitude 14°20,070′E, and altitude 456 m.a.s.l.), Maroua, Far North region of the same country in December 2011. The identity of the plant species was confirmed at the National Herbarium in Yaoundé, Cameroon, where a voucher sample was deposited under the registration number of 20532/SRF-CAM. The leaves were dried in a room under ambient condition, then pulverized with an electric grinder, and screened using a 0.4-mm mesh size sieve. The resulting powder was stored at –18°C in a deep freezer until needed for extraction.

Extraction and fractionation. The method of Gueye et al[29] was used for extraction and fractionation of the leaf powder of *B. dalzielii*. The process was carried out in the laboratory of the Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Nigeria, during the period from March to May 2012. The initial extraction was processed with the methanol solvent to obtain the residue called methanolic crude extract (MCE). To obtain the MCE, 1200 g of the powder of the plant was macerated in 2500 mL of methanol for 72 hours at room temperature, and then the maceration was filtrated using filter paper, Whatman No. 1. The residue of maceration was rinsed and filtrated several times with the fresh methanol until a clear phase was obtained. The filtrate was placed in a rotary evaporator apparatus, set at 60°C to obtain a residue called crude extract.

For fractionation (Fig. 1), 200 g of the crude extract of this plant was separated successively by the method of differential solubility in four solvents of different polarity: *n*-hexane, chloroform, ethylacetate, and methanol solvents. The crude extract was mixed with silica gel (70–260 mesh size) and macerated in *n*-hexane and then filtered with a Whatman No. 1 filter paper after phase separation. The fraction of *n*-hexane and maceration (1) were recovered. Maceration (1) was dried in the open air and then soaked in chloroform; phase chloroform fraction filtrated and maceration (2) were also recovered. Maceration (2) after drying in open air was soaked in ethyl acetate; phase ethylacetate fraction filtered and maceration (3) were also recovered. Maceration (3) was finally taken up in methanol to recover the polar compounds in the methanol fraction after filtration.

Each fraction was concentrated using rotary evaporator, and the yield of each of the solid fraction gotten was calculated according to the formula below and then stored at –4°C in a refrigerator until needed for bioassays.

Collection and rearing of mosquito species. The larvae of *A. gambiae* were collected from water sewage in gutters in February 2013 of Awka market, Anambra, Nigeria, and reared following the protocols of Walton[30] and Das et al.[31] To start the colony, larvae were kept in plastic trays containing tap water. All the experiments were carried out at 27 ± 2°C and 75%–85% relative humidity under 12:12 light and dark cycles. Larvae were fed a diet containing crayfish and biscuit in the ratio of 3:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in insect cages (30 cm × 30 cm × 35 cm) from where adults emerged. Adults were maintained in cages and were continuously provided with 10% sucrose solution in a jar with a cotton wool. On day 5, the adults were given a blood meal from a guinea pig shaved and placed on the top of the cages overnight. A beaker with 100 mL of tap water lined with filter paper was kept inside the cage at oviposition. Third generation was used for mosquitocidal tests.

The larvae of *C. quinquefasciatus* were collected from the culture of the National Arbovirus Research Center, Enugu, Nigeria, in January 2013. Our mosquito culture was maintained in the insectary of the Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu, Anambra, Nigeria,

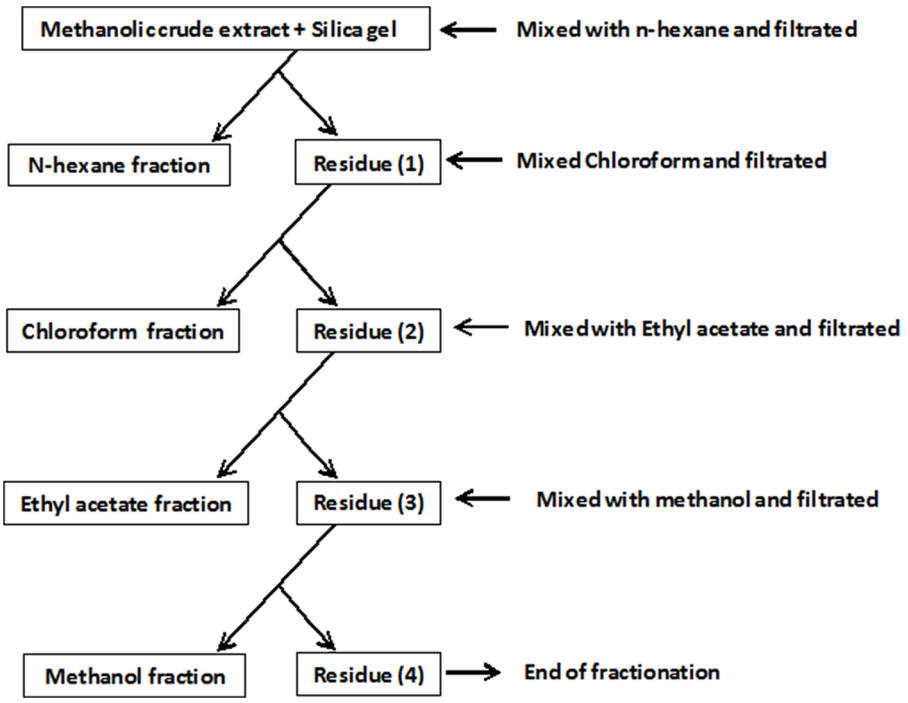

Figure 1. Diagram illustrating the steps of fractionation process.

under fluctuating temperature and relative humidity and 12L:12D photoperiod. Larvae were reared in plastic trays containing tap water, and the rearing water was changed every two days. The larvae were fed on the grower *chicken feed* (mixture of maize + wheat + soya + fish + others), and the colony was kept at 25 ± 2°C with 80%–90% relative humidity under a photoperiod of 12L:12D until the formation of pupae. The pupae were transferred to cups containing tap water and placed in the cage (30 cm × 30 cm × 35 cm) where the adults emerged. The ovitraps were kept in the cages; the eggs were collected and transferred to plastic trays.

Ovicidal test. For ovicidal activity, the method of Kumar et al[32] was followed. The freshly laid eggs were collected by providing ovitraps in mosquito cages kept two days after the female mosquitoes were given a blood meal. A total of 100 gravid female mosquitoes were placed in a screen cage where 10 oviposition cups were introduced for oviposition, 30 minutes before the start of the dusk period. The eggs were laid on the filter paper, Whatman No. 1, provided in the ovitrap. Other cups (250 mL in volume) were filled with 100 mL of test solutions of 125, 250, 500, 1000, and 2000 ppm concentration of plant extract/fractions of *B. dalzielii*, while one was filled with 99 mL of distilled water mixed with 1 mL of emulsifier (Tween-80), which served as the control. A commercial synthetic mosquitocide, Warrior™ (2,2-dichlorovinyl dimethyl phosphate [DDVP], 2000 ppm), widely used in Nigeria and Cameroon against mosquitoes was used as a positive control at the recommended concentration of 2000 ppm. A minimum of 100 eggs were used for each treatment, and the experiment was replicated four times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with tap water for hatching assessment, after counting the eggs under microscope. The percent egg mortality was calculated on the basis of nonhatchability of eggs with unopened opercula 48 hours posttreatment.[33]

Larvicidal test. The larvicidal activity of the extract and fractions of *B. dalzielii* were evaluated against two major urban mosquito vectors *A. gambiae* and *C. quinquefasciatus* according to the method described by World Health Organization.[34] The extract and fractions were dissolved in 0.5 mL of Tween-80. The concentrations of 312.5, 625, 1250, and 2500 ppm of extract/fractions of the plant were prepared in the volume of 100 mL with tap water in 250 mL beakers. The negative control consisted to add 0.5 mL of Tween-80 to 99.5 mL of tap water for extract or fractions, respectively. Warrior™ (DDVP) was used at the recommended concentration of 2000 ppm as a positive control. Twenty-five fourth instar larvae were transferred into each prepared concentration solution, and four replicates were maintained for each concentration. Mortality was recorded after 24 hours, and percent-corrected mortality was determined using Abbott's formula.

Pupicidal test. The pupicidal effect was assessed according to the method used by Ashfaq and Ashfaq.[35] Twenty-five freshly emerged pupae (six hours) were transferred into beakers of 250 mL volume, containing 75 mL of tap water. The extract/fractions of the plant used were dissolved in Tween-80 as emulsifier and then added with tap water to make up to 100 mL, corresponding to the concentrations 312.5, 625, 1250, and 2500 ppm of extract/fractions of *B. dalzielii*.

The volume of 0.5 mL of Tween-80 was added to 99.5 mL of tap water to constitute the negative control of extract/fractions. Warrior™ (DDVP) was used at the recommended concentration as a positive control. Each treatment was replicated four times, and the number of emerged adults for each replication was recorded after 48 hours.

Statistical analysis. The percentage of egg hatchability and percentage mortality data for the larvae or pupae were subjected to the analysis of variance procedure using the Statistical Package for the Social Science (SPSS 16.0). Tukey's test at $P = 0.05$ was applied for mean separation. Probit analysis[36] (Finney 1971; SPSS 16.0) was applied to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) hatchability of eggs, 48 hours posttreatment; larvae and pupae, 24 hours after treatment application. Abbott's formula[37] (Abbott 1925) was used to correct the control mortality when mortality in the control was comprised between 3% and 10% before probit analysis and analysis of variance.

Results

The effects of extract and fractions of *B. dalzielii* on egg hatching of *A. gambiae* and *C. quinquefasciatus* are presented in Figure 2. Overall, the egg hatchability percent decreased with ascending concentration of the botanicals. At the highest tested concentration (2000 ppm), the MCE of *B. dalzielii* reduced the percent of *A. gambiae* and *C. quinquefasciatus* eggs that hatched from 100% in the control to 9.67% ($F = 1088$, $P < 0.001$) and 63.33% ($F = 199.88$, $P < 0.001$), respectively. The *n*-hexane fraction caused the highest reduction of egg hatchability percent values, from 100% to 5% ($F = 1194$, $P < 0.001$) for *A. gambiae* and 48.67% ($F = 579.56$, $P < 0.001$) for *C. quinquefasciatus* at the highest tested concentration. The egg hatchability reduction percent of *A. gambiae* and *C. quinquefasciatus* attributed to the chloroform fraction of the plant from 100% to 8.67% ($F = 1136$, $P < 0.001$) and 52.33% ($F = 656.54$, $P < 0.001$), respectively, at 2000 ppm. The lowest IC_{50} values were obtained with the *n*-hexane fraction for both *A. gambiae* (1896 ppm) and *C. quinquefaciatus* (1896 ppm) (Table 1).

In general, the larvae of *A. gambiae* and *C. quinquefasciatus* were susceptible to the extracts and fractions of the leaves of *B. dalzielii* (Fig. 3). For *A. gambiae*, the MCE of the plant caused a significant mortality ($F = 8.39$, $P < 0.01$) to the larvae, which ranged from 6.67% with the lowest concentration (312.5 ppm) to 92% with the highest concentration (2500 ppm). Among the fractions obtained from the MCE, 100% mortality of the larvae was achieved by *n*-hexane fraction at 1250 and 2500 ppm, while 100% and 97% mortality were recorded with chloroform fraction at 2500 and 1250 ppm, respectively. Low mortality of 16% and 6.67% was also, respectively, registered with the ethylacetate and methanol fractions. Among the products of this plant, *n*-hexane fraction was the most effective against *A. gambiae* larvae after 24 hours with LC_{50} = 385.9 and 721.6 ppm, followed by chloroform fraction with the LC_{50} = 499.4 and 987.5 ppm (Table 2).

From the results of larvicidal activity of *B. dalzielii* leaf methanolic extracts or fractions against *C. quinquefasciatus* fourth instar larvae, only two fractions and the MCE, as well as DDVP used as a positive control, demonstrated larvicidal efficacy (Fig. 3). At the highest tested concentration (2500 ppm), a mortality of 22.67% was registered with MCE, and after fractionation, 40% and 28% mortalities were caused, respectively, by *n*-hexane and chloroform fractions. The larvae of *C. quinquefasciatus* were more susceptible to the fraction of *n*-hexane with LC_{50} = 3394.9 ppm compared to chloroform fraction with the value of LC_{50} = 5268.5 ppm (Table 2).

Apart from ethylacetate and methanol fractions of *B. dalzielii*, in which no activity was recorded, the methanolic crude extract and *n*-hexane and chloroform fractions demonstrated a significant concentration-dependent efficacy on the pupae of *A. gambiae* and *C. quinquefasciatus* (Fig. 4). The MCE of *B. dalzielii* caused mortality, ranging from 5% to

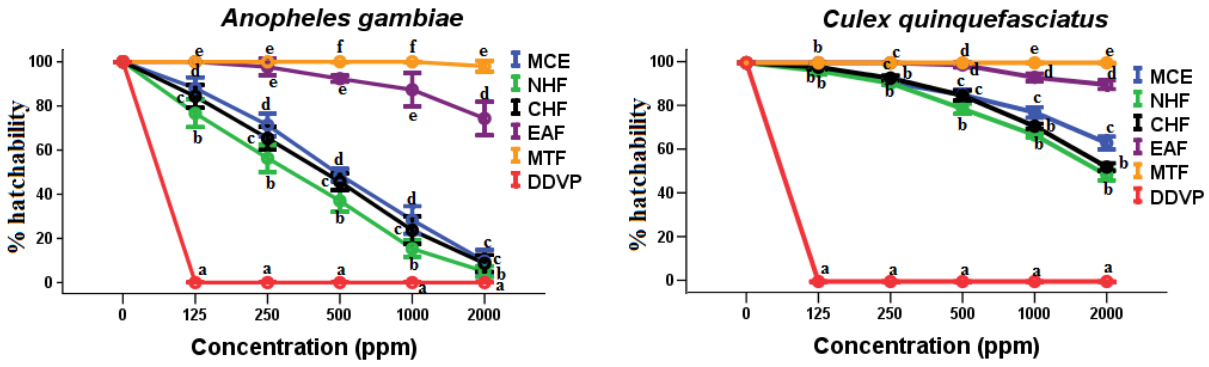

Figure 2. Ovicidal activity of leaf extract and fractions of *B. dalzielii* against *A. gambiae* and *C. quinquefasciatus* in the laboratory (25 ± 2°C, 72% ± 5% relative humidity), 48 hours postexposure. Polygon representing vertically, mean ± SE of extract/fractions and DDVP for each concentration followed by the same letter did not differ significantly according to Tukey's test at $P = 0.05$.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction.

Table 1. IC_{50} and IC_{90} values (ppm) at 48 hours posttreatment of the fractions of *Boswellia dalzielii* leaf extracts against eggs of *Anopheles gambiae* and *Culex quinquefasciatus* under laboratory conditions (25 ± 2°C, 72% ± 5% relative humidity).

MOSQUITO SPECIES	EXTRACT/FRACTIONS	SLOPE ± SE	R^2	IC_{50} (95% FL)	IC_{90} (95% FL)	χ^2
Anopheles gambiae	MCE	2.01 ± 0.09	0.86	483.78 (445.31–525.43)	2105 (1810.96–2501.65)	3.90^{ns}
	NHF	1.94 ± 0.10	0.77	306.74 (279.41–334.95)	1400 (1218.56–1647.91)	4.03^{ns}
	CHF	1.93 ± 0.09	0.83	416.86 (382.13–453.90)	1923 (1656.72–2292.06)	3.32^{ns}
	EAF	1.54 ± 0.15	0.96	5220 (3867.80–7969.66)	35700 (19860.96–83451.53)	8.82^{ns}
	MTF	1.30 ± 0.73	0.65	1.33E5 (–)	1285E6 (–)	7.58^{ns}
Culex quinquefasciatus	MCE	1.27 ± 0.11	0.95	3664 (2813.30–5220.62)	37760 (21263.93–83726.07)	7.43^{ns}
	NHF	1.49 ± 0.10	0.96	1896 (1617.10–2303.21)	13800 (9594.17–22074.10)	2.90^{ns}
	CHF	1.61 ± 0.11	0.98	2181 (1855.36–2660.62)	13590 (9549.52–21460.89)	2.39^{ns}
	EAF	1.72 ± 0.26	0.90	9923 (5996.72–24574.76)	30640 (22797.08–279104.05)	9.22^{ns}
	MTF	–	–	–	–	–

Notes: $^{ns}P > 0.05$. –, the values have not been determined because of the low or not egg inhibition.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction; FL, fiducial limit; IC, inhibition concentration.

49% ($F = 624.72$, $P < 0.001$) for *A. gambiae* and 0% to 22% ($F = 997.44$, $P < 0.001$) for *C. quinquefasciatus* pupae. After fractionation of the crude extract, the highest tested concentration (2500 ppm) of *n*-hexane fraction achieved 75% and 42% pupal mortalities of *A. gambiae* and *C. quinquefasciatus*, respectively. At the same concentration, the chloroform fraction of *B. dalzielii* caused 50% and 36% pupal mortalities, respectively, to *A. gambiae* and *C. quinquefasciatus*. A 100% pupal mortality of the two mosquito species was accomplished by the positive control (DDVP at 2000 ppm). By comparing the two fractions of *B. dalzielii* having pupicidal activity, the *n*-hexane fraction with LC_{50} values of 1226 and 3146 ppm, respectively, against *A. gambiae* and *C. quinquefasciatus* was more potent than the chloroform fraction with LC_{50} values of, respectively, 2499 and 4324 ppm on the pupae of the two mosquito species (Table 3). Moreover, *n*-hexane fraction was more effective on *A. gambiae* ($LC_{50} = 1226$ ppm) compared to *C. quinquefasciatus* ($LC_{50} = 3146$ ppm) pupae.

Discussion

In recent years, plant-based products extracted with different solvents (eg, hexane, chloroform, benzene, petroleum ether, benzene, ethyl acetate, methanol, and water) from the leaves were tested for toxicity at ovicidal, larvicidal, pupicidal, adulticidal, repellent, oviposition deterrent, and insect growth regulatory activities against mosquito species.[38–40] In this study, the MCE and its four fractions obtained from the leaves of *B. dalzielii* showed a significant concentration-dependent activity on eggs, larvae, and pupae of *A. gambiae* and *C. quinquefasciatus*. The effectiveness of this plant could be attributed to the presence of phytochemical components that act as insecticides.[41] These phytochemical components include alkaloids, flavonoids, tannins, terpenoids, phenolic group, fats, and oils found in the leaf MCE of the plant.[28]

The MCE of *B. dalzielii* reduced the percent of *A. gambiae* and *C. quinquefasciatus* eggs that hatched from 100% in the negative control to 09.67% and 63.33%, respectively, at 2000 ppm.

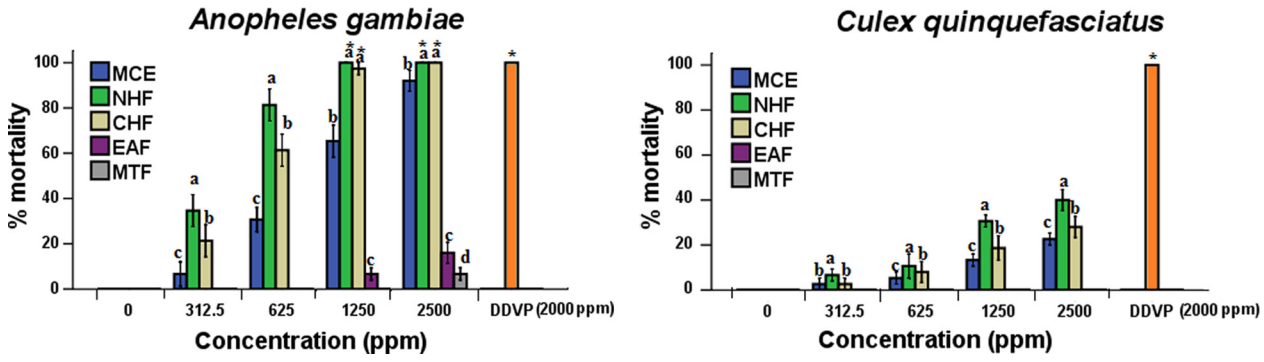

Figure 3. Larvicidal activity of *B. dalzielii* leaf extract/fractions against *A. gambiae* and *C. quinquefasciatus* in the laboratory (25 ± 2°C, 72% ± 5% relative humidity), 24 hours postexposure. Histogram representing mean ± SE of extract/fractions and DDVP for each concentration followed by the same letter or the symbol * did not differ significantly according to Tukey's test at $P = 0.05$.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction.

Table 2. LC_{50} and LC_{90} values (ppm) at 24 hours of the fractions of *Boswellia dalzielii* leaf extracts against the fourth instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus* in the laboratory (25 ± 2°C, 72% ± 5% relative humidity).

MOSQUITO SPECIES	EXTRACT/FRACTIONS	SLOPE ± SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
Anopheles gambiae	MCE	3.18 ± 0.15	0.97	918.5 (863.2–977.7)	2326.1 (2103.8–2615.2)	16.61^{ns}
	NHF	4.72 ± 0.31	0.82	385.9 (364.0–407.2)	721.6 (669.9–789.4)	12.96^{ns}
	CHF	4.33 ± 0.24	0.88	499.4 (472.8–526.6)	987.5 (914.3–1081.2)	17.54^{ns}
	EAF	2.51 ± 0.34	0.89	5924.7 (4523.7–9246.7)	19235.5 (11629.6–45099.8)	10.68^{ns}
	MTF	2.24 ± 0.56	0.70	12849.4 (6821.7–77850.6)	48082.7 (16702–1018878.8)	6.81^{ns}
Culex quinquefasciatus	MCE	1.35 ± 0.17	0.98	8798.5 (5845.1–16848.2)	77762.3 (34366.3–292652.7)	8.33^{ns}
	NHF	1.49 ± 0.14	0.95	3394.9 (2763.0–4485.3)	24650.6 (15222.2–48878.5)	13.89^{ns}
	CHF	1.59 ± 0.17	0.95	5268.5 (3584.3–10478.2)	33683.8 (15174.2–149875.2)	18.41*
	EAF	–	–	–	–	–
	MTF	–	–	–	–	–

Notes: $^{ns}P > 0.05$; $^{*}P < 0.05$. –, the values have not been determined because of the low or not mortality of larvae.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction; FL, fiducial limit; LC, lethal concentration.

Similarly, a complete inhibition of egg hatching of *A. gambiae* with methanolic extract of *Hyptis suaveolens* was reported by Ivoke et al.[42] Govindarajan et al[43] recorded similar findings in which the methanol leaf extract of *Coccinia indica* imposed zero egg hatchability (100% mortality) at 150 ppm for *C. quinquefasciatus*. The complete ovicidal activity of the methanol extract of *Cardiospermum halicacabum* was also attained at 300 ppm against *C. quinquefasciatus*.[38] The plant-based products might block the micropyle region of the egg, thereby preventing the exchange of gases, ultimately killing the embryo in the egg itself. The disturbance with egg cytoplasm was reflected in the form of dead eggs with black spot stage due to the arresting of further development of embryo inside the egg.[44,45]

Among the fractions, the nonpolar *n*-hexane fraction was the most effective on the eggs of the two mosquito species, with *A. gambiae* eggs being more susceptible than those of *C. quinquefasciatus*. The hexane and benzene extracts of *Acalypha alnifolia* tested for ovicidal activity against *A. aegypti*, *A. Stephensi*, and *C. quinquefasciatus*, exerted 100% mortality (zero hatchability) at 500 ppm.[46] These different extracts were more toxic to *A. gambiae* eggs than the eggs of other mosquito species. The *n*-hexane fraction, which is oily in nature, might easily penetrated through the micropyle and, consequently, might cause death or disturb the development of embryo inside the egg.[45]

The susceptibility of larvae to botanical insecticides depends in general on the extract or fraction, the concentration applied, and the mosquito species tested in this study. In our results, larvae of *A. gambiae* (LC_{50} = 385.9 ppm) were more susceptible to *n*-hexane fraction of plant used than *C. quinquefasciatus* (LC_{50} = 3394.9 ppm) larvae. Similar results were observed by Subarani et al,[47] where the larvae *A. stephensi* (LC_{50} = 70.80 ppm) were more sensitive to *Catharanthus roseus* methanol extract than *C. quinquefasciatus* larvae (LC_{50} = 94.20 ppm). Azokou et al[48] reported high larvicidal activity against resistant and sensitive larvae of *A. gambiae* and

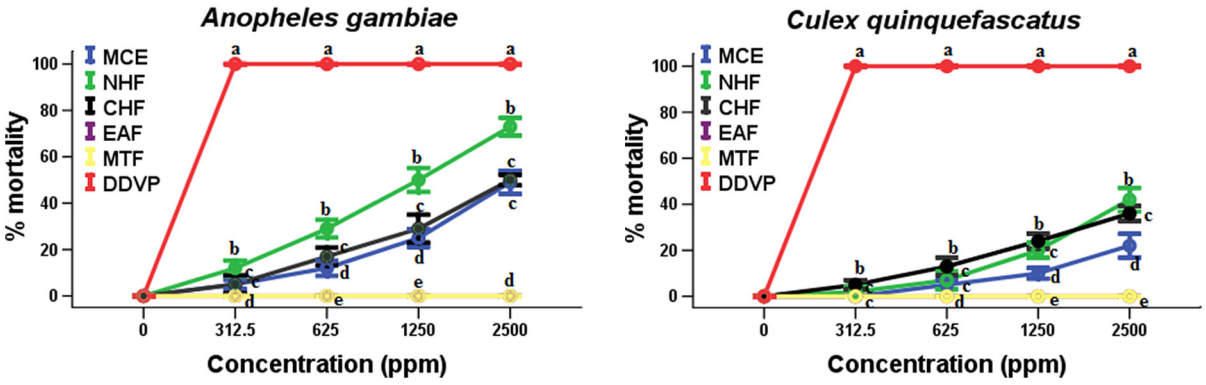

Figure 4. Pupicidal activity of *B. dalzielii* leaf extract/fractions against *A. gambiae* and *C. quinquefasciatus* in the laboratory conditions (25 ± 2°C, 72% ± 5% relative humidity), 24 hours postexposure. Polygon representing vertically, mean ± SE of extract/fractions and DDVP for each concentration followed by the same letter did not differ significantly according to Tukey's test at $P = 0.05$.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction.

Table 3. LC_{50} and LC_{90} values (ppm) (95% fiducial limits) at 24 hours posttreatment of the fractions of *Boswellia dalzielii* leaf extracts against pupae of *Anopheles gambiae* and *Culex quinquefasciatus* in the laboratory (25 ± 2°C, 72% ± 5% relative humidity).

MOSQUITO SPECIES	EXTRACT/FRACTIONS	SLOPE ± SE	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
Anopheles gambiae	MCE	1.83 ± 0.12	0.96	2706 (2373.83–3177.92)	13620 (10112.35–20010.68)	12.76^{ns}
	NHF	1.96 ± 0.11	0.93	1226 (1131.18–1335.54)	5519 (4587.83–6916.99)	10.77^{ns}
	CHF	1.73 ± 0.12	0.93	2499 (2194.17–2928.27)	13760 (10120.36–20469.88)	19.40^{ns}
	EAF	–	–	–	–	–
	MTF	–	–	–	–	–
Culex quinquefasciatus	MCE	1.79 ± 0.18	0.90	6540 (4997.08–9619.64)	34060 (20182.98–73304.46)	16.85^{ns}
	NHF	2.08 ± 0.14	0.96	3146 (2650.16–3957.44)	12990 (9023.48–21761.75)	20.81^{ns}
	CHF	1.38 ± 0.12	0.91	4324 (3448.96–5872.56)	36580 (21792.25–75533.20)	10.62^{ns}
	EAF	–	–	–	–	–
	MTF	–	–	–	–	–

Notes: $^{ns}P > 0.05$. –, the values have not been determined because of the low or not mortality of pupae.
Abbreviations: MCE, methanolic crude extract; NHF, *n*-hexane fraction; CHF, chloroform fraction; EAF, ethylacetate fraction; MTF, methanol fraction; FL, fiducial limit; LC, lethal concentration.

C. quinquefasciatus tested with hexane and chloroform fractions of *Cissus populnea*, *Cochlospermum planchonii*, and *Phyllanthus amarus*. These authors also noticed that both fractions were more toxic to *A. gambiae* larvae than *C. quinquefasciatus* larvae. The potential effect of *n*-hexane fraction might be attributed to the oily aspect of this fraction, which could easily penetrated through phospholipidic membrane of larvae and causing dysfunction of organs followed by death of the mosquito larvae.

Our results show that fractions of *B. dalzielii* caused a significant concentration-dependent mortality of *A. gambiae* and *C. quinquefasciatus*. This in line with the findings of Prabhu et al,[49] where a significant pupicidal effect of the plant extracts of *Moringa oleifera* against *A. stephensi* and pupal mortality of greater than 70% was recorded.

The positive control (DDVP 2000 ppm) caused 100% mortality to the mosquito pupae; while the extracts and fractions from *B. dalzielii* leaf manifested only moderate pupicidal activity. The most potent plant product, *n*-hexane fraction, caused 73% mortality of *A. gambiae* and 43% mortality of *C. quinquefasciatus* at 2500 ppm. The situation that mosquito larvae feed when pupae do not feed at that development stage might be the cause of the low activity of plant extracts and fractions. The only ways to attain pupae are through dermal or respiratory routes that could explain the moderate mortality of pupae in this study. Krishnappa et al[50] reported also similar observation when they tested ethyl acetate extract of *Gliricidia sepium* on larvae (92.2% mortality) and pupae (25.48% mortality) of *A. stephensi* at 100 ppm.

From this study, *B. dalzielii* methanol leaf extract, *n*-hexane, and chloroform fractions were most potent against *A. gambiae* and *C. quinquefasciatus*. This observation is also comparable to the effect of hexane, chloroform, and ethyl acetate leaf extracts of *Croton sparciflorus* (Euphorbiaceae) tested against the pupae of *C. quinquefasciatus* at 1000 ppm, where hexane fraction, which killed 89% pupae after 24 hours, was the most effective treatment.[51] Moreover, *A. gambiae* pupae were more susceptible to *n*-hexane fraction than *C. quinquefasciatus* pupae. This finding coincides with the results of Sivagnaname and Kalyanasundaram[52] who reported that *A. stephensi* pupae were 1.4 times more susceptible to the methanolic leaf extract of *Atlantia monophylla* than *C. quinquefasciatus* pupae.

In regression analysis model using result data from the biological experiments, the results are favorably attributed to the efficacy of the products tested in the case of $R^2 \geq 0.6$.[53] But in this present investigation, all R^2 values of plant-based products of the plant species against eggs, larvae, and pupae of mosquito species tested were ≥0.6, confirming the efficacy of our products tested. Moreover, almost all the chi-square (χ^2) values of the plant products were not significant, implying an approximation of the regression models to the theoretical models concerning the effectiveness of the products against mosquito species.[36]

Conclusion

From our findings, methanol extract, *n*-hexane, and chloroform fractions of *B. dalzielii* showed a significant ovicidal, larvicidal, and pupicidal activity against *A. gambiae* and *C. quinquefasciatus*. However, only the *n*-hexane fraction obtained after fractionation of the crude methanolic extract was potent enough and, thus, might be considered in the control of the immature stages of the two mosquito species, in small volume aquatic habitats or breeding sites of limited size around human dwellings.

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Author Contributions

Conceived and designed the experiments: LY, COE, and ENN. Analyzed the data: LY. Wrote the first draft of the article: LY. Contributed to the writing of the article: COE and ENN. Agreed the article results and conclusions: COE and ENN. Jointly developed the structure and arguments for the article: COE and ENN. Made the critical revisions and approved the final version: ENN. All authors reviewed and approved the final article.

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