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Alstonia boonei De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease

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ABSTRACT

Objective: To evaluate the insecticidal potential of *Alstonia boonei* (*A. boonei*) oils and derivatives against different life stages of a malaria vector, *Anopheles gambiae*.

Methods: The leaf, stem bark and root bark of *A. boonei* were collected from an open field and air dried before being blended to fine powder. Oils from this plant were extracted by cold extraction and were prepared at different concentrations. Contact toxicity of *A. boonei* was tested against the larvae and pupae of the insect while smoke toxicity of the plant materials in form of mosquito coil was tested against the adult insect.

Results: Alstodine recorded the highest insect mortality rate and the order of susceptibility of the life stages of the insect to the plant was pupae < adult < larvae. Alstodine recorded the highest repellant activity (100%) after 4-5 h of application. However, all the treatments achieved high repellency (above 70%) after 6-7 h of application compared with the control. The formulated mosquito coil (smoke) of *A. boonei* oil extracts and derivatives showed high rate of protectability as they achieved above 55% protection. Moreover, alstodine (83.22%) showed the greatest smoke toxicity effect on the insect as it recorded almost the same percentage protection as the positive control (Raid synthetic insecticide) which recorded 83.56% protection.

Conclusions: This present study has proven *A. boonei* oil extracts and derivatives as a potential botanical insecticide which could serve as a new thoroughfare of mosquito control. Moreover, the order of effectiveness of the plant can be arranged thus: alstodine > alstonine > stem bark extract > leaf extract > root bark extract.

1. Introduction

Mosquitoes are rural-urban insect which had been noted for their high prevalence in many developing countries where insect pest management is still minimal. Mosquitoes of different species including *Aedes aegypti*, *Anopheles dirus, Culex quinquefasciatus, Aedes albopictus, Anopheles funestus, Anopheles arabiensis, Anopheles annularis, Anopheles culicifacies, Anopheles stephensi* and *Anopheles gambiae* (*An. gambiae*) among others have been noted to be vector of different types of diseases among which malaria is the most prevalent in developing nations[1-9]. The high prevalence of malaria disease in Nigeria has been reported and the disease has been the leading cause of morbidity and mortality in the country [10,11]. In fact, 50% of Nigeria population suffers at least one episode of malaria each year and this has been posting negative effect on the economic growth of the country[10,12,13].

Therefore, the control of the causative agent/vector of this disease becomes imperative in order to reduce its prevalence among the citizens of the country and other developing countries. Since mosquitoes have been noted to be the major vector of malaria borne diseases, different types of synthetic chemical insecticides such as deet, IR3535 and KBR 3023 have been employed as repellent against this insect. However, the unfriendly effect of most of these past advocated synthetic chemical insecticides leads the insect pest managers of the world to comb for alternative ways of scheming this disease causing insect. Before the discovery of the popular synthetic chemical insecticides in the late 1930s, tars, smokes, plant oils and other

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modalities have been used as repellent of mosquitoes in many parts of the world[14].

Botanical insecticides have been gaining more attention and popularity as government of many developed countries are putting embargo on the use of many conventional chemical insecticides. This increasing popularity of plant base insecticides has been associated with their no or little mammalian toxicity eco-friendly. Also, they have been noted to be easily biodegradable and cheap[9,15-17]. Many botanical oils such as *Curuma longa, Cymbopogon winterianus, Ocimum americanum, Nepeta cataria, Viola odorata, Melaleuca quinquenervia, Azadirachta indica, Citrus medica* and *Murraya koenigii* have been used against wide range of mosquito species[1,7,8,18-21]. However, many of these popular botanical species which have been used for the control of mosquitoes were not popular among Nigerians and since botanicals and their derivatives still remain the promising alternative to obviate the use of synthetic chemical insecticides, there is need to search for other local Nigerian plants that could be useful in the control of this infamy insect. Moreover, tropical regions of the world including Nigeria have been noted to be well endowed with plant species that contain myriad of chemicals that are insecticidal in nature[22-27].

Alstonia boonei De Wild (Apocynaceae) (*A. boonei*) is a medicinal plant which has been used as herbal medicine for the treatment of malaria, rheumatism, asthma, toothache and ulcer[28]. Alstonine, alstodine and porphine are the active compounds found in the plant[29,30]. This plant has been proven insecticidal against different stored product insect pests[31]. Therefore, this present work evaluated the insecticidal efficacy of *A. boonei* crude oil extract, alstodine and alstonine against different life stages of mosquito.

2. Materials and methods

2.1. Collection and rearing of mosquito larva and pupa

Mosquitoes' baits consisting of shallow containers with a large surface area and which are opaque in colour were established in the Hatchery Laboratory, Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria. The opaque coloured container was filled with rain water in order to mimic mosquito's natural breeding environment and also to attract adult mosquitoes for oviposition. Small quantity of industrial yeast was sprinkled on the surface of the water and allowed to decompose slowly as this will nourish the developing larva. Wild mosquitoes were allowed to freely visit the bait and to lay eggs. Afterward the containers bearing mosquitoes larvae and pupae were transferred to the laboratory, identified and maintained at temperature of (28 ± 2) ℃ and (75 ± 5)% relative humidity.

2.2. Collection of plant materials

The leaf, root and stem bark of *A. boonei* were collected from permanent site of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The plant was identified in the Department of Plant Science and Biotechnology of the University. The two derivatives used (alstodine and alstonine) were bought from a pesticides store in Lagos, Nigeria.

2.3. Extraction of plant materials

The leaf, stem bark and root of *A. boonei* were air dried in the laboratory and were grounded into fine powders using an electric blender (Binatone Model BLG 400). The powders were further sieved to pass through 1 mm^2 perforations[32], thereafter stored in separate plastic containers with tight lids and stored in a refrigerator at 4 ℃ prior to use.

Acetone extracts of leaf, stem bark and root of *A. boonei* were made using cold extraction method. About 100 g of each of the powder were soaked separately in an extraction bottle containing 100% acetone. The mixtures were stirred occasionally with a glass rod and extraction was terminated after 72 h. Filtration was carried out using a double layer of Whatman No. 1 filter paper and acetone evaporated using a rotary evaporator at 30-40 ℃ with rotary speed of 3 to 6 r/min for 8 h. The resulting extract was air dried in order to remove traces of solvent. The crude extract was kept in a dark bottle labeled and preserved in the refrigerator till further use.

Alstodine and alstonine which are derivatives of *A. boonei* were bought from a chemical store at Surulere, Lagos, Nigeria and were kept in an air tight bottle inside refrigerator until use.

2.4. Effect of A. boonei extracts on larvae and pupae of An. gambiae

Larvicidal and pupacidal activity of the plant extracts was carried out at different concentrations by preparing the required stock solutions following the standard procedure[33]. The desired concentrations were achieved by adding 1.0 μg of the crude extract from leaf, root and stem barks as well as alstodine and alstonine to 100 mL of distilled water. From this, five concentrations of 1%, 2%, 3%, 4% and 5% of the plant extracts and its derivatives were prepared. The treatments were separately added to 2.5 L of water inside a bowl and yeast powder was added in order to provide source of food for the introduced larvae. Twenty larvae and pupae of *An. gambiae* were separately introduced into the treated water and untreated water was set as control. Each treatment was replicated five times. Mortality was observed over 24 h after the introduction of larvae and pupae to notice recovery; a recovery time of 5 min was allowed[7,33]. The larva mortality in treatments was corrected for the controls[34]. Larvae and pupae were counted as dead when they were not coming to the surface for respiration and were insensitive to probe[7,35].

2.5. Fumigant effect of plant extracts on adult An. gambiae

The method described by Akinkurolere *et al.* was adopted to evaluate the effect of the plant and its derivative on the insect with

little modification[7]. Twenty adults were placed inside a test-tube and plugged with cotton wool. Strips of filter papers (3 cm × 3 cm) were soaked in varying concentrations of extracts and the derivatives and then suspended in the test-tube. Each treatment was replicated five times. Mortality was recorded 3 h after application.

2.6. Repellent activity of A. boonei extracts and its derivatives

Repellent activity of leaf, stem bark and root of *A. boonei* and its derivatives (alstonine and alstodine) was tested using the method described by Murugan *et al*[36,37]. Human volunteers were used to test how the plant and its derivatives can protect against mosquito bite. Hundred 3-4 days old blood starved female *An. gambiae* that are sterile were kept in a net cage. The forearms of each volunteer were cleaned with isopropanol. After air-drying the forearm, 25 cm^2 of skin of each of the arm was exposed, and the remaining areas were covered with hand glove. The extracts and the two derivatives were dissolved in isopropanol and each treatment was prepared at 1%, 2%, 3%, 4% and 5% concentrations. Both the control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 5 min every 60 min for 6 h. Each treatment was replicated five times. The percentage protection was calculated by using the formula below:

$$\%\text{protection} = \frac{\text{number of bites on control arm - number of bites on treated arm}}{\text{number of bites on control arm}} \times 100$$

2.7. Mosquito coil toxicity assay of A. boonei and its derivatives

The leaf, stem bark and root of *A. boonei* extract and its derivatives were used in form of smoke to mimic synthetic chemical mosquito coils. The method described by Prabhu *et al.* was adopted to prepare the mosquito coils with minor changes[37]. A semi-solid paste material which served as a mosquito coil was formulated by separately thoroughly mixed with 10 mL of 50% concentrated plant materials, 5 g coconut shell, and charcoal powder each and distilled water. The formulation was allowed to dry under shade and was about 0.6 cm in thickness. Two control treatments were made. The first control coils were made without the extracts as well as alstonine and alstodine while the second control was made by using synthetic chemical insecticide (Raid) which served as positive control. The second control served as positive control to compare the effectiveness of botanical source mosquito coil and commercial coils. The experiments were conducted in a glass chamber of 120 cm × 80 cm × 40 cm. A window of 40 cm × 20 cm was situated at the mid bottom of one side of the chamber. Hundred 3-4 days old blood starved adult female *An. gambiae*, fed with sucrose solution, were released into the chamber. A belly shaven albino rat was kept tied inside the cage in an immobilized state. The experimental chamber was tightly closed. The experiment was replicated five times on separate days, including control mosquitoes of the same age groups. The data were obtained and average values were subsequently used for calculations. After the experiment over fed and unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of adult mosquito was calculated by using the formula below:

$$\%\text{Protection} = \frac{\text{Number of unfed mosquitoes in treatments - Number of unfed mosquitoes in control}}{\text{Number of mosquitoes treated}} \times 100$$

2.8. Statistical analysis

All the data obtained were subjected to One-way analysis of variance at 5% significant level and means were separated with Duncan's new multiple range test using SPSS version 17. Also data, obtained from mosquito's mortality, were subjected to regression analysis to calculate the LC_{50} of the extracts as well as the derivatives using probit analysis[38].

3. Results

3.1. Mortality of An. gambiae larvae treated with A. boonei oil extracts and its derivatives

Table 1 presents the effect of *A. boonei* oil extracts and its derivatives on larvae of *An. gambiae*. The mortality of the insect larvae varied with the type of plant parts used as well as increase in concentration of the oils. Only the alstodine, a derivative of *A. boonei* was able to achieve 100% insect mortality with 24 h of application and its effect was significantly ($P < 0.05$) different from others. Moreover, all the plant parts achieved above 50% mortality of *An. gambiae* except root extract which only achieved 42.50% mortality at 2% concentration. At all levels of concentration, the effect of the plant parts and the two derivatives were significantly ($P < 0.05$) different from the control.

Table 1
Percentage mortality of larvae of *An. gambiae* treated with different concentrations of *A. boonei* extracts and its two derivatives.

Plant materials	Concentrations (%)				
	1	2	3	4	5
Leaf	40.00 ± 4.08^b	52.00 ± 4.08^b	72.50 ± 7.50^c	88.00 ± 4.08^c	96.00 ± 0.24^c
Stem bark	65.00 ± 2.89^c	77.50 ± 0.50^b	88.00 ± 0.00^c	92.00 ± 0.00^{cd}	100.00 ± 0.00^c
Root	30.00 ± 4.08^b	42.50 ± 2.50^c	67.50 ± 2.50^b	77.50 ± 2.50^b	86.80 ± 0.14^b
Alstodine	88.00 ± 0.24^d	100.00 ± 0.00^e	100.00 ± 0.00^d	100.00 ± 0.00^d	100.00 ± 0.00^c
Alstonine	78.60 ± 0.22^d	89.00 ± 0.12^d	100.00 ± 0.00^d	100.00 ± 0.00^d	100.00 ± 0.00^c
Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

Each value is a mean ± standard error of five replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

3.2. Mortality of An. gambiae pupae treated with A. boonei oil extracts and its derivatives

Mortality of *An. gambiae* pupae exposed to oil extracts and two derivatives of *A. boonei* at different concentrations are presented in

Table 2. All the oil extracts and its derivatives significantly exerted high mortality rate on the insect pupae. However, the effect of the oils were concentration dependent. Only alstodine was able to achieve 100% insect mortality within 24 h of application at 3% concentration and its effect was significantly ($P < 0.05$) different from others. However, none of the oil extracts was able to achieve 100% pupae mortality even at higher concentrations of 4% and 5%.

Table 2

Percentage mortality of pupae of *An. gambiae* treated with different concentrations of *A. boonei* extracts and its two derivatives.

Plant materials	Concentrations (%)				
	1	2	3	4	5
Leaf	27.50 ± 2.88^{b}	37.50 ± 2.50^{b}	46.50 ± 7.50^{b}	56.00 ± 4.08^{b}	70.26 ± 4.08^{c}
Stem bark	52.50 ± 1.33^{c}	62.00 ± 4.08^{c}	68.78 ± 4.08^{c}	82.00 ± 0.16^{c}	88.00 ± 0.00^{d}
Root	20.00 ± 0.45^{b}	29.50 ± 2.50^{b}	38.00 ± 4.08^{b}	47.50 ± 2.50^{b}	60.00 ± 2.89^{b}
Alstodine	75.80 ± 0.88^{c}	90.20 ± 0.18^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Alstonine	68.00 ± 2.44^{d}	84.00 ± 2.24^{d}	88.00 ± 2.24^{d}	94.26 ± 0.88^{d}	100.00 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean±standard error of five replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

3.3. Fumigant toxicity of A. boonei oil extracts and its derivatives against adult An. gambiae

The fumigant toxic effect of *A. boonei* different plant parts and derivatives against adult *An. gambiae* are presented in Table 3. The results showed that none of the extracts and the derivatives were able to achieve 100% insect mortality at lower concentrations (1% and 2%). However, alstodine achieved the highest mortality of 85.00% and 96.20% at 1% and 2% concentrations respectively and this was significantly ($P < 0.05$) different from others at these levels of concentrations. Only alstodine and alstonine achieved 100% adult insect mortality at 3%, 4% and 5% concentrations and their effects were significantly ($P < 0.05$) different from oil extracts and the control.

Table 3

Percentage mortality of adult of *An. gambiae* treated with different concentrations of *A. boonei* extracts and its two derivatives.

Plant materials	Concentrations (%)				
	1	2	3	4	5
Leaf	35.62 ± 0.82^{b}	44.50 ± 0.88^{b}	66.60 ± 2.66^{b}	76.00 ± 4.08^{b}	88.26 ± 4.08^{c}
Stem bark	64.50 ± 1.44^{c}	74.00 ± 1.02^{c}	78.68 ± 1.24^{c}	82.00 ± 0.28^{c}	96.00 ± 0.13^{d}
Root	28.80 ± 0.24^{b}	34.62 ± 2.50^{b}	54.00 ± 1.25^{b}	47.50 ± 2.66^{b}	78.00 ± 1.24^{b}
Alstodine	85.00 ± 0.28^{c}	96.20 ± 0.18^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Alstonine	76.20 ± 2.88^{d}	94.00 ± 2.24^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean±standard error of five replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

3.4. LC_{50} of A. boonei oil extract and derivatives in An. gambiae after 24 h

Table 4 shows that lower amounts of alstodine (0.20%, 0.38% and 0.18%) is required to achieve 50% mortality in adult, pupae and larvae of *An. gambiae* respectively. Compared to the amount of extracts required, the two derivatives showed more entomotoxic efficacy than the oil extracts of the plant. However, fiducial limits revealed that a lower amount of alstodine was required to cause 50% mortality in larvae (0.04-0.32) of *An. gambiae* when compared to the amount needed for adult (0.06-0.34) and pupae (0.12-0.64) respectively. This further revealed that the *An. gambiae* larvae were more susceptible to *A. boonei* oil extracts as well as its derivatives than pupae and adults. The effectiveness of the *A. boonei* extracts and derivatives can be arranged in the following order: alstodine > alstonine > stem bark oil extract > leaf oil extract > root bark oil extract.

Table 4

LC_{50} of *A. boonei* extracts and derivatives required achieving 50% mortality in *An. gambiae* after 24 h post treatment.

Mosquito life stages	LC_{50} (%) of plant materials				
	Leaf	Stem	Root	Alstodine	Alstonine
Adult	2.08 (1.20-2.96)	0.82 (0.26-1.38)	2.14 (0.84-3.44)	0.20 (0.06-0.34)	0.23 (0.12-0.34)
Pupae	3.06 (2.46-3.66)	1.02 (0.20-1.84)	4.01 (2.35-5.67)	0.38 (0.12-0.64)	0.40 (0.24-0.56)
Larvae	1.38 (0.82-1.94)	0.80 (0.11-1.49)	2.64 (1.43-3.85)	0.18 (0.04-0.32)	0.22 (0.14-0.30)

Values in parenthesis represent 95% fiducial limits.

3.5. Repellant activity of A. boonei extracts and its derivatives against An. gambiae

The repellant activity of *A. boonei* extracts and derivatives in terms of percentage protection against *An. gambiae* are presented in Table 5. Among the treatments, alstodine showed the highest repellant activity against adult *An. gambiae* at all hours of exposure. Within 1-2, 2-3 and 3-4 h of exposure, only alstodine at 4% concentration achieved complete protection but its effect was not significantly ($P > 0.05$) different from its concentration at 3%, 4% and 5% of alstonine. However, at all levels of concentration and period of exposure, the two *A. boonei* derivatives were significantly ($P < 0.05$) different from the oil extracts of the leaf, stem bark and the root bark of the plant except at 6-7 h of exposure where the lower concentrations (1% and 2%) of the two derivatives were not relatively different from the higher concentrations of the stem bark oil extract. Moreover, all the extracts of *A. boonei* and its derivatives were significantly ($P < 0.05$) different from the control.

3.6. Smoke toxic effect of A. boonei and its derivatives against An. gambiae

Table 6 presents the smoke toxic effect of *A. boonei* oil extracts and derivatives against adult *An. gambiae*. None of the treatments and the positive control (Raid insecticide) was able to prevent the adult mosquito from feeding. However, the positive control had the lowest number of fed insect (8.86), which was significantly ($P < 0.05$) diferent from other treatments except alstodine which recorded 9.20. In the same manner, the positive control recorded the highest number of unfed mosquito and its effect was considerably different from other treatments except alstodine. Furthermore, none of the treatments was able to achieve 100%

Table 5

Repellant activity of *A. boonei* extracts and its derivatives against *An. gambiae*.

Plant materials	Concentrations (%)	Percentage protection at different hours					
		1-2	2-3	3-4	4-5	5-6	6-7
Leaf	1	84.24 ± 0.28^{b}	84.00 ± 0.88^{c}	83.26 ± 2.04^{c}	80.00 ± 0.00^{c}	78.66 ± 0.42^{bc}	76.82 ± 0.23^{bc}
	2	84.88 ± 0.23^{b}	84.26 ± 1.88^{c}	84.04 ± 2.22^{cd}	82.66 ± 0.24^{c}	82.00 ± 0.88^{cd}	78.96 ± 1.24^{cd}
	3	85.42 ± 0.44^{b}	85.24 ± 0.18^{cd}	84.76 ± 0.82^{cd}	84.24 ± 1.22^{c}	82.44 ± 1.24^{cd}	80.16 ± 0.56^{de}
	4	87.66 ± 1.24^{bc}	86.48 ± 0.24^{cd}	86.12 ± 1.00^{de}	84.98 ± 0.24^{cd}	84.68 ± 0.65^{d}	84.02 ± 0.88^{e}
	5	89.84 ± 0.18^{c}	88.64 ± 0.53^{d}	87.92 ± 0.88^{de}	86.64 ± 2.43^{d}	85.42 ± 0.23^{d}	84.88 ± 1.12^{e}
Stem bark	1	86.82 ± 0.24^{bc}	86.16 ± 1.64^{cd}	86.04 ± 0.23^{de}	85.24 ± 0.18^{d}	84.16 ± 1.33^{d}	82.64 ± 0.88^{de}
	2	88.12 ± 1.36^{c}	88.00 ± 0.64^{de}	86.82 ± 1.23^{de}	86.12 ± 2.44^{d}	84.98 ± 0.24^{d}	84.22 ± 1.33^{ef}
	3	89.06 ± 1.22^{cd}	89.00 ± 0.28^{de}	88.84 ± 0.56^{e}	86.44 ± 0.64^{d}	86.00 ± 0.00^{de}	84.96 ± 2.26^{ef}
	4	90.34 ± 0.58^{d}	89.82 ± 0.24^{de}	89.14 ± 2.14^{e}	88.00 ± 0.56^{d}	86.84 ± 0.24^{de}	86.04 ± 1.24^{f}
	5	90.68 ± 0.34^{d}	90.46 ± 1.64^{e}	89.92 ± 1.53^{e}	88.46 ± 0.28^{d}	86.88 ± 1.22^{de}	86.44 ± 2.26^{f}
Root	1	80.98 ± 0.26^{b}	79.86 ± 3.24^{b}	77.96 ± 0.56^{b}	76.24 ± 1.00^{b}	74.42 ± 0.28^{b}	70.24 ± 0.16^{b}
	2	82.62 ± 1.48^{b}	82.42 ± 0.43^{bc}	82.04 ± 1.23^{c}	80.12 ± 0.88^{c}	76.24 ± 2.14^{b}	72.24 ± 1.24^{b}
	3	86.44 ± 0.64^{bc}	86.12 ± 0.13^{cd}	85.67 ± 2.04^{cd}	84.88 ± 0.23^{cd}	82.68 ± 0.33^{cd}	78.46 ± 2.18^{cd}
	4	88.24 ± 0.88^{c}	88.00 ± 0.86^{de}	86.86 ± 1.55^{cd}	86.16 ± 1.64^{d}	85.78 ± 2.33^{d}	82.40 ± 0.22^{e}
	5	88.56 ± 0.68^{c}	88.18 ± 1.64^{de}	88.16 ± 1.55^{ef}	87.66 ± 0.24^{d}	85.92 ± 0.24^{d}	84.14 ± 0.28^{ef}
Alstodine	1	95.92 ± 0.44^{e}	94.44 ± 2.12^{f}	94.16 ± 1.00^{g}	94.00 ± 0.00^{ef}	90.00 ± 0.00^{f}	88.86 ± 2.68^{fg}
	2	97.22 ± 1.33^{de}	96.14 ± 0.28^{fg}	94.66 ± 2.34^{g}	94.12 ± 0.16^{ef}	92.44 ± 2.12^{f}	90.04 ± 0.56^{gh}
	3	98.88 ± 0.24^{ef}	98.16 ± 1.22^{g}	97.44 ± 1.26^{gh}	96.42 ± 0.28^{f}	94.66 ± 2.34^{fg}	94.44 ± 0.24^{hi}
	4	100.00 ± 0.00^{f}	100.00 ± 0.00^{g}	100.00 ± 0.00^{h}	98.68 ± 0.12^{fg}	98.16 ± 0.12^{g}	96.68 ± 0.32^{ij}
	5	100.00 ± 0.00^{f}	100.00 ± 0.00^{g}	100.00 ± 0.00^{h}	100.00 ± 0.00^{fg}	98.78 ± 0.22^{g}	98.22 ± 1.24^{j}
Alstonine	1	90.82 ± 2.33^{d}	90.04 ± 1.22^{e}	89.94 ± 0.82^{ef}	88.92 ± 2.42^{d}	86.14 ± 1.13^{de}	84.46 ± 1.24^{ef}
	2	92.04 ± 0.23^{d}	91.89 ± 1.43^{e}	91.24 ± 1.22^{fg}	90.46 ± 1.13^{de}	88.96 ± 0.24^{e}	86.02 ± 0.88^{fg}
	3	96.24 ± 0.26^{e}	94.88 ± 0.12^{f}	94.48 ± 0.59^{g}	94.12 ± 0.33^{ef}	92.62 ± 0.12^{f}	90.12 ± 0.88^{gh}
	4	98.96 ± 1.67^{ef}	98.62 ± 0.28^{g}	98.44 ± 2.16^{h}	96.68 ± 0.24^{f}	96.16 ± 0.88^{g}	92.82 ± 0.42^{gh}
	5	100.00 ± 0.00^{f}	100.00 ± 0.00^{g}	100.00 ± 0.00^{h}	98.78 ± 2.14^{fg}	96.28 ± 2.56^{g}	95.16 ± 2.63^{hij}
Control		0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is a mean ± standard error of five replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

protection but they all achieved above 55% protection. Control II (positive control) achieved the highest level of protection (53.56%) but its effect was not significantly ($P > 0.05$) different from that of alstodine which also achieved 83.22% protection. Thus, the order of effectiveness of the smoke of *A. boonei* extracts and derivatives can be arranged as following: alstodine > alstonine > stem bark > leaf > root bark.

Table 6

Smoke toxic effect of *A. boonei* and its derivatives against *An. gambiae*.

Plant materials	Total number of insects	Fed mosquitoes	Unfed mosquitoes	%Protection
Leaf	100	29.84 ± 0.02^{c}	70.16 ± 0.23^{c}	62.58 ± 0.88^{c}
Stem	100	24.62 ± 1.00^{c}	75.38 ± 0.56^{c}	67.80 ± 0.88^{d}
Root	100	34.20 ± 0.12^{d}	65.80 ± 0.24^{b}	58.22 ± 0.12^{b}
Alstodine	100	9.20 ± 0.12^{a}	90.80 ± 0.43^{e}	83.22 ± 1.24^{f}
Alstonine	100	14.66 ± 0.24^{b}	85.34 ± 0.12^{d}	77.76 ± 0.42^{e}
Control I	100	92.42 ± 0.22^{e}	7.58 ± 0.25^{a}	0.00 ± 0.00^{a}
Control II	100	8.86 ± 0.02^{a}	91.14 ± 0.82^{e}	83.56 ± 0.56^{f}

Each value is a mean±standard error of five replicates. Means followed by the same letter along the column are not significantly different ($P > 0.05$) using Duncan's new multiple range test.

4. Discussion

The public awareness of the perils associated with use of chemical insecticides has created an avenue for botanical source insecticides to gain more popularity in the global world insecticides market. However, the need for search of underutilized potential botanicals that could profoundly contend with many active chemical insecticides is a major concern. This is because many successful botanical insecticides that are in market are associated with some cons that may thwart their widespread use in future[27,39]. *A. boonei* is a medicinal plant with many insecticidal attributes but they are underused.

The results obtained in the research showed that oil extracts from the leaf, stem bark and root bark of *A. boonei* as well as its two derivatives (alstodine and alstonine) had a considerable effect on all life stages of *An. gambiae*. However, the effectiveness of *A. boonei* extracts and derivatives was concentration and insect life stages dependent. The larval stage of *An. gambiae* appeared to be the most susceptible to *A. boonei* extracts while the pupae appeared to be most tolerant to the plant oils. Nevertheless, the oils and the two derivatives recorded high insect mortality within 24 h of application. The effect of the oils and derivatives of *A. boonei* on the insect survival could be due to their ability to disrupt the normal respiratory activity of *An. gambiae*. Botanical source insecticides have been noted to have a considerable effect on the normal respiration of insects as many of them have a knack to block the respiratory organ (spiracle) of insects[7,9,27]. Therefore, the ability of the tested plant and its derivatives to exert high mortality of *An. gambiae* could be linked to their ability to block the insects spiracles. The oils and derivatives of this

plant may have also affected the swimming ability of the larvae and pupae of the insect as suggested by Bhattacharya *et al.* that botanical oils have a considerable effect on the swimming ability of larvae and pupae of mosquito and reduction in their surviving rate[40]. The results obtained in this study also proven the high efficacy of *A. boonei* oil extracts and its derivatives as a good mosquito repellant as they all achieved above 70% protection against mosquito bite 7 h after application. This result acquiesced with the result of Singh and Mittal in which leaf extract of *Blumea lacera* at 6% concentration recorded 78.8% and 76.2% of *Anopheles stephensi* and *Culex quinquefasciatus* respectively after 6 h[41]. Furthermore, the results obtained from the smoke experiment showed that *A. boonei* could effectively contend with many synthetic chemicals popularly used in many developing nations as there was no significant differences between the alstodine (a derivative of *A. boonei*) and the positive control (Raid, synthetic insecticide).Moronkola *et al.* reported the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides, terpenoids and steroids in *A. boonei* crude extracts[30,42,43]. All these phytochemicals present in *A. boonei* had been reported to disrupt growth and reduced larva survival as well as disruption of life cycle of insects[44]. Therefore, this could also contribute to the high effectiveness of *A. boonei* extracts and derivatives against *An. gambiae*. *A. boonei* is a medicinal plant which has been found to heal diseases including malaria, rheumatism, asthma, toothache and ulcer[42,45]. Also, this plant is found to have anti-inflamatory, analgesic and antipyretic activities. The stem bark of the plant had been used as antivenom for snake bite and as well as used after delivery to aid removal of placenta[42]. This potential plant had also been proven insecticidal against stored grains beetles[31,32]. However, despite the potency of this plant, less attention has been given to it compared with other popular botanicals such as *Azadirachta indica*, *Nacotiana tabacum* and *Eugenia aromatica* which had been incorporated into pest management programme. The result of this present study has proven *A. boonei* oil extracts and derivatives as a potential botanical source insecticide which could serve as a new boulevard of insect control. Since this plant has shown a great insecticidal potential against mosquito as it was able to vie with the synthetic insecticide used in the research (Raid-positive control), it could be integrated into malaria vector management strategy. Moreover, the order of effectiveness of the plant can be arranged as following: alstodine > alstonine > stem bark extract > leaf extract > root bark extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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