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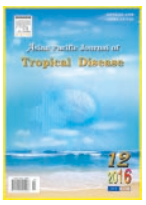

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Repellent activity of the creams formulated from *Annona senegalensis* and *Boswellia dalzielii* leaf fractions and essential oils against *Anopheles gambiae* (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate the repellent efficacy of the creams formulated from methanol extract and *n*-hexane, chloroform, ethyl acetate and methanol fractions as well as essential oils of *Annona senegalensis* (*A. senegalensis*) and *Boswellia dalzielii* (*B. dalzielii*) leaves against the malarial vector *Anopheles gambiae* (*An. gambiae*) in the laboratory.

Methods: The efficacies of 25% w/w active ingredient creams formulated from the plant-based products were tested. Different concentrations of the creams, ranging from 2.0 to 12.0 mg/cm were applied on the exposed dorsal hand area (25 cm^2) of volunteers. The treated hands were submitted to 50 caged blood-starved females of *An. gambiae* for 3 min after every 30 min until 180 min.

Results: Total protection of up to 120 and 60 min without bites of *An. gambiae* were recorded with *n*-hexane creams applied at 12 mg/cm^2 respectively for *A. senegalensis* and *B. dalzielii*. The essential oil creams of the two tested plants applied at 6 mg/cm^2 protected volunteers up to 120 min without mosquito bites. The commercial Odomos cream (12% N,N-diethyl-3-methylbenzamide) tested as the positive control at 6 mg/cm^2 protected volunteers from mosquito bites up to180 min.

Conclusions: These results suggest that the cream formulated from the *n*-hexane fraction of *A. senegalensis* and essential oil creams of *A. senegalensis* and *B. dalzielii* leaves have the potential of a natural herbal source for the development of new, safe and eco-friendly repellent products to prevent *An. gambiae* bites.

1. Introduction

Mosquitoes belonging to the genera of *Anopheles*, *Aedes* and *Culex* have tormented humans with their bites and the transmission of some deadly diseases such as malaria, Dengue fever, Chikungunya, yellow fever and lymphatic filariasis[1]. In Africa, several *Anopheles* mosquito species are malaria vectors with the species *Anopheles gambiae* Giles (*An. gambiae*) being the most important and widely distributed one[2]. According to the World Health Organization[3], 584 000 people died in 2013 from malaria worldwide with 90% of these deaths occurring in Africa. In Cameroon, malaria is by far the leading cause of morbidity (15.6%) and mortality (13.0%)[4].To reduce the risk of malaria transmission in human populations, antimalarial drugs and vector control measures are emphasized[5]. Unfortunately, the disease still remains a threat for human health because of the unavailability of antimalarial vaccines and the inadequacy of vector monitoring in Africa. However, a suitable vector control measure for malaria prevention would be a part of the solution[6]. Among malaria control methods, the use of repellents is attractive, because it reduces contacts between mosquitoes and their hosts, and in turn, would lower the rate of disease transmission in many instances[7]. In this line, synthetic repellent products containing N,N-diethyl-3-methylbenzamide (DEET) which constitutes excellent repellent agents against mosquitoes and other biting insects are predominant in the market[8]. Unfortunately, these DEET based repellent scan cause irreversible damage to the ecosystem, as some of them are non-biodegradable. They may also cause skin irritations and unpleasant smell as well as discomfort owing to an oily feeling to some users[9,10]. The health and environmental problems associated with the use of synthetic repellents have forced researchers to seek for repellents based on other less hazardous chemicals. Therefore,

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The study protocol was performed according to the Helsinki declaration and approved by Anambra State University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria Ethics Review Committee. Informed written consent was obtained from the students (2 women and 2 men without their identities) of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Agulu, Awka, Nigeria.

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much interest is being focused on repellents of plant origin, because they are more biodegradable and less harmful to humans and domestic animals. Thus, several plant-based repellent formulations including inter alia *Cymbopogon citratus*, *Eucalyptus globulus*, *Curcuma longa*, *Azadirachta indica*, *Mentha piperita*, *Tribulus terrestris*, *Blumea lacera* were effective against *Anopheles*, *Aedes* and *Culex* mosquito species[11].

Annona senegalensis (*A. senegalensis*) (Annonaceae) is a bushy shrub or small tree, mostly found in Savannah and parts of the tropical rain forest regions of Africa[12]. As an insecticide, the leaf powder of *A. senegalensis* was toxic to *Sitophilus zeamais* Motsculsky (Coleoptera: Curculionidae)[13] and *Tribolium castenum* Herbst (Coleoptera: Tenebrionidae)[14]. Gueye *et al.*[15] reported the insecticidal activity of extract and essential oils of this plant against the groundnut weevil *Caryedon serratus* (Olivier) (Coleoptera: Bruchidae). Salifou *et al*.[16] reported the effectiveness of *A. senegalensis* against fleas and lice in poultry. On mosquito species, larvicidal activity of the fractions of this plant was demonstrated against *An. gambiae*[17] and *Aedes* aegypti (L.) (*Ae.* aegypti)[18].

Boswellia dalzielii (*B. dalzielii*) (Burseraceae) is a tree that grows up to 13 m in height, found in Savannah regions, and is locally abundant in central and west of Africa[19]. The larvicidal activity of the fractions of this plant was reported against immature stages of *An. gambiae* and *Culex quinquefasciatus* Say (*Cx. quinquefasciatus*) [17,20].

The objective of this study was to evaluate the repellent efficacy of the creams formulated from the leaf extracts/fractions and essential oils of *A. senegalensis* and *B. dalzielii* against adult female *An. gambiae*.

2. Materials and methods

2.1. Plant materials

The green leaves of *A. senegalensis* were collected from Dang (latitude 7°24'9.49" N, longitude 13°32'8.70" E and altitude 1 093 m above sea level), Ngaoundéré in the Adamaoua region of Cameroon on November 2011, while *B. dalzielii* leaves were collected from Midjivin (latitude 10°10'8.00" N, longitude 14°20'0.70" E and altitude 456 above sea level), Maroua, Far North region of the same country on December 2011. The plants were identified by Pr. Mapongmetsem Pierre Marie, a botanist of the Department of Biological Sciences in University of Ngaoundéré in Cameroon, and the identity were confirmed at the National Herbarium in Yaoundé, Cameroon, where voucher samples were deposited under the registration number 7783/SRF-CAM and 20532/SRF-CAM for *A. senegalensis* and *B. dalzielii*, respectively. The leaves were dried in a room under ambient conditions, then pulverized with an electric grinder and screened using 0.4 mm mesh size sieve. The powders were then stored at –18 ˚C in a deep freezer until they were needed for bioassay.

2.2. Extraction and fractionation

Extraction and fractionation of the leaf powders of *A. senegalensis* and *B. dalzielii* were 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, 1 200 g of the powders of each plant were macerated in 2 500 mL of methanol for 72 h at room temperature and then the maceration was filtrated using filter paper Whatman No.1. The residue of the maceration was rinsed and filtered several times with the fresh methanol until a clear phase was obtained. The filtrate was submitted to a rotary evaporator apparatus to obtain a residue called crude extract.

For the fractionation process, the method of Gueye *et al*.[15] was used. A total of 200 g of the crude extract of each plant was separated successively by the method of differential solubility in four solvents of different polarity: *n*-hexane, chloroform, ethyl acetate and methanol solvents. The crude extract was mixed with silica gel (70–260 mesh size) and macerated in *n*-hexane, then filtered with Whatman No.1 filter paper after phase separation. *n*-Hexane fraction 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 the open air was soaked in ethyl acetate; phase ethyl acetate 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 a rotary evaporator and then stored at –4 ˚C in a refrigerator until needed for bioassay.

2.3. Extraction of essential oils

The finely-ground plant materials were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at –4 ˚C in a refrigerator until needed for bioassay.

2.4. Preparation of the repellent cream

The repellent cream for bioassay was formulated following the method used by Adeniran and Fabiyi[21]. Twenty-five percent (w/w) of the repellent cream product of each plant extract/fraction or essential oil were formulated. Pure white soft paraffin (8.0 g) was weighed in a 250 mL glass beaker and heated in a water bath at a temperature of about 50 ˚C. At this stage, 2.0 g of *A. senegalensis* or *B. dalzielii* extract/fraction or essential oil was added separately and mixed suitably. The mixture in a screwed covered bottle was stirred properly to ensure that the sample was uniformly mixed with the molten stage of the cream.

2.5. Collection and rearing of An. gambiae strain

The larvae of *An. gambiae* were collected from water sewage in gutters on February 2013 at Awka market in Anambra State of Nigeria and identified by the experts of the National Arbovirus Research Center, Enugu, Nigeria. To start the colony, the 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-dark cycles. Larvae were fed a diet containing crayfish and biscuit in a 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) where the adults emerged. The adults were maintained in cages and were continuously provided with 10% sucrose solution in a jar with a cotton wool. On day five, the adults were given a blood meal from a guinea pig shaved and placed on top of the cages overnight for blood feeding by females. Beaker with 100 mL of tap water lined with filter paper was kept inside the cage for oviposition. The sixth generation from the colony was used for the experiments.

2.6. Repellent test

To carry out the repellent test, ethical was obtained approval from Anambra State University Teaching Hospital, Amaku, Awka, Anambra State, Nigeria Ethics Review Committee. The acceptance with the reference number of ANSUTH/AA/ECC/39 was granted after the submission of the detailed research proposal of the present project to the Ethics Review Committee for proper study. Experiments were carried out against *An. gambiae* under ambient conditions according to the method used by the World Health Organization[22]. Repellent effects of each essential oil cream formulation were evaluated at the doses of 2.0, 4.0 and 6.0 mg/cm^2 while the extract/fractions cream repellents at the doses of 4.0, 8.0 and 12.0 mg/cm^2 by using the human bait method. A commercial repellent cream Odomos (12% DEET) was used as the positive control, while white soft paraffin was used as the negative control. The test was run during night time from 18.00 to 05.00 Greenwich mean time +1. The arms of three human volunteers were cleaned thoroughly with distilled water before the application of the repellent cream. Both arms were covered with rubber glove and a window area of 5 cm × 5 cm was opened on the dorsal part of the hand of the volunteers. The left hand was used for treatment and the right as control. After applying the test repellent, the volunteer was instructed not to rub, touch or wet the treated arm. The right hand, which acted as a control was treated only with white soft paraffin and was exposed for up to 30 s in a mosquito cage (30 cm × 30 cm × 35 cm) containing 50 nulliparous female mosquitoes (5–7 days old). If at least two mosquitoes landed on or bit the control arm, the repellency test was then continued for 3 min after every 30 min until 180 min. Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. An attempt of the mosquito to insert its stylet was considered as a bite. The experiments were repeated three times in separate cages and in each replicate different volunteers were used to minimize bias caused by the effect of skin differences on repellency. The percentage protection was calculated by using the following formula:

Protection (%) = [(NC – NT)/NC] × 100

Where NC was the number of mosquitoes that bit on the control area and NT was the number of mosquitoes that bit on the treated areas of the volunteer.

2.7. Statistical analysis

The percentage of the repellent protection data were subjected to the ANOVA procedure using SPSS 16.0. Turkey's test at $P = 0.05$ was applied for mean separation. Abbott's formula[23] was used for control repellency correction when the repellent activity in the control was between 3% and 10% before ANOVA analysis.

3. Results

The repellent activity of *A. senegalensis* extract and fractions cream formulations against adult females of *An. gambiae* were presented in Table 1. In general, the creams formulated with the extract and fractions of this plant caused a significant concentration-dependent repellent activity against adult females of *An. gambiae*, which declined with time post-exposure. The MCE cream formulation provided 100% protection without bites within 30, 60 and 90 min at 4.0, 8.0 and 12.0 mg/cm^2, respectively. After splitting the MCE of *A. senegalensis*, up to 120, 120 and 60 min of protection time without *An. gambiae* female bites were noted respectively for the concentrations 12.0, 8.0 and 4.0 mg/cm^2 with *n*-hexane fraction cream. With the chloroform fraction cream, total protection (100% repellency) without bites of *An. gambiae* were observed for 30 and 60 min at the concentrations 8.0 and 12.0 mg/cm^2, respectively. No repellent activity was observed with ethyl acetate and methanol fraction creams of this plant. The positive control (Odomos) achieved complete protection against *An. gambiae* bites, even 180 min post-exposure.

Table 1

Repellent activity of *A. senegalensis* leaf extract and fractions against *An. gambiae* in the laboratory [(25 ± 2) ℃, 72% ± 5% relative humidity].

Extracts/ fractions	Time (min)	Repellency concentration (%)				F value
		0.0 mg/cm^2	4.0 mg/cm^2	8.0 mg/cm^2	12.0 mg/cm^2	
MCE	30	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	60	0.00 ± 0.00^c	92.74 ± 2.90^b	100.00 ± 0.00^a	100.00 ± 0.00^a	341 3.00***
	90	0.00 ± 0.00^d	74.56 ± 0.76^c	90.45 ± 4.14^b	100.00 ± 0.00^a	139 8.00***
	120	0.00 ± 0.00^d	61.89 ± 1.67^c	76.27 ± 2.70^b	89.43 ± 3.24^a	917.19***
	150	0.00 ± 0.00^d	36.36 ± 4.32^c	54.62 ± 5.04^b	75.04 ± 9.73^a	87.79***
	180	0.00 ± 0.00^d	8.93 ± 2.33^c	23.73 ± 2.67^b	35.13 ± 1.60^a	192.07***
n-Hexane fraction	30	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	60	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	90	0.00 ± 0.00^c	93.20 ± 2.40^b	100.00 ± 0.00^a	100.00 ± 0.00^a	501 2.00***
	120	0.00 ± 0.00^c	86.32 ± 1.70^b	100.00 ± 0.00^a	100.00 ± 0.00^a	959 3.00***
	150	0.00 ± 0.00^d	77.55 ± 2.12^c	93.31 ± 0.45^b	96.47 ± 3.57^a	542 1.00***
	180	0.00 ± 0.00^c	53.44 ± 1.38^b	93.31 ± 0.45^a	94.66 ± 4.64^a	100 9.00***
Chloroform fraction	30	0.00 ± 0.00^c	90.67 ± 4.68^b	100.00 ± 0.00^a	100.00 ± 0.00^a	129 6.00***
	60	0.00 ± 0.00^d	64.18 ± 9.47^c	80.02 ± 3.13^b	100.00 ± 0.00^a	225.94***
	90	0.00 ± 0.00^d	38.06 ± 9.04^c	59.81 ± 8.49^b	81.32 ± 5.63^a	77.87***
	120	0.00 ± 0.00^d	22.72 ± 7.72^c	34.07 ± 4.18^b	62.52 ± 5.42^a	76.19***
	150	0.00 ± 0.00^d	7.75 ± 2.85^c	12.01 ± 0.43^b	45.83 ± 1.81^a	425.36***
	180	0.00 ± 0.00^c	3.18 ± 2.76^c	09.92 ± 3.18^b	16.58 ± 2.86^a	25.12***
Ethyl acetate fraction	30	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	60	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	90	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	120	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	150	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	180	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
Methanol fraction	30	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	60	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	90	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	120	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	150	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	180	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
Odomos	180	0.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-

Mean ± SE in the same row followed by the same letter do not differ significantly at $P = 0.05$. Each datum represents the mean of three replicates (Turkey's test). ***: $P < 0.001$.

The repellent effect of *B. dalzielii* leaf extract/fractions cream formulations against adult females of *An. gambiae* was presented in Table 2. The cream formulations of this plant showed a significant repellent activity against the female mosquito, which increased with concentrations but reduced with time post-exposure. The cream formulated from the MCE of *B. dalzielii* completely protected the skin against the bites of *An. gambiae* for 30 and 60 min when applied at 8.0 and 12.0 mg/cm^2, respectively. After fractionation, *n*-hexane fraction cream formulation totally protected the skin from the bites of the mosquito for 30, 60 and 60 min at the dose of 4.0, 8.0 and 12.0 mg/cm^2, respectively. The chloroform fraction cream formulation achieved complete protection of the skin only within 30 min at 12.0 mg/cm^2. As with the case of *A. senegalensis*, ethyl acetate and methanol fractions of *B. dalzielii* did not show any repellent effect against *An. gambiae* adult females. The commercial repellent (Odomos) used as the positive control accomplished total protection from the bites of *An. gambiae* for 180 min at the dose of 4.0 mg/cm^2.

The repellent activity of the creams formulated from the leaf

essential oils of *A. senegalensis* and *B. dalzielii* against *An. gambiae* adult females increased with ascending concentration but declined with time post-exposure (Table 3). At the concentrations 2.0, 4.0 and 6.0 mg/cm^2, the cream formulation of *A. senegalensis* essential oil completely protected the skin from mosquito bites for 60, 90 and 120 min, respectively. For *B. dalzielii* cream formulation at the concentrations of 2.0, 4.0 and 6.0 mg/cm^2, complete protection of the skin from the bites of *An. gambiae* female adults was achieved for 60, 90 and 120 min, respectively. The commercial cream repellent (Odomos) achieved complete protection up to 180 min from the bites of *An. gambiae* adult females at the dose of 4.0 mg/cm^2.

Table 2

Repellent activity of *B. dalzielii* leaf extract and fractions against *An. gambiae* in the laboratory [(25 ± 2) °C, 72% ± 5% relative humidity].

Extracts/ fractions	Time (min)	Repellency concentration (%)				F value
		0.0 mg/cm^2	4.0 mg/cm^2	8.0 mg/cm^2	12.0 mg/cm^2	
MCE	30	0.00 ± 0.00^c	88.03 ± 1.90^b	100.00 ± 0.00^a	100.00 ± 0.00^a	7771.00***
	60	0.00 ± 0.00^d	54.25 ± 3.87^c	89.24 ± 3.76^b	100.00 ± 0.00^a	836.33***
	90	0.00 ± 0.00^d	43.27 ± 4.55^c	77.37 ± 3.58^b	94.88 ± 1.63^a	579.89***
	120	0.00 ± 0.00^d	41.09 ± 5.82^c	61.33 ± 1.96^b	87.55 ± 3.53^a	325.93***
	150	0.00 ± 0.00^d	31.35 ± 4.85^c	46.23 ± 4.66^b	76.25 ± 2.34^a	237.76***
	180	0.00 ± 0.00^d	23.90 ± 0.50^c	31.10 ± 3.98^b	53.64 ± 1.84^a	300.56***
n-Hexane fraction	30	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	60	0.00 ± 0.00^c	96.47 ± 3.57^b	100.00 ± 0.00^a	100.00 ± 0.00^a	2301.00***
	90	0.00 ± 0.00^c	69.53 ± 4.54^b	89.44 ± 3.37^a	93.51 ± 3.39^a	519.12***
	120	0.00 ± 0.00^d	46.05 ± 1.08^c	64.93 ± 2.17^b	80.60 ± 3.64^a	763.79***
	150	0.00 ± 0.00^d	36.94 ± 3.24^c	63.75 ± 4.51^b	70.88 ± 4.17^a	256.43***
	180	0.00 ± 0.00^d	32.52 ± 1.71^c	38.27 ± 2.09^b	49.43 ± 4.02^a	231.17***
Chloroform fraction	30	0.00 ± 0.00^b	90.68 ± 4.35^a	97.74 ± 1.96^a	100.00 ± 0.00^a	1229.00***
	60	0.00 ± 0.00^c	79.24 ± 3.68^b	90.80 ± 2.03^a	94.57 ± 1.99^a	1105.00***
	90	0.00 ± 0.00^d	41.22 ± 3.64^c	52.91 ± 2.65^b	65.53 ± 2.85^a	340.85***
	120	0.00 ± 0.00^c	27.39 ± 3.37^b	31.02 ± 3.08^b	44.01 ± 3.58^a	121.90***
	150	0.00 ± 0.00^d	22.88 ± 1.88^c	26.42 ± 1.31^b	33.29 ± 2.90^a	183.05***
	180	0.00 ± 0.00^c	0.00 ± 0.00^c	05.71 ± 1.86^b	25.79 ± 2.93^a	149.28***
Ethyl acetate fraction	30	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	60	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	90	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	120	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	150	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	180	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
Methanol fraction	30	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	60	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	90	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	120	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	150	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
	180	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	-
Odomos	180	0.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-

Mean ± SE in the same row followed by the same letter do not differ significantly at $P = 0.05$. Each datum represents the mean of three replicates (Turkey's test). ***: $P < 0.001$.

Table 3

Repellent activity of the creams formulated with essential oils of *A. senegalensis* and *B. dalzielii* against *An. gambiae* in the laboratory [(25 ± 2) °C, 72% ± 5% relative humidity].

Mosquito species	Time (min)	Repellency concentration (%)				F value
		0.0 mg/cm^2	2.0 mg/cm^2	4.0 mg/cm^2	6.0 mg/cm^2	
A. senegalensis	30	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	60	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	90	0.00 ± 0.00^b	97.53 ± 2.18^a	100.00 ± 0.00^a	100.00 ± 0.00^a	6171.00***
	120	0.00 ± 0.00^c	90.29 ± 1.39^b	93.61 ± 0.24^b	100.00 ± 0.00^a	13590.00***
	150	0.00 ± 0.00^b	82.73 ± 4.68^a	86.04 ± 5.46^a	88.44 ± 5.34^a	477.66***
	180	0.00 ± 0.00^b	78.73 ± 3.34^a	82.91 ± 2.31^a	88.44 ± 3.08^a	268.78***
B. dalzielii	30	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	60	0.00 ± 0.00^b	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	-
	90	0.00 ± 0.00^c	94.18 ± 1.21^b	100.00 ± 0.00^a	100.00 ± 0.00^a	19730.00***
	120	0.00 ± 0.00^d	88.36 ± 2.43^c	94.24 ± 1.82^b	100.00 ± 0.00^a	2919.00***
	150	0.00 ± 0.00^c	75.13 ± 3.30^b	89.38 ± 3.94^a	90.75 ± 3.68^a	558.09***
	180	0.00 ± 0.00^c	67.73 ± 4.07^b	84.92 ± 0.95^a	85.57 ± 1.72^a	967.05***
Odomos	180	0.00 ± 0.00^c	96.47 ± 3.57^b	100.00 ± 0.00^a	100.00 ± 0.00^a	2301.00***

Mean ± SE in the same row followed by the same letter do not differ significantly at $P = 0.05$. Each datum represents the mean of three replicates (Turkey's test). ***: $P < 0.001$.

4. Discussion

During the last two decades, many plant species were screened for their repellent properties against mosquitoes with the interest in that direction increasing year by year. Plant-based products in different formulations for topical or fumigant application have been used for their repellent effects against mosquitoes bites[24-26]. Repellent properties against the malaria vectors, *Anopheles* mosquito species, were reported in several plant species. Among the plants reported, the more significant inter alia were *Azadirachta indica* products against *Anopheles* spp.[27], *Chenopodium ambrosioides* essential oil against *An. gambiae*[28], *Lantana camara* against female *Anopheles* mosquitoes[29], *Cymbopogon citratus* against *Anopheles arabiensis*[30], *Ocimum* and *Hyptis* species against afro-tropical vectors of malaria[31], *Ocimum canum* against *Anopheles stephensi* (*An. stephensi*)[32]. In the present study, the repellent activity of the two plant species against adult *An. gambiae* might be attributed to their richness in phytochemicals, such as alkaloids, saponins, flavonoids and tannins, *etc.* in the extracts/fractions[26] and citronellal, cadinol, thymol, geranial, germacrene, *etc.* in the essential oils[33,34]. These plant-based product creams may act as olfactory masking agents and gustatory stimulants[35] or like ion channel modulators[36] and ligands that bind to the specific region of the olfactory receptor neurons[37], which therefore constitute an obstacle for the recognition of hosts by the female mosquitoes.

In the present investigation, the creams formulated with methanol extract, *n*-hexane and chloroform fractions as well as essential oils of the tested two plant species showed a significant repellent activity against *An. gambiae* adult females. The repellent efficacy of these different plant product creams increased with increasing dosages and decreased with time post-application. However, *Polygala arvensis* methanol extract dissolved in isopropanol manifested strong repellent action as it provided 100% protection against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* up to 280 min[38]. The ethanolic crude extract of *Celosia argentea*, *Anthocephalus cadamba*, *Gnetumula*, *Solena amplexicaulis* and *Spermacoce hispida* achieved 100% protection against *An. stephensi*, *Ae. aegypti* and *Culex tritaeniorhynchus* adult females, in terms of repellency, up to 120 min of exposure periods[39]. Repellent activity test for cream formulation with *Tagetes erecta* and *Callistemon brachyandrus* showed 89.87%, 87.50% and 90.00% protection against *An. stephensi*, *Culex infulus* and *Ae. agepyti* for 6 h, respectively[40].

Among extracts and fractions cream formulations of the two plants used in the present study, *n*-hexane fraction cream of *A. senegalensis* at 12 mg/cm^2 exhibited the highest protection time, up to 2 h against *An. gambiae* adult females. Similarly, the hexane seed extract of *Solanum nigrum* showed strong a repellent activity against adult *An. stephensi* up to 81% in 6 h[41]. The result is also comparable to the protection time of 273 and 165 min at 5 mg/cm^2 recorded with hexane extract of the marine sponge *Cliona celata* against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively[42]. The effectiveness of *n*-hexane fraction cream could be linked in part to the presence of essential oil in the extract because of the ability of n-hexane solvent to extract mainly oils[43] and essential oils are well documented to possess repellent properties[44].

In the present investigation, creams formulated with essential oils of *A. senegalensis* and *B. dalzielii* and applied at 6 mg/cm^2 repelled up 120 min without bites from the adult females of *An. gambiae*. Similarly, the essential oil of *Tagetes minuta* provided repellency up to 90% protection for 2 h against *An. stephensi*[31]. The topical formulation from lemongrass oil exhibited an average of 3 h protection for 0.5 g dose and 5 h for 1.5 g dose against biting from mosquitoes[21]. In like manner, the essential oil of *Cymbopogon nardus* in oleaginous ointment (Vaseline) provided above 80% relative protection for a period of 4 h[30]. Likewise, the volume of 0.5 mL of *Hyptis sauveolens* essential oil as active ingredient in 20 g of cream repelled mosquitoes between 9–10 h[45]. Repellent activity of patchouli alcohol formulated from essential oil of *Pogostemon cablin* and tested at 2 mg/cm^2 concentration provided 100% protection for 280 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*[46]. Neem oil showed higher repellent activity for 300 min at 0.6 mg/cm^2 concentration against *An. stephensi*[47]. The formulation containing 30% of *Ocimum gratissimum* essential oil provided also protection against mosquito bites from *Anopheles funestus* for 84.2%–96.6%, *An. gambiae* for 73.7–91.5% and *Cx. quinquefasciatus* for 89.5–91.5%[31]. The differences in repellency could be attributed to the variation of chemical constituents of different plant essential oils because phytochemical components of the plant oil depended on the plant parts harvested, the geographical location where the plants were grown and the oil extraction method[48].

With reference to the repellent effect of the two plant species, only the cream formulated from methanol extracts, *n*-hexane and chloroform fractions as well as the essential oils showed activities. The highest protection of 120 min without the bites of *An. gambiae* was recorded with *n*-hexane cream of *A. senegalensis* at the dose of 12 mg/cm^2 and essential oils creams of the two plants applied at 6 mg/cm^2. Their average repellent protection time was less than or equal to 2 h, a level that is not recommended for use in high disease transmission areas. However, the short protection time of these plant creams may be overcome by their frequent reapplication, especially in scenarios where vector-borne pathogen risk is low. Moreover, plant-based products used as repellents are less toxic to humans and domestic animals and are easily biodegradable. Therefore, *n*-hexane fraction of *A. senegalensis* and essential oil formulated creams of *A. senegalensis* and *B. dalzielii* may be used to protect humans against mosquito bites for at least 2 h.

Conflict of interest statement

We declare that we have no conflict of interest.

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