

Preliminary study on mosquito repellent and mosquitocidal activities of *Ocimum gratissimum* (L.) grown in eastern Nigeria

Evangeline T. Oparaocha^a, Iraneus Iwu^b & J.E. Ahanaku^c

^aDepartment of Public Health Technology, ^bIndustrial Chemistry Department, ^cDepartment of Prosthesis and Orthopedic Technology, Federal University of Technology, Owerri, Imo State, Nigeria

Abstract

Background & objectives: The study examined the mosquito-repellent and mosquitocidal activities of the volatile oil of *Ocimum gratissimum* at three different locations (World Bank Estate, Ihitte and Umuekunne) in Imo State, eastern Nigeria, with the purpose of sourcing for mosquito repellent that is cheap, abundant, environment and user-friendly.

Methods: Four different lotions; 20% (v/v) and 30% (v/v) concentrations each of the extracted volatile oil in two natural oil bases (olive and palm kernel) were made and six volunteered human baits were used to evaluate the mosquito repellent and mosquitocidal activities of the stock materials at the three different centres from September to November 2008.

Results: Topical application of each of the four different lotions significantly ($p < 0.05$) reduced the biting rate of mosquitoes in all the three locations tested. The 30% (v/v) concentration in olive oil base exhibiting highest average percentage repellencies of 97.2, 95.7 and 96.3% at World Bank Estate, Ihitte and Umuekunne centres respectively while the 20% (v/v) concentration in palm kernel oil base had the least repellency of 36.3, 41.6 and 36.3%, respectively. The other two formulations had values ranging from 67.8 to 80% in the three locations. The 30% (v/v) concentration in both olive and palm kernel oil bases afforded all night protection against mosquito bites in all the centres, and demonstrated fast knockdown and paralyzing effect on few mosquitoes at the urban centre (World Bank Estate).

Interpretation & conclusion: The study confirms that *O. gratissimum* grown in eastern Nigeria has mosquito-repellent and mosquitocidal potentials and the formulations could be used to reduce human-mosquito contacts and hence mosquito-borne diseases and irritations caused by their bites.

Key words Eastern Nigeria; mosquitoes; mosquitocidal; *Ocimum gratissimum*; repellent

Introduction

Malaria transmitted by *Anopheles* (Diptera: Culicidae) mosquitoes is the leading cause of morbidity and mortality in Nigeria, and in Africa as a whole it causes about 1–2 million deaths annually^{1–2}. In south-eastern Nigeria, *Anopheles gambiae* is the major vector transmitting malaria followed by *An. funestus*³. In recent times, the use of environment friendly and biodegradable natural insecticides of plant origin to control insect vectors of diseases is gaining ground⁴.

These botanical insecticides have been found to be effective, user-friendly and inexpensive⁵.

In south-eastern Nigeria, *Ocimum gratissimum* (L.) commonly called ‘nchu anwu’ meaning, mosquito repellent is cultivated around houses. It is a herb, which grows up to six feet high with an erect stem. Ethnomedicinally, it is used as remedy for cold and catarrh, stomach pain, diarrhoea and piles^{6–8}. Other documented ethnopharmacological activities of the herb include; antipyretic⁹, diuretic, laxative and

hepatoprotective¹⁰ and treatment for mental illness¹¹. It is also used as food spices by the people¹².

Research reports on the insect repellent and insecticidal activities of *O. gratissimum* in the field and laboratory are varied^{2,13–18}, and Padilha de Paula *et al*¹⁹ as well as Usip *et al*²⁰ have tried the activity on mosquitoes and black flies respectively using human baits. This study examined the mosquito-repellent and mosquitocidal activities of *O. gratissimum* grown in eastern Nigeria in order to verify the folkloric claim that it has mosquito repellent property. Additionally, the study utilized natural oils; olive and palm kernel used locally for cooking and cosmetic purposes to formulate the volatile oil into lotions to improve its acceptability and preclude any adverse effect that could emanate from use of synthetic bases.

Material & Methods

Study location: The study was conducted in three different locations within Imo State of Nigeria. They are: World Bank Housing Estate in Owerri Municipal Council, Umuekunne-Umuagwu in Ohaji-Egbema Local Government Area (LGA) and Ezinihitte in Ezinihitte LGA. All these places lie within the rainforest zone of the south-eastern Nigeria with typical tropical climate. The temperature range during the study period was 35–40°C.

Aside from World Bank Housing Estate that is within the Owerri Urban, the other two places are rural farming communities with thickets and cash crops that have very broad leaves planted around houses. The city is also noted for stagnant water collections in gutters and potholes as well as refuse dumps littered here and there. Such practices promote proliferation of mosquitoes. And as the investigators were inspecting houses to be enlisted for the research in the urban center, mosquitoes were found inside bathroom floors with netted doors and windows, and around water closets and septic tanks even by November when the population density suppose to decrease appreciably. All the spots chosen had high rate of mosquito bites.

Collection of plant samples and extraction of the volatile oil: The green leaves of *O. gratissimum* were collected from a vegetable garden in the World Bank Housing Estate, Owerri, Nigeria. The leaves were identified by a botanist and pulverized into power using a Q-link electrical grinding machine after being air-dried. The oil was extracted with methanol using Soxhlet Extractor and separated from the methanol through distillation. The volume of oil obtained from 1000 g of pulverized dried leaves was 105 ml, giving a percentage yield of 10.5%. All the oil to be used for the experiment was extracted from the same stock.

Formulation of the volatile oil into lotions: Two natural oil bases were used: olive oil (Base A) and palm kernel oil (Base B). Four different formulations were made: 20% (v/v) and 30% (v/v) of the oil in Bases A and B. The 20% concentration of each oil is designated '1' while the 30% is designated '2'. Both bases used were obtained from the pure stock; olive oil was obtained from a super market and palm kernel oil from a freshly distilled homemade stock. Fresh stock of each formulation was made each month without alteration of concentrations.

Ethical clearance: Ethical clearance was obtained from the University Ethical Committee before commencing the investigation. Informed and free consent was obtained from the volunteers before they were recruited for the study.

Selection of subjects: In each of the three locations, six human baits were used, working in pairs; two pairs worked in the morning and evening and one pair at night. Since it has been established that only about 20% in a population receive intensive bites from mosquitoes due to chemical makeup and other factors²¹, the investigators selected only those who claim to experience much mosquito bites naturally. With the help of the University Director of Health Services, subjects were given prophylactic malaria drugs before the investigation and they continued it throughout the period of investigation and two weeks after the exercise.

Mosquito repellent test procedure: For each of the three pairs in each location, one acted as a negative control; only the base was given him to rub while 2 ml of the test lotion was given to the other to rub on the exposed parts of the body (the arms, legs and on the face). The pairs that worked in the morning and evening sessions stayed outside the house at about 5 m distance from each other from 0600 to 0800 and from 1730 to 1930 hrs while the pair for the night stayed in different rooms in the same house.

The pairs for the morning and evening sessions worked for four consecutive days, twice a month and rotated lotion type. They were told to note the number of flies resting on the exposed parts of their body by catching them with inverted cylinder tube. Each fly is collected with a different tube. For those working at night, since they were not expected to get up from bed to catch mosquitoes, a grading system was introduced to them to record palpable bites and/or number of times they were woken up by the buzzing sound of mosquitoes as below; 0 bites/buzzing sound (no bite), 1–2 bites/buzzing sound at the same time and woken up to three times (mild bite), 3 and above bites at the same time and woken up more than three times (heavy bites). Total bites for each month (16 h) of morning and evening as well as the three-month totals were calculated for each lotion type for the treated and control. For whole night bites a summary of their reports was made.

Mosquitocidal test procedure: The method utilized was very simple, the subjects were advised to note any dead mosquito or any immobilizing effect of the cream on the fly. The investigators confirmed the action before recording.

Preliminary entomological study: Randomized selection and identification of species of mosquitoes collected from each location were made with the help of a medical entomologist. As the investigators had no mind of making detailed entomological study, the mosquitoes were not dissected.

Data analysis: The repellency for the treated and

the control in each locality was compared using the two-way analysis of variance (ANOVA), while percentage repellency was calculated according to Sharma and Ansari²². The contingency between location and the activity of test lotion was verified with Chi-square.

Results

The monthly and quarterly diurnal biting rate of mosquitoes on both treated and control baits at different concentrations and formulations and at different locations is shown in Table 1. The results showed significant ($p < 0.05$) reduction in the biting rate for the four different formulations at the three locations (World Bank Estate, Ihitte and Umuekunne), with 30% concentration (v/v) in olive oil base having the highest impact (287 vs 8; 443 vs 19 and 491 vs 18), for the three-month totals, while 20% (v/v) in palm kernel oil had the least (273 vs 174; 428 vs 250 and 501 vs 319), respectively (Table 1).

The results on the percentage repellency showed values ranging from 36.3 to 97.2% for the three-month averages, with the 30% in olive oil base having the highest values (97.2, 95.7 and 96.3%), while the 20% in palm kernel base had the least (36.3, 41.6 and 36.3%) in the World Bank Estate, Ihitte and Umuekunne study locations respectively (Table 2). The 20% concentration in olive oil base showed repellency of 74–81.6%, and 30% concentration in palm kernel oil base showed 67.8–86.5% repellency from the three locations. Chi-squared analysis of the impact of location on the activity of the volatile oil showed that the results from the three localities did not differ significantly ($p > 0.05$).

Impact on whole night biting rate showed that all the formulations had some positive impact on reducing night biting rate, but 30% formulation in both the bases seem to have afforded all night protection against the bite of mosquitoes (Table 3) in all the locations studied.

Observation on the mosquitocidal impact of the for-

Table 1. Monthly and quarterly diurnal mosquito biting rate (16 h/month) on treated and untreated individuals by lotion type and location

Lotion type/Month	World Bank Estate		Ihitte		Umuekunne	
	Treated	Control	Treated	Control	Treated	Control
20% (v/v) of <i>O. gratissimum</i> in palm kernel oil (Base B1)						
September	74	111	98	160	56	92
October	51	86	72	145	60	100
November	49	76	75	123	52	95
Quarterly total	174	273*	245	428*	168	287*
30% (v/v) of <i>O. gratissimum</i> in palm kernel oil (Base B2)						
September	29	123	35	157	38	93
October	28	87	36	150	37	175
November	10	74	25	110	27	134
Quarterly total	67	284*	96	417*	102	402*
20% (v/v) of <i>O. gratissimum</i> in olive oil (Base A1)						
September	27	121	40	170	61	209
October	26	100	27	147	39	171
November	15	75	27	125	28	137
Quarterly total	68	296*	94	442*	128	517*
30% (v/v) of <i>O. gratissimum</i> in olive oil (Base A2)						
September	5	124	9	171	8	195
October	3	86	6	148	7	172
November	0	77	4	124	3	124
Quarterly total	8	287*	19	443*	18	491*

**p* <0.05

mulations showed that only 30% formulations in both bases had some mosquitocidal action by causing fast knockdown paralyzing effect on some mosquitoes that were at close range to treated human bait's body. The affected mosquitoes were knocked-down fast on the ground. The impact is likely to be due to airborne effect of the volatile oil. In all, only seven mosquitoes demonstrated this fast knockdown effect of the volatile oil during the three-month study and the observation was made only in the World Bank Estate, Owerri. Randomized identification of the collected mosquitoes showed that 89.3% were *Anopheles gambiae*, while 10.7% were *An. funestus*, no other *Anopheles* species was seen.

Discussion

The results of this investigation confirmed that *O. gratissimum* grown in eastern Nigeria has insect-re-

pellent activity and could be used effectively to control the nuisance and adverse health impact of mosquito bites particularly at 30% (v/v) in both the olive and palm kernel oil bases. The findings are in conformity with the observations of earlier researchers who tried the insect repellent activity of the volatile oil against mosquitoes^{16,19}, and against blackflies²⁰. The disparity found in the percentage repellency of the formulations of the volatile oil at 30% (v/v) and 20% (v/v) concentrations in olive oil and palm kernel oil tend to suggest that palm kernel oil presented some hindrances to the full expression of the repellent activity of the volatile oil.

The mosquitocidal action of the volatile oil was expressed only in 30% (v/v) concentration in olive oil and palm kernel oil in the urban city but not in the rural farming communities. All the mosquitoes that were so affected were *An. gambiae*. The relative

Table 2. Monthly and quarterly percentage repellency of the volatile oil by lotion type and location

Lotion type/ Month	Percentage repellency in each of the locations		
	World Bank Estate	Ihitte	Umuekunne
20% (v/v) of <i>O. gratissimum</i> in palm kernel oil (Base B1)			
September	33.6	38.8	35.4
October	41.1	46.9	36.9
November	35.5	37	39
Quarterly average	36.3	41.6	36.3
30% (v/v) of <i>O. gratissimum</i> in palm kernel oil (Base B2)			
September	76.4	77.7	80.3
October	67.8	76	78.9
November	86.5	77.3	80
Quarterly average	76.4	77	79.7
20% (v/v) of <i>O. gratissimum</i> in olive oil (Base A1)			
September	76.4	76.5	70.8
October	74	81.6	77.2
November	80	78.4	79.6
Quarterly average	77	78.3	75.2
30% (v/v) of <i>O. gratissimum</i> in olive oil (Base A2)			
September	96	94.7	96
October	96.5	95.9	95.9
November	100	96.8	97.6
Quarterly average	97.2	95.7	96.3

Table 3. Impact of lotion type on all night biting rate of mosquitoes by locations

Lotion type	World Bank Estate		Ihitte		Umuekunne	
	T	C	T	C	T	C
Base B1	+	++	+	++	+	++
Base B2	0	++	0	++	0	++
Base A1	0	++	+	++	+	++
Base A2	0	++	0	++	0	++

0 = No bite; + = Mild bite; ++ = Heavy bite; T = Treated; C = Control.

small number of affected mosquitoes may be due to the fact that most of them were repelled before coming within reasonable close range to treated human baits. The reason for the selective mosquitocidal impact on the few mosquitoes could not be established.

The results also indicated that the test oil afforded whole night and to a greater extent, dawn and dusk protection at 30% (v/v) concentration in both the olive and palm kernel oil bases. Malaria mosquitoes bite mainly during night, dusk and dawn. Consequently, the oil could be used as an alternative, relatively safe, natural, insect-repellent to protect people from mosquito bites at night when they are on bed, and at dawn and dusk when often due to poor housing and harsh climatic conditions as well as social and religious responsibilities stay outside their houses. Since the extraction technique is simple and the bases as well as the plants are abundant, the local people could be taught how to prepare the lotions. The formulation could equally help to supplement the protection afforded by window and door nets for those who because of cost and/or odour of permethrin do not like to sleep under insecticide-treated bednets²³. The colour, and consistency of the lotions remained unchanged during the three months study. This is an added advantage showing that the natural bases used were as good as synthetic liquid paraffin used by earlier researchers like Usip *et al*²⁰.

Oral interview of the subjects used for the study showed that there were no adverse impact in the form of rashes, irritation, pains or other skin problems during the three-month study. Documented research reports equally confirmed that the volatile oil of *O. gratissimum* has low acute toxicity, no mutagenic risks and is not irritable on the skin^{10,19-20}.

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Corresponding author: Evangeline Toochi Oparaocha, Department of Public Health Technology, School of Health Technology, Federal University of Technology, Owerri, Imo State, Nigeria.
E-mail: tochievan@yahoo.com

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