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Preliminary Study on Bioactivity of *Anopheles* Mosquito Exposed to *Ocimum gratissimum* Formulated Candles

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Abstract: The mortality of *Anopheles* mosquito exposed to varying concentrations of crude and oil extracts of *Ocimum gratissimum* was assayed. Erratic, non-directional and loss of balance during flight and finally culminating in death was observed in both crude and oil extracts of the plant. The lethal concentrations of crude and oil extract were determined to be 5.9g/50g of wax and 0.000068g/50g of wax respectively. The crude and oil extract were found to be effective in the control of *Anopheles* mosquito with the oil extract been more toxic. The mortality rate of the oil and crude extract of *Ocimum gratissimum* were observed to be dose dependent. The results indicate that both extracts have a toxic effect on the larvae but the highest mortalities were recorded in the oil extract.

Keywords: Bioactivity, Candles, *Ocimum gratissimum*, Anopheles

1. Introduction

Mosquitoes are the major vectors for the transmission of diseases (such as dengue fever, chikungunya, yellow fever and malaria) in the tropical and subtropical countries which causes debilitating effects to humans [1]. There are approximately 3,500 species of mosquitoes grouped into 41 genera and human malaria is transmitted only by females of the genus *Anopheles*. Of the approximately 430 *Anopheles* species, only 30-40 species transmit malaria in nature. *Anopheles* mosquito is one of the vectors that transmit malaria parasite, belonging to the genus *Plasmodium* [2].

Ocimum gratissimum is an aromatic perennial shrub belonging to the family Lamiaceae. It is commonly known as scent leaf. *O. gratissimum* is found in the tropical and warm temperate regions such as India and Nigeria. In Nigeria it is commonly known as Scent leaf or "Nchuanwa" in Igbo, "Effirin" in Yoruba, and "Dai doyatagida" in Hausa [3, 4]. The plant is used as a food spice [5] and for the treatment of many infections [6].

Resistance to existing commercial insecticides and undesirable side effects from synthetic insecticides has necessitated research on insecticides derived from plant extracts. Aside this, the existing insecticides are out of reach for the very poor because of cost. Since the raw materials and technology required are readily available and abundant in the environment a lot of plant based repellant formulations such as cream formulations [7, 8] and essential oils [9] have emerged in the commercial market. Despite these advances, deployment of the products to rural remote areas where they are most needed remain a huge problem. We report bioassay findings on test candles formulated with *Ocimum gratissimum* for effectiveness in controlling mosquitoes, with a view of deploying active phytochemical agents in widely used items in resource poor communities.

2. Materials and Methods

2.1. Study Area

This study was conducted in Lokoja which is located at longitude 7° 49' North and latitude 6° 45'East in Kogi State. Average maximum temperature of 33.2°C and average minimum temperature of 22.8°C. Moderate rainfall which last for four to five months and also a dry season which

exceeds the rainy season. Lokoja serves as a confluence for two most prominent rivers in Nigeria; River Niger and River Benue and several tributaries.

2.2. Preparation of Plant Samples

Fresh *O. gratissimum* leaves were obtained from the wild within Lokoja. The plant was identified at the herbarium section of the Department of Biological Sciences, Federal University Lokoja. Voucher numbers were allotted a sample of the plant and kept in the herbarium. The collected plant samples were thoroughly rinsed with distilled water and air dried at room temperature [32°C – 34°C] for two weeks. The dried leaves were pulverized using an electric blender.

2.3. Ethyl Acetate Crude Extract of *Ocimum gratissimum*

Two hundred [200] grams of pulverized plant samples were placed in a glass container. The samples were soaked with ethyl acetate in a ratio of one gram of samples is to 1mL of ethyl acetate [1:1] and were left for 72 hours and then filtered. The resulting filtrates were then concentrated using a rotary evaporator.

2.4. Ethyl Acetate Oil Extract of *Ocimum gratissimum*

Two hundred [200] grams of the pulverized plant samples were loaded into a soxhlet extractor and 500ml of ethyl acetate added to the still pot of the extractor. The oil was extracted within 2 hours at room temperature. The extract obtained was dispensed in a beaker and left for complete evaporation at room temperature. The oil obtained was stored in a beaker and stored at room temperature until when needed.

2.5. Collection of Mosquito Larvae

Mosquito larvae were collected at the onset of the rainy season from pools of still clean water at various locations within the Federal University Lokoja campus. The larvae collected were transferred in a porcelain bowl containing clean water to the laboratory for identification.

2.6. Identification of Mosquito Larvae

Hand lens was used to distinguish the larvae of mosquitoes for other aquatic insects that may have been collected. The absence of legs, siphon, presence of a thorax wider than the head or abdomen and the presence of three distinct body regions [head, thorax and abdomen] were the basis for identification of mosquito larvae. Larva lying parallel to the water surface was classified as *Anopheles* larvae.

2.7. Formulation of Candles

Fifty grams [50g] of crude extract and [50mls] oil extract of the plant was measured using a digital weighing balance. The 50g of wax was measured for each extract concentrations and melted in a container. A thermometer was placed inside the melting wax to ensure that a temperature of 170 degree centigrade is maintained. The temperature was constantly monitored to ensure it is revolved around 170 degree centigrade. The mold was heated at 150 degree, and removed from oven. A liberal amount of hot glue was added on the bottom of the wick and then placed in the middle of the mold. The already measured extract and melted wax was poured into the mold and allowed to set on a flat surface. The negative control candle was also set up by pouring melted wax into molds containing glued wick. The candles set up as control had no trace of extract.

2.8. Bioactivity

The efficacy of the plant extracts against *Anopheles* mosquito was evaluated. The mosquitoes was exposed to a wide range of test concentrations and observed after 30mins, after which a range of four concentrations [0.5g, 1.0g, 1.5g, and 2.0g for crude extract; 0.5ml, 1.0ml, 1.5ml, 2.0ml for oil extract] were used for the assay. For each test concentrations and control experiment, 50 mosquitoes were transferred into net cages [measuring 50cm x 50cm]. Behavioural changes and possible mortality of the mosquitoes were observed after 30mins across the treatments.

Data were presented as mean ± standard deviation [SD]. Data were analyzed using One-way Analysis of Variance [ANOVA]. *P-value* of less than 0.05 was declared as statistically significant using SPSS statistical analysis. Probit analysis for lethal calculation [LC_{50}] for the oil and crude extracts was computed using data obtained from the mortality figures in all the treatments.

3. Results and Discussion

Behaviours such as erratic, non-directional and loss of balance during flight were observed in both the crude and oil extract. This could probably be a display of knock down [KD] effects resulting from physical contact of the mosquitoes with chemical vapour particles emanating from the extract formulated candles. These changes in behavior were not observed in the control treatment.

The highest percentage mortality [68%] was recorded in the treatment with the highest concentration of the crude extract while that of the oil extract recorded eighty-two [82%] percentage mortality as shown on tables 1 and 2. The differences in percentage mortality noticed between the crude and oil extract can be associated possibly to the lethal concentrations, with the oil extract being more toxic than the crude extract. In both cases, the mortality rate was dependent on the concentrations of the extract and exposure time of the mosquitoes to the extracts [Tables 1 and 2], though at 2.0g of crude extract the mortality rate remained the same at from 25 minutes to 30 minutes of exposure.

Similar studies using ethyl acetate, hexane, chloroform, water and acetone extract of *Ocimum gratissimum* has been shown to have larvicidal and pupicidal activity against *culex quinquefasciatus* [10]. The phenols, flavonoids and tannins which have been reported in previous phytochemical analysis [11] of the plant may be the bioactive compounds that acted

as potential toxic agents against the *Anopheline* mosquito vectors. The varying results obtained in lethal concentration and lethal times are probably due to the differences in levels of concentration among the insecticidal ingredients of the plant. Therefore we document that crude and oil extracts when used to formulate candles exhibit adulticidal effect on *Anopheles* mosquitoes. This could be used in resource poor communities where electricity is not available and the only main stay source of light are candles.

The lethal concentration for the crude extract was determined to be 5.9g/50g of wax [Table 1] while that of oil extract was determined to be 0.000068g/50g of wax [Table 2]. The oil extract of *Ocimum gratissimum* was found to be more toxic on *Anopheles* mosquitoes than the crude extract based on the differences of the lethal concentrations values. Kazembe and Chaibva [12] have also reported similar observations with *ocimum americanum*. The linear regression values showed positive relationships between the mortality and concentration of the crude and the oil extract [Figures 1 and 2], indicating that mortality increased with an increase in concentration of extracts. Similar studies on *Culex gelides and Culex quinquefasciatus* by Chinnaperuma and Abdul [11] using hexane, ethyl acetate and methanol extracts of *Ocimum gratissimum and Ocimum gratissimum* have shown also reported adulticidal activities.

Table 1. *Relative mortality of different concentrations of crude extract of Ocimum gratissimum against Anopheles mosquito.*

Conc	No. of mortality		Percentage [%] of mortality						LC_{50}
	Control	Treated	5mins	10mins	15mins	20mins	25mins	30mins	
0.5g	2 ±1.2	5.3±2.3	0	0	2	6	8	16	
1.0g	2 ± 1.2	12 ±4.2	4	4	6	8	18	32	5.9g /50g of
1.5g	2 ±1.2	19.7±5.3	4	14	14	14	30	42	wax
2.0g	2 ±1.2	42 ±8.4	10	18	40	48	68	68	

The changes across the treatment were significant [$p < 0.05$] and the mortality was dose dependent.

Table 2. *Relative mortality of different concentrations of oil extract of Ocimum gratissimum against Anopheles Mosquitoes.*

Concentration	No. of mortality		Percentage [%] of mortality						
	Control	Treated	5mins	10Mins	15Mins	20Mins	25mins	30mins	LC_{50} Value
0.5ml	5.3±3.1	59±7.7	6	16	22	40	42	42	
1.0ml	5.3±3.1	47.3±8.9	8	22	34	42	52	55	0.000068ml/50g
1.5ml	5.3±3.1	35 ± 6.6	12	34	42	52	68	76	of wax
2.0ml	5.3±3.1	28 ± 5.9	26	46	62	64	74	82	

The changes across the treatment were significant [$p < 0.05$] and the mortality was dose dependent

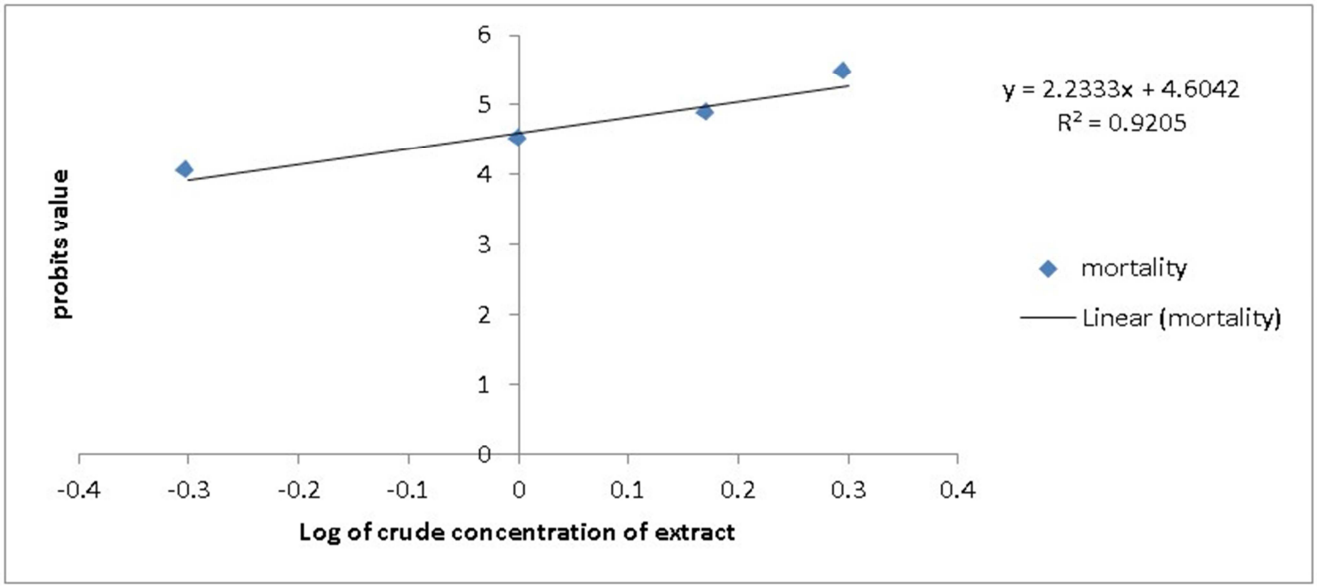

Figure 1. *Probit analysis of crude extract treatment of Ocimum gratissimum on Anopheles mosquitoes.*

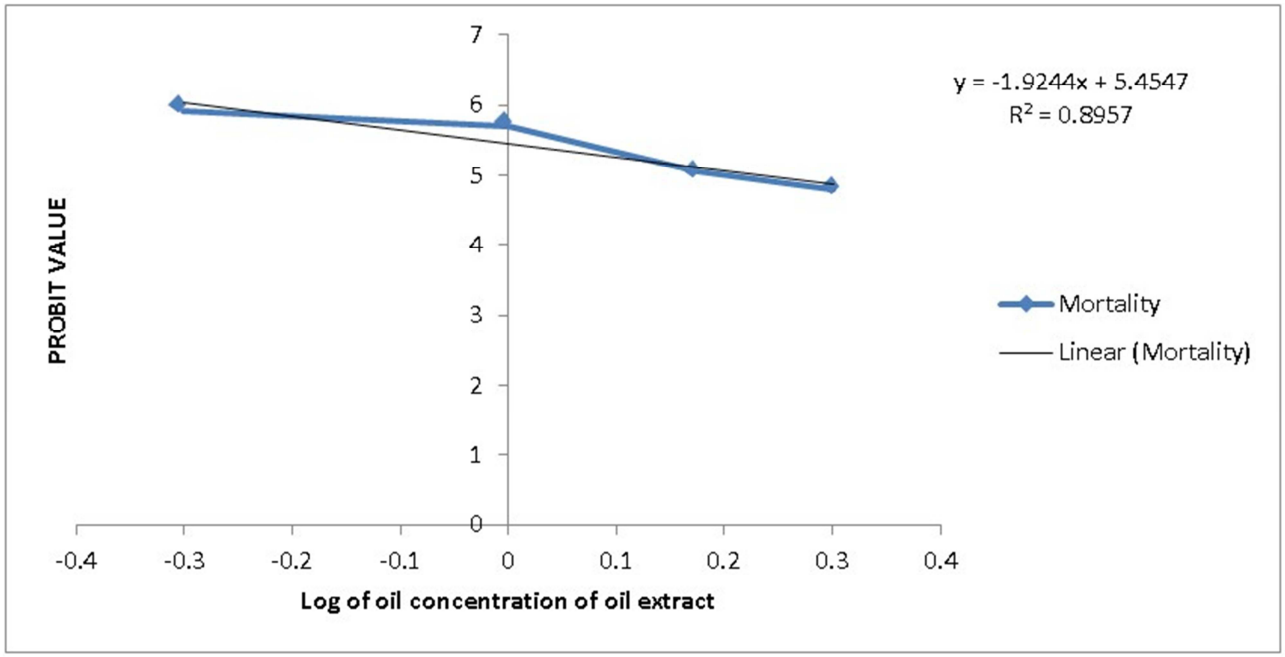

Figure 2. *Probit analysis of oil extract treatment of Ocimum gratissimumon Anopheles mosquitoes.*

4. Conclusion

This study showed that *Ocimum gratissimum* which is very abundant in the environment when used to formulate candles exhibits activity on *Anopheles* mosquito and hence, a better approach which is safer and more cost effective in the control of *Anopheles* mosquitoes and spread of Malaria. The extracts could also be further tested for adulticidal properties probably at lower concentrations.

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