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Research Paper

# Bioassay of Herbal Mosquito Repellent Formulated from the Essential Oil of Plants.

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## ABSTRACT

Malaria, Dengue fever and Filariasis, are serious public health problem in tropical regions, especially in Africa and Asia and are transmitted through mosquito bites. For effective control of these diseases, therefore, there is need to prevent individual from mosquito bites. The effective and safe method is the use of mosquito repellent naturally obtained from medicinal plants instead of commonly available synthetic insecticides and repellents such as Organo Phosphorus Carbamate, *N, N*-Diethyl-3-methylbenzamide (DEET), and Dichloro Diphenyl Trichloroethane (DDT) which are carcinogenic and non eco-friendly. In this study, the repellency activities of four formulated herbal mosquito repellents from the essential oils of some selected MAPPs of Nigeria were evaluated. The essential oils were extracted by hyrodistillation method at 50°C using all glass Clevenger apparatus. These were then stored at 4°c in the refrigerator pending further studies.Graded concentrations (6%, 8%, 10% and 12%) of the essential oils in a complex solution of polyethylene glycol, ethanol and water were prepared and their mosquito repellency activities was carried out in the laboratory against Standard Kisumu Strains of *Anopheles gambiae*. The result showed that both 8% and 10% formulations have the most promising activity exhibiting 68-95% repellency activities lasting for 2hours. The standard (odomos® mosquito repellent cream) exhibited 75-100% repellency activities which also lasted for 2hours as compared to 8% and 10% formulations. The present study demonstrates the potential for using essential oils from medicinal plants as mosquito repellent.

**Keywords**: Malaria; Anopheles gambiae; Essential oil; Repellents.

## INTRODUCTION

Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases like Malaria, Filariasis, Japanese Encephalitis (JE), Dengue fever and Yellow fever. Thus one of the approaches for control of these mosquito borne diseases is the interruption of disease transmission by either killing the mosquitoes or preventing them from biting individuals. In the past

and before the discovery of synthetic organic insecticides, herbal products such as Nicotine from tobacco leaves *(Nicotiana tabacum*), Anasbasine and Lupinine (alkaloids extracted from Russian weed *Anabasis aphylly*), Rotenone from *Derris elliptical* and Pyrethrums from *Chrysanthemum cinererifolium* flower of proven potentials have been playing an important role as natural mosquito repellent or insecticide in the interruption of the transmission of mosquito borne diseases both at the individual and the community level.

Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides (Campbell, 1993), (Hartzell, 1941), (Jacobson, 1971). However, the extensive use of synthetic insecticides during the last five decades has resulted in environmental hazards and also in the development of physiological resistance in major vector species. This has therefore necessitated the need for research and development of environmentally safe, biodegradable, low cost, indigenous method for vector control, which can be used with minimum care by individual and communities in specific situation (Mittal, et al., 2003).

For this study, six Nigerian Medicinal, Aromatic and Pesticidal Plants (MAPPs) (*Eucalyptus globulus, Citrus sinensis, Cymbopogon citratus, Ocimum basilicum,* H*yptis suaveolens,* and *Azadirachta indica*) were collected for the preparation of essential oil used in the formulation of four herbal mosquito repellents. The selection of these medicinal plants was based on their availability as raw materials, scientific evidence and the folkloric use as mosquito repellents (Olawore, et al., 2003), (Oyedele, et al., 2002), (Evans, 2000). While the present study is a product driven research which has not been carried out on these six plants, the aim of this study is to therefore formulate potent herbal mosquito repellent from these six identified medicinal plants and investigate biologically their repellency effects which may further lead in the nearest future to the development of environmental friendly and effective herbal mosquito repellent for the control and prevention of malaria infections.

## MATERIALS AND METHOD

***Sample collection*:** The plant samples - *Eucalyptus globulus, Ocimum basilicum* (sweet basil), *Cymbopogon citratus* (lemon grass), *Citrus sinensis* (sweet orange), *Azadirachta indica* (Neem) and *Hyptis suaveolens* (scent leaf) were collected in November, 2010 in the Nigeria Natural Medicine Development Agency's Botanical Garden, Epe, Lagos, identified and voucher in the Botany Department, University of Lagos, Nigeria. Voucher specimens (LUH4685, LUH4686, LUH4687, LUH4688, LUH4689, LUH4690) kept in the herbarium. The leaves were removed and then slightly washed to remove sand and other physical contaminants. The leaves were then reduced to a suitable size to avoid the escape of the essential oil and loaded them in the extraction flask.

***Extraction*:** Essential oil from the fresh leaves and peels of the plants samples were obtained using Hyrodistillation method (BP, 1980). The oils extracted were stored in an appropriate sample bottles at a temperature of $4^{o}$C till the period of formulation and repellency testing.

***Formulation*:** Graded concentrations of the plants' samples essential oil extracts (6%-12%) were prepared by mixing different amount of the individual essential oil extracts from the six plant samples used in this study in a complex solution of polyethylene glycol, ethanol and water. These concentrations were selected based on some preliminary information corroborated by an earlier studies carried out by (Oyedele, et

al., 2000), (Aisien, et al., 2004) and (Oyewole, et al., 2008) and were basically aimed at determining the effective concentrations which may be used in future development of an effective herbal mosquito repellent. The entire formulated herbal mosquito repellents were placed in screwed-cap vials and kept in the freezer at a temperature of $4^{o}$C till further studies.

***Breeding of Mosquitoes*:** Susceptible Standard Laboratory Strains (Kisumu Strain) of adult anopheles mosquito specie (*Anopheles gambiae*) were raised in a netted cage of size (25 x 25 x 25cm) under laboratory conditions (25-30$^{o}$C) from the larval colony. Adult female mosquitoes were fed regularly with blood from exposed skin of live animals (guinea pig) and the males with a 10% sugar solution.

***Preliminary Test*:** A range finding test was conducted to select the best out of the four repellent formulations (6%, 8%, 10% and 12%) prepared for repellency evaluation. This experiment was conducted using one hundred and sixty (160) adult anopheles mosquitoes of age ranging between 6-10 days old. The experiment was conducted in duplicates using four human volunteers and eight netted mosquito cages each of which contains 20 adult mosquitoes that have been starved for 24hours prior to the time the test was conducted. 1ml of each of the repellent formulation was rubbed on the forearms of the human volunteers. The mosquitoes were first tested for their readiness to bite by exposing the untreated arm for 10Secs after they were blown off. The treated forearm of each of the volunteers was then exposed to the cage containing the adult mosquitoes for 3mins. After which it was withdrawn and returned back 30mins later. This process was repeated at 30mins interval until the repellent was no more effective. The experiment was conducted in the night with protection period ranging from 3min-2hs (i.e. 3min, 30min, 1h and 2h).

***Standard Test*:** This test was designed to establish and estimate dose response lines and effective dose of the two individual formulations (8% and 10%) that passed the preliminary test corresponding to 50% ($ED_{50}$) protection from mosquito landing and / or probing as compared to the effective dose of the standard (Odomos® mosquito repellent cream) containing 12% N, N-diethyl-m-toluamde (DEET) corresponding to 50% ($ED_{50}$) protection from mosquito landing and / or probing. The test is also designed to estimate the complete protection time of the individual repellent formulation, which is the time between the application of the repellent and the first mosquito landing and / or biting. The experiment was conducted on a separate day for each of the two repellent formulations using one hundred and sixty (160) adult female anopheles mosquitoes of age range between 6-10days and was kept in eight mosquito netted cages with each containing 20 adult female anopheles mosquitoes. Before the test was conducted, mosquitoes were fed with the blood from the animal skin and then starved for 24hs. The test was carried out in the dark in quadruplicates using the human – bait (WHO, 2009). Eight human volunteers were used for this experiment. 1ml of each of the sample was rubbed on the forearm of the four volunteers while 1g of the standard was rubbed on the arm of another four human volunteers. The untreated arms of the volunteers were used as control. Again, the readiness of the mosquitoes was tested by exposing the untreated arm of the volunteers for about 10Secs. The test was then conducted at the interval of 3min, 30min, 1h, and 2h. The percentage repellency was determined using the method of (Abdelkarim, et al., 2006).

## RESULT AND DISCUSSION

According to tables 2 and 3, all the four repellent preparations showed significant degree of repellency by exhibiting averagely, 95% repellency activity in the first

3mins of exposure time and an average of 50% repellency activity at the end of two hours exposure period. The two best formulations (8% and 10%) were chosen based on their ability to repel at 100% in their first 3mins and more than 60% at the end of 1h exposure period. The 6% preparation was also effective but not as effective as 8% and 10% at the end of 2hrs repellency test while 12% was least effective at the end of the 2h protection period. Based on this fact, the standard test was conducted on the 8% and 10% repellent formulations using one hundred and sixty female anopheles mosquitoes. The repellency activity of the two repellent formulations was compared with a commercial mosquito repellent cream (Odomos®) containing 12% N, N-diethyl-m-toluamide (DEET). In this study, 1ml of the repellent preparation was compared with 1g of the standard and hence used to determine the effective dose ($ED_{50}$) of the repellent preparations.

According to tables 4 and 5 and tables 6 and 7, the two repellent formulations (8% and 10%) exhibited a great significant repellent activity at 94% in 3minutes. However, the 10% preparation was found to be more effective than the 8% repellent formulation. In 1h of exposure time, 10% repellent preparation exhibited 83.33% while 8% exhibited 76.47% activity. The 10% repellent preparation was also more effective at the end of the 2h exposure time exhibiting 72.22% while 8% had 64.70% at this period.

In this study, the standard has also been found to be effective. However, there was a little significant different between the repellent activity of the standard and the test samples. The standard exhibits 100% repellent activity at the end of the first 3minutes exposure time and 76.47% at the end of the 2hours exposure time while both the test samples compete favorably with the standard at the end of each test. For instance, at the end of the 2hours exposure time, the 10% preparation was able to repel up to 72.22% while the standard has 76.47%. Also at the end of 1hour exposure time both the 10% repellent preparation and the standard exhibited 83.33% and 88.24% repellent activity respectively. Similar study conducted by (Oyedele, et al., 2000) showed the repellency activity of ointment and cream from lemon grass oil against *Aedes aegypti* at a concentration of 15%v/w to be 2-3hs. Other study by (Aisien, et al., 2004) also showed that volatile oils of *Citrus sinensis*, *Ageratum conyzoides*, *Cymbopogon citratus*, *Callistemon rigidus* and *Ocimum gratissimum* repel blackfly, *Simulium damnosum*, the vector of human onchocerciaisis up to 2hs at a concentration of 10 and 20%v/v with liquid paraffin as carrier. The result obtained in this study has answered three questions usually come up in the bioassay of mosquito repellent. The two formulations (8% and 10%) compared with the standard (Odomos® mosquito repellency cream) repel the mosquito specie (*Anopheles gambiae*) used for the repellency assay with an effective dose of 1ml while protection period lasted for 2hours.Though, this study has showed that the standard was a little bit more effective than the two repellent formulations, however, the standard was prepared using 12% DEET while the more effective sample contains 10% of the essential oil mixtures from the plant. Hence if the standard was prepared at the same concentration as the sample, the sample might be more effective.

## CONCLUSION

Four different formulations from the combination of the essential oil from six plant samples of different families have been evaluated in this study. The mosquito bite deterrent effect of 8% and 10% essential oil formulations are very promising for topical use. However, the 10% formulation have more repellency effect than 8%

formulation and also compete favorably when compared with the commercially available standard (Odomos® mosquito repellent cream**).**Though, investigation on the dermatological effect on the skin of the two promising formulations has not been conducted in the course of this study, however, for the period the test was conducted and even several hours after the experiment, no skin irritations was experienced by the volunteers used in this study. The 2hours repellency activity of the two formulations however is low. Though, many factors might have played a role here. These may include: the type of vector the repellent is tested on, the age of the vector, the period the plant samples were collected, use of natural or synthetic fixation agents and the geographical location of the plant samples (Barnard, et al., 2004)**.**

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**Table-1: % Composition of individual repellent formulation in a complex solution of Polyethylene glycol, Ethanol and Water.**

| Repellent formulation | % Composition of essential oil extracts from plant samples | | | | | |
|---|---|---|---|---|---|---|
| | ***A. indica*** | ***H. suaveolens*** | ***O. gratissimum*** | ***C. sinensis*** | ***E. globulus*** | ***C. Cymbopogon*** |
| 6% | 2.4% | 0.1% | 1.0% | 0.5% | 1.0% | 1.0% |
| 8% | 2.0% | 0.1% | 1.0% | 0.9% | 2.0% | 2.0% |
| 10% | 2.5% | 0.3% | 1.6% | 0.6% | 2.5% | 2.5% |
| 12% | 3.0% | 0.5% | 2.0% | 1.0% | 3.0% | 2.5% |

**Table-2: Mean number of mosquitoes landing/biting the control and treated arm.**

| Repellent concentrations | Designed cages | Average no of mosquitoes landing or/ biting the treated and the control arms of the human volunteers at specific period. | | | | |
|---|---|---|---|---|---|---|
| | | **Control** | **3min.** | **30min.** | **1h.** | **2h.** |
| 6% | A1<br>A2 | 16.0 ± 0.0 | 1.0 ± 0.1 | 6.0 ± 0.0 | 7.0 ± 0.5 | 8.0 ± 0.0 |
| 8% | B1<br>B2 | 18.0 ± 0.5 | 0 | 4.0 ± 0.5 | 5.0 ± 0.6 | 9.0 ± 0.0 |
| 10% | C1<br>C2 | 18.0 ± 0.0 | 1.0 ± 0.1 | 4.0 ± 0.0 | 6.0 ± 0.6 | 6.0 ± 0.5 |
| 12% | D1<br>D2 | 16.0 ± 0.0 | 1.0 ± 0.1 | 3.0 ± 0.6 | 5.0 ± 0.5 | 9.0 ± 0.0 |

**Table-3: Percentage of individual formulation at each exposure time.**

| Sample concentration | Exposure time | Total No. of mosquitoes in a cage | Total No. of mosquitoes landing or biting the treating arm | Percentage protection |
|---|---|---|---|---|
| 6% | 3min. | 40 | 1 | 93.755 |
| | 30min. | 40 | 6 | 62.50% |
| | 1h. | 40 | 7 | 52.94% |
| | 2h | 40 | 8 | 50% |
| 8% | 3min | 40 | 0 | 100% |
| | 30min | 40 | 5 | 72.22% |
| | 1h. | 40 | 6 | 66.67% |
| | 2h. | 40 | 9 | 50% |
| 10% | 3min | 40 | 1 | 94.44% |
| | 30min | 40 | 4 | 77.77% |
| | 1h. | 40 | 7 | 61.11% |
| | 2h. | 40 | 8 | 55.56% |
| 12% | 3min | 40 | 1 | 93.75 |
| | 30min | 40 | 4 | 75% |
| | 1h. | 40 | 6 | 62.50% |
| | 2h. | 40 | 9 | 43.75% |

**Table-4: Mean number of mosquitoes landing/biting the treated and control arm using 8% formulation and the standard (Odomos ®).**

| Repellent concentrations | Designed cages | Average no of mosquitoes landing or/ biting the treated and the control arms of the human volunteers at specific period. | | | | |
|---|---|---|---|---|---|---|
| | | Control | 3min. | 30min. | 1h. | 2h. |
| 8% | $Rs_1$<br>$Rs_2$<br>$Rs_3$<br>$Rs_4$ | 17.0 ± 0.3 | 1.0 ± 0.5 | 2.0 ± 0.6 | 3.0 ± 0.6 | 6.0 ± 0.8 |
| Standard | $Ro_1$<br>$Ro_2$<br>$Ro_3$<br>$Ro_4$ | 16.0 ± 0.5 | 0.0 ± 0.1 | 1.0 ± 0.8 | 1.0 ± 0.8 | 3.0 ± 0.5 |

**Table-5: Mean number of mosquitoes landing/biting the treated and control arm using 10% formulation and the standard (Odomos ®).**

| Repellent concentrations | Designed cages | Average no of mosquitoes landing or/ biting the treated and the control arms of the human volunteers at specific period. | | | | |
|---|---|---|---|---|---|---|
| | | Control | 3min. | 30min. | 1h. | 2h. |
| 10% | $Rs_1$<br>$Rs_2$<br>$Rs_3$<br>$Rs_4$ | 18.0 ± 0.3 | 1.0 ± 0.6 | 1.0 ± 0.5 | 2.0 ± 0.9 | 5.0 ± 0.3 |
| Standard | $Ro_1$<br>$Ro_2$<br>$Ro_3$<br>$Ro_4$ | 17.0 ± 0.0 | 0.0 ± 0.1 | 0.0 ± 0.3 | 1.0 ± 0.4 | 4.0 ± 0.2 |

**Table-6: Percentage repellency at each exposure time for 8% and standard (Odomos ®).**

| Repellent sample | Exposure time | Total no of mosquitoes in a Cage | Total no of mosquitoes landing or biting the treating arm | Percentag protectio |
|---|---|---|---|---|
| 8% | 3min. | 80 | 1 | 94.11% |
| | 30min. | 80 | 3 | 82.35% |
| | 1h. | 80 | 4 | 76.47% |
| | 2h. | 80 | 6 | 64.70% |
| Standard | 3min. | 80 | 0 | 100% |
| | 30min. | 80 | 3 | 81.25% |
| | 1h. | 80 | 2 | 87.50% |
| | 2h. | 80 | 4 | 75% |

**Table-7: Percentage repellency at each exposure time for 10% and standard (Odomos ®).**

| Sample concentra tion | Exposure time | Total no of mosquitoes in a cage | Total no of mosquitoes landing or biting the treating arm | Percentag protectio |
|---|---|---|---|---|
| 10% | 3min. | 80 | 1 | 94.44% |
| | 30min. | 80 | 2 | 88.89% |
| | 1h. | 80 | 3 | 83.33% |
| | 2h | 80 | 5 | 72.22% |
| Standard | 3min. | 80 | 0 | 100% |
| | 30min. | 80 | 0 | 100% |
| | 1h. | 80 | 2 | 88.24% |
| | 2h. | 80 | 4 | 76.47% |