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Mosquito repellency and knockdown effect of a plant based formulation

Musau, J. K.[1*], Mbaria, J. M.[1], Nguta, J.M.[1], Mbaabu, M.[2], Kiama S.G[2]
[1]*Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya*
[2]*Department of Veterinary Anatomy and Physiology University of Nairobi, P.O. Box 30197-00100, Nairobi, Kenya*

Abstract: Personal protection against mosquito bites is an efficient way of preventing mosquito borne diseases. Six plants namely *Tagetus minuta* L., *Adansonia digitata* Linn. , *Ocimum suave*, *Plectranthus barbatus* A., *Azadirachta indica* A. Juss. , *Lantana camara* L were identified for this study. They were formulated in petroleum jelly into formulations of 10% and 20%. When tested on human subjects, they exhibited mosquito repellency effect similar to or greater than *N*-diethyl-3-methylbenzamide (DEET) which is the normally accepted positive control. The knockdown effects of the formulations against the mosquitoes were similar or greater than citronella oil. The formulations can be used as alternatives to synthetic repellents which are mostly expensive for everyday use and toxic especially to children.

Key words: *repellency, knockdown, Aedes aegypti, DEET, formulation*

I. INTRODUCTION

Mosquitoes transmit diseases that cause high rates of morbidity and mortality in human. The effects of these diseases transcend human affliction to economic loss and social disruption in the poorest countries of the world [1, 2]. Mosquito control methods are habitat change, biological control, physical control and chemical control including individual safeguards from the mosquitoes [3]. Physical control and habit change are unattainable since it is impractical to eliminate all aquatic habitats while others are sources of water and food [4]. Biological control is difficult as mosquito predators also prey on beneficial insects. The predators cannot be used in polluted and/or temporary water areas. Frequent use of insecticides for mosquito control has resulted in vector resistance for all classes of insecticides and undesirable effects on non-target organisms [5, 6]. Among the approaches for control of these mosquito-borne diseases is the interruption of the disease transmission by killing or preventing mosquitoes from biting human being [7]. This can be achieved by use of repellents. Use of insect repellents is one of the most efficient ways to prevent disease transmission by biting insects, particularly by mosquitoes because it reduces man-vector contact [8], and minimizes the discomfort of insect bites [9]. Synthetic repellents are expensive for everyday use and there are concerns about their toxicity and safety [3]. Long term exposure of new born babies and children to pyrethoid based mosquito repellents is associated with clinical, biochemical and neurological effects [10]. *N*-diethyl-3-methylbenzamide (DEET) dissolves synthetic fabrics and plastic on eyeglasses and watches. Doubts about its effectiveness and safety have been expressed by service members of the US military, its largest number of users [11].

Compared to synthetic repellents, plant based repellents are simple, cost-effective and readily available [12]. They are also environmentally safe, degradable and target specific [13]. They are widely accepted by the public even though very few of them have been evaluated for toxicity [14]. The aim of this study was to develop a plant based mosquito repellent that is effective and safe to use.

II. MATERIALS AND METHODS

2.1 Ethical consideration

For use of human subjects in the repellency testing of the formulations, the study protocol was submitted to the Kenyatta National Hospital / University of Nairobi ethical review committee (KNH/UoN ERC) where approval was granted under protocol number P357/05/2015.

2.2 Selection of plants

Six plants were identified for this study based on ethnobotanical and ethnopharmacological surveys carried on Msambweni district of Kwale county [15], in addition to comprehensive review of relevant literature on ethnomedicinal plants in the Kenya's south coast that have been reported to have repellency activity against mosquitoes. The plants were *Tagetus minuta* L., *Adansonia digitata* Linn. , *Ocimum suave*, *Plectranthus barbatus* A., *Azadirachta indica* A. Juss. , *Lantana camara* L. Traditional herbal practitioners helped in initial

identification and field collection. Further identification was done by a plant taxonomist at the department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi where voucher specimens were deposited.

2.3 Preparation of plant material and extraction

The plants' parts used were leaves. They were scrutinized for any foreign matter or moulds then cleaned with distilled water. They were then be chopped into small pieces and air dried under shade at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The dried plant material was ground to fine powders using a laboratory mill. The obtained powdered plant material was packed in 500gram portions and stored in clean air tight paper bags [16].

2.4 Extraction

One thousand grams (1000 grams) of each of the plant powder was extracted separately in conical flasks by use of acetone. Analytical grade acetone was added until the powder was fully submerged. Thereafter, thorough stirring was done to ensure proper mixing and then shaking be done regularly to allow percolation for four days. On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask and acetone removed in a rotary evaporator at 60^0c and collected for recycling. The resultant viscous substance was dried and stored in amber in a refrigerator at $^+4^0$C pending formulation.

2.5 Formulation of the test extracts

The test extracts were formulated in pure petroleum jelly. The formulations tested were made into concentrations of 10% and 20% of plant extracts in petroleum jelly. To make 10% of the formulation, fifty four (54) grams of the pure petroleum jelly was weighed and transferred to a clean 100ml beaker. The beaker with the pure petroleum jelly was then placed in a water bath at 80 °C and stirred with a stirring rod until it was fully melted. One gram (1 gm) of each of the six plant extracts was then added to the melted jelly and stirred continuously until it mixed fully with the petroleum jelly. On complete mixing, the resultant formulation was stored at $+4^o$C awaiting repellency testing. To make 20% of the formulation, forty eight (48) gram of pure petroleum jelly was melted in a beaker using water bath at 80 °C. Two grams (2 gm) of each of the six plant extracts added to the melted petroleum jelly and stirred continuously until full mixing. The resultant formulation was stored at $+4^o$C awaiting repellency testing.

2.6 Test mosquitoes

The mosquitoes used for the laboratory repellent bioassay were 3-7 day old laboratory-bred adult females of *Aedes aegypti*. Prior to the time of tests, they were starved for 24 hours and provided with only water. Tests were conducted in triplicate [17].

2.7 Cage tests

They were performed in 40 x 40 x 40 cm cages made of aluminium sheet at the bottom, Pyrex window screen on sides and top, and a cotton stockinet sleeve for access on the front [18]. Tests were performed in a dark room with red light as the only source of illumination to maintain a 12:12 (Light: Dark) photoperiod and room temperature of 27 ± 2^o C with relative humidity of 80% maintained by use of an electric fan heater [19]. Active female host-seeking *A. aegyptiae* mosquitoes aged 3-7 days were collected from stock population using an aspirator and starved for the preceding 24 hours. Fifty test mosquitoes were used in each of triplicates [18, 19, 20]. Acetone/petroleum jelly mixture (1:1) was negative control while 20% DEET (*N, N*,-diethyl-3 methyl benzamide) was the positive control.

Volunteers who had avoided use of fragrance, any mosquito repellent, perfumed soap or tobacco for atleast12 hours prior and during the experiment were used for the experiments [18,19]. The forearm, from the elbow to the wrist (~696.6 cm^2) was washed with water and left to dry then test sample applied as evenly and as thinly as possible. The rest of the hand from the wrist to the fingers was covered with latex glove to prevent the mosquitoes from biting [19]. Acetone/petroleum jelly mixture was applied on the other forearm that had been prepared as above, and served as negative control.

The volunteer's forearm then was inserted into the cage for 3 minutes. The number of mosquitoes that landed on or probed during this period were counted and shaken off before they can imbibe any blood [18, 19, 21].

Percent protection efficacy (PE) was calculated using the formula:

PE = (C-T)/C x 100

Where: C and T are the mean numbers of mosquitoes that landed on the control and test arm respectively [22].

2.8 Determination of knockdown effect of formulated product

Knockdown effect of the formulations was determined using WHO bioassay method [23], with slight modifications [24]. Tests were done in triplicates with positive and negative controls. Filter papers were treated

with the formulated product and then air dried. Each was inserted into a chamber shown below. Twenty five active *A. aegyptiae* mosquitoes aged 3-7 days that had not been blood-fed were selected using an aspirator from the stock populations of adult mosquitoes and used for this test. They were placed in each of the chamber with filter papers with different concentrations of formulated product for 1 hour. Untreated filter papers were negative control while citronella oil at concentrations of 500 mg/m^2 was positive control.

After one hour the mosquitoes were transferred to different holding chambers that had cups with 10% sucrose solution for the mosquito to feed. SPSS V22 was used for data analysis. Mortality and recovery within 24hours was scored and time taken to knock down 90% of the population (KD90) and 95% confidence interval was calculated per treatment. Comparison was done between the controls and treatments and considered statistically significant at $P \leq 0.05$.

III. RESULTS

3.1 Repellency testing

The two formulations exhibited repellency greater than or similar to *N*-diethyl-3-methylbenzamide (DEET), the positive control. Formulation A which contained 10% of the plant extracts had repellent effect similar to *N*-diethyl-3-methylbenzamide (DEET) while formulation B comprising of 20% of the plant extracts and offered 100% protection. This was greater than that was offered by *N*-diethyl-3-methylbenzamide (DEET) even though the difference in activity of the formulated products and *N*-diethyl-3-methylbenzamide (DEET) was not statistically significant. When compared to Ballet® mosquito repellent jelly, they showed a better repellency effect. The difference in activity between the formulated products and Ballet® mosquito repellent jelly was significant, $P<0.001$.

3.2 Knockdown effects

The knockdown effect of the two formulations was greater than Ballet® mosquito repellent jelly and the difference was significant ($P<0.001$). The knockdown effect of formulation A was similar to citronella oil while that of formulation B was greater than for citronella oil but the difference was not stastically significant.

Tables 1 and 2 below show the percent protection and knockdown

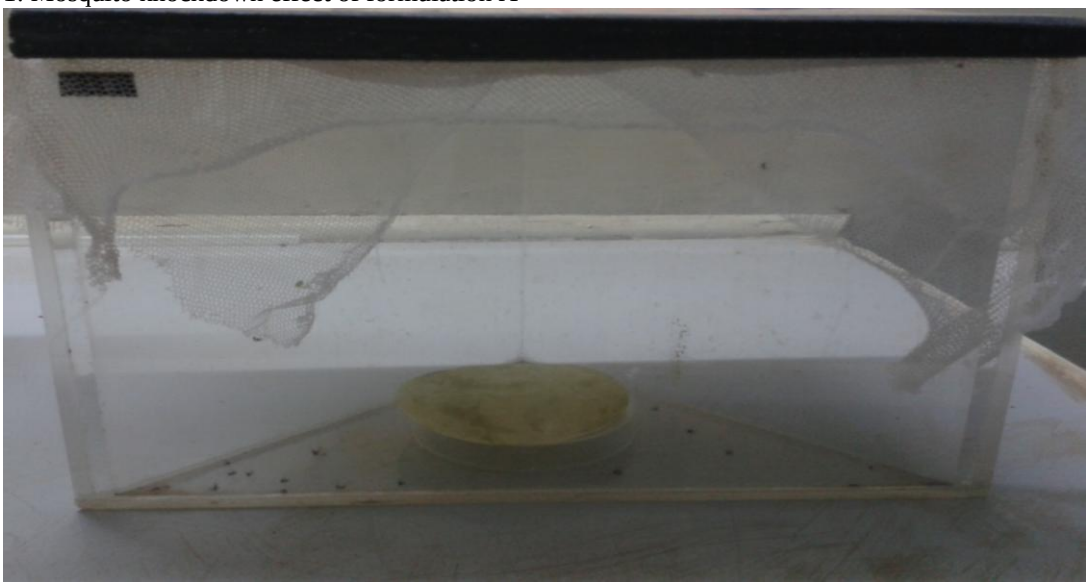
Table 1: Percent (%) protection of formulated products compared to DEET and Ballet® mosquito repellent

Treatment	% Protection
Product A (10%)	98.33±1.67
Product B (20%)	100.00±0.00
Ballet® mosquito repellent	75.00±4.07
Ethanol (Negative control)	0.00±0.00
DEET 20 %(Positive control)	98.33±1.67
P-value	<0.001

Table 2: Knock down effect of formulated products, Ballet® mosquito repellent, and citronella oil

Treatment	% Knockdown
Product A (10%)	93.33±3.33
Product B (20%)	96.67±3.33
Ballet® mosquito repellent	35.67±8.82
Negative Control	13.33±3.33
Citronella oil	93.33±3.33
P-value	<0.001

Plate 1: Mosquito knockdown effect of formulation A



All mosquitoes were dead/ immobile in the first three minutes. They never recovered even when they were transferred to the recovery chamber

IV. DISCUSSION

Since mosquitoes were identified as vectors of malaria and yellow fever in the late nineteenth century by British and U.S. military officers [25], they have been known to transmit many other diseases such as dengue and dengue hemorrhagic fever, rift valley fever, filariasis, West Nile Virus, Japanese encephalitis and other arboviruses. These diseases are important causes of morbidity and mortality in developing countries [26]. As they bite, they introduce foreign proteins with their saliva thus stimulate allergic reactions and localized irritation which may be secondarily infected with bacteria due to scratching that ensues. At the same time, the human skin is compromised leading to a possibility of secondary infection with bacteria [27].

Personal protection is important in controlling mosquito borne diseases through interrupting killing or preventing mosquitoes from biting human being [7, 28]. Shortcomings of synthetic repellents such as *N*,*N*-diethyl-3-methylbenzamide (DEET) are many [11,29,30].

From the results, the formulated products had repellency and knockdown effect suggesting there was synergism among the plant extracts. The knockdown effect of both formulations was equal or greater than that of citronella oil which was the positive control. Similarly, the percent protection offered by the each of the two formulations was equal or greater than that of *N*,*N*-diethyl-3-methylbenzamide (DEET) the positive control. The drawback of using plant-based repellents is that many of them are made up of relatively volatile constituents and are generally not effective over long periods of time and as such require frequent reapplication [31]. Formulation with petroleum jelly could have contributed to the improved activity of the formulations. Many researchers have reported improved repellency effective over several hours with addition of a base or fixative materials [32]. If a volatile compound is combined with a non-volatile substance, it is possible to block insect attack both on the air and the skin surface [33].

In this study, Ballet® mosquito repellent jelly offered 75% protection. This is in agreement with a previous finding that Ballet® does not provide complete protection against mosquitoes [34]. Therefore, Ballet® mosquito repellent jelly must be applied several times to protect against mosquitoes. The developed formulations can be used as alternatives to the more expensive synthetic formulations as they offer a unique way of protection against mosquitoes in the form of great repellent effect and knockdown activity.

V. CONCLUSION

From the results, the two formulated products have excellent repellency and knockdown effect due to synergism among the plants' extracts. The formulated products can be used as mosquito repellent agents as alternatives to synthetic mosquito repellents. Formulating the products with a fixing agent, in this case the petroleum jelly enhanced the activity of the formulations by preventing loss of active compounds as has been observed by other researchers. Further research is necessary to evaluate the activity of the formulated products in both semi field and field trials.

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