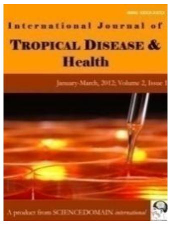

International Journal of TROPICAL DISEASE & Health
6(3): 73-79, 2015, Article no.IJTDH.2015.041
ISSN: 2278–1005

SCIENCEDOMAIN *international*
www.sciencedomain.org

Bioprospection for Repellent Effect of Natural Volatiles from *Ocimum suave* Willd Growing in Dar es Salaam, Tanzania against Anopheles Mosquitoes

Wilson Leonidas[1,2*], Judith Kagondi Shipili[1,2], Josephat A. Saria[1], Yohana Lawi[1], Eliningaya J. Kweka[3,4], Frank Magogo[3], William N. Kisinza[3] and Hamisi M. Malebo[2]

[1]Faculty of Science, Technology and Environmental Studies, Open University of Tanzania, P.O.Box 23409, Dar es Salaam, Tanzania.
[2]Department of Traditional Medicine Research, National Institute for Medical Research, P.O.Box 9653, Dar es Salaam, Tanzania.
[3]Amani Medical Research Centre, National Institute for Medical Research, P.O.Box 81, Muheza, Tanzania.
[4]Tropical Pesticides Research Institute, Division of Livestock and Human Disease Vector Control, P.O.Box 3024, Arusha, Tanzania.

Authors' contributions

This work was carried out in collaboration between all authors. Authors WL, JKS, JAS, YL, EJK, FM, WNK and HMM designed the study, wrote the protocol and performed experiments, carried out data analysis, literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2015/14710
Editor(s):
(1) Anthony R. Mawson, Public Health & Director Institute of Epidemiology & Health Services Research, Jackson State University, USA.
Reviewers:
(1) Francis W. Hombhanje, Centre for Health Research & Diagnostics, Divine Word University, Papua New Guinea.
(2) Maureen Isabel Leyva Silva, Department of Vector Control, Institute of Tropical Medicine "Pedro Kouri" Cuba.
(3) Talal Hassan AL-Harazi, Department of Medical Microbiology and Parasitology, Taiz University, Yemen.
(4) Peter Githaiga Mwitari, Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Kenya.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=912&id=19&aid=7651

Original Research Article

Received 16th October 2014
Accepted 18th November 2014
Published 6th January 2015

**Corresponding author: Email: wilsonmahene@gmail.com;*

ABSTRACT

Essential oil was extracted from *Ocimum suave* Willd leaves by water distillation and tested for repellency effectiveness against *Anopheles gambiae* adult mosquitoes. The percentage yield of the essential oil was 0.2%. Six concentrations of *O. suave* essential oil were applied on human skin of four volunteers, and the repellency effectiveness which was analyzed by PoloPlus (LeOra software version 1.0, 2002-2014), revealed promising RC_{50}, RC_{75}, RC_{90} and RC_{99} with their confidence limits as 0.1161 mg/cm^2 (0.02067 - 0.1767 mg/cm^2), 0.2823 mg/cm^2 (0.22328 - 0.3654 mg/cm^2), 0.4319 mg/cm^2 (0.35226 - 0.58862 mg/cm^2) and 0.98934 mg/cm^2 (0.54731 - 0.99972 mg/cm^2), respectively. *O. suave* essential oil exhibited high Anopheles mosquitos' repellency effectiveness which merits further scientific attention for the development of natural repellents for the control of malaria and other mosquito borne diseases. These findings provides a scientific evidence and base for formulation for further mosquito repellency semi-field and field trials for the development of cheaper and affordable new mosquito repellent product(s) to meet human healthcare needs in the prevention and control of malaria and other mosquito transmitted infections.

Keywords: Ocimum suave; essential oil; Anopheles gambiae; repellency.

1. INTRODUCTION

Mosquitoes are not only a nuisance to humans as they bite them to get their blood meal but, also transmit disease causing parasites and viruses in the process. Female mosquitoes are responsible for the transmission of a number of human blood-borne diseases. Mosquitoes have been shown to transmit the mostly fatal and disabling diseases, the most prevalent being malaria, bancroftian filariasis, yellow fever, dengue fever and several arbovirus infections [1]. The transmission of these infections to humans is a function of the availability of competent mosquito vectors; hence, disease control initiatives targeting mosquitoes remain the most successful mechanism for the prevention and control of malaria, bancroftian filariasis, yellow fever, dengue fever and several arbovirus infections [2]. An estimated 3.4 billion people are at risk of malaria, of which 1.2 billion are at high risk of malaria morbidity and death [3]. There were an estimated 207 million cases of malaria in 2012 (uncertainty range: 135 – 287 million) and an estimated 627,000 deaths (uncertainty range: 473,000 – 789,000) [3]. Ninety percent of all malaria deaths occur in sub-Saharan Africa [3]. In 2012, malaria killed an estimated 482,000 children under five years of age majority of deaths occurred in sub-Saharan Africa. This translates to 1,300 children deaths every day, or one child dying almost every minute [3].

Resistance to drugs and pesticides is taking malaria-control and eradication measures to even more demanding levels. The observed resistance shows strong positive correlation with heavy reliance on the pesticides and drugs currently on the market. In some parts of the world where malaria was once almost eradicated using synthetic pesticides such as dichlolodiphenyl trichloroethane (DDT) and antimalarial drugs, malaria has returned with new features not witnessed during the pre-eradication time. These include vector resistance to pesticides and malaria parasite resistance to drugs [4]. In Africa and some other parts of the world, the heavy reliance on pyrethroids has led to the emergence of pyrethroids-resistance in mosquitoes in malaria-endemic settings [5]. The vector resistance to pesticides challenge requires integrated approach that ensures that malaria parasites are not transmitted to humans.

The 2012 WHO Malaria Report estimates that, malaria related illness and mortality have been dramatically declined by the integrated efforts including the distribution of long lasting insecticide treated mosquito nets (LLINs), efficient case management with potent antimalarials, source reduction and selective household spraying [6]. This depicts that in the current context, where mosquitoes' multiplication and resistance to pesticides is increasing in response to the prevailing climate change and the use of pesticides available on the market,an integrated approach in dealing with the malaria problem is now becoming an inevitable scientific endeavor. This entails, by *bioprospection, discovery of new compounds with more pesticidal and repellency effectiveness, oviposition deterrence and mosquito attractants to* be part of the integrated vector control initiative that will be applied based on local studies.

Moreover, environmental concerns that synthetic chemicals contaminate the environment due to their poor biodegradability and their direct toxicity to humans and other non-target organisms have made humans to dislike their use, thereby calling for search for safer pesticides and repellents of natural sources that are more biodegradable, more target specific and relatively of low toxicity as one of the constituents of the integrated approach in the fight against mosquito borne diseases, malaria in particular [7].

The biting behavior of Anopheles mosquitoes is intriguing as at the moment they have been shown to bite people before retiring to bed or wait and bite when people get out of the net at night even when insecticide treated nets (ITNs) are used; hence this challenge calls an additional mosquito control tool to completely curtail human-mosquito contacts when not under ITNs. Measures to prevent humans from mosquito bites are currently the most important measure for the control of malaria2. In comparison to vaccines and chemoprophylaxis as means of personal protection, repellents are an inexpensive and practical means of personal protection against nuisance and disease vector bites, in conjunction with other or when other control measures are not practical [8-10]. The mosquito repellent products commonly available on the market contain *N*,*N*-diethyl-3-toluamide (DEET) as the active ingredient [11]. However, DEET has operational disadvantages due to its allergic reactions, toxicity to man and is a good solvent for synthetic materials and plastics [12]. Hence, there is a need for research to identify and develop safer and effective natural repellents [2]. A number of plant species have previously been investigated for their effects against mosquitoes such as toxicity, oviposition deterrent and repellency [13].

Natural anti-mosquito agents have been used in Africa and other parts of the world since antiquity. These include *Ocimum suave*, *O. gratissimum*, *O. tenuiflorum, Lantana camara, Lippia javanica* and *Hyptis suaveolens* [2,10]. In this bioprospection initiative for eco-friendly, safer and effective natural repellents we have put on test essential oil from *Ocimum suave* Willd as a possible candidate for incorporating into an integrated malaria vector control initiative, as additional means of malaria prevention and control, and to provide a useful complement to insecticide treated nets (ITNs). *O. suave* Willd belongs to the genus *Ocimum* in the family Lamiaceae. *O. Suave* is an aromatic shrub growing in high attitude in Tanzania and other countries. The genus *Ocimum* comprises about 200 species of herbs and shrubs distributed in Africa, Asia, Central and Southern America [14]. Major chemical compounds known to occur in the genus include: flavonoid glycosides, essential oils such as ambrosial, β-bisabolene, (E)-α-bisabolene, methyl chavicol, 1,8-cineole, eugenol, α-terpineole, linalool, (z)-cinammic acid methyl ester and camphor. Important biological activities of the genus has been reported as antimicrobial, antispasmodic, antihelmintic, antiviral, larvicidal, antipyretic antiaflatoxigenic [14].

2. MATERIALS AND METHODS

2.1 Study Area

The laboratory study was carried out at Ubwari laboratory of the Amani Centre of the National Institute for Medical Research (NIMR) situated 1 km near Muheza town.

2.2 Plant Materials

Leaves of *O. suave* were collected from Pugu forest near Dar es Salaam in Tanzania. A qualified taxonomist from the University of Dar es Salaam in Tanzania located the plants for harvesting. Voucher specimens were prepared and deposited at the herbarium in the Department of Botany at the University of Dar es Salaam in Tanzania.

2.3 Hydro-Distillation

The extraction process was carried out in the Phytochemistry Laboratory at the Department of Traditional Medicine Research in NIMR in Dar es Salaam, Tanzania.The fresh leaves were grinded using an electric blender and immediately transferred into a round bottomed flask and mounted on Clevenger apparatus for hydro-distillation to obtain essential oil. Temperature was first set at 100°C and later, after boiling was reduced to 50 °C. The condensed vapor and afforded oil was separated using a separating funnel by taking advantage of their mutual immiscibility.

The percentage yield was determined using the formula:

$$\textbf{Percentage yield} = \frac{\text{Weight of essential oil}}{\text{Weight of leaves}} \times 100$$

2.4 Laboratory Based Mosquito Repellency Assay

Adult *Anopheles gambiae* aged between 5 and 9 days were obtained from a laboratory colony maintained at 27±2°C and 70-80% relative humidity. Larvae were fed a 3:1 mixture of dog biscuits, and yeast powder. 12:12 (light:dark) photoperiod was maintained. Adults were provided with a 10% sucrose solution. Observations of repellency were made using female mosquitoes starved for the preceding 7 hours. Serial dilutions of candidate repellent were made with ethanol to identify effective dose range. Four volunteers applied incremental doses of 0.028, 0.056, 0.121, 0.186, 0.251 and 0.316g/590cm^2 were applied on the test forearm. A single test comprised of continuous use of the same mosquitoes by the same volunteer and was completed in one day. Two replicates of this process were obtained using different batches of *An. gambiae* mosquitoes in two days (each replicate test for one extract was completed in one day). 1 ml of ethanol was applied evenly using a syringe to a corresponding area between the wrist and elbow of approximately 590 cm^2 was used as the site of application and allowed to dry (for 1 min) and the arm was inserted into a cage containing 50 mosquitoes. As negative control was used 1 ml of ethanol. Six concentrations of 0.028, 0.056, 0.121, 0.186, 0.251 and 0.316g/590cm^2 were evaluated which were dissolved in ethanol and applied in the same manner as the control. Before insertion of the arm into the cage containing 50 female mosquitoes, the hands were protected by medical gloves. The forearm applied with diluents (ethanol) was inserted into the cage first and the number of mosquitoes that landed in three minutes period was recorded. During testing, the volunteers were advised to avoid movement of the arm. The control forearm was carefully withdrawn and this arm was then treated with the lowest dose of repellent in 1 mL alcohol and allowed to dry for one minute. The treated arm was placed in the cage for another three minute period and observed for mosquito landings. The number of landings was also recorded. This procedure was repeated for each additional incremental repellent dose. Successive tests were carried out one after the other without delay and the repellent dose at each test was calculated as the sum of the doses applied to arrive at the cumulative dose for each test.

In the control experiment, 1 mL ethanol was applied on the other forearm (left arm) and allowed to dry. This forearm was inserted in the cage for three minutes to verify that the number of landings is approximately ≥10 per one minute, as was observed at the beginning of the experiment. If the rate was <10 females in one minute, the results of this experiment were discarded. Data analysis was carried out using by PoloPlus (LeOra software version 1.0, 2002-2014), employing probit analysis model, to obtain RC_{50}, RC_{75} and $RC_{99,}$ and their confidence limits.

Percentage repellency was calculated according to WHOPES as [15]:

$$\text{Mosquito repellency (\%)} = \frac{100\ (1 - T)}{C}$$

Where:

T = Number of probing mosquitoes on treated arm;
C = Number of probing mosquitoes on control arm.

3. RESULTS AND DISCUSSION

The yield of *O. suave* oil was 0.2%. The six different concentrations of *O. suave* essential oil provided percentile repellence concentrations, $RC_{(\%)}$ (percentile effective doses) as summarized in Table 1 below. It was observed that increasing concentration of the repellent oil led to increased percentage repellency. The results revealed promising RC_{50}, RC_{75}, RC_{90} and as 0.1161 mg/cm^2, 0.2823 mg/cm^2, 0.4319 mg/cm^2 and 0.98934 mg/cm^2, respectively.

This is clearly depicted by the regression line which appears to be sigmoid in nature (Fig. 1). The fact that the essential oil is obtained by using a simple technology that requires no intensive training, and that raw materials are readily available in rural areas, this product promises to be cost effective and hence accessible to even the poverty stricken households in rural areas. Previous studies have shown that *O. suave* essential oil can afford up to 74% repellency efficacy [10]. The study reported the concentration of the essential oil in formulations used and not the amount of the oil per unit skin surface area. This study goes an extra mile to show the concentration of the essential oil on human skin (amount of essential oil per skin surface area) that confers the herein claimed percentage protection at various concentrations of the essential oil within the tested concentration (dose) range (Fig. 1). In this study we have demonstrated concentration dependency mosquito repellency activity as the concentration of the essential oil was increased more was the repellency activity observed. In a previous study, *O. suave* essential oil repelled and killed all stages of the tick *Rhipicephalus appendiculatus* [16]. The principal repellent components in *O. suave* oil were previously reported as eugenol, methyl eugenol, linalool, cis-β-ocimine, and linalyl acetate [17]. The *O. suave* essential oil exhibited higher *Anopheles* mosquitos' repellency effectiveness in this study which merits further scientific attention for the development of natural repellents for the control of malaria and other mosquito borne diseases.

These findings provides a scientific evidence and base for formulation for further mosquito repellency semi-field and field trials for the development of cheaper and affordable new mosquito repellent product(s) to meet human healthcare needs in the prevention and control of malaria and other mosquito transmitted infections. The ethanolic solutions of essential oil of *O. suave* showed a directly proportional relationship between concentration and percent repellency, making this plant an option to continue studying as an alternative to control *An. gambiae*.

Table 1. Percentile repellence concentrations of *O. suave* essential oil

RC_{50}	Lower limit (mg/cm^2) / Upper limit (mg/cm^2)	**RC_{75}**	Lower limit (mg/cm^2) / Upper limit (mg/cm^2)	**RC_{90}**	Lower limit (mg/cm^2) / Upper limit (mg/cm^2)	**RC_{99}**	Lower limit (mg/cm^2) / Upper limit (mg/cm^2)	**SLOPE (SE)**	X^2
0.1161	0.02067 / 0.1767	0.2823	0.22328 / 0.3654	0.4319	0.35226 / 0.58862	0.68934	0.54731 / 0.99972	0.041+- 0.008	2.697

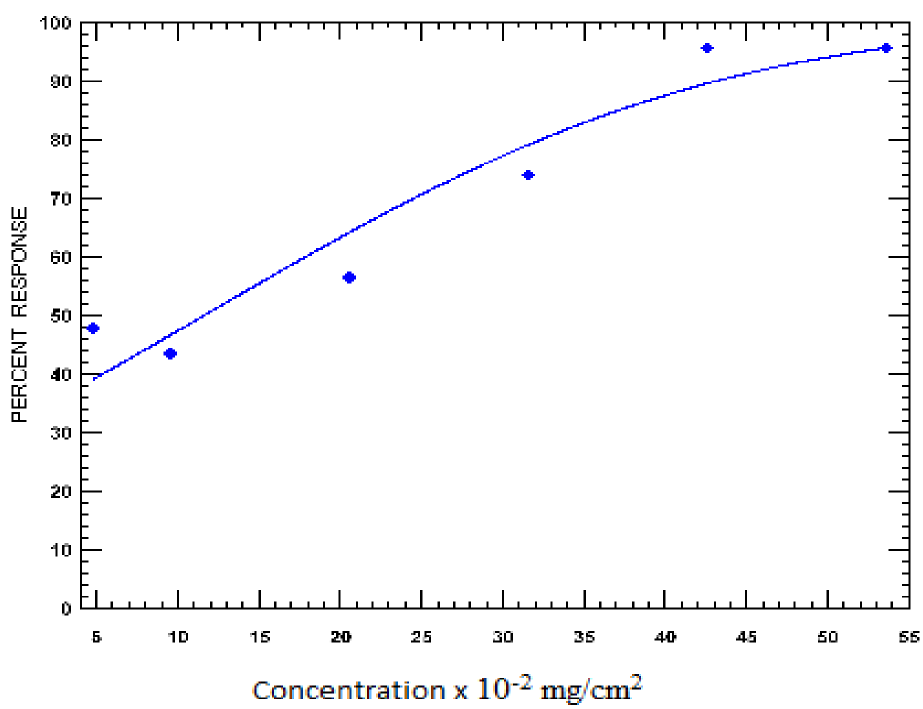

Fig. 1. Regression line showing percentage response (repellency) of *O. suave* essential oil an *An. gambiae* to at 3 min

4. CONCLUSION

Our findings confirm the effectiveness of *O. suave* traditionally used in Tanzania by communities as mosquito repelling plant. Further research on the essential oil should include 1) toxicological and safety assessment of extracts 2) testing against other mosquito species including wild population 3) investigating complete protection time and the best formulation (concentration, solvent and other additives, etc.) to make the essential oil unfold its full potential effectiveness both in terms of efficacy and complete protection time 4) repellency testing on other arthropods 5) compete protection time testing 6) Description of the mode of action.

CONSENT

Not applicable.

ETHICAL APPROVAL

The research protocol was approved by the Faculty of Science, Technology and Environmental Studies of the Open University of Tanzania, P.O. Box 23409, Dar es Salaam, Tanzania.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. White GB. In: Manson's tropical disease 20th Edition, ELBS low price edition. London; 1973.
2. Malebo HM, Imeda C, Kitufe NA, Katani SJ, Sunguruma R, Magogo F, Tungu PK, Nyigo VA, Wiketye V, Mwaiko GL, Ogondiek JW, Mbogo GP, Mhame PP, Matata DZ, Malima R, Magesa SM, Massaga JJ, Malecela MN and Kitua AY. Repellence effectiveness of essential oils from some Tanzanian *Ocimum* and *Hyptis* plant species against afro-tropical vectors of malaria and lymphatic filariasis. Journal of Medicinal Plants Research. 2013;7(11): 653-660. DOI:10.5897/JMPR012.732. Available:http://www.academicjournals.org/JMPR
3. World Malaria Report. World Health Organization, Geneva, Switzerland; 2012.
4. Sharma VP. Re-emergence of malaria in India. Indian Journal of Medical Research. 1996;103:26–45.
5. Karunamoorthi K, Sabesan S. Insecticide resistance in insect vectors of disease with special reference to mosquitoes: A potential threat to global public health. Health Scope 2013;2(1):4–18. Available:http://dx.doi.org/10.5812/jhs.9840
6. World Malaria Report. World Health Organization, Geneva, Switzerland; 2013.
7. Karunamoorthi K. Medicinal and aromatic plants: A major source of green pesticides/risk-reduced pesticides. Journal of Medicinal and Aromatic Plants. 2012;1: e137. Available: http://dx.doi.org/10.4172/2167-0412.1000e137
8. WHO. International Travel and Health Vaccination Requirement and Health Advice. Geneva, Switzerland: World Health Organization; 1995.
9. Gupta RK, Rutledge LC. Role of repellents in vector control and disease prevention. American Journal of Tropical Medicine and Hygiene. 1994;50(6, supplement):82–86.
10. Malebo HM, Nguruwe R, Lugimbana L, Sambu E, Malecela E, Kamugisha ML, Senkoro K, Magesa SM. Efficacy of *O. suave* volatile oil formulation against man-biting mosquitoes in Muheza, north-east Tanzania. Tanzania Health Research Bulletin. 2005;7:25-30.
11. Hayes WJ, Laws ER. Handbook of pesticide toxicology: General principles; 3rd edition. Academic Press, San Diego. 1991;1:1576.
12. Thavara U, Tawatsin A, Chompoosri J, Suwonkerd W, Chansang U, Asavadachanukorn P. Laboratory and field evaluations of the insect repellent 3535 (ethyl butylacetylaminopropionate) and deet against mosquito vectors in Thailand. Journal of American Mosquito Control Association. 2001;17(3):190-5.
13. Moore S, Lenglet A. An overview of plants used as insect repellents. In: Traditional Herbal Medicines for Modern Times: Traditional Medicinal Plants and Malaria. Edited by: Willcox M, Rasoanaivo P & Bodeker G. CRC Press LLC, Washington, D.C. 2004;22:344-359.
14. Bhasin M. *Ocimum*- Taxonomy, medicinal potentialities and economic value of essential oil. Journal of Biosphere. 2012;1:48-50. ISSN 2278 – 3342.

15. World Health Organization 2009. Guidelines for efficacy testing of mosquito repellents for human skin WHO/HTM/NTD/WHOPES/2009.4. City: World Health Organization; 2009. Guidelines for efficacy testing of mosquito repellents for human skin WHO/HTM/NTD/WHOPES/2009.4.
16. Mwangi EN, Hassanali A, Essuman S, Myandat E, Moreka L and Kimondo M. Repellant and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. Experimental Applied Acarology. 1995;19:11–18.
17. Chogo JB, Crank G. Chemical composition and biological activity of the Tanzanian plant *Ocimum suave.* Lloydia. 1981;44:308–311.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=912&id=19&aid=7651