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Research Article
Repellent and Mosquitocidal Properties of Smoke from Oil and Non-polar Extracts of *Ocimum viride* Leaf Against *Aedes aegyptii*

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Abstract

Background and Objective: *Ocimum viride* leaf extracts exhibit mosquito repellent activities. However, the duration of its protection is not reported. This study therefore assessed and compared the protective times, percent flying inhibition and minimum inhibitory concentration of oil and non-polar extract of *Ocimum viride* leaf against *Aedes aegyptii*. **Materials and Methods:** Five different concentrations (50, 40, 30, 20 and 10%) each of the two extracts dissolved in acetone were formulated and incorporated into a burning material made of coconut shell flour, potato starch and sodium nitrate. A blank burning material which contained none of the extracts was formulated as control. Ten mosquitoes released into a test cage were exposed to smoke from the burning material. The repellence and insecticidal activities of the oil, non-polar extract and control were monitored at 5 min interval over a period of 30 min. The protective time (Pt) and percent flying inhibition for each treatment were determined and analyzed with ANOVA. **Results:** Non-polar and oil extracts offered dose-dependent repellent potential but no mosquitocidal activities. The oil exhibited significantly longer protective time ($p<0.05$) [$Pt (oil) = 0.4400x+2.0$, $Pt (non\text{-}polar) = 0.006429x^2-0.1957x+2.3$] and flying inhibition ($p<0.001$) than the non-polar extract with minimum inhibition concentrations 2.17 and 5.62%, respectively. **Conclusion:** Oil from *Ocimum* leaf repelled *Aedes aegyptii* better than its non-polar extract at minimum concentration and offered higher protective time.

Key words: *Ocimum viride*, mosquitocidal, repellent, protective, *Aedes*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aedes aegyptii is a known vector of several debilitating diseases including dengue fever, Chikungunye, yellow fever and Zika[1,2]. Both sexes of *Aedes* are nuisant to humans: The males produce irritating buzzing sounds and the saliva injected by females during their blood meal cause severe itch[3,4]. Population of *A. aegyptii* like other mosquito species are controlled by destruction of potential breeding sites, bio-control, larviciding or adulticiding using pesticides such as dichlorodiphenyltrichloroethane (DDT), trapping of adults and commonly, use of suitable insect repellents[5].

Insect repellents could be of natural or synthetic origin. These synthetic repellents such as N, N-diethyl-3-methylbenzamide (DEET), picaridin (2-(2-hydroxyethyl) and piperidinecarboxylic acid (1-methylpropyl ester) and pyrethroids[6,7] are incorporated in aerosol sprays, burning materials in the form of coil, creams and bed nets[8]. They are not wholly protective owing to lapses in their usage. Pyrethroid-insecticide treated bed nets, for example, only protect their users whilst they habit in it[9]. The nets are also misused by some farmers for hedges for their crop farms as well as catch nets during fishing[10]. Mosquito larvae's exposure to rain water washes from these nets increases their chances of developing resistance to these synthetic repellents[11,12]. In addition, long exposure of humans to smoke from burning mosquito coils is associated with ailments such as breathing problems, headache, eye irritation, bronchial irritation, lung cancer and skin reaction[13,14]. Mostly these coils weaken the mosquitoes for as long as they burn. The mosquitoes resume flight after metabolizing the active ingredients and recurrence of this makes mosquitoes grow resistant to synthetic repellents overtime.

In many countries, *Aedes aegyptii* has developed insecticide resistance to synthetic insecticides[15] unlike natural repellents. These natural repellents have also been used for medicinal purposes for a long time because they do not pose hazard of toxicity to human or domestic animals and are also biodegradable[16]. An example of plant with mosquito repellent property is *Ocimum.* Members of the genus *Ocimum* (family Lamiaceae) is an aromatic annual and perennial herb with numerous culinary, horticultural and ethnomedicinal benefits. Ethnomedicinally, *Ocimum* plant is used as remedy for cold and catarrh, stomach pain, diarrhea and piles. It also has antipyretic, diuretic, laxative and hepatoprotective properties[17]. The plant parts are burnt by traditional users to repel mosquitoes but duration of protection is not known. Similar studies using natural repellents mostly report on repellent and insecticidal activities of various non-polar fractions of the leaf and not the oil. Also, percentage inhibitions of the non-polar extracts are reported but little is known about how long the extract incapacitates the insects (protective time). In this study, the repellent and/or insecticidal quality of oil and non-polar extract of *Ocimum viride* leaf incorporated in a burning material, against one of the species of mosquitoes, *Aedes aegyptii* were assessed and compared. Percent flying inhibition and protective times of the oil and non-polar extract were determined and also, compared to determine which of the two offered more protection.

Non-polar extract and oil from *Ocimum viride* leaf have similar minimum inhibition concentration and protective time.

MATERIALS AND METHODS

This study was conducted in the Department of Conservation Biology and Entomology, University of Cape Coast (U.C.C), Ghana within a period of 4 months (February-May, 2017).

Collection and preparation of test material: *Ocimum viride* leaves were freshly collected in U.C.C. Botanical gardens, washed in tap water and distilled water and subsequently air-dried at room temperature. The plant was authenticated by the School of Biological Sciences Herbarium, University of Cape Coast, Ghana.

Extraction of non-polar and oil extracts: About 50 g of the processed leaves were pulverized and mixed with 250 mL of 99.8% ethanol. The ethanol-pulverized leaf mixture was shaken in mechanical shaker at 300 oscillations per min for 48 min. Solvent extraction was done thrice on the ethanol-pulverized leaf mixture using 90 mL each of petroleum ether (40-60°C) and distilled water. About 692 g of the processed leaves were placed in a round-bottomed flask containing 50 mL of distilled water. The mixture was steam distilled and the cloudy distillate collected in a beaker. Solvent extraction was performed thrice on the distillate using 20 mL of petroleum ether. The beakers containing the ethereal extract and distillate were put on water bath at 60°C to vapourize the ether leaving the non-polar and oil extracts, respectively. The mass and percentage yields of the residue were computed.

Preparation of test material: Various concentrations each of non-polar and oil extracts (10, 20, 30, 40 and 50%) were formulated by dissolving 0.1, 0.2, 0.3, 0.4 and 0.5 mL of leaf extracts in 0.9, 0.8, 0.7, 0.6 and 0.5 mL of acetone, respectively. Test material was prepared as suggested by Elsner *et al.*[18]. A carrier starch water paste consisting of 3 g potato starch dissolved in 3 mL of 60°C water, 6 g of coconut shell flour, 0.5 g of $NaNO_3$, 4.5 mL of 100°C distilled water and 1 mL of extract concentration were prepared. Thin sheets of test material were formed by placing the paste in a 1.0 cm width ×1.0 cm height×20 cm length mould. The moulds were dried for 3 days and subsequently cut into pieces weighing 1.5 g. A blank strip containing 4 g potato starch, 6 g coconut shell and 0.5 g $NaNO_3$ was also formulated.

Cage test experiment: Larvae of *Aedes* mosquitoes were collected from a breeding site and subsequently cultured in the lab till adulthood under temperature of 27±2°C and relative humidity, 75±5%. Ten adult *Aedes aegyptii* mosquitoes (Nc) were released into a glass cage of dimensions 22×22×22 cm. About 0.2 mL of acetone was added to ground test material in a Petri dish and mixed thoroughly. The soaked material was set on fire and upon quenching of the fire, the burning material was introduced into the test cage. The number of mosquitoes which remained in flight (Nt) upon treatment introduction was recorded at 5 min interval for 30 min. The percentage biting protection was calculated using the modified Abbot's formula[19]:

$$\text{Flying inhibition (FI) (\%)} = \frac{\text{Nc} - \text{Nt}}{\text{Nc}} \times 100$$

The protective time (PT) which refers to the minimum period for any of the repelled mosquito to resume flight was recorded.

Minimum flying inhibitory concentration (MFIC): Minimum flying inhibitory concentration for 30 min inhibition was determined for non-polar and polar extracts by plotting a scatter plot with natural logarithm of the concentrations on the x-axis and flying inhibition (%) on the y-axis. A line of best fit was drawn and the x-intercept (flying inhibition (%) = 0) was determined for the various times. The value for antilog of the x-intercept was considered as the minimum flying inhibitory concentration for that time.

Statistical analysis: The mean percentage flying inhibition and mean protective time for each treatment were computed and statistically compared using two-way ANOVA (Analysis of Variance) using Minitab 17 statistical software. A p-value less than 0.05 was considered significant.

RESULTS

Extraction of oil and non-polar materials: The steam distillation process using a lot of leaves yielded a thick yellowish liquid (oil) of mass and volume, 3.3 g and 3.0 mL, respectively. The density and percentage yield of the oil were 1.1 g mL^{-1} and 0.484%, respectively. The non-polar extraction yielded a greenish liquid of mass and volume, 11.2 g and 13.6 mL, respectively. The density and percentage yield of the non-polar extract were 0.823 g mL^{-1} and 9.067%, respectively.

Protective time (PT): The mean protective time of oil extract was significantly greater than that of non-polar extract ($p = 0.000$) (Table 1, 2). There was no significant difference among the protective times with respect to their concentrations ($p = 0.074$) (Table 2) though there was dose dependent decrease in protective time: $PT_{50}>PT_{40}>PT_{30}>PT_{20}>PT_{10}$ ($p = 0.000$) (Table 1).

Flying inhibition (% FI): The mean percentage of flying inhibition for oil and non-polar extracts were 70.0% and

Table 1: Protective time (min) of oil and non-polar extracts of *Ocimum viride* leaf

Concentration (%)	Oil/min	Non-polar/min
50	20	9.0
40	15	4.0
30	13	2.0
20	5	2.0
10	3	0.5

Table 2: Two-way ANOVA of protective time (min) of *Ocimum viride* leaf extracts

Sources	DF	SS	MS	F-value	p-value
Extracts	1	148.225	148.225	14.50	0.019
Conc.	4	203.900	50.975	4.99	0.074
Error	4	40.900	10.225		
Total	9	393.025			

S: 3.198, R-Sq: 89.59%, R-Sq(adj): 76.59%

Table 3: Two-way ANOVA of Flying inhibition (%) of *Ocimum viride* leaf extracts

Sources	DF	SS	MS	F-value	p-value
Extracts	1	1041.67	1041.67	24.41	0.000
Conc.	4	17866.67	4466.67	104.69	0.000
Time	5	6688.33	1337.67	31.35	0.000
*Extracts conc.	4	66.67	16.67	0.39	0.813
*Extracts time	5	288.33	57.67	1.35	0.284
*Conc. time	20	653.33	32.67	0.77	0.722
Error	20	853.33	42.67		
Total	59	17458.33			

S: 6.53197, R-Sq: 96.89%, R-Sq(adj): 90.83%

61.67%, respectively. Analysis of variance of data on percentage of flying inhibition showed that the mean flying inhibition (%) of oil extract was significantly greater than that of non-polar extract (p = 0.000). In each extract, there was significant dose-dependent increase in flying inhibition (%): $FI_{50}>FI_{40}>FI_{30}>FI_{20}>FI_{10}$ (p = 0.000). There was a significant decline in percent flying inhibition with increase in time.

Minimum flying inhibition concentration (MFIC): The minimum flying inhibitory concentration for non-polar and oil extracts were 5.62% (v/v) and 2.17% (v/v), respectively. The equations involving the percentage of flying inhibition (y) and natural logarithm (x) of the concentrations for oil and non-polar extracts were y = 23.342x-18.098 [R^2 = 81.50%] and y = 29.998x-51.797 [R^2 = 84.52%], respectively.

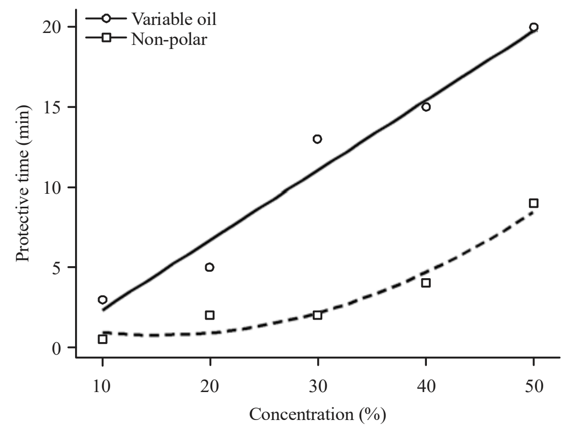

Fig. 1: Regression fit for protective times (Pt) of Ocimum viride leaf extracts at various concentrations (x). Regression equations are as follows: Pt (oil) = 0.4400x+2.0, Pt (non-polar) = $0.006429x^2$-0.1957x+2.3]

DISCUSSION

Ocimum viride is an aromatic perennial herb with ethnopharmacological properties such as being used as remedy for cold and catarrh, stomach pain, diarrhoea and piles. Additionally, it has antipyretic, diuretic, laxative and hepatoprotective properties[17]. In this study, the insecticidal and mosquito repellent properties of oil and non-polar extracts of *O. viride* leaves were investigated. The cage test experiments with the test material yielded no mosquitocidal but repellent effect. The non-polar and oil extracts may therefore contain active ingredients which could possibly alter the physiology of *Aedes aegyptii* and subsequently knocking them down but not out. Similar results in which acetone and hexane fractions of *Clausena anisata* leaf extract exhibited repellent but no adulticidal activity against *A. aegyptii* was reported by Mukandiwa *et al.*[20]. *Ocimum canun* leaf extracts (acetone, hexane and chloroform fractions) rather exhibited adulticidal quality against *A. aegyptii*. Differences in results is attributable to amount of active compounds, geographical location in which the plant grew and/or seasons[17,21] the leaves were collected. Also, burning exhausts the amount of active components quicker than allowing the oil or extract to vapourize on its own. The method of burning the extract or oil was chosen because it reflects the mode in which local communities use these repellents.

Oil from *O. viride* leaf extract recorded a significantly higher mean percentage flying inhibition (FI) and longer protective times (PT) than the non-polar extract. This observation concord with findings of Oshaghi *et al.*[22] which reported significantly higher repellent effect of essential oils from *Citrus limon* (Rutaceae) and *Melissa officinales* (Labiatae) against *Anopheles stephensi*. Non-polar extract is made up of oil and other non-polar compounds. Therefore, it could be possible that the oil contains higher amount of active ingredient responsible for the mosquito repellent effect than the non-polar extract. The active ingredients present in *Ocimum* essential oils which could be responsible for mosquito repellent or knock off effect are thymol and eugenol[23].

A regression equation was modelled to predict the protective time (min) that a 1% concentration of oil and non-polar extracts could offer (Fig. 1). About 1% concentration of oil and non-polar extracts could offer 2.44 min [Pt (oil) = 0.4400x+2.0] and 2.11 min [Pt (non-polar) = $0.006429x^2$-0.1957x+2.3] protective time, respectively. Thus, with higher concentration of 100%, 46 min protective time could be offered for oil extract whilst 100% concentration of non-polar extract could offer approximately 47 min protective time. These times could be enough for any individual using this extract to rid the room of these mosquitoes either by sweeping or physically killing them.

The phytochemical constituents of *Ocimum viride* is unknown, however, phytochemical screening of crude extract of *Ocimum urticifolium* leaf revealed the presence of tannins, glycosides, saponins, flavonoids, steroids, terpenoids and phenols[24]. Analysis of oil from *Ocimum urticifolium* showed a total of 22 components, with the abundance of monoterpene and sesquiterpenes[24] to be 98.99%. The mechanism of action of the hypothetical active ingredient(s) within the oil and non-polar extracts of *Ocimum viride* is/are also not established. However, essential oils of *Ocimum kilmanda scharicum* block electrophysiological responses of olfactory sensory neurons to attractive odours in *Anopheles gambiae*[25]. Other possible mechanisms include illiciting lethargic response[26] such as serving as a neurotoxin and inhibiting growth regulators of processes such as chitin synthesis[27], normal growth and endocrine system[28]. *Aedes aegyptii* may have detoxified, sequestered or excreted[29,30] by activation of cytochrome p450 monooxygenases, esterases and enzyme-catalyzed conjugation of metabolites into more soluble and excretable forms[30,31]. It is predicted the mosquitoes thus resumed their flight activity when the intoxicant has been fully metabolized and rid off their body, the duration of which determines the protective time for the repellent, be it, oil or non-polar extract.

CONCLUSION AND RECOMMENDATION

Oil and non-polar leaf extracts of *O. viride* exhibited repellence but not insecticidal property against *Aedes aegyptii.* The oil recorded a lower minimum inhibitory concentration and a higher protective time than non-polar extract. Thus, oil from *O. viride* better reduce *Aedes aegyptii* human contact than non-polar extract. Toxicological effects of oil on vital organs such as heart, lung and lung be investigated to ascertain the effective dose which will be minimally toxic. Studies need to be conducted on the exact mechanisms of action the oil employs to knockdown *Aedes aegyptii* .

SIGNIFICANCE STATEMENT

The study discovered that minimum inhibitory concentrations of oil and non-polar extract against *A. aegyptii* were 2.17 and 5.62%, respectively. About 1% concentration of oil and non-polar extract, when burnt, could offer 2.44 min and 2.11 min protective time, respectively. This information is useful for production of burning material such as mosquito coil with *Ocimum viride* as active ingredient.

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