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Screening of Locally Available Lemon Grass (*Cymbopogon citratus*) for Repellency Action against *Aedes aegypti*

Egbuche, C. M[*1]., Enyinnaya, J. O[1]., Nwankwo, E. N[1]. and Okonkwo, N. J[1].

[1]Department of Parasitology and Entomology, Nnamdi Azikiwe University Awka Anambra State, Nigeria.

*Email: cm.egbuche@unizik.edu.ng

ABSTRACT

In Nigeria, re-emergence of diseases caused by *Aedes* mosquito borne pathogens, and challenges associated with existing control tools occur. This necessitates an improved insecticide-based tool for use in Integrated Vector Management. So, repellent action of *Cymbopogon citratus* essential oil (EO) on *Aedes aegypti* (Linn.) was tested. The EO from *C. citratus* plant sourced from Awka, was extracted by hydro distillation, and the phytochemicals analyzed by Gas Chromatography-Mass Spectrometry. Repellent action of the EO on unfed *Ae. aegypti* was assessed using "WHO arm-in-cage" method. In triplicate, arms of human volunteers were treated with different concentrations (50.0, 25.0, 12.5, 6.25 and 3.125 % v/v) of the EO and submitted to 25-caged, unfed *Ae. aegypti* for 1 minute in every 30 minutes. β-cedrenes, kaempferol, and thujopsene dominated the 13 phytochemicals identified. The standard (Odomos®) and all EO concentrations recorded 100 % repellency on application. At 30 minutes, $EC_{99.9}$ value was25.0 %, concentrations ≥ 62.5 % v/v gave 100 % repellency ($p > 0.05$). At 60 minutes, $EC_{99.9}$ value was 30.68 %, concentrations ≥ 12.5 % v/v gave 100 % repellency ($p > 0.05$). At 90 to 120 minutes, 50 % concentration maintained 100 % repellency ($p < 0.05$), $EC_{99.9}$ value was 48.79 % at 120 minutes. Recording 120 minutes protection time with no adverse effect, 50 % concentration showed equal potency as Odomos®. Research to isolate the active ingredients in the EO for incorporation into beneficial health and economic products is desirable.

Key words: Repellency, *Cymbopogon citratus*, *Aedes aegypti*, Awka, Anambra State

INRODUCTION

As vectors of disease pathogens that cause many fatalities worldwide, mosquitoes are of great public health importance (El-Sheikh *et al*., 2016). Among them, the Genera *Aedes* are well known for transmission of pathogens that cause dengue fever, yellow fever, Chikungunya fever, Zika virus disease and Japanese encephalitis (Renugadevi *et*

al., 2012). The global incidence of dengue has grown dramatically with about half of the world's population now at risk (WHO, 2022). Dengue fever is known to occur in Nigeria with increased risk of transmission (Omatola *et al.,* 2020). The magnitude of this disease is unclear as it is often mistaken for malaria or other febrile illnesses and secondly, vast majority of cases are asymptomatic or mild and self-managed (WHO, 2022). Between the years 2017 to 2018, 163 Yellow Fever cases were confirmed in 17 States in Nigeria, with Anambra State recording one case; also in May 2019, three new confirmed cases from Institute Pasteur (IP) Dakar were received from Onitsha North Local Government Area in Anambra State (NCDC, 2019). Chikunguya appears to be a neglected disease in Nigeria as a systematic review of eligible articles conducted by Abdullahi, *et al.* (2020) showed serological evidence of Chikunguya Virus (CHIKV) infection in some zones of Nigeria.

The abundance of *Aedes* species that are known vectors of disease-causing pathogens has been reported in Anambra State of Nigeria (Egbuche *et* al., 2016; Egbuche *et al.,* 2021). *Aedes* mosquitoes bite indoors and outdoors, thereby defying efforts in the use of Long-Lasting Insecticide Nets (LLINs) for their control. There is therefore needed to intensify efforts towards achieving elimination of mosquito borne diseases in Nigeria. The use of repellents for personal protection against mosquito bite is accepted as part of integrated mosquito-borne disease control program (Amer and Mehlhorn, 2006). This is important because certain activities can keep people outdoors especially at night in Nigeria (Ezihe *et al.,* 2020). In use include crude plant extracts, essential oils (EOs) and isolated compounds. Researchers have validated some plants such as lemon grass (*Cymbopogon Citratus)*, scent leaf (*Ocimum gratissimum*), neem tree (*Azadiractha indica*), cloves (*Syzygium aromaticum)*, peels of citrus fruits (*Citrus* species), bush tea (*Aspalathus linearis*, thyme (*Thymus vulgaris*), and eucalyptus (*Eucalyptus* species) as having repellency activities against mosquitoes (Adeniran and Fabiyi, 2012; Esimone *et al*., 2011; Maia and Moore, 2011; Naseem *et al.,* 2016; Ranasinghe *et al*., 2016). Research has also shown that some of these plants show no adverse effect on humans and can be used to treat skin diseases and other conditions(Adeniran and Fabiyi, 2012; Keziah *et al*., 2015; Moghimipour *et al*., 2009).Nevertheless, new and/or improved insecticide based tools to be developed for the elimination of mosquito borne diseases needs to focus on the susceptibility of local vectors to such insecticides (WHO, 2020). Therefore, the present study screened locally available lemon grass (*Cymbopogon citratus*) for repellency activities against *Aedes* mosquitoes found in Awka, southeast Nigeria.

MATERIALS AND METHODS

Experimental Design

This was a longitudinal study laid out in a Completely Randomized Design (CRD) to determine the repellent effect of the Essential oil (EO) of *Cymbopogon citratus*, and its immediate adverse effects on humans. In the study, five differing concentrations of the EO were prepared and used as treatment; each replicated thrice. A standard / positive control (Odomos®, containing 12 % DEET) and negative control (Only 70 % Ethanol applied) were equally used. Percentage repellency, Effective Concentration ($EC_{99.9}$), protection time and the adverse skin reactions of the essential oils were all determined in this study. The independent variables were the different concentrations of the essential oils,

while the dependent variables were the percentage repellency and protection time.

Recruitment of human volunteers for the repellency bioassay

Fourteen (14) volunteers for the study were students of Nnamdi Azikiwe University, Awka, Anambra State. Five (5) volunteers were assigned to the treatment groups (i.e., one to each of the five EO concentrations), one (1) to the negative control, one (1) to positive control, and seven (7) to observer group (i.e., an observer to each set-up for record taking).

Inclusion criteria: Literate students:

i. who exhibited mild or no sensitivity to mosquito bites?
ii. of both genders aged between 18 and 40 years
iii. whose attractiveness to the target species was verified before repellency test.
iv. willing to submit to brief query about personal condition.
v. willing to refrain from use of perfumed products, alcoholic beverages or smoking after 9 pm the evening preceding the efficacy test and throughout that test,
vi. able to withstand biting insects on skin or otherwise effectively monitor and remove biting insects that contact skin.

Exclusion criteria: Any students known to be:

i. hypersensitive to mosquito bites.
ii. allergic to insect repellents or common cosmetics.
iii. sensitive to any of the test material ingredients.
iv. in poor physical condition.
v. in contact with insect repellent a day prior to the efficacy test.
vi. pregnant or lactating.

Ethical Approval and Informed Consent

Ethical for the study was obtained from Ethics Review Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka; Anambra (Ref.: COOUTH/CMAC/ETH.C/Vol.1/FM:04/234). All volunteers were informed about the objectives, procedures, potential risks and benefits of the study. Before the participants were selected for the study, they were given a questionnaire (containing information for determining inclusion or exclusion in the study) to complete; and a written informed consent was also collected. Volunteers were also assured that all the information obtained from them shall be kept confidential.

Collection and Identification of the plant materials

The fresh leaves of *Cympopogon citratus* (Lemon grass) were harvested in Science Village, within Nnamdi Azikiwe University premises Awka Anambra State. The plant was authenticated by a Botanist in the Department of Botany, Nnamdi Azikiwe University. The voucher sample was deposited in the Herbarium of the Botany Department as NAUH-187^{A}.

Collection, rearing and identification of *Aedes aegypti* mosquitoes for Repellency test.

In collaboration with the National Arbovirus and Vector Research Center (NAVRC) Enugu, Eggs of *Aedes* mosquitoes were collected and reared in the laboratory unit of the Department of Parasitology and Entomology; Nnamdi Azikiwe University, Awka, following WHO (2012) method. The *Aedes* mosquitoes were specifically identified as *Aedes aegypti* using the identification guide by Gillett (1972) and the colony maintained at 32±4^{o}C, 83±3% RH,

with a photo period of 12:12 hour (Light: Dark).

Preparation and Extraction of Essential Oil from *Cympopogon citratus*

The fresh plant materials were cut into pieces using a knife, and then the extraction procedure was carried out in accordance with Elyemni *et al*. (2019). The essential oils from the plant materials were extracted by hydro distillation using a Clevenger-type apparatus for 1 hour. Under optimal operating conditions, a quantity of 100 g of each sample was added to 200 ml of distilled water in a 500 ml flask. Then, the flask was placed on a balloon heater for 1 hour. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential oil was collected, dried under a glass desiccators using silica gel as desiccant, and stored in amber bottles to avoid photo-oxidation until used for analysis.

Phytochemical Analyses of the Essential Oil from *Cympopogon citratus*

This was carried out at Springboard Research Laboratory Awka, Anambra State. The chemical composition of the essential oil and their molecular weights were determined by Gas Chromatography coupled with Mass Spectrometry (GC/MS) following Elyemni *et al*. (2019).

Preparation of Different Concentrations of the EO from *Cympopogon citratus*

The essential oils of *Cymbopogon citratus* and *Ocimum gratissimum* were regarded as 100% concentration. Then, five (5) different concentrations viz., 50% v/v, 25% v/v, 12.5% v/v, 6.25% v/v and 3.125% v/v were constituted using 70 % ethanol as the diluent. The density of the plant essential oil (1.04 g/ml) was determined thus: $Density\ of\ EO = \frac{W1}{W2}$, where W1 (0.48 g) and W2 (0.46 g) are weights of equal volume (1 ml) of EO and water respectively in an empty EDTA bottle weighing 2.02 g. With that, the volume of essential oil to be used in preparing the different concentrations was also determined. A negative control was set using ethanol without any trace of essential oil. Odomos® (12% DEET, Batch No: BSL 1812, Mfd: December 2021, Expiry Date: November 2026, NAFDAC Registration No.: A5-0710, Mfg. Lic No.: NH/176), a conventional mosquito repellent was procurred from Blessed Fex Scientific Company, Bridge Head Market Onitsha, Anambra State, Nigeria, and used as the positive control/standard.

Preparation of the adult female *Aedes* Mosquitoes for Repellency Test

The adult female mosquitoes (n = 25), 5–7day old, free from pathogens (since they were reared in the laboratory), were placed in screened cages (30 cm × 30 cm × 30 cm), with a mosquito netting on top and two sides and a cotton stockinet sleeve on one side used to introduce and retrieve mosquitoes using a mouth aspirator. The mosquitoes were fed on 10% sugar solution and starved for 8-24 hours prior to the experiments.

Preparation of Human Volunteers for Repellency Test

Seven human volunteers were used in the experiments while a single observer was assigned to each set up for independent record taking. The test subjects all wore long rubber gloves that covered their forearms, after washing their forearms with odourless soap. An exposure area (25 cm^2) was cut open on rubber disposable hand gloves. The cut edges were lined with masking tape (Keziah *et al*., 2015).

Bioassay for determination of percentage repellency, protection time, and adverse skin reaction from the EO

Repellent effects of the EO were evaluated at concentrations of 50% v/v, 25% v/v, 12.5%, 6.25% v/v and 3.125% v/v using the WHO arm-in-cage method based on WHO protocol WHO (2009) with modifications as used by (Keziah *et al*., 2015). *Aedes aegypti* mosquitoes bite during the day; hence the experiment was carried out between 1:00pm and 4:00pm Nigerian time. Before each test, the arms of the human volunteers were washed with odourless soap and thoroughly rinsed with distilled water and allowed to dry for 10 min before the application of the EO. Both arms of the seven human volunteers were covered with rubber glove and a window area of 5 cm x 5 cm (25 cm^2) was opened on the dorsal part of the hand of the volunteers as the exposure area. Each test repellent formulation and 70 % ethanol (100µl) were applied to the marked area of one hand of each volunteer while Odomos® (12% DEET); a conventional mosquito repellent cream of ~10 $mg/cm^2$was applied as the positive control/standard.

Before the start of each exposure period, the bare hand of the test volunteers, used as control for each volunteer, was exposed for up to 1 minute in a mosquito cage (30 cm × 30 cm × 30 cm) containing 25 unfed, 5-7 days old, pathogen female mosquitoes as used by Costa *et al*. (2017). If at least two mosquitoes land and/or probe the arm, the repellency test was then carried out (Younoussa *et al*., 2016). This was done to ensure that the mosquitoes were host seeking. Then, each volunteer put the test hand into the mosquito cage for the first 1 min. The experiments were also conducted in triplicate in separate cages. This procedure was repeated at 30-minute interval, up to 120 minutes of exposure. The number of mosquito landing and/or probing was counted over 1 min in every 30 min interval. The percentage repellency was calculated by using the formula,

$Repellency\ (\%) = \frac{C-T}{C} \times 100$, Where C is the number of mosquitoes landing and/or probing on the control and T is the number of mosquitoes landing and/or probing on the treated areas of the volunteer (Tawatsin *et al*., 2001; Thavara *et al*., 2001).

The protection time was determined by looking at the pattern of mosquito bite at given intervals, being a modification of Younoussa *et al*. (2016) where protection time was estimated to be less than the value of the first interval with less than 100 % corrected repellency effect.

Determination of adverse effects of the EO on the human volunteers

Skin of each volunteer was observed daily for 3 weeks duration of the test for skin allergic reactions, irritation, skin sensitization, skin keratinization, and spots appearance, which could be caused by application of the EO.

Data Analysis

The data from this study was summarized in Tables. Percentage repellency was then used to compare the performance of different concentrations of the EO at a given time interval, using One-Way ANOVA at P=0.05. A Student-Newman-Keuls (SNK) test was applied for mean separation. The protection time was summarized using descriptive statistics. The statistical package used was SPSS version 25.0.

RESULTS

Phytochemical compounds in *Cymbopogon citratus* Essential oil

Qualitative and quantitative phytochemical screening of *Cymbopogon citratus* (Lemon Grass) essential oil revealed that thirteen

compounds were present namely: thujopsene, alpha-pinene, camphor, beta-pinene, pentane, lunamarin, alpha-cedrenes, beta-cedrenes, kaemferol, alpha-cedrol, gamma-terpinene, genaniol, and terpinolene. Beta-cedrenes (15.9824 ppm) was the most abundant, followed by kaemferol (3.7223 ppm) and thujopsene (3.2519 ppm), with lunamarin (0.0000 ppm) and gamma-terpiene (0.0000 ppm) as the least abundant (Table 1).

Table 1: Compounds (ppm) present in essential oils of *Cymbopogon citratus*

Component (ppm) → ↓	**Retention**	**Area**	**Height**	**External**
Thujopsene	0.123	3359.9492	360.197	3.2519
Alpha-pinene (α-pinene)	2.700	2436.2185	191.759	1.3562
Camphor	5.793	5819.4134	456.746	1.8211
Beta-pineane (β-pineane)	8.436	4721.4984	371.183	0.1041
Pentane	10.793	5439.4994	375.479	0.2389
Lunamarin	12.450	6916.0478	372.761	0.0000
Alpha-cedrenes (α-cedrenes)	29.116	5616.7994	440.911	0.0174
Beta-cedrenes (β-cedrenes)	19.756	3930.5748	309.144	15.9824
Kaempferol	21.553	5025.1523	394.848	3.7223
Alpha-cedrol (α-cedrol)	24.580	4953.9757	389.494	0.5190
Gamma-terpiene (γ-terpiene)	31.363	4199.9884	329.743	0.0000
Genaniol	39.900	2206.4580	173.478	0.0279
Terpinolene	41.836	3159.5580	248.559	0.1387
Total		**57785.1333**		**27.1799**

Repellency and Effective Concentration (EC) of *Cymbopogon citratus* EO against adult female *Aedes aegypti*

Immediately after application of different concentrations of the EO and the standard, 100 % repellency was observed as no mosquito was found landing on the participants except in the negative control (Table 2). At 30 minutes of application, $EC_{99.9}$ value was 25.0 % and 0.33±0.33 mosquitoes were observed to land only on the participants that applied 3.125 % of the EO, resulting in 90.09 % repellency. However, there was no significant difference ($p = 0.452$) in the repellency of 3.125 % concentration of the EO, when compared with the effects of other concentrations and the standard/ Positive control (Table 2). At 60 minutes of application, $EC_{99.9}$ value was 30.68 % and0.67±0.67 (94.97 % repellency) mosquitoes landed on the participants that applied 3.125 % of the EO and 0.33±0.33 (97.52% repellency) mosquitoes on the participants that applied 6.25 % of the EO. However, the difference on the number of landing mosquitoes observed at 60 minutes was not significant ($p = 0.552$). At 90 minutes, an aberrant $EC_{99.9}$ value of 118817.39 % was obtained considering that only the least concentration (3.125 %) and the highest concentration (50.0 %) used recorded no landing mosquito, just as the

standard (Odomos®), though the difference when compared with other concentrations used was not significant (p = 0.081). At 120 minutes, $EC_{99.9}$ value was 48.79 % and only 25.0 % and 50.0 % concentrations recorded no landing mosquito, just as the standard (Odomos®). There was a significant difference when compared with other concentrations used (p = 0.015).

Table 2: Repellency and Protection time of *Cymbopogon citratus* EOs against *Aedes aegypti*

Treatment↓		Time (Minutes)→ Concentration↓	0	30	60	90	120
			Numbers (Mean±SE) of mosquito landing/probing				
Essential oils of *C. citratus*	1.	3.125 (% v/v)	[a]0.00±0.00	[a]0.33±0.33	[a]0.67±0.67	[a]0.00±0.00	[a]1.00±0.58
	2.	6.25 (% v/v)	[a]0.00±0.00	[a]0.00±0.00	[a]0.33±0.33	[a]2.00±1.00	[b]3.00±1.00
	3.	12.5 (% v/v)	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.33±0.33	[a]0.67±0.33
	4.	25.0 (% v/v)	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.33±0.33	[a]0.00±0.00
	5.	50.0 (% v/v)	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00
Positive Control	6.	12% DEET	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00	[a]0.00±0.00
Negative Control	7.	70% ethanol	0.67±0.33	3.33±0.33	13.33±1.3	13.00±1.1	13.00±2.0
			Repellency (%) of *C. citratus* EOs against mosquitoes				
Essential oils of *C. citratus*	1.	3.125 (% v/v)	100.00	90.09	94.97	100.00	92.31
	2.	6.25 (% v/v)	100.00	100.00	97.52	84.62	76.92
	3.	12.5 (% v/v)	100.00	100.00	100.00	97.46	94.85
	4.	25.0 (% v/v)	100.00	100.00	100.00	97.46	100.00
	5.	50.0 (% v/v)	100.00	100.00	100.00	100.00	100.00
Positive control	6.	12.0% DEET	100.00	100.00	100.00	100.00	100.00
Negative Control	7.	70.0% ethanol	97.32	86.68	46.68	48.00	48.00
		P-value	-	0.452	0.552	0.081	0.015
		$EC_{99.9}$	-	25.0	30.68	118817.39	48.79

Values of number of mosquitoes landing are mean of three independent determinations. Columns sharing different superscripts are significantly different (p > 0.05) by one-way ANOVA.

Protection Time of *Cymbopogon citratus* EOs as Mosquito Repellents against *Ae. aegypti* bites

The protection time using different concentrations of the plant EO was estimated from Table 2. The first interval that had less than 100 % repellency effect with 3.125 % concentration (see circled value in Table 2) was 30 minutes, giving a protection time of < 30 minutes. The first interval that had less than 100 % repellency effect with 6.25 % concentration (see circled value in Table 2) was 60 minutes, giving a protection time of < 60 minutes. The protection time for 12.5 % and 25.0 % concentrations were the same, < 90 minutes since the first interval that had less than 100 % repellency effect with them (see circled values in Table 2) was 90 minutes. At the

end of the proper repellence experiments, the essential oil of *Cymbopogon citratus* showed total protection (100 % repellency) up to 120 min at 50 % concentration.

Adverse effect of *Cymbopogon citratus* EO on the skin of the test participants

No adverse effects on the exposed skin of test subjects were observed throughout the 3-weeks post-treatment period. However, only a mild hot and itching sensation which quickly subsided was recorded from some of the test subjects after the application of the repellent oils (Table 3).

Table 3: Immediate effects of *Cymbopogon citratus* EOs on test participants

Skin Reaction	Test volunteers	Number with visible skin reactions
Quickly subsiding mild-Hot sensation	5	2
Quickly subsiding slight Itching	5	1
Spot appearance	5	0
Skin keratinization	5	0
Skin sensitization	5	0

Numbers of test volunteers was based on the number that used the test repellent.

DISCUSSION

This work has validated the potency of the essential oil of *Cymbopogon citratus* as a plant-based repellent for the control of *Aedes aegypti*. Phytochemical screening showed that essential oil of *Cymbopogon citratus* from Awka contained at least one of the major phytochemicals (bioactive) constituents, such as beta-cedrenes, thujopsene, and alpha-pinene (Table 1). However, there are clear variations between the present findings and the chemical composition of the essential oils of *Cymbopogon citratus* reported in earlier studies. Solórzano-Santos and Miranda-Novales, (2012) identified citral ($C_{10}H_{16}O$) as the major volatile constituent while the present revealed that β-cedrenes, kaemferol, and thujopsene, were the most abundant compounds, with a sizeable quantity of camphor and α-pinene. According to Ogede and Abdulrahman (2022), citral, mycrene, and thujene were the three main substances found in the *Cymbopogon citratus* essential oil that was harvested in Nigeria. Additional compounds such as geranial, neral, β-myrcene, citronellene, limonene oxide, geraniol (7.3%) and linalool were the major components found in the leaf of *C. citratus* grown in Kaduna, North Central Nigeria, while α-pinene was detected in small quantity (Olayemi, 2017). The present study is consistent with the findings of Olayemi (2017) that a sizable amount of α-pinene was present in EO of *C. citratus*. The presence of monoterpenes (such as camphor and α-pinene) and sesquiterpenes (such as β-cedrenes and thujopsene) reported in the present study corroborates the findings of other researchers (Gnankiné and Bassolé, 2017; Jaenson *et al*., 2006; Nerio *et al*., 2010; Park *et al*., 2005) that the repellent properties of several EOs frequently appear to be associated to the presence of monoterpenes, monoterpenoids, and sesquiterpenes.

Pinto *et al*. (2015) has shown that plant genotype, soil, geographical and agronomic conditions, especially soil, may all have a role in the quantity and chemical composition range of EO of plants of the same species in different regions. Kumar *et*

al. (2011) was of the same view and emphasized that the primary elements in the EO typically remain the same with mere changes in their concentration levels. The present study has revealed that *C. citratus* locally grown in Awka Nigeria possess some of the primary elements in the EO but with different concentration levels in line with the observations of Pinto *et al*. (2015) and Kumar *et al*. (2011). The EO in the present study may also contain some secondary metabolites whose active ingredients have potential insecticidal properties and could be viable substances for the production of novel mosquitoes and other insect repellants. In fact, the 100% repellency in 60 minutes of application with EO concentrations ≥12.5%, with at most $EC_{99.9}$ value of 48.79%, as well as 100% repellency at 50% concentration for up to 120 minutes are pointers to repellent property of *C. citratus* EO that was comparable to those of positive control Odomos® (Table 2).

Furthermore, the significant repellency activity shown by different concentrations of EO against *Ae. Aegypti* could be attributed to the presence of β-cedrenes, kaemferol, and thujopsene which were most abundant in the essential oil of *C. citratus.* Camphor, a known *Ae. aegypti* repellent (Hwang *et al*., 1985), was also expected to be partly responsible for the mosquito-repellent activities observed in the present study. High repellent activity of *C. citratus* essential oil against *Ae. aegypti* at concentrations ≥12.5% agrees with earlier reports by Phasomkusolsil and Soonwera (2010), and Phasomkusolsil and Soonwera (2011) though Soonwera and Phasomkusolsil (2014) later described a lower repellent activity of *C. citratus* essential oil against the *Ae. aegypti* mosquito.

Because of the highly volatile nature of EOs, it has been suggested that the improvement on protection time of 120 minutes observed with *C. citratus* EO at 50% concentration, can be achieved using a base or fixative substance, such as vanillin, petroleum jelly, mustard, or coconut oils (Das *et al*., 2003; Keziah *et al*., 2015; Tawatsin *et al*., 2001). However, the effectiveness and longevity of repellent products could dependent on a number of variables, like the type of repellent (active ingredients and formulation), the mode of application, environmental factors (temperature, humidity, and wind), the attractiveness of particular people to insects, loss due to removal by perspiration and abrasion, the sensitivity of the insects to repellent, and insect biting density (Ahmad *et al*., 2011; Govindarajan, 2011; Singha and Chandra, 2011). Additionally, the outcomes of tests can be impacted by variations in the tested mosquito species, such as their age, body size, and adult density in test cages (Barnard *et al*., 1998).

That no adverse effects were noticed in the tested human participants three weeks following the application of the EO (Table 3) have somehow established confidence and safety in the application of the EO from *C. citratus* as insect repellants. Further research is therefore needed to isolate the active constituents from the essential oil so that their effectiveness as mosquito repellents could be thoroughly evaluated and incorporated into potential products with health and economic benefits.

CONCLUSION

Control of mosquitoes using locally available plant material is promising with *C. citratus*. Regardless that mosquito bites indiscriminately; the overall pattern of repellency recorded with different concentrations of the EO applied was

uniform; and interestingly 50 % concentration of the EO recorded 100 % repellency up to 120 minutes.

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Conflict of interest
We declare no conflict of interest**.**

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