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Natural Anophelin mosquito repellents and phytochemical analysis of ethanol and hexane leaf extracts from four plant species

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Abstract

Currently mosquitoes control is the cornerstone to minimize the rising number of mosquito borne diseases. There is an urgent need looking for alternatives to the current reliance on synthetic insecticides for the vectors control. Application of active toxic agents from plant extracts as an alternative control strategy was available from ancient times. These are non-toxic, easily available at affordable prices, biodegradable and show broad-spectrum target-specific activities against different species of vectors. Natural products with repellency properties are urgently needed. An insect repellent works by masking human scent, and a number of natural and synthetic mosquito repellents were studied. The main aim of this study was to identify the phytochemicals and compare their potentials as mosquito repellents from the ethanol (Et. OH) and hexane (hex) leaf extracts (LEs) of *Ocimum basilicum*, *Coleus forskohlii*, *Eucalyptus camaldulensis* and *Cymbopogon flexuosus* under laboratory conditions at 50 and 100% concentrations. Phytochemical analysis showed that alkaloids, saponins, flavonoids, terpenes, tannins and terpenoids were present/or absent in the 4 plant-LEs. At 50% concentration of Et. OH extracts, *C. forskohlii* exhibited higher repellency potential on *Anopheles gambiae* with protection time of 137.3 min, while *O. basilicum*, *E. camaldulensis* and *Cy. flexuosus* registered protection time of 30.6, 15.3 and 19 min, respectively. At 100% concentration of ethanol-extracts, *C. forskohlii* caused the highest protection time against the vector with protection time of 182 min. *O. basilicum, E. camaldulensis* and *Cy. flexuosus* were equally less potent against the insect, with protection time 42.6, 32.6 and 28 min, respectively. Regarding hex-extract, at 50% concentration of *C. forskohlii,* the highest repellency potential , with protection time of 174 was registered, while *O. basilicum*, *E. camaldulensis* and *Cy. flexuosus* registered protection times of 44, 18 and 28.6 min, respectively. However, the 100% concentration of Et. OH-extracts of, *C. forskohlii* and *O. basilicum* exhibited the highest protection time, with protection times of 228 and 116min, respectively. *E. camaldulensis* and *Cy. flexuosus* were less potent, with protection times 28.6 and 54.6 min, respectively. The EOs of *C. forskohlii* and *O. basilicum* L EOs proved to have potentials as repellency agents against *Anopheles*. GC-MS analysis of the extracts identified the compounds of both solvents extracts and the terpens were the major compounds. According to the results, further studies are required, especially on the extracts of basil and coleus since they proved to be effective.

Keywords: Mosquito repellent, Anopheles, Essential oils, Sweet Basil, Wild Thyme, Kafure, Lemon Grass

Introduction

Soonwera and Phasomkusolsil [1] stated that mosquitoes are pestiferous insects to public health; transmit several deadly dangerous diseases, *e.g.* dengue (DF), filariasis, malaria, yellow fever (YF) and Japanese encephalitis (JE). Several researchers reported that every year at least 500 million people in the world suffer from one of the tropical diseases that include DF, malaria and filariasis [2, 3].

Salih *et al*., [4] reported mosquitoes are among the most disturbing blood -sucking insects afflicting human beings. The mosquito female's behavior, involving feeding on human blood, is responsible for the transmission of a number of diseases. One to two million deaths is reported annually, due to malaria worldwide.

Pest/vector management is facing economic and ecological challenges worldwide, due to human and environmental hazards caused by majority of the synthetic pesticides. Identification of novel effective insecticidal compounds is essential to combat the increasing resistance rates. Botanical pesticides have long been touted as attractive alternatives to synthetic pesticides for pest/vector management, because botanicals reputedly pose little threat to the environment or to human health. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests/vectors continues to expand, yet only a handful of botanicals are currently used in agriculture, public health, industrialized world, and there are few prospects for commercial development of new botanical products.

The medical vectors control is considered the most important corner-stone to prevent the diseases; however, the control is either by the synthetic pesticides or the repellents that prevent the biting of the mosquitoes. Personal repellents are substances applied to skin, clothing, or other surfaces to repel or discourage insects and other arthropods, *e.g*. ticks, from feeding on humans. Repellents help people to avoid bites from mosquitoes, and other biting arthropods that may transmit diseases, and allow people to engage freely in outdoor activities. Insect repellents may also be used to exclude insects from an area [5].

With increasing public concern regarding pesticide safety and problems of insecticide resistance, new safer active ingredients are becoming necessary to replace the existing compounds. Furthermore, the use of repellents in an integrated pest/vector management (IPM/IVM) program has been ignored to a large extent [5]. Salih *et al*., [4] reported that the personal protection from mosquito bites is currently the most important measures to control mosquito transmitted diseases. The mosquito repellent products commonly available on the market contain N, N-diethyl- 3-toluamide (DEET) as the active ingredient. The synthetic repellents are not safe for human, especially children, because they may cause skin irritation, hot sensation rashes or allergy [6].

Recently, as results of the public health concern on the safety of many synthetic products, many research scientists renewed the interest on the use of natural products from plant origin for mosquito's management [1]. In addition, plant essential oils (EOs), in general, have been recognized as an important natural resource of insect repellents. The plant EOs base repellents according to Soonwera and Phasomkusolsil [1] are effective for mosquito control, and are environment friendly.

Vector borne disease such as malaria is a major global health problem; half of the world's populations, mostly the poorest countries living in the tropical world's are at risk of malaria. The disease caused by *Plasmodium* parasites transmitted by infected female Anopheles mosquitoes bit is serious problem in Africa, where one in every five (20%) childhood deaths is due to malaria. Most malaria cases and deaths are in sub-Saharan Africa Most malaria cases and deaths are in sub-Saharan Africa [7].

Therefore, herbal insect repellents gained importance recently; detailed knowledge concerning them is still lacking. As a result, it is high time to launch extensive search to explore eco-friendly biological/botanical materials for controlling pests. Curtis [8] Choochote *et al.*, [9] reported that EOs have been investigated and described as potentially natural sources of insect repellents. The effective repellency against female *Anopheles* results varied with different species of the plant and the concentration/dose. According to Trongtokit *et al*., [10] study on *An. stephensi* in Brazil using *Cymbopogon nardus* citronellal 40% EOs was applied topically, reported 100% protection for 7-8 hr. The present study investigated the potential of sweet basil (*Ocimum basilicum* L), wild thyme (*Coleus forskohlii)*, Kafure (*Eucalyptus camaldulensis)* and, lemon grass (*Cymbopogon flexuosus)*, LEs as mosquitoes repellents.

Curtis [8] stated in many parts of the world, plant derived natural products have traditionally been used as repellents against insects bites. Particularly essential oils have been investigated and described as potentially natural sources of insect repellents it is time to search for effective alternatives to synthetic repellents as reported by Choochote *et al.,* [9]. Many research studies were conducted on various medicinal plants and the effective repellency against female anopheles mosquitoes, the result is varying according to the plant and the concentration. The present study investigates to figure out the effectiveness of *Ocimum basilicum* L., *Coleus forskohlii*, *Eucalyptus camaldulensis* and, *Cymbopogon flexuosus Steud*, (leafs) extract as mosquitoes repellent.

Materials and Methods

Collection of plant material and extraction

Fresh leaves of the four above-mentioned plants were collected from Gezira State, Sudan. The collected leaves were washed, shade-dried and powdered. The powder was extracted in ethanol (Et. OH) and hexane (hex) by soaking in Et. OH, and clevenger apparatus was used for preparation of hex- extract; 50 g of each plant leaves powder (LP) was extracted separately in conical flasks by Et. OH. Analytical grade Et. OH was added until the LP was fully submerged, thoroughly stirred to ensure proper mixing, and subjected to shaking at regular intervals to allow percolation for 4 days. On the 5th day, the extracts were filtered using Whatman No.1 filter paper into another conical flask and the Et. OH was evaporated. The resultant viscous substance was dried and stored in amber-colored vials in a refrigerator at 4^{o}C pending formulation. For hex-extracts the EO was isolated by steam-distillation, using a clevenger apparatus for 6 hr; moisture in the oil was removed by anhydrous sodium sulfate and stored at 5^{o}C for further use.

Formulation of plant leaves extracts

The plant leaves extracts to be tested were formulated in pure fragrance-free Vaseline jelly. The formulations tested were made into two concentrations of 50% and 100%. To make 50% of the formulation, 5g of the pure Vaseline jelly was weighed and transferred to a clean 100 ml beaker, placed in a water bath at 80°C, stirred with a rod up to the melting point. Five g of each of the 8 extracts was then added to the melted jelly, followed by continuous stirring to be completely mixed with the jelly. The resultant 8 formulations of eight plant extracts were stored at 4°C for the bioassay. To make 100% of the formulation, 1 g of pure Vaseline jelly was melted as above; 10 g of each of the 8 extracts were added and treated as above. The two concentrations (50 and 100%) were used in bioassay.

Anopheline mosquito

The *Anopheles gambiae* (3-7 days old) was obtained from Blue Nile National Institute for Communicable Disease (BNNICD) insectary, University of Gezira, colony maintained at 12: 12 LD photoperiod, 70±10% R.H. and 24±2°C. The adult females of Anopheles used for the bioassay before any blood meal. Females were starved for 24 hr and provided with only 10% sugar solution in water. Tests were conducted in triplicate 25 individual of starved adult mosquitoes rearing cage was used and topical treated arm with plant extract exposed to the mosquitoes in the cage to observe the mosquitoes bite.

Bioassay test

The reared mosquitoes were taken from the insectary of Blue Nile Institute for Communicable Disease (BNICD) (24±2C° and 80% RH) and the experiment was conducted in the insectary lab of BNICD, the arm cage method was used. Three gram of formulated plant leaves extract was applied onto the forearm of a human subject. The treated arm was exposed until the first bite to 25 female mosquitoes, in a (252x3.14x60 cm3) cage, and any mosquitoes landing and biting were counted. After any bite the time was recorded at which the first bites occurred the bioassay terminate and repeated for the other 50 mosquitoes to make triplicate at successive time intervals. For the negative and positive control, the arm with Vaseline jelly and with DEET was used respectively. Numbers of mosquitoes landing and biting in a minute were recorded. The application of plant extract was used following Frances methods [11]. Two concentrations (50 and 100%) for each plant, ethanol and hexane extract and mixture of ethanol and hexane for each plant to account the number of mosquitoes land and the complete protection time (CPT) in the treated area (20-15cm2), with 3g formulated plant extract.

Phytochemical Analysis

The phytochemical analysis was carried out at chemistry lab, Faculty of Agricultural Sciences, University of Gezira by using the method of Balbaa [12] and Harborne [13] to investigate the presence of the saponins, tannins, sterols, triterpens, flavonoids, terpenoids and alkaloids.

Gas chromatographymass spectrometry

The qualitative and quantitative analysis of the sample was carried out by using Gas Chromatography-Mass Spectrometer (GC/MS-QP2010-ultra) from japans Simadzu company, with the serial number 020525101565SA and capillary column (Rtx-5ms-30m[2E3?]0.25mn[2E3?]0.25µm).The sample was injected by using split mode, Helium as A carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10°C/min to 300°C as a final temperature degree with 5minutes hold time, the injection port temperature was 300°C as the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 29 minutes. Identification of the components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the national institute of the standards and technology (NIST).

Data Analysis

For each plant species, the number of mosquitoes landed on the arm was counted at different time intervals and the Percentage Repellency (PR) per each plants leaf extract/solvent/concentration was determined using the following equation (1) according to Teklani and Perera [14] as follows:

$$PR = \frac{NIU - NIT}{25\ (IS)} 100 \quad (1)$$

Where:

- PR=Percentage repellency.
- NIU=Number of individual in the untreated group.
- NIT=Number of individual in the treated group.
- IS=Number of insects used.

At least triplicates were carried out for all the plants leaves extracts for selected extracts concentration with significant mosquito repellent activity.

Results

Phytochemical analysis

Phytochemical analysis of ethanolic and hexane extracts of the four plants leaves were carried out to identify the presence of Alkaloid, Tannin, Saponin, Terpenoids, Flavonoids and terpens. In this study four plant species (Basil, Wild Thyme, Kafure and Lemon Grass) belonging to different families were collected. Most of these plants were reported to treat a variety of diseases in traditional medicine system.

Ethanol Extracts

The preliminary phytochemical screening had shown the presence of Alkaloid, Tannin, Flavonoids, Saponin and Terpens in the leaves extracts the summary of the results are presented in **Table 1**. The Alkaloid, Tannins, saponin and Terpens were detected in the Basil leaves extract; Alkaloid, Tannins, and Terpens in Wild Thyme leaves extract; Alkaloid, Tannins, Flavonoids and saponins in Kafure leaf extract and Alkaloid,Tannins, and saponins in Lemongrass,

Table 1. The phytochemical constituents (PC)of ethanol (E) and hexane (H) leaf extracts from the studied four plants.

PC	Oc		Col		Eu		Cy	
	E	H	E	H	E	H	E	H
Alkaloid	+	+	+	-	+	+	+	+
Tannins	+	+	+	-	+	+	+	+
Flavonoids	-	-	-	-	+	+	-	-
saponins	+	-	-	-	+	+	+	+
Terpens	+	-	+	+	-	-	-	-

Oc=Ocimum, Col=Coleus, Eu=Eucalyptus, Cy= Cymbopogon.

(+)= Presence, (-) = Absence

Hexane extract

The preliminary phytochemical screening had shown the presence of Alkaloid, Tannin, Flavonoids, Saponin and Terpens in the hexane leaves extracts the summary of the results are presented in **Table 1**. In Basil leaves extract Alkaloid, Tannins was only detected. While in Wild Thyme leaves extract terpens only was detected. In Kafure leaves extract Alkaloid, Tannins, Flavonoids and Saponins were detected and Terpens is absent. In Lemongrass extract Alkaloid, Tannins, and Saponins were detected.

Bioassay

The results of bioassay were collected as a complete protection time (CPT), and the number of mosquitoes landing for the concentrations (50 and 100%), ethanol, hexane and hexane plus ethanol extract. Ethanol extracts presented in **Tables 2** and 3 shows that Wild Thyme 50% and 100% were recorded the highest protection time 137.3 and 182 min respectively compare to the other plant extracts, with percent repellency 92 and 89% respectively. For the hexane extracts presented in **Tables 4** and 5 prove that Thyme 50% and 100% were recorded the highest protection time 124 and 228 min respectively compare to the other plant extracts, with percent repellency 90 and 92% respectively. Regarding the combination of the two extracts presented in **Tables 6** and 7 also the Wild Thyme 50% and 100% were recorded the highest protection time 182 and 215 min respectively compare to the other plant extracts except Basil in 50% recorded higher protection time (86 min), with percent repellency 84 and 88% respectively. By the way the selected four leaf plant extracts offers protection against *An. arabiensis* adult female mosquitoes but varied in terms of repellency up to 228 minutes of exposure periods recorded by Wild Thyme.

Chemical constituents detected in Kafure leaf extracts

Table 8 shows the chemical constituent of Kafure leaves etha- nol and hexane extracts; their concentrations, peak area, retention time and chemical group of each compound. A total of 13 compounds detected in ethanol and hexane extract which represent 63.63% and 68.54% for ethanol and hexane extract, respectively. Eucalyptol was the main compounds (33.11% and 26.93%), respectively. **Table 9** shows the chemical constituent of Kafure leaf's ethanol extract; their concentrations, peak area, retention time and chemical group of each compound. A total of 20 compounds detected in ethanol extract and represent 36.37% of the total of the extract. Globulol 9.94% and Squalene 8.10% were the main compounds. **Table 10** shows the chemical constituent detected in Kafure leaf's hexane extract; their concentrations, peak area, retention time and chemical group of each compound. A total of 25 compounds detected in hexane extract, as follows, where Globulol was the main compound (18.06%).

Discussion

The synthetic chemicals used to control insects leads to several documented environment and human health hazards. An alternatives method suggested using natural products that possess good efficacious results and environmental friendly. Among those alternative methods the use of essential oils from plants belonging to several plant species subjected intensive studies which provide successful results against field crop pests and public health pests and disease vectors control, including mosquitoes (*Anopheles, Culex and Aedes*). These EOs were extensively tested by several scientists for their repellent properties as valuable natural resources and to elucidate their chemical and physical properties, in addition to their modes and sites of action, formulation, toxicological profiles, side-effects, etc... Today medical vector management has to face environmental and economic costs.

Table 2. Spatial repellent activity of 50% and 100% evaluated plants leaves ethanol extract against Anopheles mosquito based on the number of landed mosquitoes (NLM) and protection time per minute (PT).

Treatment	50%								100%							
	NLM				PT				NLM				PT			
	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean
Control	25	24	24	24.33	-	-	-	-	25	24	24	24.33	-	-	-	-
DEET	8	9	5	7.33	72	91	121	94.66	8	9	5	7.33	72	91	121	94.66
Wild Thyme	1	2	1	1.3	113	122	177	137.3	2	3	1	2	210	154	182	182
Sweet Basil	2	3	1	2	25	36	31	30.6	4	2	6	4	42	31	45	42.6
Kafure	4	2	1	2.3	15	10	21	15.3	2	3	4	3	25	32	41	32.6
Lemongrass	2	3	5	3.3	15	17	25	19	4	2	3	3	31	25	28	28

Table 3. Percent repellency (PR) of 50% and 100% of evaluated plants leaves ethanol extract against Anopheles mosquito based on the number of landed mosquitoes (NLM).

Treatment	50%					100%				
	NLM				PR	NLM				PR
	1st	2nd	3rd	mean		1st	2nd	3rd	mean	
Control	25	24	24	24.33	-	25	24	24	24.33	-
DEET	8	9	5	7.33	68%	8	9	5	7.33	68%
Wild Thyme	1	2	1	1.3	92%	2	3	1	2	89%
Sweet Basil	2	3	1	2	89%	4	2	6	4	81%
Kafure	4	2	1	2.3	88%	2	3	4	3	85%
Lemongrass	2	3	5	3.3	84%	4	2	3	3	85%

Table 4. Spatial repellent activity of 50% and 100% evaluated plants leaves hexane extract against Anopheles mosquito based on the number of landed mosquitoes (NLM) and protection time per minute (PT).

Treatment	50%								100%							
	NLM				PT				NLM				PT			
	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean
Control	25	24	24	24.33	-	-	-	-	25	24	24	24.33	-	-	-	-
DEET	8	9	5	7.33	72	91	121	94.66	8	9	5	7.33	72	91	121	94.66
Wild Thyme	1	2	2	1.6	191	142	189	174	1	2	1	1.3	230	208	246	228
Sweet Basil	3	2	5	3.3	46	36	50	44	2	1	3	2	90	118	140	116
Kafure	4	6	8	6	18	21	15	18	3	1	2	2	36	21	28	28.3
Lemongrass	3	4	6	4.3	22	36	28	28.6	2	3	5	3.3	59	41	63	54.3

Table 5. Percent repellency according to the number of landed mosquitoes (NLM) of 50% and 100% percent of evaluated plants leaves hexane extract against Anopheles mosquito.

Treatment	50%					100%				
	NLM				PR	NLM				PR
	1st	2nd	3rd	mean		1st	2nd	3rd	mean	
Untreatedcontrol	25	24	24	24.33	-	25	24	24	24.33	-
DEET	8	9	5	7.33	68%	8	9	5	7.33	68%
WildThyme	1	2	2	1.6	90%	1	2	1	1.3	92%
SweetBasil	3	2	5	3.3	84%	2	1	3	2	89%
Kafure	4	6	8	6	73%	3	1	2	2	89%
Lemongrass	3	4	6	4.3	80%	2	3	5	3.3	84%

Table 6. Spatial repellent activity and protection time per minute (PT)of 50 and 100% of evaluated plants leaves hexane and ethanol extract mixture against Anopheles mosquito.

Treatment	50%								100%							
	NLM				PT				NLM				PT			
	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean	1st	2nd	3rd	mean
Control	25	24	24	24.33	-	-	-	-	25	24	24	24.33	-	-	-	-
DEET	8	9	5	7.33	72	91	121	94.66	8	9	5	7.33	72	91	121	94.66
Wild Thyme	2	3	5	3.3	164	194	190	182.6	4	2	1	2.3	211	233	201	215
Sweet Basil	4	1	3	2.6	48	51	31	43.3	2	6	4	4	66	94	101	87
Kafure	4	6	7	5.6	17	11	19	15.6	6	5	8	6.3	20	15	23	19.3
Lemongrass	6	3	4	4.3	15	24	19	19.3	1	2	4	3.3	27	20	31	26

Table 7. Percent repellency (PR) according to the number of landed mosquitoes (NLM) of 50% and 100% of evaluated plant leaves hexane and ethanol extracts mixture against Anopheles mosquito.

Treatment	50%					100%				
	NLM				PR	NLM				PR
	1st	2nd	3rd	mean		1st	2nd	3rd	mean	
DEET	8	9	5	7.33	68%	8	9	5	7.33	68%
Wild Thyme	2	3	5	3.3	84%	4	2	1	2.3	88%
Sweet Basil	4	1	3	2.6	86%	2	6	4	4	81%
Kafure	4	6	7	5.6	74%	6	5	8	6.3	72%
Lemongrass	6	3	4	4.3	80%	1	2	4	3.3	84%

The Synthetic repellents DEET and pyrethroids containing formulations commonly sold in markets and insecticides sales vender. The side effects of these two chemical groups was reported by several research scientists, accordingly plant-based repellents not only minimize the use of synthetic chemicals and economic cost and save huge amount of money spent on synthetic compounds, also protects the environment, health of users. The plants screened for phytochemical analysis seemed to have the potential to act as a source of useful chemicals and also to improve and help in the health status of the people exposed to mosquito's bits (**Table 1**). This study justifies the use of plant species in medicinal field to protects human from mosquito's bits and hence minimize the malaria spreading. There are different plants which have Mosquito repellent activity and this activity affected with species the environment and other factors. And the chemical constituent of these show different results on the mosquito repellent. The different plant extract contains different chemical constituent shows different results on the mosquito repellent activities (**Tables 2-7**).

This study investigated the mosquito spatial repellent effi-

cacy of the basil, wild thyme, kafure and lemongrass, (ethanol, hexane and mixture of both ethanol extract and hexane extract). The present study results provided information and observations on the repellency activity of tested four plant species LEs against *Anopheles* mosquitoes. Mosquitoes usually make surreptitious landings on exposed skin to feed this why most people wearing protective clothing stands as a good measure of preventing them from vector contact. Application of these plants LEs prevented landing attempts and bites by mosquitoes; this verifies already documented evidence that some plants LE is a potent mosquito repellent.

Sweet basil

O. basilicum EOs are materials that are traditionally used in Sudan the mosquitoes by burning leafs and flowers. However, Coker *et al.,* [15] stated that certain characteristics, *e.g.* volatility, limited its effectiveness. Active ingredients, e.g. terpenes (2° metabolites) and oil components are responsible for the repellency activity. For a plants material to be valuable as a mosquito repellent it must effectively prevent insect bites on the treated area for several hours, and it must work in different environmental conductions, should be environmental- friendly when applied to human skin, it must acceptable and having a pleasant odor, it should also be harmless to clothing, it should have a relatively low cost and effective against other biting insects such as flies. Basil leaves extract provide different spatial repellency against mosquitoes with different concentration, in ethanol, hexane and mixture of ethanol and hexane extract (**Tables 2, 4 and 6**). Ethanol extract provided 30.6 and 42.6 min 32 and 45% for 50% and 100% ethanol extract respectively and the hexane extract was provided 44 and 116 min and evaluated with 46% and 122% for 50% and 100% respectively when compared with DEET, while the mixture of ethanol and hexane extract shows that basil was provides the protection time in both (50% and 100%) was 43 min and 87 min respectively and this repellency act as 45% and 91.9% when compared with standard mosquito repellent DEET (94.6 min protection time) respectively (**Tables 3, 5 and 7**). Basil hexane leaves extract provide the highest repellency against mosquitoes (116 min), Baba *et al.,* [16] conclude that hexane basil leaves extract provided 183 min and 303 min CPT from concentration 50% and 100% respectively (**Tables 4 and 6**). In this study; the presence of ethanol extract in the mixture was reduced the repellency of mixture from the 116 to 87 min in 100% concentration; so the tannin and alkaloids may responsible for the repellent activity in hexane extract according to the phytochemical analysis (**Table 1**). The phytochemical analysis was preceded an Alkaloids, Tannins, saponins and terpens were found in the Ethanol Extract, While Alkaloid and Tannins only detected in Hexane extract..

Wild thyme

Ethanol extract, the wild thyme was provides the highest protection time in both (50 and 100) %137.3 min and 182 min respectively when compared with other plant ethanol extract, and repellency at 154% and 192%, respectively when compared with standard mosquito repellent-DEET (94.6 min protection time). Active movement away from treated arm within minute of application was observed in wild thyme, and this strong repellent activity may caused by terpens group which appear in ethanol and hexane extract (according to phytochemical analysis) as main compounds and thymol (monoterpenoid) was highest concentration (54.96% and 57.42%) in ethanol and hexane extract respectively according to GC-MS analysis, and this was confirm by the study done by Pandey *et al.,* [17] they prove the activity of thymol that repel *An. stephensi* adults at the dose of 25.0 mg/mat after 1 h duration, while same degree of repellency was obtained by the oil at the dose of 55.0 mg/mat, which indicating thymol has double activity than the essential oil obtained from seeds of *Trachyspermum ammi* (Linn.). In present study beside the alkaloids and tannins present in ethanol extract only (phytochemical analysis), the hexane extract of the wild thyme is provides the highest protection time in both (50 and 100) % 174 min and 228 min respectively when compared with other plant leaves extracts and this highest CPT and repellency as 183% and 241% respectively, when compared with standard mosquito repellent-DEET (94.6 min CPT). Regarding the mixture of ethanol and hexane extract of each of the tested plants, wild thyme is provides the highest protection time in both (50 and 100%) 182.3 min and 215 min with spatial repellency at 193% and 227% respectively, compared to DEET. The main compounds detected in ethanol and hexane extract was thymol which shows considerable anti-feedant activities against insects in many studies conducted by Gonzalo *et al*., [18] and Won *et al.,* [19] shows that the wild thyme essential oil was rich source monoterpenes, including thymol, p-cymene, carvacrol, linalool, and *o*-terpinene, were assessed to determine their repellent activities to the mosquitoes. *o*-Terpinene had a potent spatial repellent activity with a protection rate of essential oil topical treatment while the carvacrol and thymol showed an equivalent level of repellency. The Wild thyme was the best one compared with the rest plants it was provided highest CPT; in both ethanol and hexane extract. However, the hexane 100% was provided the protection until 228 min, this period even longer than DEET (2.4 times). According to phytochemical analysis the spatial repellency of Wild thyme could be rise from terpens mainly because the hexane extract shows the terpens only. although provided the highest CPT and the ethanol extract which contain terpens beside the alkaloids and tannins show low CPT. Our study show that thymol and eucalyptol (monoterpenoid) was main compounds in either ethanol and hexane extract and its concentration was corresponding with spatial repellency period, the repellency of plant extract could be due to the eucalyptol and this was proved by James *et al.,* [20] they found that mosquito feeding and ovipositional repellency due to the presence of major monoterpenoid in the chemical volatile oil (*i.e.,* 1,8-cineole (eucalyptol) identified in plant extract of *Hemizonia fitchii* (Asteraceae).

Phytochemicals are the principal active components that are

Table 8. Chemical constituent detected appear in the GC-Mass chromatogram in both ethanol and hexane extract of Kafure leaf's and area percentage of peaks.

ID	Name	Area% Ethanol	Area% Hexane
1.	Alpha-Pinene	3.38	6.77
2.	D-Limonene	0.19	0.55
3.	Eucalyptol	33.11	26.93
4.	Gamma.-Terpinene	0.30	0.47
5.	Pinocarvone	1.06	1.94
6.	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	0.25	0.37
7.	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	0.18	0.24
8.	Copaene	0.19	0.42
9.	Alloaromadendrene	0.39	0.26
10.	Caryophyllene	0.76	0.92
11.	Aromandendrene	7.02	7.07
12.	Alpha.-Guaiene	1.60	2.37
13.	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	15.20	20.23

Table 9. Chemical constituent detected appear in the GC-Mass chromatogram in ethanol extract of Kafure leaves and area percentage of peaks.

ID	Name	Peak Area(%)
1.	alpha-Phellandrene	0.79
2.	p-Cymene	1.78
3.	3-Methyl-4-cyclohexene-1,2-dicarboxylic anhydride	0.20
4.	Benzene, 1-methyl-4-(1-methylethenyl)-	0.67
5.	Isoledene	0.11
6.	1-Tridecene	0.12
7.	Bicyclo[2.2.2]octa-2,5-diene, 1,2,3,6-tetramethyl-	0.15
8.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	0.04
9.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	0.18
10.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)-	0.21
11.	(S)-(-)-4-Isopropenyl-1-cyclohexene-1-carboxylic acid	1.98
12.	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	1.42
13.	Guaia-1(10),11-diene	1.37
14.	7-epi-cis-sesquisabinene hydrate	0.79
15.	Globulol	9.94
16.	Phytol, acetate	3.03
17.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.55
18.	9-Eicosyne	1.04
19.	n-Hexadecanoic acid	3.90
20.	Squalene	8.10

believed to exhibit the medicinal activity of the plants and possibly the repellent activity of the oils. According to Banthorpe [21] and Heinrich *et al.,* [22] terpenoids could be responsible for the flavor of fruits, the fragrance of the flowers and the quality of agricultural products. Therefore Coker *et al.,* [15] prove that the presence of terpens is speculated to be associated with fragrance material and spatial repellent activity of oils.

Kafure

Kafure leaves extract provided different CPT by (50% and 100%) concentration, in ethanol; hexane and ethanol and hexane extract (mixture) (**Tables 2, 4 and 6**). Ethanol extract provide 15 min and 32 min by 50% and 100% concentration respectively. However the hexane extract provide 18 min and 28 min complete protection by 50% and 100% concentration respectively when the mixture provided 15 min and 19 min complete protection by 50% and 100% concentration respectively. The result shows that hexane extract was slightly stronger repellent than the ethanol and the mixture; the phytochemical analysis shows the presence of the: saponins, tannins, and alkaloids and absence of triterpens in both ethanol and hexane extract; while the GC-MS detected some of monoterpenes like euclyptol, (Cyclic) 33.11% and 26.93% and 2-Naphthalenemethanol, decahydro-alpha, alpha, 4a-trimethyl-8-methylene-, [2R-(2alpha, 4aalpha, 8abeta)]-15.20% and 20.23%, in ethanol and hexane extract respectively, and Globulol was 9.94% in ethanol extract and hexane extract was contained (-)-Globulol 18.06% so this variety may respon-

Table 10. Chemical constituents detected appear in the GC-Mass chromatogram in hexane extract of Kafure leaves and area percentage of peaks.

ID	Name	Peak Area(%)
1.	Camphene	0.14
2.	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	0.07
3.	beta-Pinene	0.22
4.	beta-Myrcene	0.06
5.	o-Cymene	3.58
6.	Doconexent	0.20
7.	Butanoic acid, 3-methyl-, 3-methylbutyl ester	0.28
8.	alpha-Campholenal	0.14
9.	Terpinen-4-ol	1.58
10.	L-.alpha.-Terpineol	1.67
11.	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2-en-2-yl)-	0.19
12.	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	0.08
13.	Citral	0.15
14.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)	0.20
15.	12-Oxatetracyclo[4.3.1.1(2,5).1(4,10)]dodecane, 11-isopropylidene-	0.17
16.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	1.38
17.	beta-Humulene	0.31
18.	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	0.22
19.	gamma-Muurolene	0.21
20.	Aromadendrene, dehydro-	0.20
21.	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	0.26
22.	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-	1.32
23.	(-)-Globulol	18.06
24.	Caryophyllene	0.47
25.	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene-, (4aS-cis)-	0.27

sible of different repellency and the 32 min was highest CPT achieved by kafure ethanol extract. In the study conducted by Fathelrahman [23] showed ethanol extract 10% concentration provide 17% repellency for 60min. the result was corresponding with this study that increasing extract concentration of kafure was increase the repellency in both extract and the kafure extract, although was weak in spatial repellency.

According to the result of GC-MS analysis of the chemical compound of kafure leaves extract of the current study (**Tables 8, 9 and 10**); the results were corresponding with the GC-MS analysis of essential oil yield and composition in *Eucalyptus* species in Montenegro coastline by Slavenko*, et al;* [24] show the presence of α-pinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, p-Cymene, Eucalyptol γ-terpinene, terpinene-4-ol, α-terpineol, aromadendrene, alloaromadendrene, also was found in this study.

According to the GC-MS analysis of the present study prove the presence of o-Cymene 3.58%, Terpinen-4-ol 1.58%, L-.alpha.-Terpineol1.67%, Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-(1-methylethenyl), L-alpha -Terpineol 1.67% [2R-(2.alpha.,4a.alpha.,8a.beta.)]-1.38%, 1H-Cycloprop[e] azulen-4-ol,decahydro-1,1,4,7-tetramethyl,[1a R (1a alpha, 4beta, 4a beta, 7alpha, 7a beta, 7b alpha)]-1.32%, (-)-Globulol 18.06%, 2-Naph- thalenemethanol, decahydro -alpha, alpha, 4a-trimethyl -8-methy- lene-,[2R-(2alpha,4a alpha, 8a beta)]- 20.23%, was found in the hexane leaves extract and not found in ethanol extract, and alpha-Pinene was 6.77% in hexane extract and 3.38% in ethanol extract.

Lemongrass

Application of *Cymbopogon* oil prevented landing attempts and bites by mosquitoes; and this verifies already documented evidence by Baldacchino *et al.,* [25] and Prabhakar *et al.,* [26] that *Cy. Citrates* extract is a potent mosquito repellent. However, the interval of protection is a matter of concern; in this study, lemon grass leaf extracts provided protection from mosquito for 3 hours after which landing attempts and bites were experienced. This observation corroborates previous records of plants essential oils by Bhupen *et al.,* [27].

According to Bhupen *et al.,* [27] has demonstrated the essential oils from a variety of plant species been used as insect repellents; such as *Cymbopogon*. In the present study Lemongrass leaves extract provided different CPT with different concentration (50% and 100%) in ethanol, hexane and ethanol and hexane extract (mixture). Ethanol extract provided 19 min and 28 min by 50% and 100% concentration respectively (**Tables 2 and 4**). However the hexane extract provide 28 min and 54 min complete protection by 50% and 100% concentration respectively (**Table 6**). While the mixture provided 19 min and 26 min complete protection by 50% and 100% concentration respectively.

The 54 min was maximum CPT provided by lemon grass (hexane extract) with spatial repellency is equal 57% compared with DEET, and also was low when compared with wild thyme and basil extract. In phytochemical studies both ethanol and hexane extract was showed the Alkaloid, tannins and saponins while the terpens and flavonoids were absent and those groups were important as insect repellent. Although monoterpenes was detected by the GC-MS as main compounds as 2,6-Octadienal, 3,7-dimethyl-, (Z)-in ethanol extract and 2,6-Octadienal, 3,7-dimethyl-, (Z)- and 2,6-Octadienal, 3,7-dimethyl-, (E)- in hexane extract. In the study conducted by Vinutha *et al.,* (28) on essential oil yield from aerial part of *Cymbopogon flexuosus*; Myrcenol, Linalool, Trans-chrysanthemal, 3,6,6-Trimethyl -cyclohex-2-enol, Citronellal, (-)-Isopinocampheol, 1-Pentanol, 5-cyclopropylidene-3- undecyne, 3-carvomenthenone, (Z)-linalool oxide, (furanoid), Neral, Geranial, β-Vatirenene, Citronellol, Dextro-carvone, Cycloisolongifolene, Trans-(-)-Carveol, cis-Carveol, Nerol, Oxiranmethanol, 3-methyl-3(4-mathyl-3-pentenyl), Bicyclopentylone, Geranic acid, Geranyl acetate, most of those compounds were detected in lemongrass extract in this study.

According to the result, undiluted hexane leaves extract (100%) provide maximum CPT according to the follows: 228 min wild thyme, 116 min basil, 54 min Lemongrass and 28 min kafure, thus the hexane extract was seem more stronger in repellency than the ethanolic extracts, and the *Coleus* is the best and effective as mosquitoes repellent than other plants, followed by Basil and Lemon grass and kafure.

The results of the current work indicated that the spatial repellent potential of the leaves extract oils is concentration dependent. However it is observed that all the thyme leaves extract oils indicated protection of at least 2.5 h at 50% concentration and 2.5-5 h protection at 100%. Accordingly the use of plants leaves extract essential oil in prevent mosquito bite is expected to reduce the cost and environmental effects of mosquito repellents.

According to Annamaria *et al.,* [29] showed volatile compounds detected in *Bixa orellana* samples the major volatile compound present in seed extracts proved to be source of repellent activity (mainly of sesquiterpenes, monoterpenes, arenes and as α-humulene). These volatile compound found in most of our chemical analysis to the four plants which reflects the repellent activity of the test extract especially thyme. In conclusion the preliminary findings of this laboratory evaluation bioassay of the repellent potential of the essential oils of *Ocimum basilicum* L., *Coleus forskohlii*, *Eucalyptus camaldulensis* and, *Cymbopogon flexuosus Steud*, leaves extracts have confirmed their traditional use as a broad mosquito repellent agent. Further investigations leading to identification of the lead compounds exhibiting the repellent activity is recommended. This would enhance a robust development of plant based oil for protection against mosquito bite. Basil provided considerable repellency against Mosquitoes. Wild thyme was most effective which provided 105 min CPT evaluated with 205% in compare to the control (DEET, lavender). The examined plants will be useful if the further studies have been conducted and it will replace the commercial mosquitoes repellent (DEET, lavender). Terpenoids were found in most of the extracts, but in the *C. forskohli* with the highest concentration. The presence of the (+)-4-carene, benzene, 1-methyl-3-(1-methylethyl)-, 2-naphthalenemethanol, decahydro- were related with hex- extracts of *C. forskohli* only, and it has strong repellency activity.

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