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ANALYTICAL INVESTIGATION OF THE EXTRACT OF LEMON GRASS LEAVES IN REPELLING MOSQUITO

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Keywords:

Lemon grass, *Cymbopogon citratus*, Soxhlet extraction, Gas chromatography-mass spectrophotometer (GC-MS)

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ABSTRACT: The main objective of the work was to extract the active ingredient in *Cymbopogon citratus* leave and to formulate a mosquito repellent cream naturally obtained from medicinal plants instead of commonly available synthetic repellents and insecticides such as N-Diethyl-3methylbenzamide (DEET), which are carcinogenic and non eco-friendly. The formulation of cream have smooth texture with a pH 7.30 which is non-irritant and suitable for the skin. The efficacy of the extract of Lemon grass (*Cymbopogon citratus*) was investigated on mosquito in this research using different concentrations of the oil extract. The sample (cream) with the highest concentration of the active ingredient extracted was found to be most effective in repelling mosquitoes. Ethanol and Hexane were used to extract the essential oil and Gas chromatography-mass spectrophotometer (GC-MS) was used to analyse the constituents in the extracted active oil. The most abundant constituents observed in the hexane extract are cyclotetracosane (4.05%) and naphthalene (5.03%). Hexane proved to be a better solvent by giving a percentage yield of 7.8% of essential oil while ethanol a percentage of 2.9%.

INTRODUCTION: Plant essential oils (EOs) have been widely used for many years due to their antimicrobial properties in foods and pharmaceutical products [1]. Essential oils are natural products obtained from plants. It is estimated that the global number of plants is of the order of 300,000 and about 10% of these contains essential oils and could be used as a source for their production [2].

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A large number of essential oils extracted from different families have been shown to have high repellence against arthropod species. Among the essential oil producing plants, such as *Cymbopogon* spp., *Eucalyptus* spp. and *Ocimum* spp. have been widely studied. *Cymbopogon* plants have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon [3]. *Cymbopogon* produces the most used natural repellents in the world [4]. Many extracts and essential oils isolated from these plants have been tested against different kinds of arthropods. *Cymbopogon excavatus* gave 100% repellence for 2 h, when it was evaluated in the laboratory against *Anopheles arabiensis* and its repellence decreased to 59.3% after 4 h [5].

Mosquito-borne diseases cause significant morbidity, mortality and economic burden to humankind [6]. The mosquito, *Aedes aegypti* is the major vector of yellow fever, dengue and dengue hemorrhagic fever (DHF). These mosquito-borne infections are found in tropical and sub-tropical regions around the world, predominantly in urban areas and semi-urban areas. The global incidence of dengue has grown dramatically around the world in recent decades and there are approximately 2.5 billion people at risk [7]. One of the methods available for the control of mosquitoes is the use of insecticides. In last two decades, the use of chemical insecticides in mosquito control method has resulted in instability of the environment, mosquito resistance, mosquito resurgences and toxic to nontarget organisms including natural enemies in the agriculture ecosystem [8]. Hence, it has now become important to find an alternative means of mosquito control method, which can eliminate the use of chemical pesticides [9].

Mosquitoes play an important role in the spread of vector-borne diseases like malaria, dengue, chikungunya, filariasis and Japanese encephalitis which cause thousands of deaths per year [1]. Mosquitoes are the most important and abundant pest in urban, sub- urban and rural environment. Although, chemical control provides quick mortality, resistance of mosquito against the use of insecticides have been widely reported. Moreover, chemical mosquito repellents contain toxic synthetic pyrethroids as active ingredients whose exposure to food and water is hazardous to health. In the present study, an attempt has been made to develop an eco-friendly mosquito repellent sprayed with lemon grass oil. It is an established fact and practice is that the natural mosquito repellent is more effective and keeps environment pleasant and eco-friendly. Raw materials have been selected based on experience and practice by ancestors. The formulation is safe, eco-friendly, cheap, easy to use and has maximum repellence against mosquitoes. In addition this, the mosquito repellent is less harmful to our health than the ones available in the market [10].

World Health Organization (WHO) estimates globally, shows that about 25 million people harbouring microfilaria and 19 million cases of people suffering from filarial disease manifestations were recorded in India [11, 12]. The use of chemical insecticides to control mosquito population is fast and easy to use but mosquitoes are becoming resistant with continuous use. Alternatively, natural pesticides (especially from plants) can be used as a very good substitute. Aromatic plants and their essential oils are best sources of many active compounds for multipurpose uses. Plant-based phytochemicals having mosquitocidal properties are now recognized as potent alternative insecticides to replace synthetic insecticides [13].

Apart from the hazards caused by the use of established pesticides and insecticides to man and livestock, they are also very expensive to purchase; therefore, they are no more within the reach of many Nigerians. Moreover, chemical control technology is subject to the predicament of unstable foreign exchange. In the light of these shortcomings, it becomes inevitable to research into local sources of insecticides that would be cheap and readily available to individuals. Moreover treatment using medicines of natural origin is gaining momentum nowadays on account of increasing concern about potentially harmful synthetic additives [14].

Cymbopogon citrates, commonly known as lemon grass and other *Cymbopogon* species is a tall, coarse grass with a strong lemon taste. Lemon grass is a perennial herb widely cultivated in the tropics and sub-tropics, and it designates two different species; East Indian *Cymbopogn flexuosus* (DC.) and West Indian, *Cymbopogon citratus* [15]. It is a tropical plant, grown as an ornamental in many temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom [16]. Biologically active compounds derived from selected plants species such as *Cymbopogon citrates, Ocimum gratissiumum, hyptis sauveolen*, *Acarcia Arabica*, *Azadirachta indica* and *Eleusive indica* have been commonly used in the past to control insects in many tropical counties [14, 16]. The essential oils obtained from the leaves and stems of this plant are used as remedy for several health problems like fever, throat inflammations, ears or eyes a typical example is the use of the leaves in the Eastern Nigeria to treat various heart disorders [1].

Various herbal sources with mosquito repellent activities have been claimed in various traditional resources like Ayurveda [17]. The stable fly *Stomoxys calcitrans* L. and mosquitoes are among the most damaging arthropod pest of livestock worldwide, with a high economic impact on dairy and beef cattle production. Control of stable fly populations includes various methods, such as chemical control (pesticides and repellents), cultural control (sanitation), mechanical control (trapping devices), and biological control (parasitoids and entomopathogenic fungi) [17].

The rationale for carrying out this study was to contribute to the ever growing and increasing scientific database knowledge on traditional medicine and medicinal plants via the studying the mosquitocidal activities of *Cymbopogon citrates*, a traditional medicinal plant available worldwide.

MATERIALS AND METHODS:

Procurement of Raw material: *Cymbopogan citrates* leaves were harvested and collected freshly from a native farm in Ota, Ogun State, Nigeria. The leaves were sorted to remove insects, variegated leaves and debris. They were then rinsed in water to remove dust particles after which they were allowed to air dry.

All chemicals used are of analytical grade.

Sample preparations: The Lemon grass was separated from its stalks and air-dried at room temperature after which it was cut into smaller sizes in other to fit into the extraction chamber. Soxhlet extraction method was used for the extraction of essential oil from lemongrass; hexane and ethanol were conveniently used for this extraction process. Further analysis were done on the essential oil obtained using the GC-MS which was used to characterize the compositions and concentration of elements in the essential oil.

The extraction process: The already cut leaves were weighed and placed in the thimble. The thimble was inserted in the extraction chamber placed above a flask containing the solvent. A condensing unit is attached to this setup. As the solvent boils, vapour moves up into the condenser, condenses and flows into the thimble allowing for separation. The volatile compounds which have high affinity for the solvent get attached to it and it drops back into the flask below.

250 ml of each solvent was used throughout the course of the experiment. The mass of the leaf was varied for each of the solvent from 25 g to 30 g. At the end of the experiment, the extract was distilled. The evaporator embedded in this unit removes the solvent from the extract by evaporation leaving the essential oil.

Cream production: Two phases are involved in the production of cream:

- The oil phase
- Water phase

TABLE 1: MATERIALS USED IN CREAM PRODUCTION

Materials	Amount
Oil Phase	
Lanolin	10 (ml)
Mineral oil	20 (ml)
Petroleum Jelly	10 (g)
Beeswax	8 (g)
Vitamin E oil	10 (ml)
Water Phase	
Distilled Water	25 ml
Borax	0.5 ml

Table 1 shows the materials and quantities used for the production of the cream before incorporating the essential oil. For the oil phase, mineral oil or Vitamin E, beeswax or steric acid, petroleum jelly and lanolin were mixed together in a beaker as indicated in (**Table 1)** and preheated slowly in a water bath for about 3-5 minutes with continuous stirring for proper mixing.

For the water phase, borax and distilled water were put into another beaker and also heated for about 3-5 minutes also with continuous stirring. Alternatively the distilled water can also be heated till almost boiling and stirred in baking soda.

The water phase was then slowly added to the oil phase and the mixture was continuous to ensure proper mixing using a hand blender. The oil turned white immediately to show that emulsification was achieved. Immediately the pan was placed in an ice water bath in the sink.

After cooling, the cream was then portioned into four covered cups and the active ingredient (essentials oil) incorporated at different concentrations ranging from 0.5 to 2 ml with occasionally mixing until completely cooled and homogenous.

Efficacy test of the Essential oil: Five experiments were carried out with four different concentrations of the active ingredient and one control. Samples 1, 2, 3 and 4 contained 0.5, 1.0, 1.5 and 2.0 ml of active ingredients respectively. Sample 5 was taken as the control experiment i.e. had no incorporated material in it. These experiments were carried out at night to ensure availability of mosquitoes.

RESULTS:

TABLE 2: HEXANE EXTRACTION WITH VARIED QUANTITY OF LEAF

Mass of Leaf (g)	Mass of Oil (g)	Volume of Oil (ml)	Density (g/ml)
25.0	1.58	2.60	0.608
27	1.40	2.2	0.636
30.0	0.90	1.60	0.563

TABLE 3: ETHANOL EXTRACTION WITH VARIED QUANTITY OF LEAF MASS

Mass of Leaf (g)	Mass of Oil (g)	Volume of Oil (ml)	Density (g/ml)
25.0	1.10	1.73	0.636
27	0.60	0.45	1.333
30.0	0.36	0.20	1.800

The oil yield (ml/g) was calculated using equation 1

$$Y_{oil} = \left(E/_{Ki}\right) \quad \ldots\ldots\ldots\ldots\ldots \quad 1$$

Where,

Y_{oil} is the extracted oil yield in percent (ml/g)

E is the total oil yield (g)

K_i is the total mass of leaf (ml)

Total mass of Oil yield:

Total mass of oil for Hexane extract:

Total mass of leaf: (25+27+30) g = 82g

Total Oil yield: (2.6+2.2+1.6) ml = 6.4 ml

$Y_{oil} = \left(E/_{Ki}\right)$

$\left(6.4/_{82}\right)$ = 0.0780 ml/g

Total mass of oil for Ethanol extract:

Total oil yield: (1.73+0.45+0.2) ml = 2.38 ml

$Y_{oil} = \left(E/_{Ki}\right)$

$\left(2.38/_{82}\right)$ = 0.029 ml/g

Density of oil:

Hexane:

Density= mass/volume

Mass of oil = 3.88 g

Volume of oil = 6.4 ml

Density = 3.88 / 6.4 = 0.6063g/ml

Ethanol:

Mass of oil = 2.06g

Volume of oil = 2.38ml

Density = 2.06/2.38 = 0.8656 g/ml

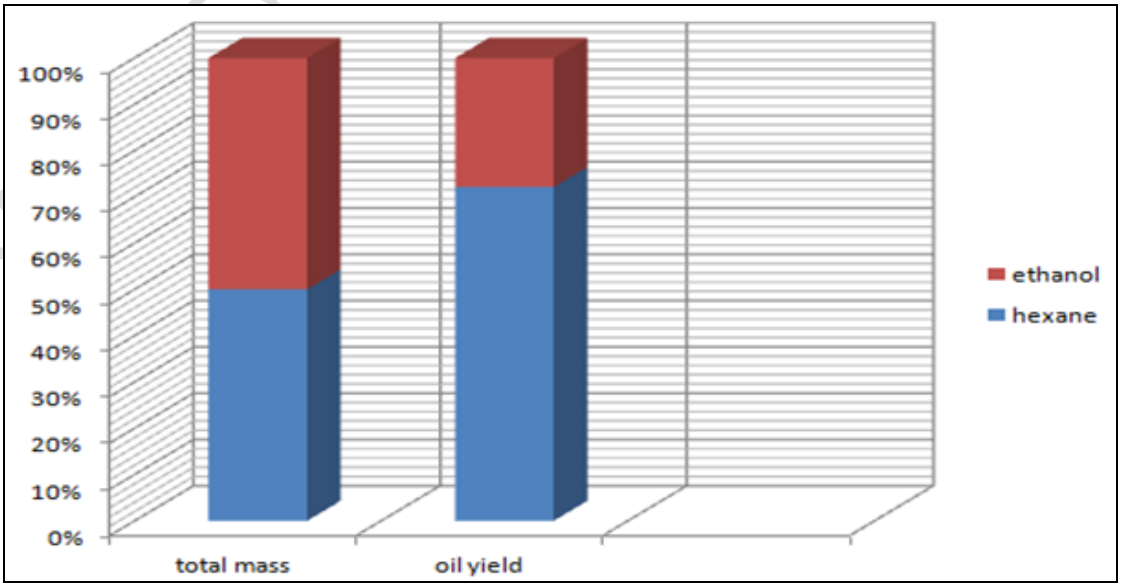


FIG. 1: TOTAL YIELD OF OIL FROM THE ETHANOL AND HEXANE

DISCUSSION: Yield obtained from using hexane as solvent was more than that obtained when ethanol was used as shown in **Tables 2** and **3**. This can be explained using solubility and polarity. The rule of solute-solvent extraction is: like dissolves like. Hence, it can be explained that lemon grass contains a lot of non-polar solutes since a higher yield was obtained.

Effect of mass on oil yield: From **Tables 2** and **3**, it was observed that as mass of the sample increases, the yield decreases which is represented by **Fig. 2**. Smaller surfaces tend to give a higher surface area for penetration of solvent which enhances extraction, therefore the smaller the mass or size, larger the surface area and the bigger the yield and vice versa.

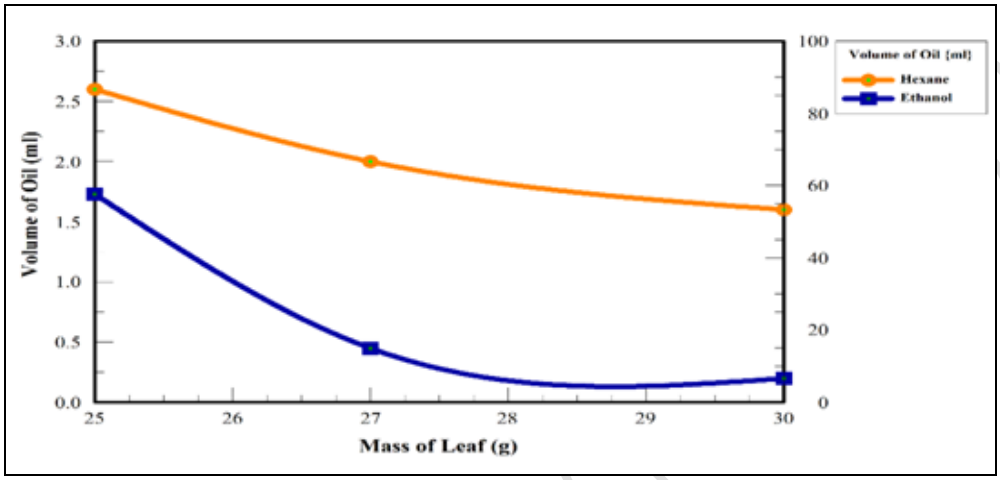


FIG. 2: VARIATION OF MASS OF EXTRACT AGAINST VOLUME OF EXTRACT

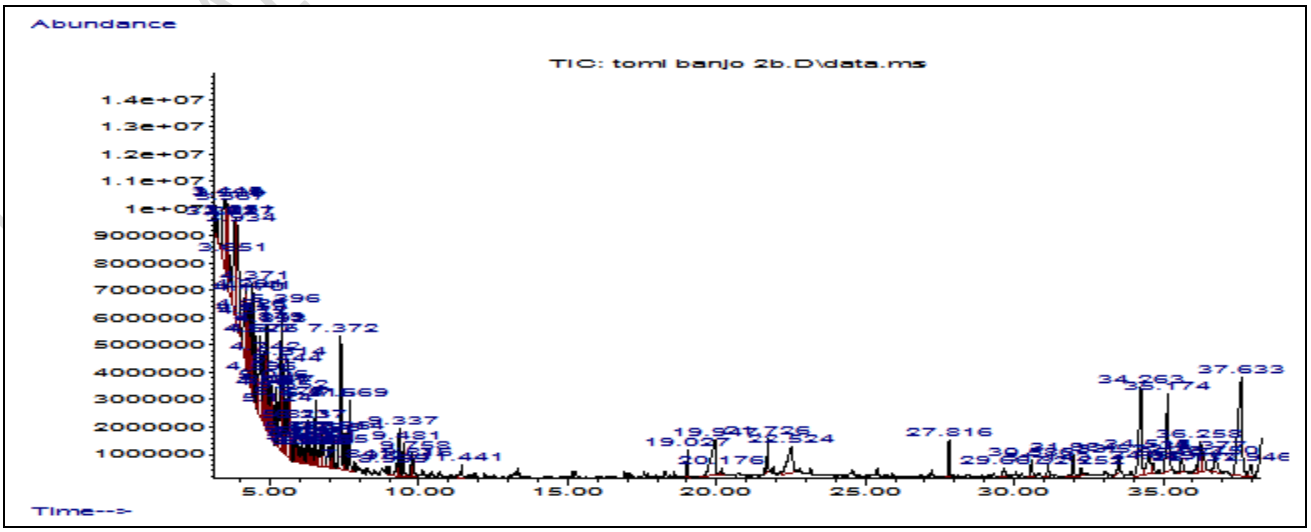
Fig. 2 clearly shows an inverse relationship between the mass of the extract (leaf) and the volume of the extract. It is a negative slope graph, hence as mass increases, volume decreases.

GC-MS Result: The chemical composition of the oil obtained was analyzed with gas chromatography-mass spectrometric technique using method of [13, 14]. Qualitative and quantitative analytical results are listed in the **Table 3** along with retention indices of the identified compounds.

The analysis of the essential oil of lemongrass leaves led to the identification of 120 constituents, with 80 constituents from hexane solvent and 41 from ethanol solvent.

Two compounds were identified as the most abundant for the hexane fraction, Naphthalene (5.03%) and cyclotetracosane (4.05%) while Benzene, 4-ethyl-1,2-dimethyl and Naphthalene, 2-methyl were also observed.

GC-MS Result for Hexane:



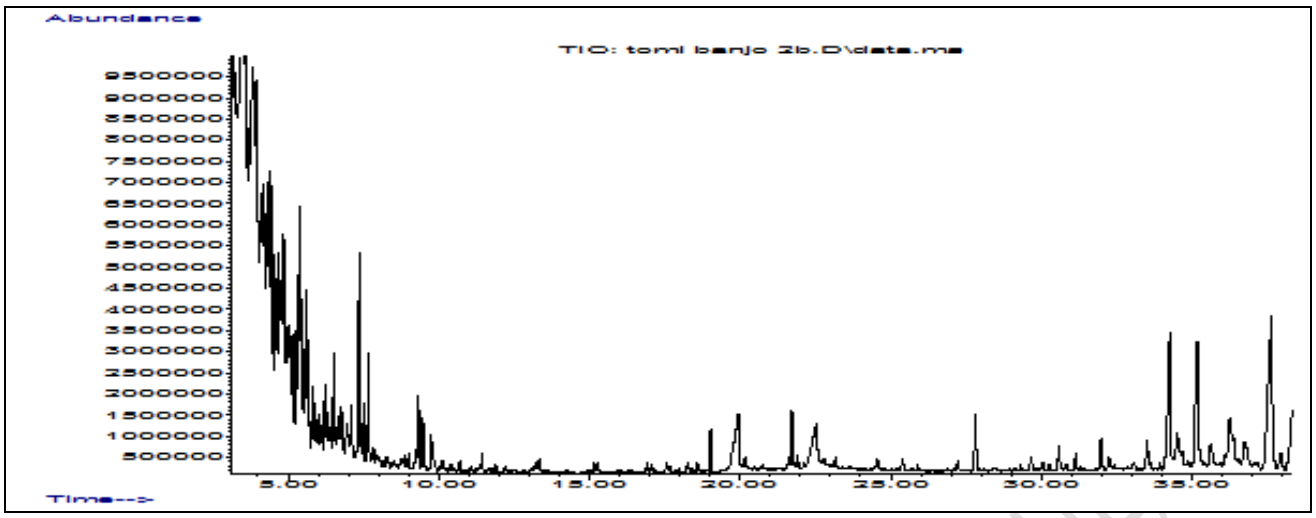


FIG. 3: THE SPECTRA OF THE ESSENTIAL OIL USING HEXANE AS SOLVENT

TABLE 3: GC-MS RESULT FOR HEXANE

S/N	Name	Retention Time [Min]	% in oil
1	Benzene, 4-ethyl-1,2-dimethyl	3.825	3.42
2	Benzene,1,2,4,5-tetramethyl	4.443	1.65
3	Trans-decalin,2-methyl	4.523	1.43
4	Benzene,2-ethenyl-1,4 dimethyl	4.678	2.18
5	Benzene,1,3-diethyl-5-methyl	4.741	2.13
6	Benzene,1,4-diethyl-2-methyl	5.124	0.49
7	Naphthalene	5.399	5.03
8	Benzene pentamethyl	5.811	0.55
9	Benzene,1-ethyl-4-(1-methylethyl)	5.936	0.31
10	1H-Indene,2,3,-dihydro-4,7 dimethyl	6.514	1.06
11	1H-Indene,2,3-dihydro-4,7 dimethyl	6.766	1.14
12	Naphthalene, 2-methyl	7.373	3.65
13	Hexadecanoic acid, methyl ester	19.028	0.59
14	n-hexadecanoic acid	19.950	2.64
15	9-octadecanoic acid (2)-,methyl	21.729	0.80
16	Cis-Vaccenic acid	22.524	2.15
17	Tetracontane,3,5,24-trimethyl	29.665	0.29
18	13-docosenamide	30.587	0.49
19	Cyclotetracosane	34.260	4.05

Cyclotetracosane: The molecular formula for this compound is $C_{24}H_{48}$ and its molecular weight is about 336.6379

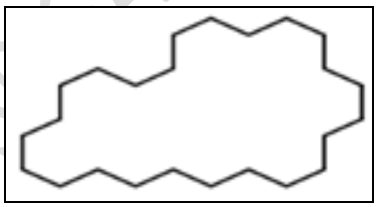


FIG. 3: MOLECULAR STRUCTURE OF CYCLOTETRACOSANE

It has a density of 0.79 g/cm^3 which is very close to the density obtained when hexane was used as solvent and a boiling point of 448.8 °C at 760 mmHg. Cyclotetracosane has a refractive index of 1.433 and its flashpoint point is 212 ^{o}C.

Naphthalene: The molecular formula for this organic compound is $C_{10}H_8$. It has a molecular weight of 128.16. It has a characteristic odour and aromatic taste. It has a boiling point of 217.9 ^{o}C and a melting point of 80.2 °C. It is soluble in ethanol and its solubility is about 7.7 g/100ml. It also has a density of about 0.9625 g/cm^3 with a refractive index of 1.5878v [22].

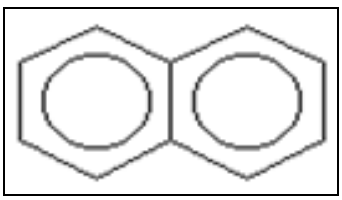
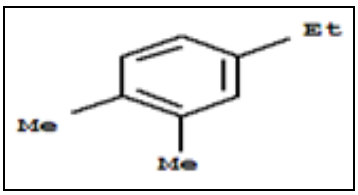


FIG. 4: MOLECULAR STRUCTURE OF NAPHTHALENE

It is the simplest polycyclic aromatic hydrocarbon and a white crystalline solid with a characteristic odour that is detectable at concentrations as low as 0.08 ppm by mass. As an aromatic hydrocarbon, naphthalene's structure consists of a fused pair of benzene rings. It is best known as the main ingredient of traditional mothballs.

Benzene, 4-ethyl-1, 2-dimethyl:



Formula: $C_{10}H_{14}$
Molecular weight: 134.2182
FIG. 5: MOLECULAR STRUCTURE BENZENE, 4-ETHYL-1,2-DIMETHYL

Benzene, 4-ethyl-1, 2-dimethyl has melting point of -67 ºC, flash point of 61.5 ºC, boiling point of 190 ºC, density of 0.867 g/cm^3 which is very close to density obtained from the essential oil when ethanol was used as solvent and refractive index 1.498v

Plant extracts and phytochemicals are good sources of repelling mosquito due to their efficiency, easy biodegradability and development of less to nontoxic products; hence they may be applied to mosquito breeding places. Many plant extracts and essential oils possess better larvicidal activity against many mosquito species. The highest mortality was observed in hexane extract. Previously, many authors reported that acetone extracts of various plants have highest larvicidal and adulticidal activity against mosquito [23, 24].

TABLE 4: EFFICACY TEST RESULT

Cream (g)	Active ingredient (ml)	Repellence
50	0.5	Repelled mosquitoes between 1-2 hours
50	1.0	Repelled mosquitoes between 3-5 hours
50	1.5	Repelled mosquitoes between 5-6 hours
50	2.0	Bite was not noticed until 7 hours after application
50	Control	Bite was felt immediately

The results obtained from **Table 4** confirms the facts that had been established by previous works in terms of efficacy of the active ingredient in *Cymbopogon citratus* [18, 19, 20, 21]. From the experimental result, it was clearly confirmed that the essential oil in *Cymbopogon citratus* extract repels mosquitoes at different concentrations. 0.5 ml concentration repelled mosquito between 1 – 2 hours of application, 1 ml repelled mosquito within 3 – 5 hours of application, also 1.5 ml repelled within 6 hours of application while 2.0 ml was found to be most effective, mosquito were not found around until after the 8th hour of application. It is clear from the result that the active ingredient in the *Cymbopogon citratus* showed a significant measure of repellence as well as toxicity to the mosquitoes. However no mosquito was not found dead.

CONCLUSION: From this research, it was evident that more yield was obtained from hexane solvent when compared with that obtained from the ethanol solvent. Therefore hexane is said to be a better solvent for extraction of lemongrass essential oil.

The technique of solvent extraction is based on the fact that like dissolves like. Hence, polar solutes dissolve in polar solvents whereas non-polar solutes dissolve in non-polar solvents. The result obtained from **Table 1** established that hexane is a non-polar solvent while ethanol is a polar solvent. So using the principle of solute-solvent extraction, naphthalene and cylcotetracosane are non-polar solutes since they dissolved in hexane.

Therefore, it can be said of the lemongrass leaf that it contains more non-polar constituents that polar ones since it contains about 80 compounds in the hexane fraction.

Cymbopogon citratus active ingredient has insecticidal Properties. Furthermore the mosquito repellent cream produced possesses repellence characteristics against mosquito. Thus the necessity to produce non-toxic, safe and biodegradable attractive and synthetic insecticide has made the *Cymbopogon citratus* to be apparent. Natural insect repellents tend to provide coverage for a shorter time, but their coverage is safer so you may find it worth the extra effort applying a bit more often.

Because of their shorter protection time, natural repellents are ideal for short evening outdoor activities like walking the dog, barbecuing, or watering the garden. This study reaffirms the possibility of using indigenous Nigerian plants with insecticidal property for the control of Mosquitoes.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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Reviewer's recommendations:

1. Specify designation and current full address of corresponding author.
2. Check for spelling, grammar and punctuation error(s).