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OIL EXTRACT FROM LOCAL LEAVES - AN ALTERNATIVE TO SYNTHETIC MOSQUITO REPELLANTS

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ABSTRACT

Apart from the fact that chemical based (synthetic) repellents have been discovered to have unfavourable effects on man and livestock, they are expensive, non-biodegradable and no longer efficient due to adaptation of mosquitoes to them. With these shortcomings, an eco-friendly plant based insecticide as an alternative is urgently needed. This study was based on using the oil extracts from local leaves namely: *Hyptis Sauveolens*, *Mentha spicata*, and *Cymbopogon citratus* leaves as mosquito repellent. The leaves' oil extracts were screened for the presence of phytochemicals (secondary metabolites only). The phytochemical screening result showed that leaves' oil extracts contained flavonoids, tannins, phenols, steroids, terpenoids, saponins, glycosides and anthraquinones. Different concentrations of the oil extracts were incorporated into the formulated body lotion/body cream. The lotion with the highest concentration of the oil extract (0.6 ml) had the highest repellence time lasting up to about eight (8) hours. The leaves' oil extract was analysed using Gas chromatography- Mass spectroscopy (GC-MS) to identify the chemical composition, and only major chemical components were reported in this work.

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Introduction

Mosquitoes are blood-sucking insects which are carriers of deadly diseases such as malaria, yellow fever, Zika virus and dengue fever [1]. Mosquito-borne diseases cause mortality and economic burden on man [2]. Major source of illness or death worldwide is diseases spread by mosquitoes [3]. More people die each year from mosquito-borne diseases than from any other single cause diseases e.g. Malaria Filariasis (disease transmitted by *Brugia malayi* and *Anopheles* mosquito), Encephalitis (this is a viral disease spread by fully grown female mosquitoes, a species of *Aedes* and *Culex*) and Yellow fever [4].

Plant essential oils, especially leaves' oil extracts have been used to a great extent for many years because of their antimicrobial quality and pharmaceutical products [5]. They have also been widely used as a medicinal and aromatic plant since the ancient times. Previous investigations reported that various plant extracts have displayed mosquito repellence activity especially larvicidal effect on *C. pipiens*, *C. quinquefasciatus*, *A. aegypti*, *A. stephensi* and *Aedes tessellatus* [6]. Many essential oils and extracts obtained from various plants have been tested on different kinds of arthropods and confirmed viable [7]. Literature revealed that plants, flowers, roots, leaves and seeds contain some active ingredients which are known as Essential oils. Many of them are odoriferous while some are volatile and ethereal which can be extracted with ether, an organic solvent) [4].

N, N-diethyl-3-methylbenzamide (DEET- synthetic repellent) is the most striking and best studied insect repellent currently available in the market. DEET based synthetic mosquito repellents cause irreversible damage on ecosystem since they contain chemicals which are non-degradable in nature [8, 9]. Insecticides made from the combination of different artificial substances

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are toxic and affect the environment by making the soil, water and air undesirable [10]. Local leaves' oil extracts especially *Mentha spicata* have been reported by various researchers as a good mosquito repellent and possess antimicrobial activity [4, 7, 11, 12, 13, 14, 15, 16].

Considerable research efforts have proved that essential oil compounds like Tulsi, Clove, Garlic, Kapoor kacheri, Lemongrass and their derivatives possess effective mosquito repellents' property [17,18]. Essential oil and leave extract may be a substitute to synthetic insecticides because they are eco-friendly, effective, easily biodegradable and inexpensive [10].

Hyptis Sauveolens

Hyptis is a genus of flowering plant in the Lamiaceae family. Hyptis is popularly known as bush mints, which are broadly spread in the tropics and warmer regions of the Americas. There are over 300 species, which may be annual or perennial, and small herb to large shrub [4].

Mentha spicata

Its English name is Spearmint which is 30–100 cm long and is described by its strong odour [19, 20]. It is known for its distinctive smell which makes it very useful as flavouring for foods. It is also commonly used as an indigenous herbal remedy. *Mentha spicata*'s leaves are used as tea infusions and spicing. Its oil extract is used to treat several diseases [21].

Cymbopogon citratus

Cymbopogon citrates is popularly known as lemon grass. Other species are tall, coarse grass with a strong lemon taste. It is a perennial herb widely cultivated in the tropics and sub-tropics, and it comprises of two different species; East Indian *Cymbopogn flexuosus* and West Indian, *Cymbopogon citratus* [22]. *Cymbopogon* is grown as an ornamental in many temperate regions with maximum height of about 1.8m and its leaves about 1.9cm widely covered with whitish blooms [7].

Cymbopogon citratus and *Ocimum* species have been broadly studied and used against mosquitoes. *Cymbopogon* plants have been locally used to repel mosquitoes in remote areas such as the Bolivian Amazon [23, 7]. The oil extract of *Cymbopogon* was discovered to produce the most viable and effective natural repellents in history [24]. [25] reported that *Cymbopogon excavatus* gave about 100% repellence for 2 hours, when it was studied in the laboratory against mosquitoes such as *Anopheles* spp, and its repellence decreased to 59.3% after 4 hours.

Literature revealed several phytochemicals which exhibit harmful effects on mosquitoes and their larva [26]. Some phytochemicals present in the leaves' oil extracts have been found to have antimicrobial, insecticidal and anticonstipative [27, 28], antispasmodial and antioxidant properties [29].

The aim of this work was to get a substitute to chemical mosquito repellents available in the market by obtaining oil extracts from three (3) local leaves through Soxhlet extraction method.



Fig. 1: *Hyptis Sauveolens*.



Fig. 2: *Mentha spicata* plant



Fig. 3: *Cymbopogon citratus*

Materials and Methods

Plant material

Fresh leaves of the plants were collected and washed with distilled/deionized water. The leaves were air dried in a room for about two weeks.

Figure 1 shows *Hyptis Sauveolens* plant, figure 2 shows the picture of freshly plucked leaves from *Mentha spicata* plant while figure 3 shows *Cymbopogon citratus* plants, respectively.

Extraction process

The Soxhlet extraction method was used with hexane as a solvent using method [11, 30]. The oil extracts were left to evaporate to be dried and stored in an airtight glass container until needed.

Cream Formulation

The method of [7] was used for the formulation of a cream/lotion, and the oil extracts of the three leaves (3) were added into the cream using various quantities.

Mosquito repellence test

The mode of the adopted testing was the open room testing. Two different rooms were used: Rat house of the Department of Applied Biology and the Environmental laboratory in the Department of Chemical Engineering of Covenant University, Ogun State, Nigeria. The rat house and the environmental laboratory were studied at night and a large amount of mosquitoes were seen to be flying, this made the rooms suitable for the efficacy test of the leaves' oil extracts. Four students were used as test subjects, with three (3) having different repellent concentrations on their hands and legs, while the remaining one was used as a control.

Phytochemical analysis

Qualitative phytochemical screening of the leaves of *Hyptis Sauveolens*, *Mentha spicata* and *Cymbopogon citratus* plant was carried out using [11] methods to test only for the presence of secondary metabolites.

Results

Table 1: Efficacy test result

Active ingredient (ml)	Oil extract from <i>Hyptis Sauveolens</i>	Oil extract from <i>Mentha spicata</i>	Oil extract from <i>Cymbopogon citratus</i>
0.2	Repelled mosquitoes within 2 hours	Repelled mosquitoes within 1 hours	Repelled mosquitoes within 1 hours
0.4	Repelled mosquitoes within 4 hours	Repelled mosquitoes within 2 hours	Repelled mosquitoes within 2-3 hours
0.6	Repelled mosquitoes within 7-8 hours	Repelled mosquitoes under 4 hours	Repelled mosquitoes within 4-5 hours

Control: Mosquitoes were seen gathering around immediately.

Table 2: Phytochemical screening analysis

Phytochemical Components	Oil extract from <i>Hyptis Sauveolens</i>	Oil extract from <i>Mentha spicata</i>	Oil extract from <i>Cymbopogon citratus</i>
Tannins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Glycosides	+	-	-
Phenols	+	+	+
Steroids	+	+	+
Saponins	+	-	+
Anthraquinones	-	-	+
Alkaloids	+	+	+

NOTE: + = present - = absent

Table 3: GC/MS Analysis

Oil extract of <i>Hyptis Sauveolens</i>	Oil extract of <i>Mentha spicata</i>	Oil extract of <i>Cymbopogon citratus</i>
Sabinene	Carvone	Carvone
Limonene	Neophytadiene	Citral
Bicyclogermacrene	Hexadecanoic acid, methyl ester	Geranial
β - phellandrene	9,12,15-octadecatrienoic acid	Neral
1,8 - cineole	Dihexylcyclopropene	Myrcene
	Phytol	

Discussion

Table 1 shows that, the higher the amount of the extract incorporated into the cream, the longer the time for which mosquitoes were repelled. Therefore, it was observed that the lowest amount of the extract in the cream which was 0.2 ml repelled mosquitoes within 1 hour before they were seen coming near the target in both oil extracts of *Mentha spicata* and *Cymbopogon citratus*, but repelled for about 2 hours in the oil extract of *Hyptis Sauveolens*. In 0.4 ml of extracts of the three leaves, *Hyptis Sauveolens* also had the highest repellence period followed by *Cymbopogon citratus*. The highest amount of extract incorporated into the cream/lotion which was 0.6 ml repelled mosquitoes within 4 -5 hours in both *Mentha spicata* and

Cymbopogon citratus, while the highest repellence in all was observed in *Hyptis Sauveolens* with repellence period of up to 8 hours. Previous research works have been carried out on using local leaves as a substitute to synthetic mosquito repellents [4, 7, 13, 14, 15, 31].

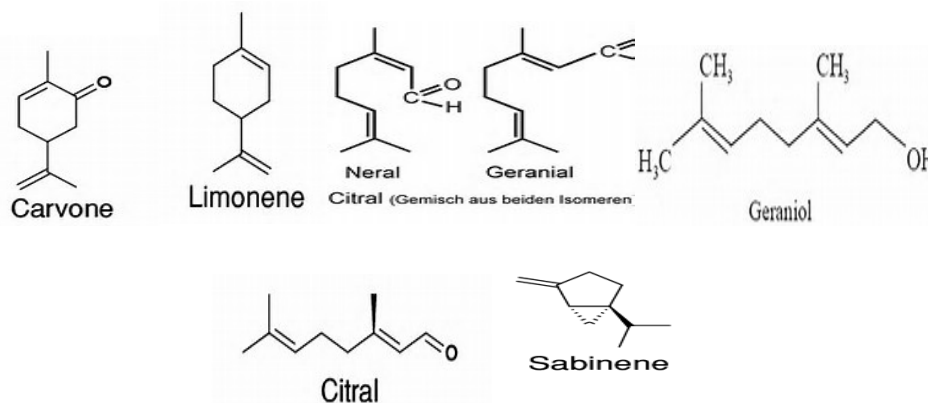
The phytochemical screening of the three local leaves indicated that they all had tannins, steroids, flavonoids, terpenoids, phenols and alkanoids, while glycosides were only discovered in the oil extract of *Hyptis Sauveolens*. Saponins were discovered only in the extract of both *Hyptis Sauveolens* and *Cymbopogon citratus*. While anthraquinones were only indicated in *Cymbopogon citratus*. Plant extracts and phytochemicals are good sources for controlling mosquitoes because of their efficiency, easy biodegradability, eco-friendliness, and may even be applied to mosquito breeding places [32, 33].

GC/MS Analysis

The most abundant composition in the oil extracts were listed in table 3 above. Carvone was found in both extracts of *Mentha spicata* and *Cymbopogon citratus* as the most abundant composition, while citral was the most abundant in the extract of *Hyptis Sauveolens*'s leaf.

Carvone is known to be a member of a family of chemicals called terpenoids. Carvone is naturally found in many essential oil extracts, but is the most abundant in the oils from seeds of caraway and spearmint. Limonene is a solvent with a good scent which naturally occurs in the rind of citrus fruit. 3,7-dimethyl-2,6-octadienal or lemonal (Citral) is either a pair, or a mixture of terpenoids with the molecular formula $C_{10}H_{16}O$. These two compounds are double bond isomers. The E-isomer is known as geranial (citral A). The Z-isomer is known as neral (citral B).

Sabinene is a naturally occurring bicyclic monoterpene with the molecular formula $C_{10}H_{16}$. It is frequently found in essential oils of a variety of plants.



Conclusion

It can be seen from this study that local leaves can be used as a substitute to non-degradable and carcinogenic synthetic mosquito repellents since they have insecticidal properties. Oil extract of *Hyptis Sauveolens* had the highest repellence activity out of the three (3) tested leave oil extracts. The produced mosquito repellent cream/lotion possesses repellence characteristics against mosquitoes. The results from the efficacy test revealed that natural insect repellents obtained from the plant extracts tend to provide coverage for a shorter time. Because of their shorter protection time, natural repellents are ideal for short evening outdoor activities like walking the dog, reading in an open space, classrooms, watering the garden and barbecuing. This study confirms and reaffirms the possibility of using indigenous Nigerian plants' oil extracts with insecticidal properties for the control of Mosquitoes.

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