



Research Article

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Phytochemical and Antimicrobial Activities of the Leaf Oil Extract of Mentha Spicata and its Efficacy in Repelling Mosquito

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ABSTRACT

Synthetic drugs and repellents have been discovered to have adverse toxicity effects apart from the fact that they are no longer efficient due to adaptation of microbes and mosquitoes to them. This study is based on using the extract from local leaves (*Mentha spicata* plant) as treatment for microbial diseases as well as mosquito repellent. Extract of leaf were studied and screened for the presence of phytochemicals (secondary metabolites) and antimicrobial properties against some fungi and bacteria viz., *Pseudomonas aeruginosa*, *Bacillus Subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Escherichia coli* and *Saccharomyces cerevisiae*. The result of the phytochemical screening revealed that the leave extract contained tannin, steroids, flavonoids, terpenoids, phenols and cardiac glycosides while anthraquinones and saponins were not determined. From the microbial analysis the zone of inhibitions indicated that the extract of *Mentha spicata* plant had strong activity against bacteria and fungi used in this analysis. *Mentha spicata* oil extract with the highest concentration when introduced into the produced cream had the highest repellency time lasting up to four [4] hours. The chemical constituents of the leaf oil extract were analyzed using Gas chromatography- mass spectroscopy (GC-MS) and the major chemical constituent identified was carvone.

Keywords: *Mentha spicata*; Phytochemical; Antimicrobial; Oil Extract; Efficacy

INTRODUCTION

Over the years, the continuous use of synthetic insecticides has resulted in resistance in mosquitoes. Mosquitoes are blood-sucking insects which are carriers of deadly diseases like malaria, yellow fever, zika virus and dengue fever [1]. Mosquito-borne diseases cause significant morbidity, mortality and economic burden to humankind [2]. Major source of illness and death all over the world is due to transmitted diseases by mosquitoes [3]. Every year about 700 million persons get affected by diseases transmitted by mosquitoes [4]. More people died every year from mosquito-borne disease than from any other single cause diseases e.g. Malaria, Filariasis (Caused by *Brugia malayi*, spread by anopheles' mosquito), Encephalitis (a viral diseases transmitted by adult female mosquito of the spp *Aedane* and *culcine*) and Yellow fever [5].

Essential oils extracted from different plant families have been shown to have high repellence against arthropod species [6]. These synthetic insecticides are toxic and affect the environment by contaminating the soil, water and air [7]. N, N-diethyl-3-methylbenzamide (DEET) is a non-natural chemical that is present virtually in all mosquito-repellent formulations in the market. DEET based synthetic mosquito repellent cause unrepairable damage to ecosystem since they contain chemicals which are not easily broken down to simpler units in nature [8,9]. Although it serves as a very excellent mosquito repellent, some concerns are associated with its use such as irritation to mucus membranes, concentrated formulations dissolve plastic and reports on some human toxicity effects have been received [10].

Effect of other essential oil producing plants, such as *Cymbopogon* spp., *Eucalyptus* spp. and *Ocimum* spp. have reported and used against mosquitoes. *Cymbopogon* leaf is locally used to repel mosquitoes in remote regions such as the Bolivian Amazon [2, 11]. The leaf oil extract of *Cymbopogon* was found to produce the most effective natural repellents in the world [12]. [13] reported that *Cymbopogon* oil extract gave 100% repellence for two [2] hours, when it was analyzed against *Anopheles* spp, its repellence decreased to 59.3% after four [4] hours. Ayurveda is another herbal source with mosquito repellent properties which have been reported in so many research works [14, 15]. Considerable research efforts have proved that essential oil compound like Tulsi, Clove, Garlic, Kapoor kacheri, Lemongrass species possesses effective mosquito repellents property [16,17]. Essential oil and extracts from plant may be a substitute to non-natural insecticides because they are effective, eco-friendly, easily biodegradable and inexpensive [7].

Mentha spicata has been used as a medicinal and aromatic plant since prehistoric times. The English name given to it is Spearmint and it is 30–100 cm long. It is characterized by its attractive strong odor [18, 19]. It is known for its distinctive smell which makes it very useful as a flavoring for foods. It is also used commonly as a domestic herbal remedy. Its leaves in its natural form can be used as flavoring, tea infusions and spicing. In addition, mint oil is used to treat several diseases [20]. Previous investigations have reported that various - *Mentha* plant spp. oil have shown larvicidal effect on *C. pipiens*, *C. quinquefasciatus*, *A.aegypti* *A. stephensi* and *Aedes tesselatus* [21].

Phytochemicals present in plant extract have been reported by several researchers to exhibit detrimental effects on insects especially mosquitoes and their larva [22]. Phytochemicals can group into two categories [23], namely primary metabolites such as Amino acids, chlorophyll etc. and secondary metabolites which are Alkaloids, flavonoids, Tannins, Saponins etc. [24]. Some of these phytochemical have been found to have insecticidal, antimicrobial, and anticonspitative properties [25, 26] antispasmodial and antioxidant activities [27].

This research was carried out to find an alternative to synthetic mosquito repellents and antimicrobial creams using natural sources instead of synthetic formulation. The study is based on using the extract from local leaves (*Mentha spicata* plant) as treatment for microbial diseases as well as mosquito repellents. Soxhlet extraction method was used using two different solvents (hexane and petroleum ether).



Figure 1: *Mentha spicata* plant

MATERIALS AND METHODS

Plant material

Fresh mint leaves were obtained commercially from Festac market, Lagos Nigeria. The leaves were washed with distilled water and air dried in a room for about two weeks.

Extraction process

The Soxhlet extraction method was used to carry out the extraction process of dried leaves using equal volumes (250ml) of the two different solvents (hexane and petroleum ether) using method [28]. The extracts were left to evaporate to dryness and stored in an airtight glass bottles until needed.

Cream Formulation

The method of [6] was used for the formulation of cream, and the oil extract of *Mentha spicata* was added in various quantity.

Mosquito repellency test

The mode of testing adopted was the open room testing. Two different rooms were used. The animal room of the Department of Applied Biology and the laboratory room in the Department of Chemical Engineering of Covenant University, Ogun State, Nigeria. Both rooms were inspected at night and a large number of mosquitoes were seen to be flying which made the rooms suitable for the test to be carried out. Four students were used as test subjects, three having the three different repellent concentrations on their hands and legs, while the fourth student was used as control.

Microorganisms

Clinical isolates of six microbes (four bacteria and two fungi) *Pseudomonas aeruginosa*, *Bacillus Subtilis*, *Staphylococcus aureus*, *Aspergillus Niger*, *Escherichia coli* and *Saccharomyces cerevisiae* were obtained from the Applied Biology and Biotechnology Unit of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria.

Determination of Antimicrobial analysis

The isolates were analyzed for viability by resuscitating them in buffered peptone water after which they were sub-cultured into nutrient agar medium and later incubated at 37° C for 24 hours. Antimicrobial activity of the extract was determined using the agar well diffusion techniques as described by [29, 30] for the study. 18 ml of Mueller Hinton agar plates (MHA oxiod) England, were inoculated with 0.1 ml of an overnight broth culture of each bacteria and fungi isolate (Equivalent to 3×10^7 cfu/ml) MF (McFarland standard) [31] in sterile petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set. Holes were bored on the plates by using standard sterile cork borer of 9 mm diameters and equal volumes of the plants extracts (1000µl) were transferred into the well with the aid of sterile micropipette. The experiments were carried out in duplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract [32]. The plates were incubated at 37°C for 24 hours until marked decline in the potency of the extracts to inhibit the growth of the test isolates was observed. The zone of inhibition was measured in millimeter (mm) and average values were calculated and recorded.

Phytochemical analysis

Qualitative phytochemical screening of *Mentha spicata* was carried out using the following methods to test only for the presence of secondary metabolites.

Test for Tannins: 0.008 M Potassium ferricyanide was added to 1 ml of the extract in a test tube. 1 ml of 0.02 M Ferric chloride containing 0.1 N Hydrochloric acid was also added. A blue-black colouration was observed.

Test for Saponins: 5 ml of distilled water was added to crude extract. The mixture was shaken vigorously with some drops of olive oil in a test tube. Stable foam was observed which an indication of the presence of saponins.

Test for Flavonoids: Crude extract was added to 5 ml of diluted ammonia solution and concentrated H₂SO₄. Yellow colouration which disappeared on standing indicates the presence of flavonoids.

Test for Terpenoids (Salkowski test): 2 ml of chloroform and 3 ml of concentrated H₂SO₄ was added to 5 ml of extract to form a layer of reddish brown colouration.

Test for Phenols: 2ml of 10% ferric chloride was added to 2ml of the leaf oil extract. A bluish green color was formed to show the presence of phenols.

Test for Steroids: To 1ml extract 1ml of chloroform and equal volume of concentrated H₂SO₄ acid was added from the walls of the test tube. A red colour in the upper layer and yellow with green fluorescence was observed to show the presence of steroids.

Test for Cardiac Glycosides: Glacial acetic was added to 1ml of the leaf extracts. Few drops of ferric chloride was added with concentrated H₂SO₄. A reddish brown at the junction of two layers was observed this indicates the presence of cardiac glycosides.

Test for Anthraquinones: 10ml of sulphuric acid was boiled with 5ml plant extract and filtered. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted out into another test tube then 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

RESULTS

Phytochemical screening

Table 1. Phytochemical composition of *Mentha spicata*

Phytochemical Components	Inference
Tannins	+
Terpenoids	+
Flavonoids	+
Cardiac glycosides	+
Phenols	+
Steroids	+
Saponins	-
Anthraquinones	-

NOTE: +: present - : absent

Antimicrobial analysis

Table 2 . Zone of Inhibition (mm)

S/N	organisms	H1	P1	C(H)1	C(P)1	C	H2	P2	C(H)2	C(P)2	C
1	Pseudomonas aeruginosa	15	17	-	-	-	14	16	-	-	-
2	Bacillus Subtilis	10	12	-	-	-	11	11	-	-	-
3	Escherichia coli	25	26	-	-	-	25	27	-	-	-
4	Staphylococcus aureus	26	27	-	-	-	25	26	-	-	-
5	Saccharomyces cerevisiae	25	24	-	-	-	24	25	-	-	-
6	Aspergillus niger	26	27	-	-	-	25	26	-	-	-

H- Hexane

P- Petroleum ether

C(H)- Hexane control

C(P)- Petroleum ether control

Mosquito repellency test

Table 3. Efficacy test result

Cream (g)	Active ingredient (ml)	Repellence
20	0.3	Repelled mosquitoes within 1 hour
20	0.5	Repelled mosquitoes within 2 hours
20	0.8	Repelled mosquitoes under 4 hours
20	Control	Bite was felt immediately

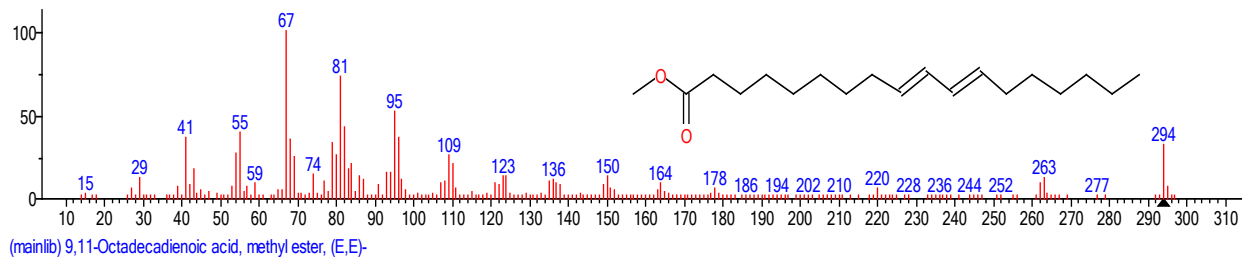
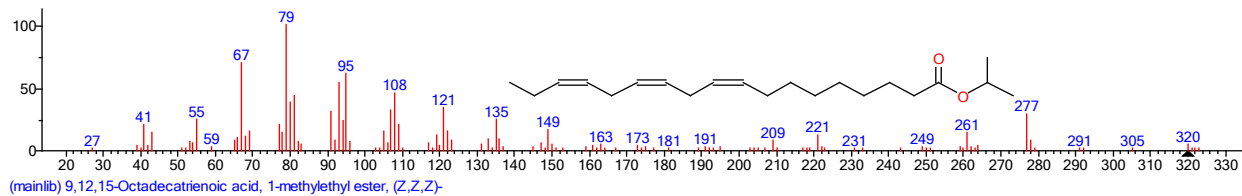
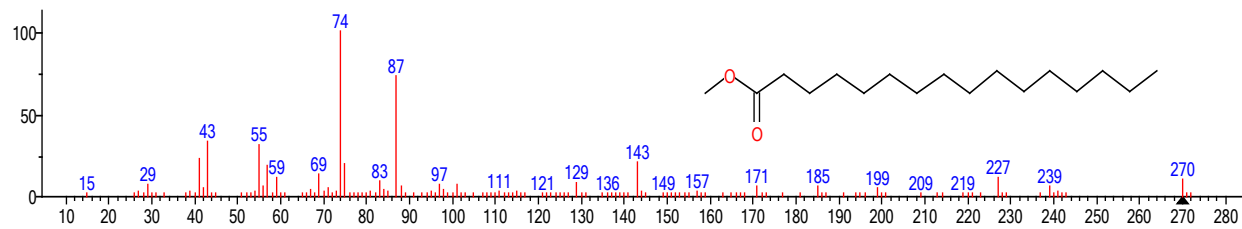
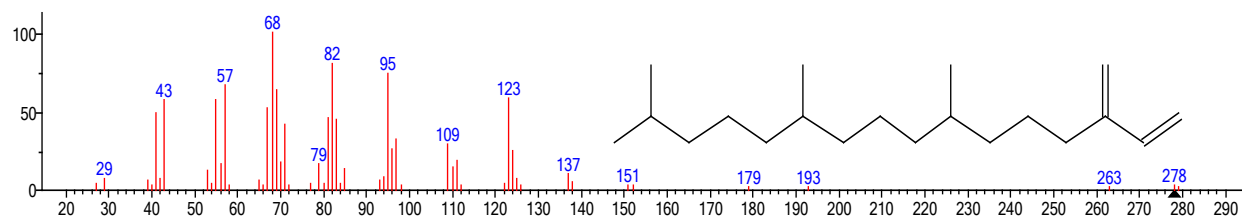
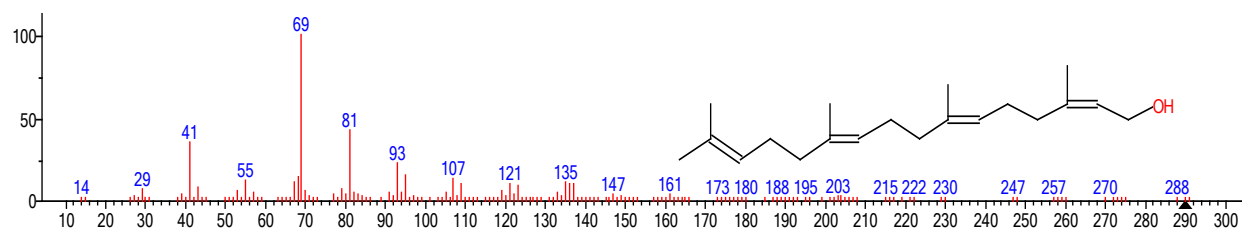
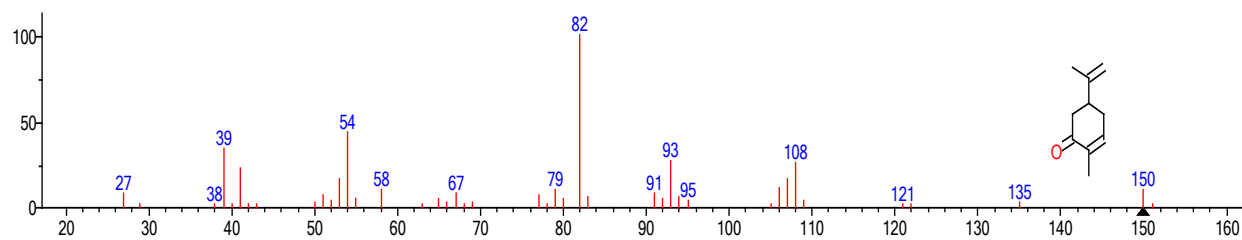
DISCUSSION

Phytochemical screening of the extract of *Mentha spicata* (Table 1) indicated that the plant had tannin, steroids, flavonoids, terpenoids, phenols and cardiac glycosides while saponins and anthraquinones were not determined. [33,34] had previously reported and established the phytochemical properties of *Mentha spicata* plant. Thus the antimicrobial activity on the microorganism tested were due to the presence of the phytochemicals earlier mentioned in the leaf extract. This study reported the leaf extract of *Mentha spicata* to be an effective inhibitor of microbial growth as they showed varying degrees of activity on the test microorganisms [Table 2].

It was observed in table 2 that *Pseudomonas aeruginosa* and *Bacillus ubtilis* resisted both extracts. (i.e. very insignificant activity by the extracts against the microbes were recorded). The growth of the other two bacteria and fungi were inhibited by the extract. The results also reported that the extract from petroleum ether had the highest activity against *Staphylococcus aureus* and *Aspergillus niger* followed by *Escherichia coli* and lastly *Saccharomyces cerevisiae*. The extract from hexane had the highest activity against *Staphylococcus aureus* and *Aspergillus niger* followed by *Escherichia coli* and lastly *Saccharomyces cerevisiae*. It was observed that the activity of the two extracts were very similar as the measurements of the zones of inhibition are very close as seen in the table of results. This shows that there is no significant difference between the compositions of the oil extract from hexane and that of petroleum ether.

From table 3, it was observed that the amount of the oil extract introduced into the cream is directly proportional to the amount of time in which the repellent lasted (i.e. the higher the amount of the extract introduced into the cream, the longer the time for which mosquitoes were repelled). Therefore, the analysis of the results showed that the lowest amount of the oil extract in the cream which was 0.3 ml repelled mosquitoes for about 1 hour, while the highest amount of extract introduced into the cream which was 0.8 ml repelled mosquitoes for about 4 hours. The results reported here were in agreement with previous work done on using local leaves to repel mosquito, [5, 6, 35, 36, 37, 38]. From the established experimental result, it was observed that oil the extract of *Mentha* plant repelled mosquito at different concentrations.

GC/MS Analysis for Hexane Extract



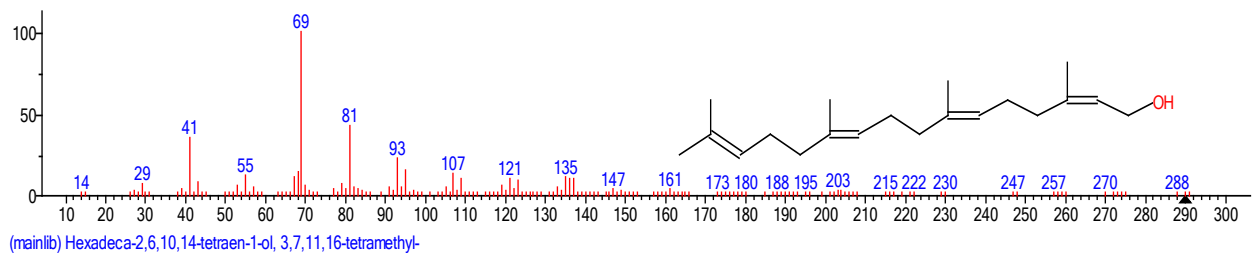
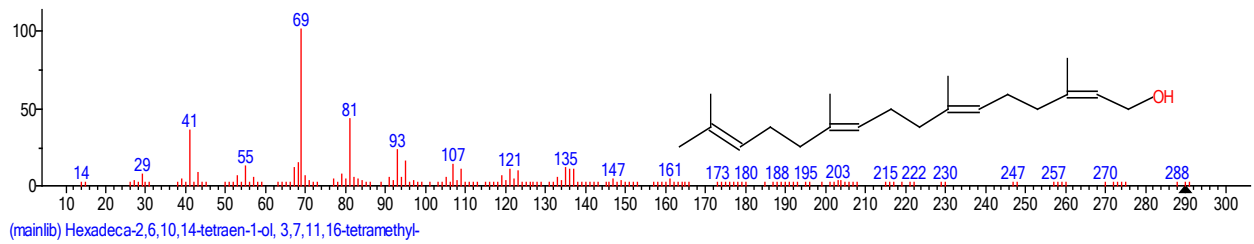
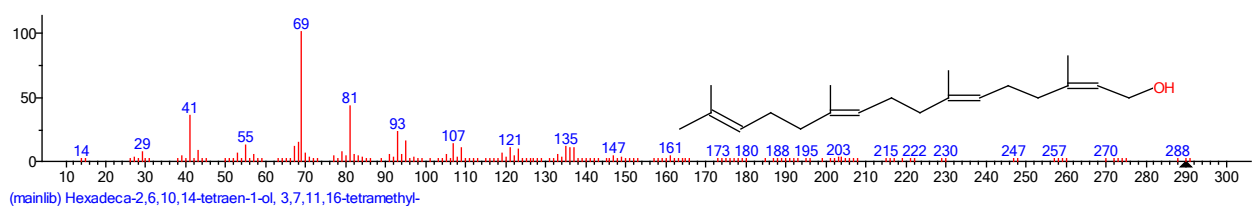
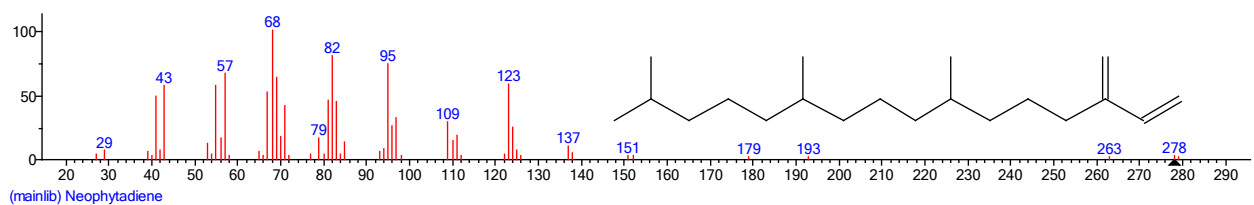
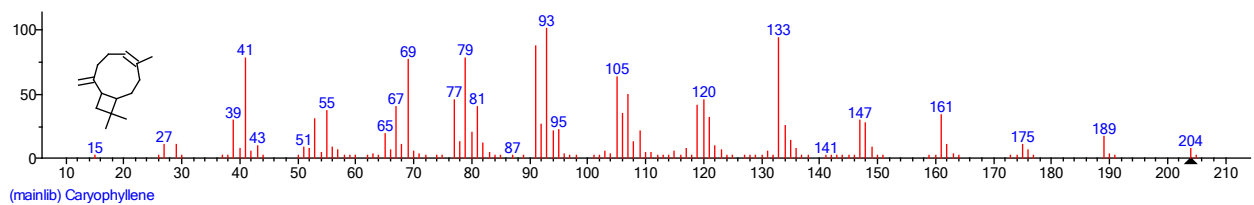
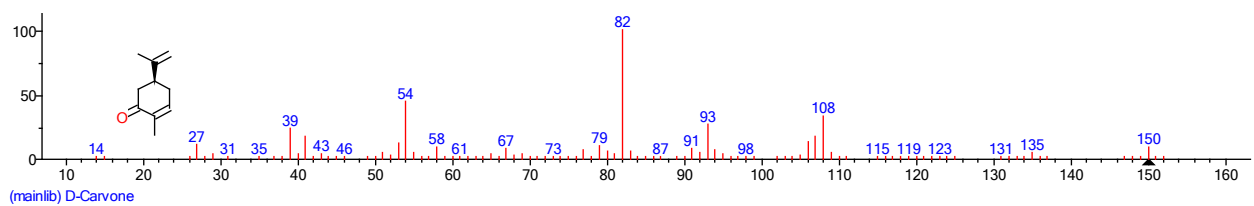


Figure 2: Mass Spectrum of fragments of most abundant chemical constituent of the Hexane extract

GC/MS Analysis for Petroleum Ether Extract



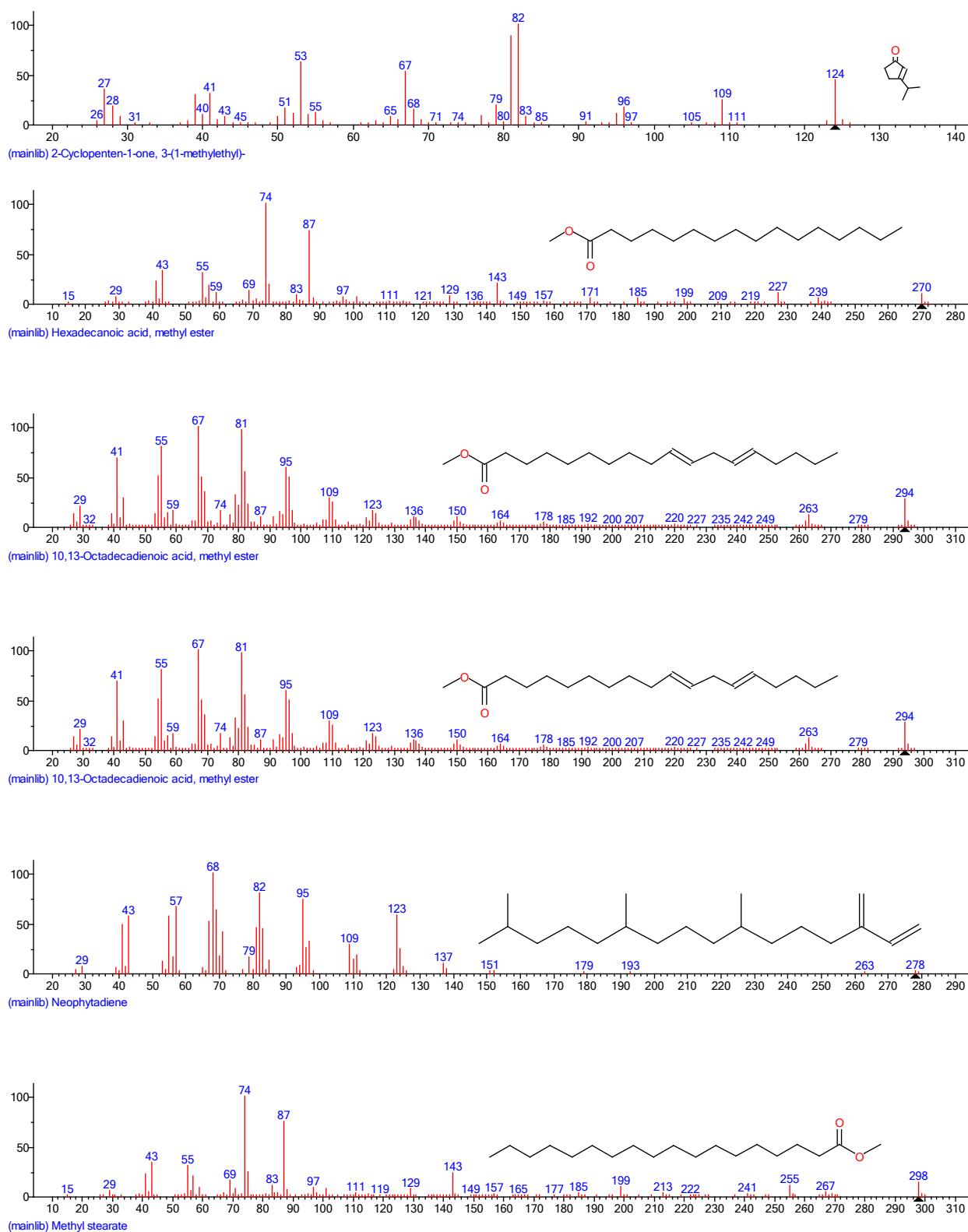


Figure 3: Mass Spectrum of fragments of most abundant chemical constituent of the Petroleum Ether extract

From the GC/MS analysis result, the most abundant component of the extracts is Carvone. Other peripheral components include;

- Neophytadiene
- Hexadecanoic acid, methyl ester
- 9,12,15-octadecatrienoic acid

Both solvent used had similar components when analyzed with GC/MS.

Carvone

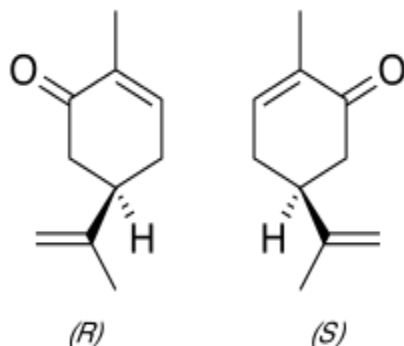


Figure 4: Structural formula of carvone

Carvone is known to be a member of a family of chemicals called terpenoids. It's preferred IUPAC name is 2-Methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-one. Carvone was found to be the most abundant component in the extract of *Mentha spicata* leaves with the area percentage of 27.68%. Carvone has been suggested as a mosquito repellent in previous years and is currently under review to be used as a pesticide commercially.

Neophytadiene

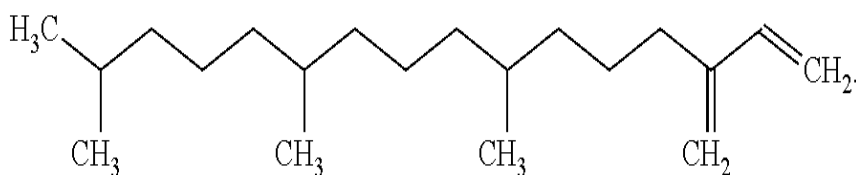


Figure 5: Structural formula of Neophytadiene

Hexadecanoic acid, methyl ester

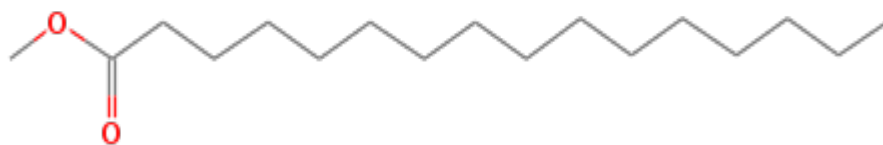
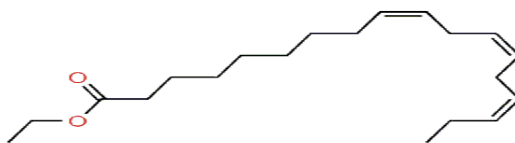


Figure 6: Structural formula of Hexadecanoic acid

It is also known as palmitic acid. It has a chemical formula $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$. It is known to be the most saturated fatty acid found in plants, animals and microorganisms.

9,12,15-octadecatrienoic acid**Figure 7:** Structural formula of 9,12,15-Octadecatrienoic acid

This is also known as alpha-Linolenic acid. It is referred to as an n-3 fatty acid. It is a carboxylic acid with an 18-carbon chain and three cis double bonds.

CONCLUSION

At the end of the study, it was concluded that the extract of *Mentha spicata* leaves can be used as treatment for microbial diseases caused by *Staphylococcus aureus*, *Aspergillus niger*, *Escherichia coli* and lastly *Saccharomyces cerevisiae*. It was also concluded that the extract of *Mentha spicata* leaves had mosquito repelling properties. This confirmed the historical use of the leaf as antibacterial agent and mosquito repellent. The active component of the extract was discovered to be Carvone.

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