Sudan University of Science and Technology

College of Graduate Studies

Extraction, Constituent and Biological Activity of some Phytochemicals

الاستخلاص والمكونات والفعاليه البيولوجيه لبعض المواد الكيميائيه النباتيه

A Thesis Submitted in Fulfillment for the Requirements of the Ph.D. Degree in Chemistry

by

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October, 2020

قال تعالى:

(اللَّهُ لَا إِلَهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ وَلَا يَئُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ (٢٥٥))

صدق الله العظيم

سورةالبقرة

Dedication

To

My parents,

Brother and Sisters

Acknowledgement

First of all I would like to thank the most Merciful, Omnipotent, and Omniscient Almighty **Allah** for the great help and blessing during my whole life and especially in this research.

It is my pleasure and honor to express my gratitude to my supervisor Professor Mohamed Abdel Karim Mohamed, who has given me much of his time for suggestions and supervision of this work. I have learned a lot from him and I hope to learn more. I was lucky to have a chance to work with him. I am very proud of being one of his students.

I am grateful to my co-supervisor Dr. Mahmoud Mohamed Ali for his advice, guidance and valuable assistance.

My deep thanks also are extended to the technical staff –Chemistry Dept. –Sudan University of Science and Technology for all facilities.

Thanks are extended to my colleages Dr. Mohamed Alaa Eldin and Amin for their infinite support.

Abstract

This research was designed to study the oils of six potential medicinal plants collected from Jordon: *Foeniculum vulgare, Pimpinella anisum, Nigella sativa, Carum carvil, Petroselinum crispum and Lepidium sativum.* GC-MS analysis of the targeted plants, was conducted and the identification of the constituents was accomplished.

Foeniculum vulgare, Pimpinella anisum, and Carum carvil oils contained the following components as major constituents in different ratios:

-9-Octadecenoic acid (Z)-, methyl ester

- 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

- Hexadecanoic acid, methyl ester

Petroselinum crispum and Lepidium sativum oils contained the following components as major constituents in different ratios:

-6-Octadecenoic acid, methyl ester, (Z)-

- 9,12-Octadecadienoic acid (Z, Z)-, methyl ester

Nigella sativa oil gave the following major constituents:

-9, 12-Octadecadienoic acid (Z, Z)-

- Oleic Acid

- n-Hexadecanoic acid

Pimpinella anisum oil showed moderate anticandidal activity. It also exhibited significant activity against Gram positive *Staphylococcus aureus, Bacillus subtilis* and Gram negative *Escherichia coli.*

Nigella sativa oil exhibited moderate activity against Gram positive *Staphylococcus aureus, Bacillus subtilis.* and it give significant effect against Gram negative *Pseudomonas aeruginosa,*

Carum carvil showed significant anticandidal activity. It also exhibited moderate activity against Gram positive *Staphylococcus aureus, Bacillus subtilis* and Gram negative *Escherichia coli.*,

المستخلص

يشتمل البحث على دراسة التركيب الكيميائي لزيوت ستة نباتات طبيه تنمو بالاردن وهذه الزيوت هي لبذور كل من الشمار،اليانسون،الكمون الاسود،الكراويه،البقدونس وحب الرشاد. كذلك يتضمن البحث دراسة حول فعالية الزيوت كمضادات ميكر وبات.

أهم المكونات في زيت بذوركل من الشمار،اليانسون والكراويه بنسب مختلفه هى:

-9-Octadecenoic acid (Z)-, methyl ester

- 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

- Hexadecanoic acid, methyl ester

واهم المكونات في زيوت بزور كل من البقدونس وحب الرشاد بنسب مختلفه هي:

-6-Octadecenoic acid, methyl ester, (Z)-

- 9,12-Octadecadienoic acid (Z, Z)-, methyl ester

اما زيت بذور الكمون الاسود اهم المكونات هى:

-9, 12-Octadecadienoic acid (Z, Z)-

- Oleic Acid

- n-Hexadecanoic acid

تناولت الدراسة أيضا فعالية الزيوت كمضاد بكتيري أو فطري.

اظهر تقييم زيت اليانسون نشاط معتدل كمضاد بكتيري وكذلك تاثير فعال كمضاد فطري. زيت الكمون الاسود اظهر نشاط قاتل كمضاد بكتيري وكذلك نشاط معتدل كمضاد فطري.اظهر زيت الكراويه نشاط معتدل كمضاد بكتيري وفطري.

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Introduction

1.1- General approach

During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world[1]. There are hundreds of significant drugs and biologically active compounds developed from the traditional medicinal plants. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic antipyretic and many other pharmacological effects[2]. Now medicinal herbs are excellent alternative to chemical drugs, one of the major reason for this is short side effect compared to chemical drugs[3,4]. Plants have all time played a vital role in the health and treatment of human society[5]. Medicinal herbs have fewer side effects than synthetic drugs and due to their antioxidant property they reduce drugs toxicity[6,7]. Also, the natural effective ingredients cause biological balance and prevent drug accumulation in body[8]. So herbal plants can be used in the treatment of different diseases [9-14]. From 422 000 flowering plants around the world, more than 5000 ones are used for medicinal purposes.

1.2- *Foeniculum vulgare*

Foeniculum vulgare (Fennel) is one of the ancient spice plants which were widely grows in arid and semi-arid and due to its economic significance and pharmaceutical industry practice, it is one of the world's main medicinal herb[15].

Fig 1-1: Foeniculum vulgare

The seed essential oil of *Foeniculum. vulgare* has also been reported to possess antibacterial activity against some human pathogenic bacteria. Ethanol and water extracts of *F. vulgare* have shown activity against *Campylobacter jejuni* and *Helicobacter pylori*. *F. vulgare* essential oil has been shown to exhibit potential for the control of mult-idrug resistant towards *Acinetobacter baumannii* infections. Some chemical constituents from *F. vulgare* have been identified as active antimicrobial principles such as a phenyl propanoid derivative – dillapional which was found to be the active antimicrobial principle of the *F. vulgare* stem. Another molecule –

scopoletin which is a coumarin derivative has been isolated from *F. vulgare* and reported to possess marginal antimicrobial effect[15].

Fennel has anti-inflammatory, antispasmodic, antiseptic, carminative, diuretic and analgesic effect and is effective in gastrointestinal disorder treatment. It has anti-ulcer and anti-oxidant properties and is used to treat neurological disorders[16,17].

1.3- *Pimpinella anisum*

Pimpinella anisum **L.,** is a plant belonging to the Umbelliferae family, and it is one of the oldest medicinal plants. It is an annual grassy herb reaching 30–50 cm in height with white flowers, and small green to yellow seeds.

Fig 1-2: Pimpinella anisum

Fig 1-3: Pimpinella anisum

This plant grows in Sudan the eastern Mediterranean region, west Asia, the Sudan, Middle East, Mexico, Egypt, and Spain[18]. *P. anisum* is primarily grown for its fruits (anise seeds). Anise seeds contain 1.5–5% essential oil and are used as flavouring, digestive, carminative, and for the relief of gastrointestinal spasms. Consumption of seeds by lactating women increases milk and also reliefs their infants from gastrointestinal problems [19]. In the food industry, anise is used as flavoring and aromatic agent for fish products, ice cream, sweets, and gums[18-20].

Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine[21]. Seeds have warm and dry nature and can increase milk production, menstruation, urine, and sweat secretion and also making good complexion. It is also effective in polishing of teeth.

In some traditional texts, anise is mentioned for melancholy, nightmare, and also in treatment of epilepsy and seizure[22,23] .

The antibacterial activities of the aqueous, 50% (v/v) methanol, acetone and petroleum ether extracts of *Pimpinella anisum* L. fruits were tested against 4 pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherchia coli, and Klebsiella pneumoniae*) by disc diffusion method. The results showed that only aqueous and methanol extracts exhibited fair antibacterial activity against all of the test bacteria and the aqueous extract was found to be more effective than methanolic extract, whereas acetone and petroleum ether extracts cannot inhibit the growth of the pathogenic test bacteria[24].

1.4- *Nigella sativa*

The seeds of *Nigella sativa* L. (Ranunculaceae), have been employed for thousands of years as a spice, food preservative and curative remedy for numerous disorders[25,26]. The historical tradition of seeds used in medicine is substantial. *N. sativa* is known to have beneficial effects on a wide range of diseases. The plant showed antiasthmatic[27], antitumor[28], antiviral[29], antibacterial[30], anti-inflammatory[31], gastroprotective[32], antimalarial[26], antihypertensive[33], antidiabetic[34], anti-atherosclerotic[35], antioxidant[36], nutritional[37] and anti-cholesteroleamic[38] properties.

Thymoquinone, the main constituent of the essential oil of *N. sativa* seeds, has been shown to exert beneficial effects on acute gastric ulcer[39]. In addition, thymoquinone and its reduced product thymohydroquinone have been reported to have an antibacterial activity and beneficial interaction with some antibiotics[40].

Fig 1-4: Negilla sativa

Diabetes mellitus (DM) is one of the most common lifestyle diseases. Diabetes had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 [41]. Prevention and control of DM is a major challenge and requires molding lifestyle towards more physical activity and less calorie intake avoiding sedentary habits. However, most people find it difficult to change their lifestyle and look for a less cumbersome alternative[38]. A

traditional component of food that can reduce appetite, glucose absorption in intestine, hepatic gluconeogenesis, blood glucose level, body weight, and can stimulate glucose induced secretion of insulin from beta-cells in pancreas[39], may prove to be useful for prevention and control of diabetes mellitus. Most of these actions have been shown by seeds of *Nigella sativa* and their constituents in animal experiments and at the same time have not exhibited adverse effects[39].

1.5- *Carum carvi*

Carum carvi **L.** belongs to the family Apiaceae. It is one of the earliest cultivated herbs in Africa, Asia and Europe.

Fig 1-5: Carum carvi

In folk medicine, this plant is used as a carminative for stomach disorders, diarrhea, and colic, as well as particularly in veterinary medicine[42]. Caraway has a long history of use as a household

remedy especially in the treatment of digestive complaints where its antispasmodic action soothes the digestive tract and its carminative action relieves bloating caused by wind and improves the appetite[43-45]. It is often added to laxative medicines to prevent griping[44]. The seed is antiseptic, aromatic, anaesthetic, anodyne, antianxiety, diuretic, mildly expectorant, fungicidal, muscle relaxant, soporific, tonic, emmenagogue, expectorant, galactogogue and stimulant[43,46]. It can be chewed raw for the almost immediate relief of indigestion and can also be made into infusions. The seeds are also used in the treatment of bronchitis and are an ingredient of cough remedies, especially useful for children and for mothers for increasing breast milk. A tea made from the seeds is a pleasant stomachic and carminative, it has been used to treat flatulent colic[46,47]. The seed is used in Tibetan medicine where it is considered to have an acrid taste and a heating potency. It is used to treat failing vision and loss of appetite[48]. An essential oil from the seed is used in perfumery, for scenting soap, as a parasiticide etc.[49]. *C. carvi* seeds are used in traditional Sudanese medicine and other folk medicines as a carminative, since it is effective against spasmodic gastrointestinal complaints, flatulence, irritable stomach, indigestion, lack of appetite, and dyspepsia in adults[50].

1.6- *Petroselinum crispum*

Petroselinum crispum (Parsley) belongs to the Apiaceae family. It is a well-known spice and vegetable. Its herb and root are widely known for their effects on digestion, stomach, kidney, blood, and liver[51]. Parsley has been claimed in Arab Traditional Medicine to possess variety of properties including laxative, diuretic and antiurolithiatic.

Fig 1-6: Petroselinum crispum

The leaves are used as hot application against inflammatory condition, mastitis and haematomata[52]. Parsley, widely used as a salad ingredient or as a healthy garnish, is capable of masking foul odors, as it has a spicy scent.

Parsley is a medicinal herb used in folk medicine remedy to decrease the blood glucose level in Turkey[53], and it shows a protective effect

against hepatic toxicity caused as a complication of diabetic state[54].The plant therapy can provide blood glucose homeostasis and can regenerate the B-cells of the endogenous pancreas[55]. Also parsley has been claimed in folk medicine to possess laxative properties attributed to the presence of some volatile oil that are more concentrated in seeds than in stems or leaves[55]. Parsley have advocated diuretic effect in folk medicine, and it is mediated through an inhibition of sodium- potassium pump that would lead to the reduction in sodium and potassium re- absorption leading, thus, to an osmotic water flow in to lumen and diuresis[56].

1.7- *Lepidium sativum*

Lepidium sativum L. is a plant in the family Cruciferae. The seeds of *L. sativum* contain essential oils, fatty oils, carbohydrate, protein, fatty acids, vitamins, flavonoids and isothiocynates glycoside[57].

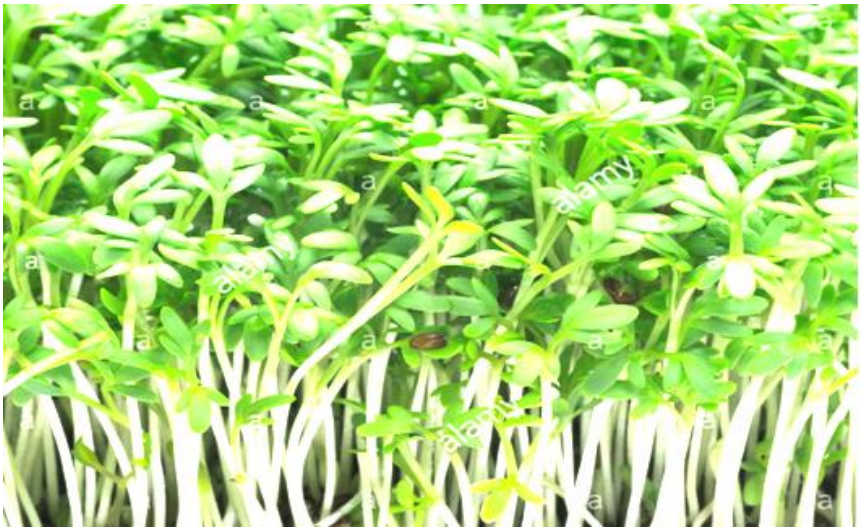


Fig 1-7: Lepidium sativum

Lepidium sativum is an annual, herbaceous edible plant that is botanically related to mustard and watercress. *Lepidium sativum* is native to Egypt and South west Asia. Locally known as “El-Rshad”. It is a fast-growing, edible herb with tangy flavor and aroma[58].

The seeds of *L. sativum* (LSS) are also used to cure throat diseases, asthma, headache, uterine tumor, nasal polyps and breast cancer. Seeds contain some metals including: silver (Ag), cadmium (Cd), iron (Fe), copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), arsenic (As), chromium (Cr), and platinum group elements. Copper and zinc are essential trace elements for living organism at low concentration (< 10 mg/L), however, they become toxic at high concentration (>10 mg/L[59].

The seeds of *L.* sativum are aperient, diuretic, tonic, demulcent, carminative, galactogogue and emmenagogue. They are boiled with milk and are used to procure an abortion. Seeds have been applied as a poultice to pains and hurts and are also used in the treatment of bacterial and fungal infections[60]. Traditional sweets for lactating mothers are prepared from the seeds. The seeds of this plant also possess rapid bone fracture healing ability.

1.8-Gas Chromatography coupled to Tandem mass spectrometry

Essential oils(EOs) analysis is based mostly on separation techniques giving the best performance, achieved by the most

effective tool. The most popular tool used by scientists for separation techniques is the chromatography and coupled to that is often the mass spectrometry for the identification of components. Analysis of EOs have recently known major developments with varying methods adapted from the conventional gas chromatography coupled to mass spectrometry technique. The driving force of this surge has been the characterization and identification of the structure of known and novel molecules. The advantage of using a gas chromatograph is that it provides the conditions required for achieving the separation of analyte components without lowering the performance of the column when it comes to more complex analysis. However, gas Chromatography can be insufficient or difficult to interpret. Presently, we have seen in the literature the use of the gas chromatography coupled to Tandem mass spectrometry. It is a powerful analytical technique which offers the possibility of detecting specific, targeted compounds whether present in large amount or in trace[61].

Following the separation by gas chromatography, the Tandem mass spectrometry operates by selecting the target ions having specific and known mass. These ions are then dislocated by collision with helium molecules. The product ion resulting from this collision gives a spectrum which confirms the target analyte as even if there is another ion with the same mass, the spectrum will be different[61].

This factor increases the selectivity of the tandem mass spectrometry. The target gas, which can be argon, xenon, helium or other (according to choice of energy desired for the collision ion dissociation process), can play an important role in the results as the pressure and temperature of the target gas affect the internal energy distribution and thus affecting also the mass spectrum. Hence, low energy target gas is less reproducible[61]. Whereas the high energy target gas for the collision ion dissociation process was found to be more reproducible and to give less rearrangement in the mass spectrum making it less complex to analyze[61]. According to the literature, gas chromatography coupled to Tandem mass spectrometry is not only commonly used for the regular analysis of EOs but it remains however an accurate tool for the separation and detection of trace elements found in a complex mixture.

1.9- Essential oils

The term essential oil is derived from the drug *(Quinta essential*), named by Paracelsus von Hohenheim of Switzerland [62]. Numerous authors have attempted to provide a definition of essential oils. The French Agency for Normalization gives the following definition, the essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or dry distillation. The essential oil is then separated from the aqueous phase by physical means [63].

The essential oil is a liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the “oil of” the plant material from which they were extracted, such as oil of clove. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majorities of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits [64].

Essential oil may occur in various parts of aromatic plants:

• Flowers, of course, including: orange, pink, lavender, and the (clove) flowerbud or(ylang-ylang) bracts,

• Leaves, most often, including: eucalyptus, mint, thyme, bay leaf,savory, sage, pine needles,and tree underground organs, e.g., roots (vetiver),

• Rhizomes (ginger, sweet flag),

• Seeds (carvi, coriander),

• Fruits, including: fennel, anise, Citrus epicarps,

• Wood and bark, including: cinnamon, sandalwood, rosewood.

1.9.1-Methods of extraction of essential oils

An essential oil may be extracted from plant raw material by several methods [65,66]

1.9.1.1-Classical and conventional methods

There are several conventional techniques used for the extraction of essential oils including the following techniques:

i)Hydrodistillation

The conventional method for the extraction of essential oils is hydrodistillation (HD), in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser. The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively[67,68].

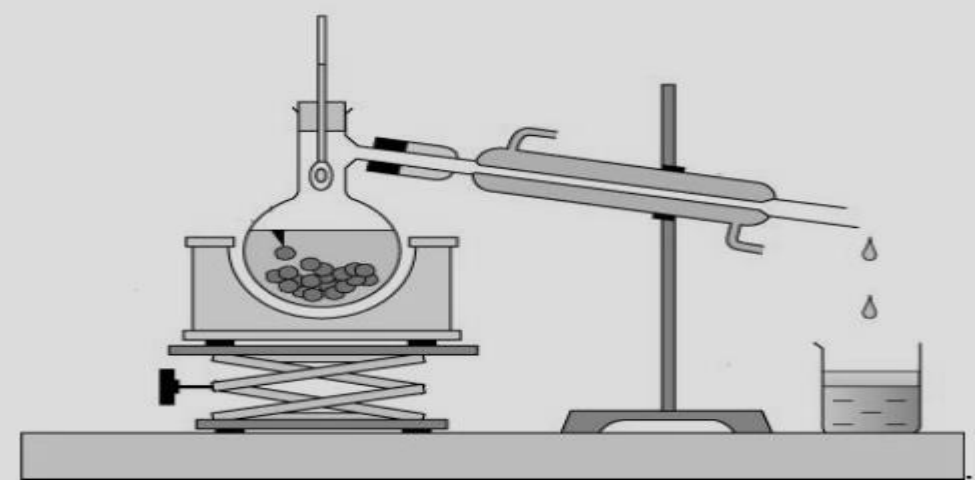


Fig 1-8: Apparatus for hydrodistillation

ii)Solvent extraction

Liquid–liquid extraction, partitioning or solvent exraction, is a method to separate a compound based on the solubility of its parts. This is done by using two liquids that don't mix, for example, water

and an organic solvent. In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent[69].

iii)Soxhlet extraction

The Soxhlet extractor was basically designed for the extraction of a lipid from a solid matrix. This technique involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid [69].

iv)Cold pressing method

During cold pressing the oil is extracted from its matrix at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils [70]. In this process, the outer layer of the plants contains the oil are removed by

scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

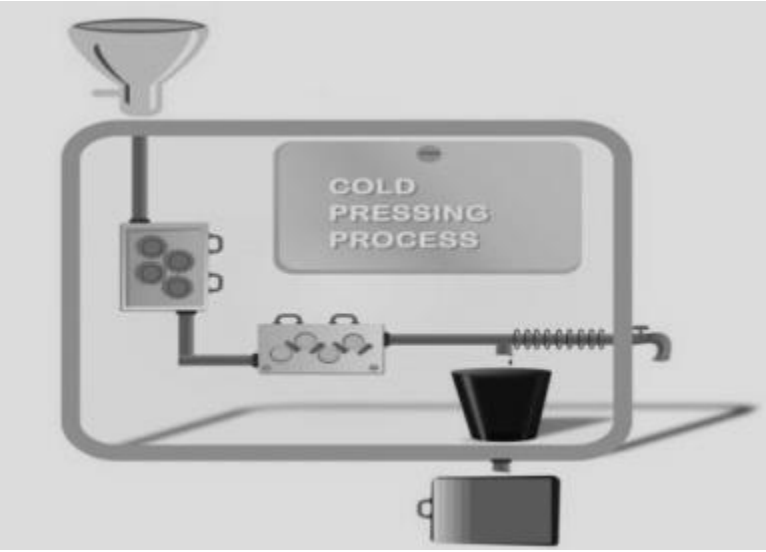


Fig 1-9: Cold Pressing Apparatus

v)Steam Distillation

The process of steam distillation is designed mainly for temperature-sensitive plant such as natural aromatic compounds. This technique is one of officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is

then condensed further and the essential oil is collected [71]. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.

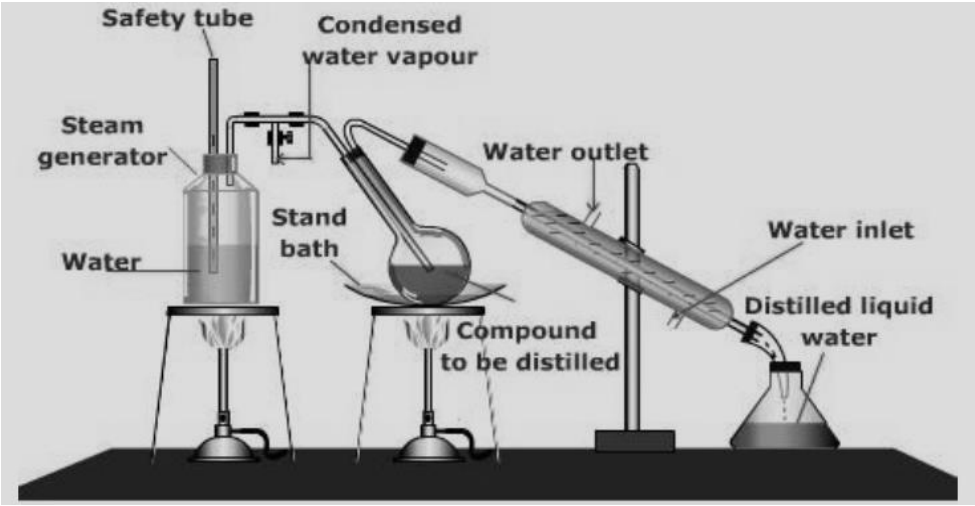


Fig 1-10: Schematic of steam distillation apparatus

A Major disadvantage of the above mentioned conventional techniques is associated with the thermo-instability of essential oils components which may undergo various reactions like hydrolysis, isomerization and oxidation as a result high applied temperature.

Some innovative extraction techniques have some advantages over the conventional methods including: reduction of extraction times, reduction of energy consumption, reduction of volumes of solvents used.

Some of these innovative techniques are highlighted hereafter:

a) Supercritical fluid extraction

During the process of supercritical fluid extraction, a supercritical fluid is employed as the extracting solvent[72]. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction[72]. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32^oC), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [72]. The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes [73]. These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods[74]. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity[75]. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of

superior quality, with better functional and biological activities [75]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

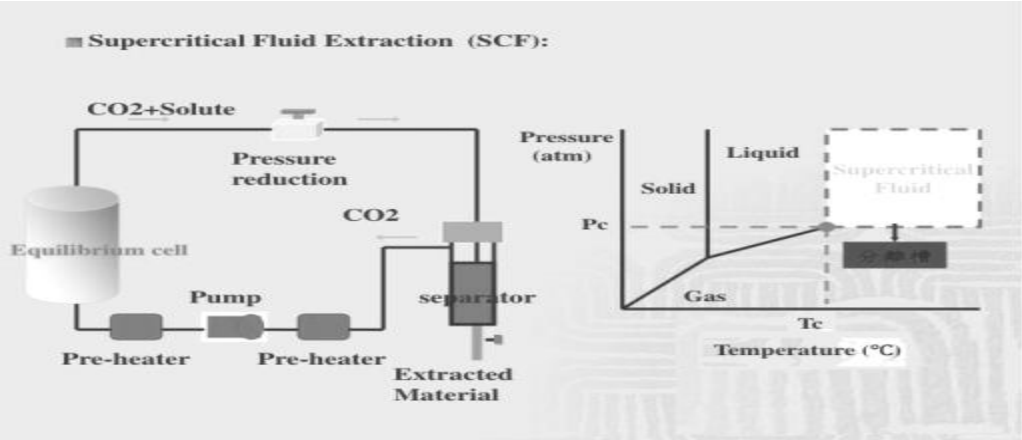


Fig 1-11: Schematic of supercritical fluid extraction apparatus

b) Microwave hydrodiffusion and gravity

Microwave hydrodiffusion and gravity (MHG) is an echo-friendly (green technique) used for the extraction of essential oils. It is originally a microwave blend- microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants [75]. Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and as it does require less energyn[76].The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental

impact and power saving [78].

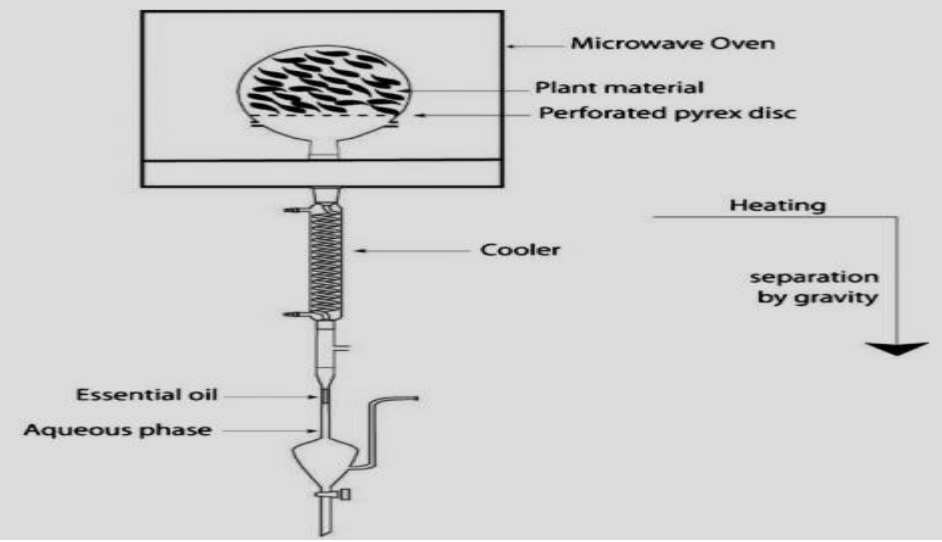


Fig 1-12: Microwave hydrodiffusion and gravity system

c)Solvent-free microwave extraction

The process of solvent-free microwave extraction (SFME) is an innovative extraction technique used for the extraction of essential oil and is accomplished by the water which exists within the matrix without using any solvent [79]. Based on the integration of dry distillation and microwave heating energy. It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent [80]. In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 hours and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation

power, temperature, and extraction time were controlled by the panel in the instrument.

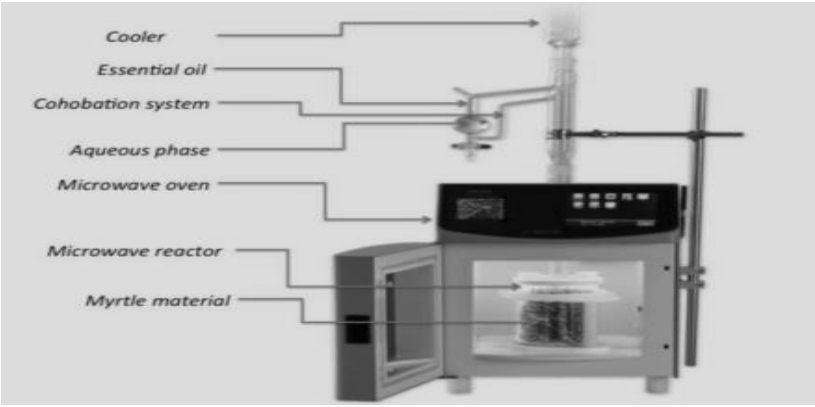


Fig 1-13: Schematic of solvent-free microwave extraction system

d)Ultrasonic-assisted extraction

The technique of ultrasonic-assisted extraction is reputed as an excellent technique that can achieve high valuable compounds and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material [81]. The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation. In these applications the power ultrasonic increases the surface wetness

evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer[82]. The plants raw material is immersed in water or another solvent (Methanol or ethanol or anyonefromthesolvents) and at the same time, it is subjected to the work of ultrasound[83]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds [84].

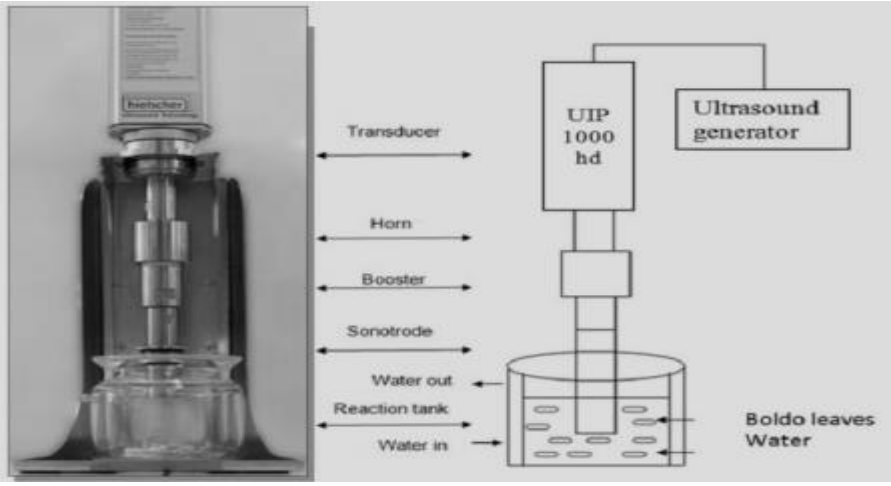


Fig 1-14: Ultrasonic-assisted extraction apparatus

e) Microwave-Assisted Hydrodistillation

The process of microwave-assisted hydrodistillation uses microwave oven in the extraction process. The output of this technique is strongly dependent on the dielectric constant of water and the sample [85]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising

microwave-assisted hydrodistillation technique (Scheme8). Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [86,87]and consuming only a fraction of the energy used in conventional extraction methods[88].The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process

many types of materials under a wide range of conditions. Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process. The principle of heating using Microwave-assisted hydrodistillation is based upon its direct impact with

polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously[89].

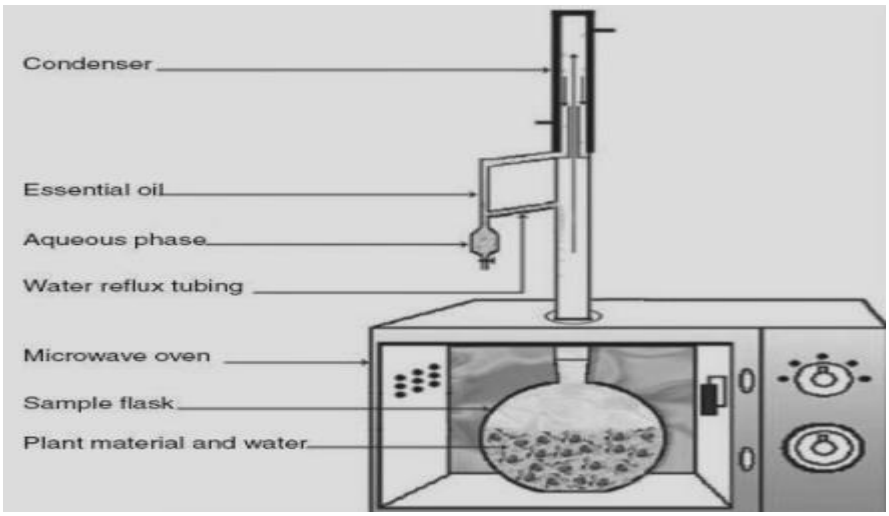


Fig 1-15: Schematic of a microwave-assisted hydrodistillation system

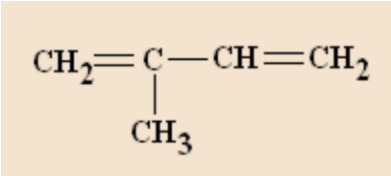
1.9.2-Chemical constituents of essential oils

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups[91]:

- -Volatile fraction: essential oil which are constituting of 90–95% of the oil in weight and containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.
- -Nonvolatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

i)Hydrocarbon

Essential oils consist of chemical compounds that have hydrogen and carbon as their building blocks. Basic hydrocarbon found in plants are built of isoprene units having the following structure[91].



(Isoprene)

ii)Terpenes

Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes, diterpenes, triterpenes and polyterpenes. Referring back to isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes[91].

iii)Monoterpenes [$C_{10}H_{16}$]

The main properetes of monotepense are analgesic, bactericidal, expectorant, and stimulant.

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C_{10}). Some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids. Examples are limonene and menthol[91].

The branched-chain C_{10} hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally occurring monoterpenes identified. Some of these being linear derivatives (geraniol, citronellol). The monoterpenes can be monocyclic like camphor – bicyclic like pinenes (**α** and **β**) or tricyclic. Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium* (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses[91].

iv)Sesquiterpenes

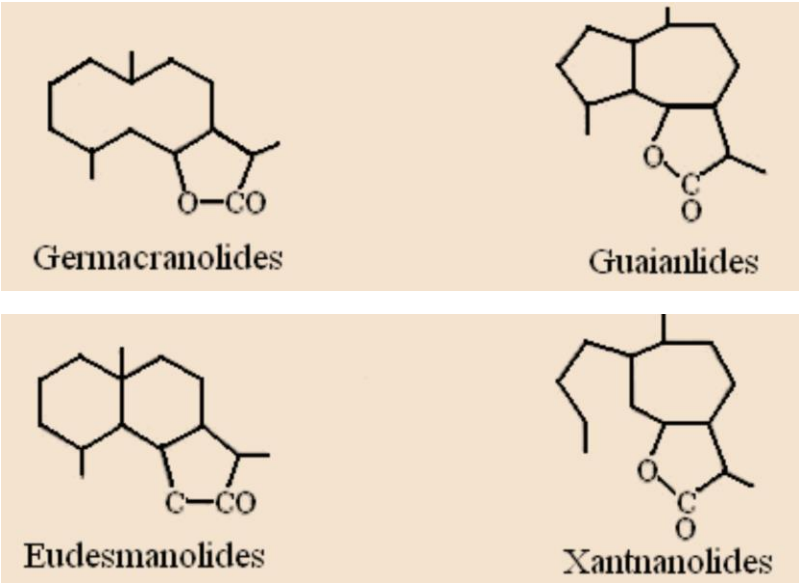
The main properties of sesquiterpenes are anti-inflammatory, anti-septic, analgesic, anti-allergic.

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and their structure may be linear, monocyclic or

bicyclic. They constitute a very large group of secondary metabolites; some have been shown to be stress compounds formed as a result of disease or injury.

Over 500 sesquiterpene lactones are known. They are particularly characteristics of the Compositae but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents.

Chemically these lactones can be classified according to their carboxylic skeletons; thus, from the germacranolides can be derved the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc[91].



v)Diterpene

The main properties of diterpense are ananti-fungal, expectorant, hormonal balancers, hypotensive.

Diterpenes are made of up four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in almost all plant families and consist of compounds having a C20 skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate [91].

vi)Alcohols

The main properties of alcohols are an antiseptic, antiviral, bactericidal and germicidal.

Alcohols are the compounds which contains hydroxyl function. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low

or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use[91].

vii)Aldehydes

The main properties of plant aldehydes are anantifungal, anti-inflammatory, anti-eptic, antiviral, bactericidal, disinfectant, sedative. Medicinally, essential oils containing aldehydes are effective in treating *Candida* and other fungal infections

viii)Acids

The main properties of plant acids are anti-inflammatory.

Organic acids may occur in their free state or are generally found in very small quantities within essential oils. Plant acids act as components or buffer systems to control acidity

ix)Esters

Esters are formed through the reaction of alcohols with acids. Essential oils containing esters are used for their soothing, and balancing effects. They are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system[91].

x)Ketones

The main properties of plant ketones are anti-catarrhal, cell proliferant and expectorant.

Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic. The most toxic ketone is thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil[91].

xi)Lactones

The main properties of plant lactone are antiinflammatory, antiphlogistic, expectorant and febrifuge.

Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action than ketones.

Aim of this study

This study was designed to fulfill the following goals:

- Extraction of oils from six plant species which are key species in Sudanese traditional medicine. These species are: Seeds of *Foeniculum vulgare*، *Pimpinella anisum* L, Nigella *sativa* L، *Carum carvi* L، *Petroselinum crispum* and *Lepidium sativum* L
- Investigation of the constituents of the oil by GC-MS analysis.
- Evaluation of the targeted oils for antimicrobial potential.

Chapter Two
Materials and Methods

Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Foeniculm vulgare, Pimpinella anism L, Nigella sativa, Carum carvi L, Petroselinum crispm and Lepidium sativum L* were purchased from the local market in Jordan and identified by direct comparison with a herbarium sample.

2.1.2- Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness)**.**

2.1.3-Test organisms

The targeted oils were screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeroginosa, Escherichia coli* and *Candida albicans*.

2.2- Methods

2.2.1-Extraction of oil

Powdered plant material (400g) was exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil.

2.2.2- GC-MS analysis

The targeted oils were analyzed by GC-MS. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness) was used. Oven temperature program and other chromatographic conditions are shown below:

Table 2-1: Oven temperature program

Rate	Temperature(°C)	Hold Time ($min.^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Table 2-2: Chromatographic conditions

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min
2.Spilt ratio	- 1.0

2.2.3-Antimicrobial assay

i)-Bacterial suspensions

One ml aliquots of 24 hours' broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

ii)-Fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

iii)-Antimicrobial test

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume micrometer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Chapter Three

Results and Discussion

Results and Discussion

This research was designed to study the oils of six potential medicinal Jordon plants, the results of this target plants studied by GC-MS, the constituents of oils show bellow are returns to: *Foeniculum vulgare, Pimpinella anisum, Nigella sativa, Carum carvil, Petroselinum crispum and Lepidium sativum*

3.1- *Nigella sativa*

3.1.1- GC-MS analysis of *Nigella sativa* oil

Nigella sativa oil was studied by GC-MS. The analysis showed 12 constituents dominated by, 9,12-octadecadienoic acid (Z,Z)- (59.95%)، oleic acid (14.86%) and n-hexadecanoic acid (11.85%).The total ion chromatogram is illustrated in Fig 3-1, while the oil components are displayed in Table 3.1.

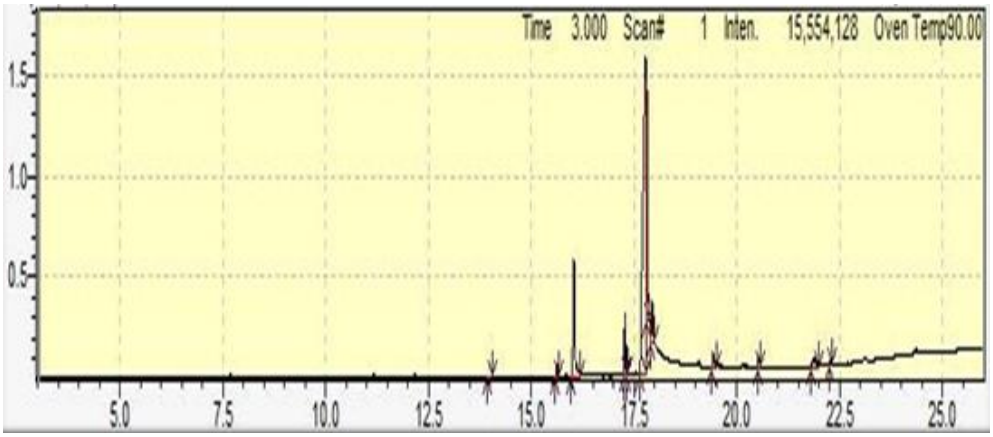


Fig 3-1: Total ions chromatograms

The mass spectrum of the major components is discussed below:

i-9, 12-Octadecadienoic acid (Z,Z)- (59.95%)

9, 12-Octadecadienoic acid (Z,Z)-

Fig 3-2 the mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-. The peak at m/z 280 which appeared at retention time (17.786) is due to M^+ [$C_{18}H_{32}O_2$]. The signal at m/z 264 is due to loss of a methyl group.

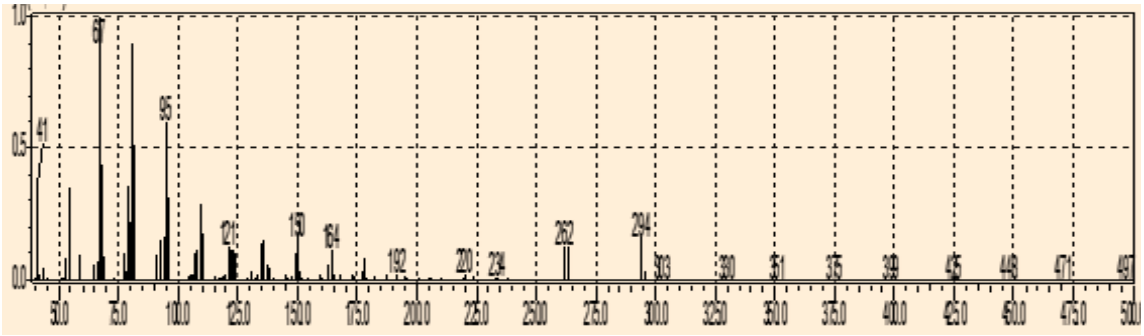
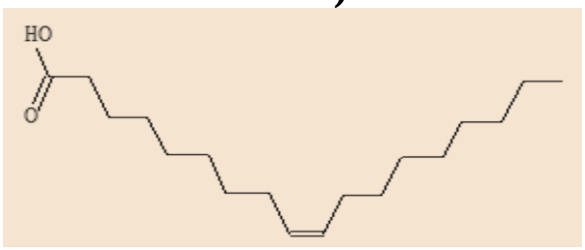


Fig 3-2: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-

ii- Oleic Acid (9-octadecenoic acid)-14.86%



Oleic Acid

The mass spectrum of oleic acid is depicted in Fig 3-3. The signal at m/z 282(retention time 17.813) corresponds $M^+[C_{18}H_{34}O_2]$ while the signal at m/z 264 is due to loss of OH group.

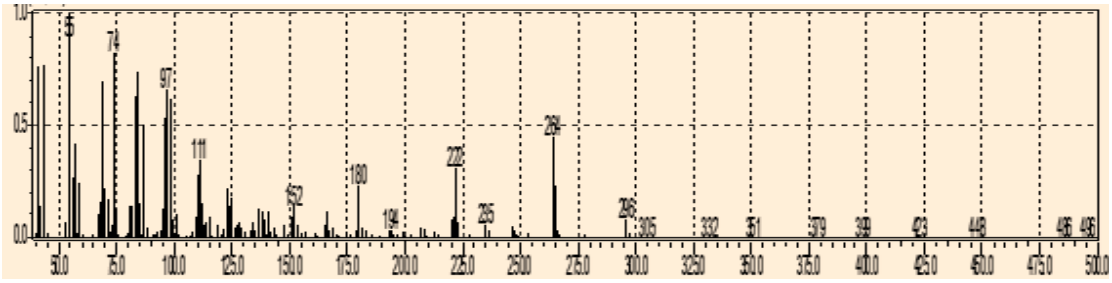
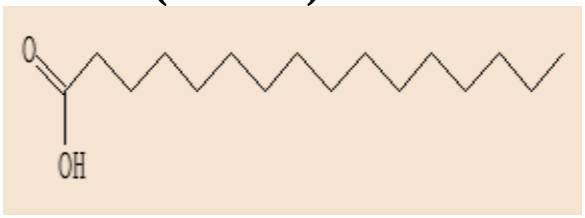


Fig 3-3: Mass spectrum of 9-octadecenoic acid (Z)-

iii-n-Hexadecanoic acid (11.85%)



n-Hexadecanoic acid

The mass spectrum of n-hexadecanoic acid is displayed in Fig 3-4. The peak at m/z 256, which appeared at retention time. 16.046 accounts for the molecular ion: $M^+[C_{16}H_{32}O_2]$. The signal at m/z·

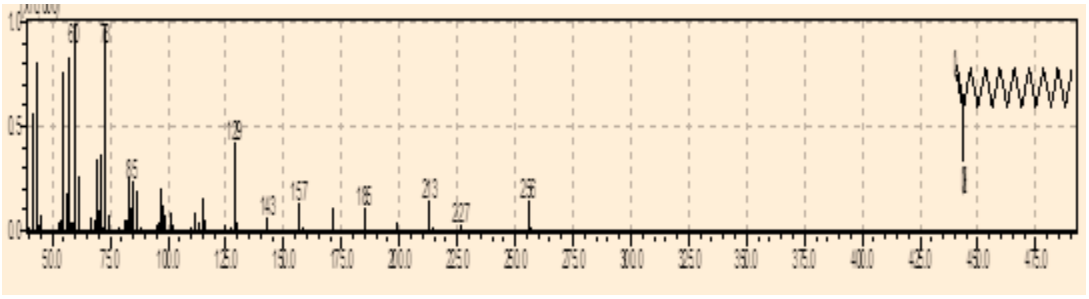


Fig 3-4: Mass spectrum of n-hexadecanoic acid

Table 3.1: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	Tetradecanoic acid	13.969	0.10
2.	Hexadecanoic acid, methyl ester	15.627	0.79
3.	n-Hexadecanoic acid	16.046	11.85
4.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.280	3.63
5.	9-Octadecenoic acid (Z)-, methyl ester	17.324	1.58
6.	9,12-Octadecadienoic acid (Z,Z)-	17.786	59.95
7.	Oleic Acid	17.813	14.86
8.	Octadecanoic acid	17.943	3.18
9.	cis-13,16-Docasadienoic acid	19.433	1.56
10	Trilinolein	20.538	0.79
11	.beta.-Sitosterol	21.894	1.43
12	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	22.285	0.28

3.1.2-Antimicrobial assay

Nigella sativa oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard pathogenic bacteria. The average of the diameters of the growth inhibition zones are displayed in Table (3.2). Results were interpreted as follows:>9 considered inactive; 9-12: weak activity; 13-18: active and <18: very active. Ampicilin, gentamicin and clotrimazole have been used as positive controls.

Table 3.2: Antimicrobial activity of the oil

T	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	10	15	--	15	--
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa:*Staphylococcus aureus*
Ec.*: Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Bs.: Bacillus subtilis

At a concentration of 100mg/ml, the oil exhibited moderate activity against Gram Positive *Bacillus subtilis* and Gram negative *Pseudomonas aeruginosa.* It also showed weak activity against Gram Positive *Staphylococcus aureus.* However, it failed to give inhibitory effect against Gram negative *Escherichia coli* and the yeast *Candida albicans*.

3.2- *Lepidium sativum*

3.2.1. GC-MS analysis of *Lepidium sativum* oil

The oil of the medicinally important plant *Lepidium sativum* was investigated. The GC-MS analysis showed 22 constituents. Major are: 6-octadecenoic acid, methyl ester, (Z)- (43.45%)، 9,12-octadecadienoic acid (Z,Z)-, methyl ester (30.47%),propanal, 2-methyl-3-phenyl- (5.76%).The total ion chromatogram is presented in Fig 3-5, while the oil components are displayed in Table 3.3.

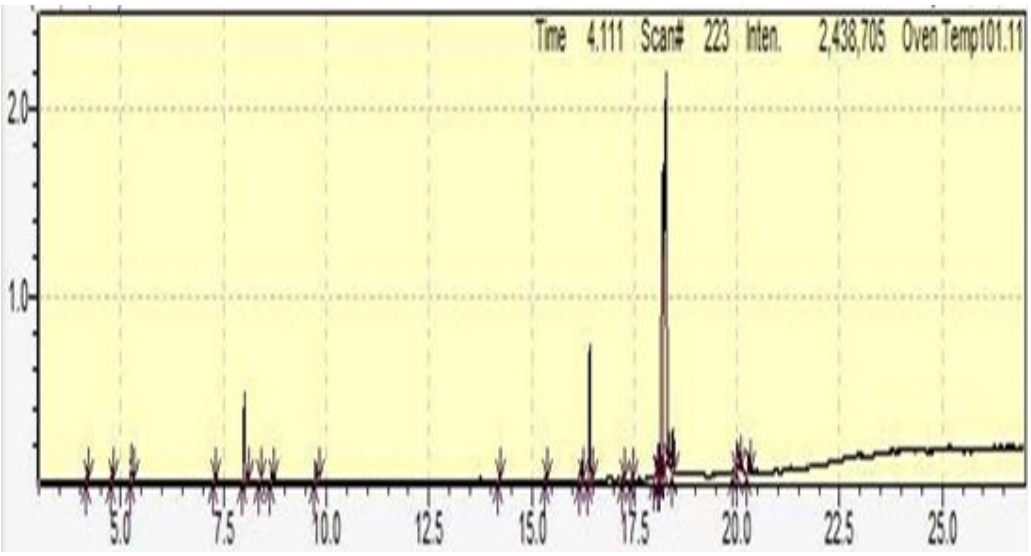
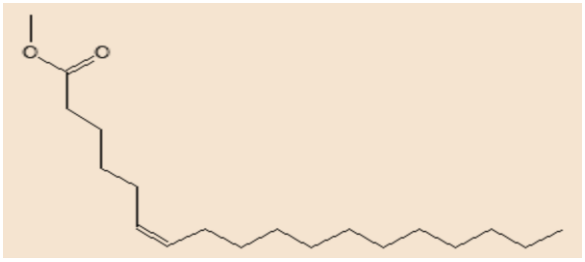


Fig 3-5: Total ions chromatograms

Mass spectra of the major components are discussed below:

i-6-Octadecenoic acid, methyl ester, (Z)- (43.45%)



6-Octadecenoic acid, methyl ester

Fig 3-6 presents the mass spectrum of 6-octadecenoic acid, methyl ester, (Z). The peak at m/z 296 which appeared at retention time (18.276) is due to M^+ [$C_{19}H_{36}O_2$]. The signal at m/z 264 is due to loss of a methoxy.

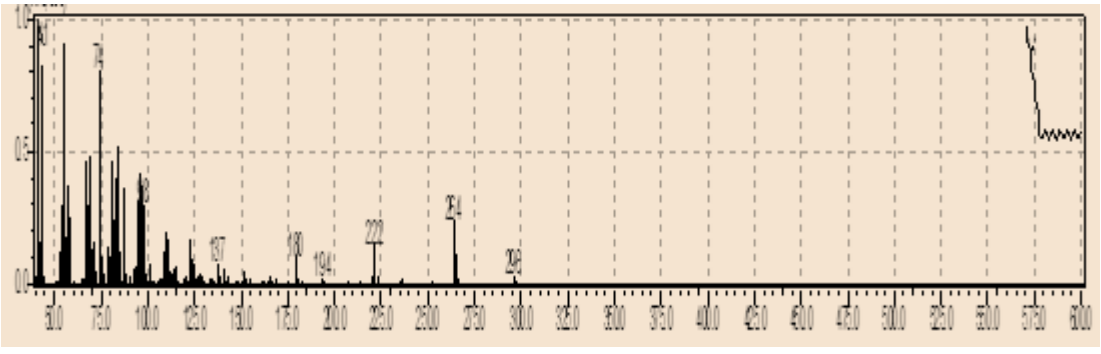
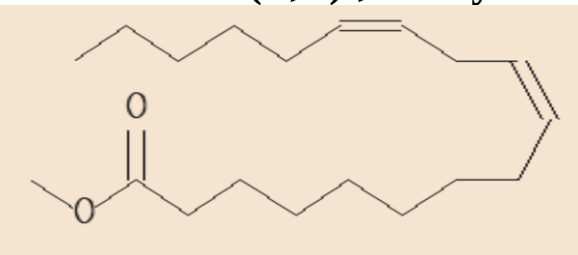


Fig 3-6: Mass spectrum of 6-octadecenoic acid, methyl ester, (Z)-

ii- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (30.47%)



9,12-Octadecadienoic acid (Z,Z)-, methyl ester

Fig 3-7 presents mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester . The peak at m/z 294 which appeared at retention time (18.198) is due to M^+ [$C_{19}H_{34}O_2$]. The signal at m/z 263 is due to loss of a methoxyl.

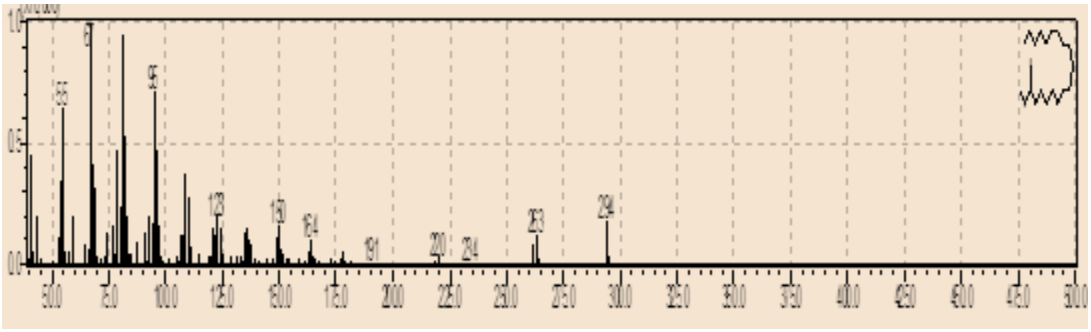
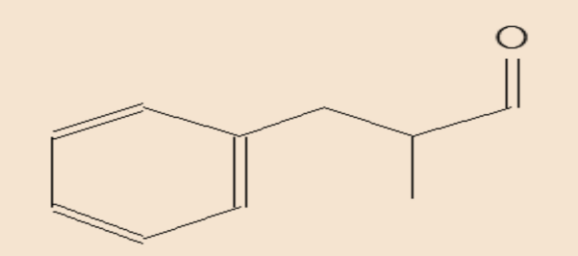


Fig 3-7: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl

C- Propanal, 2-methyl-3-phenyl (5.76%)



Propanal, 2-methyl-3-phenyl-

Fig 3-8 shows the mass spectrum of propanal, 2-methyl-3-phenyl-. The peak at m/z 148, which appeared at retention time. (8.005)

accounts for the molecular ion: $M^{+}[C_{10}H_{12}O]^{\cdot}$ The signal at m/z 133 accounts for loss of a methyl group.

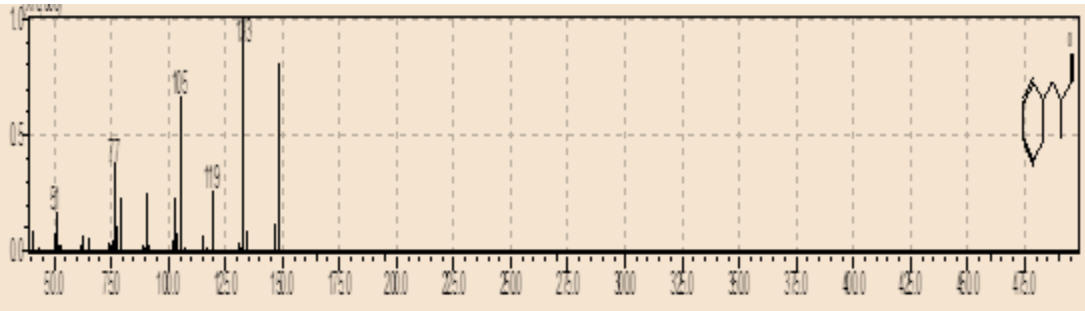


Fig 3-8: Mass spectrum of Propanal, 2-methyl-3-phenyl-

Table 3.3: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	.beta.-Pinene	4.172	0.44
2.	p-Cymene	4.782	0.43
3.	.gamma.-Terpinene	5.254	1.61
4.	Carane, 4,5-epoxy-, trans	7.286	0.20
5.	Propanal, 2-methyl-3-phenyl-	8.005	5.76
6.	Santolina triene	8.364	0.09
7.	2-Caren-10-al	8.666	0.36
8.	Hydratropamide, .alpha.-methyl-	9.741	0.82
9.	Methyl tetradecanoate	14.199	0.09
10	Pentadecanoic acid, methyl ester	15.332	0.12
11	9-Hexadecenoic acid, methyl ester, (Z)-	16.214	1.34
12	Hexadecanoic acid, methyl ester	16.417	7.67
13	cis-10-Heptadecenoic acid, methyl ester	17.230	0.29
14	Heptadecanoic acid, methyl ester	17.446	0.09
15	6,9-Octadecadienoic acid, methyl ester	18.033	0.48
16	8,11-Octadecadienoic acid, methyl ester	18.087	0.53
17	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.198	30.47
18	6-Octadecenoic acid, methyl ester, (Z)-	18.276	43.35
19	Methyl stearate	18.438	2.46
20	14,17-Octadecadienoic acid, methyl ester	19.922	0.20
21	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	20.026	1.89
22	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	20.274	1.31

Antimicrobial activity

Lepidum Sativum oil was screened for antimicrobial activity against five standard microbial strains. The diameters of the growth of inhibition zones are shown in Table (3.4). conventional termes were used for interpretation of the results: (<9mm: inative;9-12 mm: partially active;13-18mm: active; 13-18mm: active; very active). Tables (3.5) and (3.6) represents the antimicrobial activity of standard drugs.

Table 3.4: Antibacterial activity of *Lepidum Sativum* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	50	18	-	16	16	17
	25	17	-	14	15	15
	12.5	16	-	-	14	13
	6.25	12	-	-	-	12

Table 3.5: Antibacterial activity of standard chemotherapeutic agent

Drug	Conc(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30		
	20	14	25		
	10	11	15		
Gentamycine	40	25	19	22	21
	20	22	18	18	15
	10	17	15	15	12

Table 3.6: Antifungal activity of standard chemotherapeutic agent

Drug	Conc.(mg/ml)	An	Ca
Clotramizole	30	22	38
	15	17	31
	7.5	16	29

Sa*: staphylococcus*

Ec*: Escherichia coli*

Pa*: Pseudomonas aeruginosa*

Ca*: Candida albicans*

Bs*: Bacillus subtilis*

Lepidum sativum oil showed excellent activity against *Staphylococcus aureus* in the concentration range: 50-25mg/ml.It also showed very good anticandidal potential in the range 50-25mg/ml.

3.3- *Petroselinum crispum*

3.3.1--GC-MS analysis of *Petroselinum crispum* oil

Petroselinum crispum essential oil was investigated by GC-MS technique. The analysis showed 33 constituents. Major are: 6-octadecenoic acid, methyl ester, (Z)-(35.70%)، naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha.,7. alpha.,8a. beta.)]- (13.82%),9,12-octadecadienoic acid (Z,Z)-, methyl ester (11.74%) ، hexadecanoic acid, methyl ester (5.85%), ledol (5.45%).The total ion chromatogram is presented in

Fig 3-9 , while the oil components of the oil are displayed in Table 3.7.

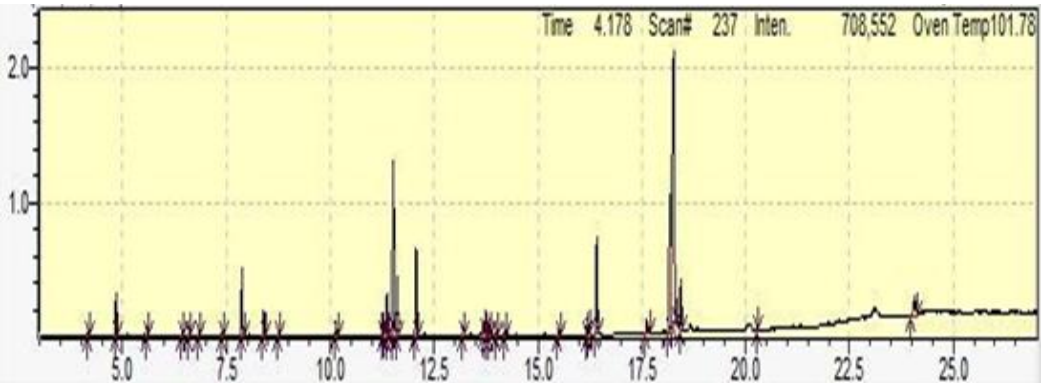


Fig 3-9: Total ions chromatograms

Mass spectra of major components are discussed below:

i)-6-Octadecenoic acid, methyl ester, (Z)-(35.70%)

6-Octadecenoic acid, methyl ester

Fig 3-10 presents the mass spectrum of 6-octadecenoic acid, methyl ester, (Z). The peak at m/z 296 which appeared at retention time (18.272) is due to M^+ [$C_{19}H_{36}O_2$]. The signal at m/z 264 is due to loss of a methoxyl.

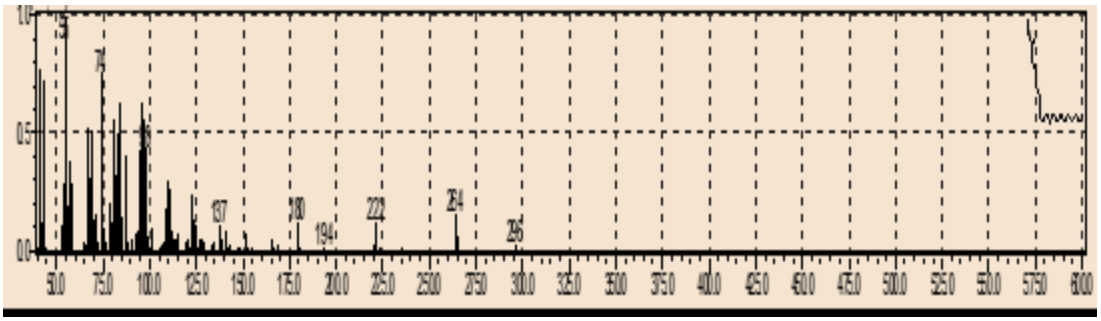
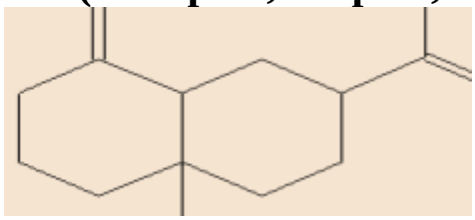


Fig 3-10: Mass spectrum of 6-Octadecenoic acid, methyl ester

ii)- Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR- (4a. alpha.,7. alpha.,8a. beta.)]- (13.82%)



Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR- (4a. alpha.,7. alpha.,8a. beta.)]-

Mass spectrum of naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR- (4a. alpha.,7. alpha.,8a. beta.)] is depicted in Fig 3-11. The signal at m/z 204(retention time:11.521) corresponds $M^{+}[C_{15}H_{24}]^{\cdot}$ The signal at m/z 161 is due to loss of an isopropyl group.

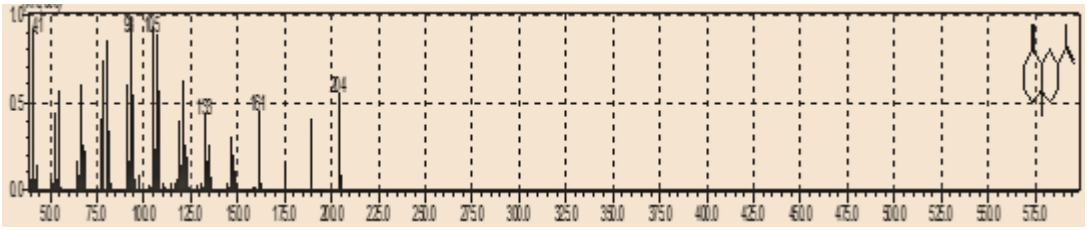
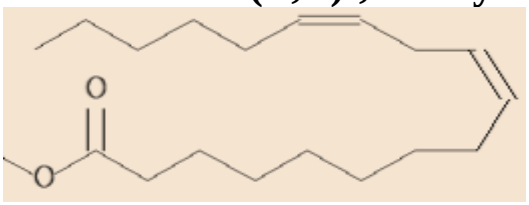


Fig 3-11: Mass spectrum of Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha.,7. alpha.,8a. beta.)]

iii)- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (11.74%)



9,12-octadecadienoic acid, methyl ester

The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is displayed in Fig 3-12. The peak at m/z 294, which appeared at retention time :18.181 accounts for the molecular ion: $M^+[C_{19}H_{34}O_2]$. The signal at m/z 263 accounts for loss of a methoxyl function.

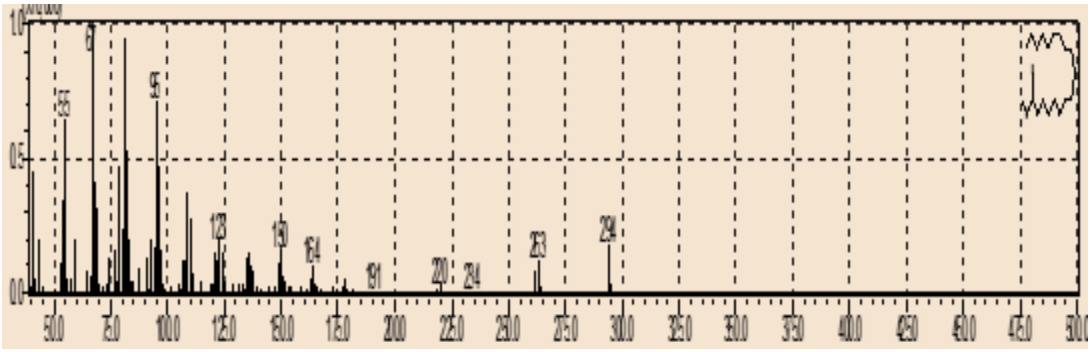
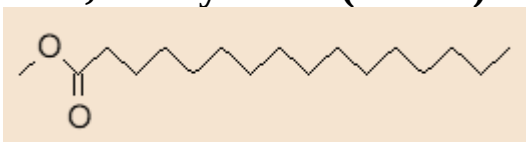


Fig 3-12: Mass spectrum of 9,12-octadecadienoic acid, methyl ester

iv- Hexadecanoic acid, methyl ester (5.85%)



hexadecanoic acid, methyl ester

The mass spectrum of hexadecanoic acid, methyl ester is shown in Fig 3-13. The peak at m/z 270, which appeared at retention time

(16.418) in total ion chromatogram, corresponds the molecular ion: $M^+[C_{17}H_{34}O_2]$. The signal at m/z 239 corresponds to loss of a methoxyl.

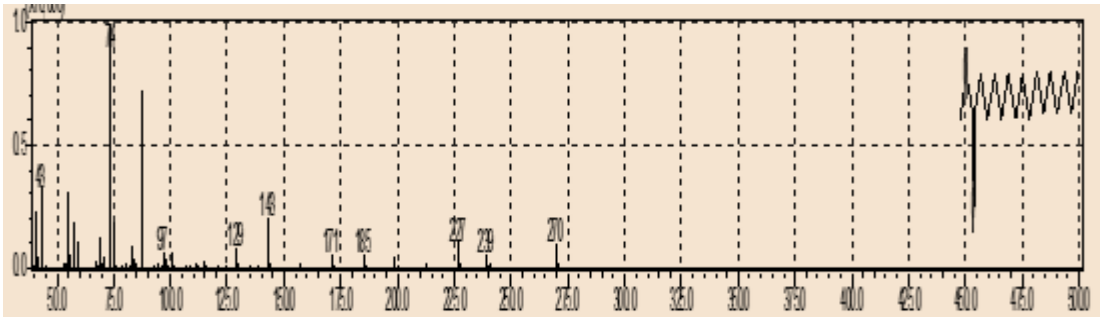
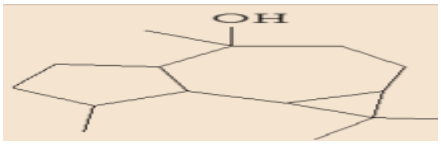


Fig 3-13: Mass spectrum of hexadecanoic acid, methyl ester

v)- Ledol (5.45%)



Ledol

The mass specrum of ledol is shown in Fig 3-14. The molecular ion $M^+[C_{15}H_{26}O]$ appeared at m/z 222 with retention time (12.066).

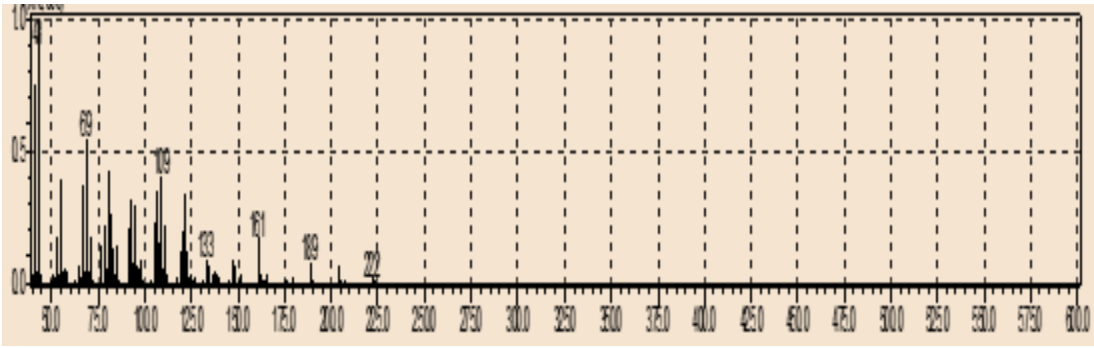


Fig 3-14: Mass spectrum of Ledol

Table 3.7: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	.beta.-Pinene	4.172	0.10
2.	D-Limonene	4.839	2.01
3.	2-Cyclohexen-1-one, 3,4,4-trimethyl-	5.591	0.36
4.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	6.439	0.18
5.	Carane, 4,5-epoxy-, trans	6.607	0.47
6.	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-	6.831	0.17
7.	4-Isopropyl-5-methylhexa-2,4-dien-1-ol	7.394	0.14
8.	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)-	7.873	4.05
9.	1-Cyclohexene-1-carboxylic acid, 2,6,6-trimethyl-, methyl ester	8.399	1.50
10	Bicyclo[3.2.0]hept-2-ene, 4-ethoxy-, endo-	8.755	0.15
11	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	10.140	0.80
12	4,5-di-epi-aristolochene	11.262	1.25
13	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	11.310	0.43
14	.alpha.-Guaiene	11.365	2.44
15	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	11.521	13.82
16	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.615	3.47
17	Ledol	12.066	5.45
18	Apiol	13.199	0.44
19	Selina-6-en-4-ol	13.683	0.69
20	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	13.740	0.25
21	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	13.827	0.15
22	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	14.000	0.15
23	Methyl tetradecanoate	14.198	0.11
24	Falcarinol	15.505	0.28
25	7-Hexadecenoic acid, methyl ester, (Z)-	16.188	0.64
26	9-Hexadecenoic acid, methyl ester, (Z)-	16.211	0.45
27	Hexadecanoic acid, methyl ester	16.418	5.85
28	2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 8,8-dimethyl-	17.628	1.18
29	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.181	11.74
30	6-Octadecenoic acid, methyl ester, (Z)-	18.272	35.70
31	Methyl stearate	18.438	2.88
32	Eicosanoic acid, methyl ester	20.282	0.33

33.	.gamma.-Sitosterol	24.071	2.37

3.4-*Carum carvi*

3.4.1- GC-MS analysis of *Carum carvi* oil

Carum carvi essential oil has been studied by GC-MS**.** The analysis revealed 23 constituents (Table 3.8). Major components are: 9-octadecenoic acid (Z)-, methyl ester (45.97%),9,12-octadecadienoic acid (Z,Z)-, methyl ester (33.90%), hexadecanoic acid, methyl ester(9.01%) and methyl stearate(4.47%).The total ion chromatogram is presented in Fig 3-15.

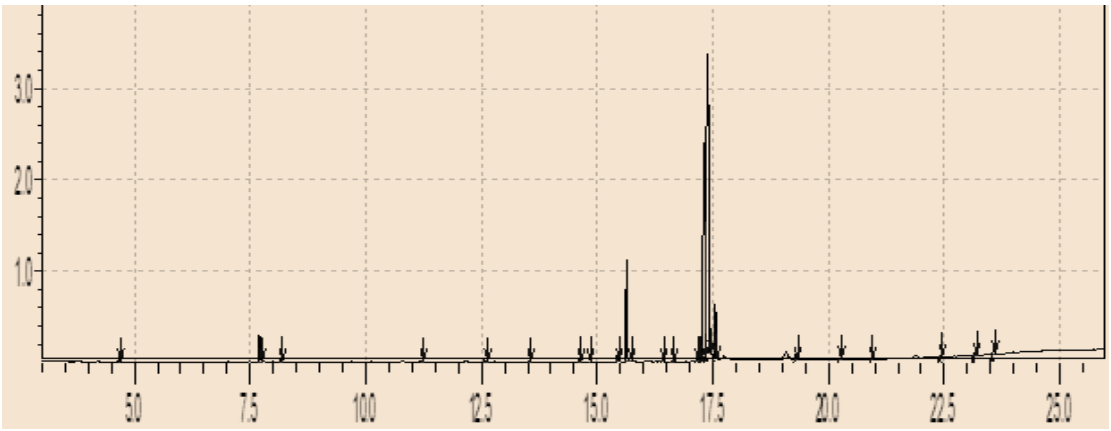
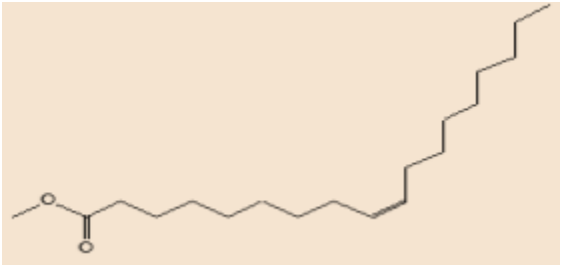


Fig 3-15: Total ions chromatograms

The mass spectra of the major components are discussed below:

i)-9-Octadecenoic acid (Z)-, methyl ester (45.97%)



9-octadecenoic acid methyl ester

Fig 3-16 illustrates the mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester. The peak at m/z 296 which appeared at retention time (17.392) is due to M^+ $[C_{19}H_{36}O_2]^+$. The signal at m/z 264 is due to loss of a methoxyl.

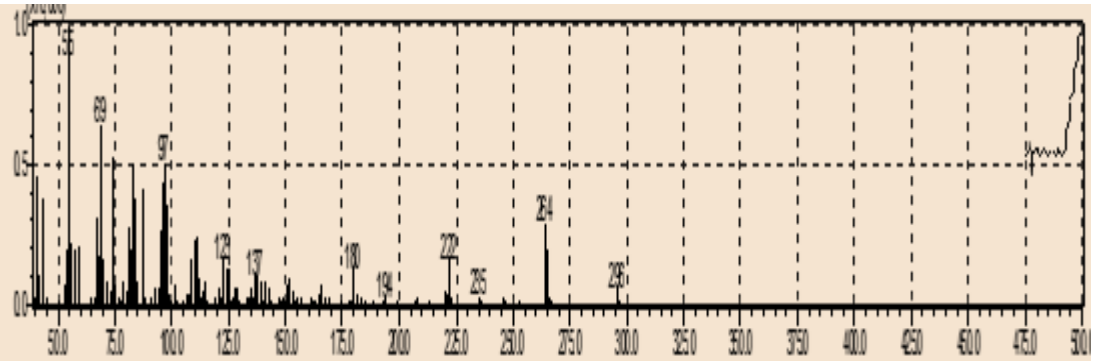


Fig 3-16: Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

ii)- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (17.392%)

9,12-octadecadienoic acid (Z,Z)-, methyl ester

Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is depicted in Fig 3-17.The signal at m/z 294. retention time (17.319) corresponds $M^+[C_{19}H_{34}O_2]^+$ .The signal at m/z 263 is due to loss of a methoxyl.

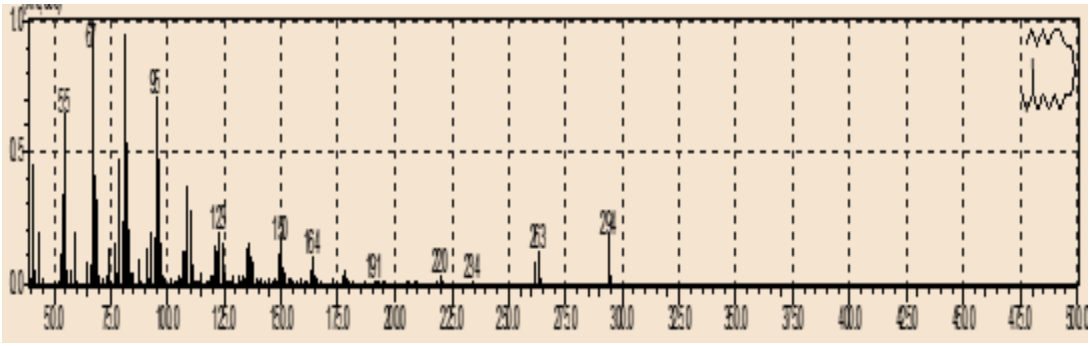
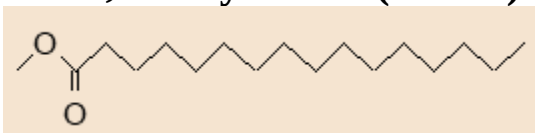


Fig 3-17: Mass spectrum of 9,12-octadecadienoic acid

iii)- Hexadecanoic acid, methyl ester، (9.01%)



Hexadecanoic acid

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig 3-18. The peak at m/z 270, which appeared at retention time. (15.636) accounts for the molecular ion: $M^+[C_{17}H_{34}O_2]^+$ .The signal at m/z 239 accounts for loss of a methoxyl.

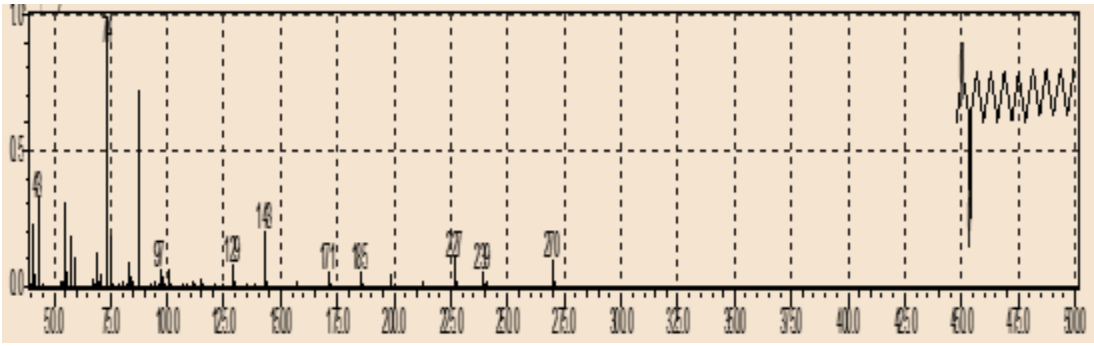


Fig 3-18: Mass spectrum of hexadecanoic acid, methyl ester

iv)- Methyl stearate (4.47%)

Methyl stearate

The mass spectrum of Methylstearate is shown in Fig 3-19. The molecular ion $M^+[C_{10}H_{18}O]^+$ appeared at m/z 298 with retention time (17.551).The signal at m/z 269 accounts for loss of (methoxyl-2H).

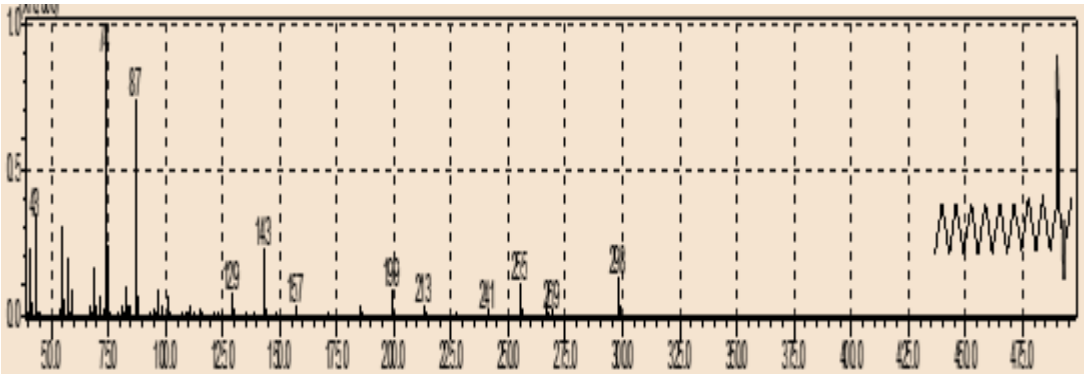


Fig 3-19: Mass spectrum of Methyl stearate

Table 3.8: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	D-Limonene	4.665	0.43
2.	(-)-Carvone	7.696	2.13
3.	1-methyl-4-(prop-1-en-2-yl)-7-oxabicyclo[4.1.0]heptan-2one	8.163	0.25
4.	Dodecanoic acid, methyl ester	11.217	0.02
5.	Apiol	12.559	0.18
6.	Methyl tetradecanoate	13.531	0.09
7.	Pentadecanoic acid, methyl ester	14.606	0.13
8.	2-Pentadecanone, 6,10,14-trimethyl-	14.825	0.02
9.	9-Hexadecenoic acid, methyl ester, (Z)-	15.438	0.67
10.	Hexadecanoic acid, methyl ester	15.636	9.01
11.	cis-10-Heptadecenoic acid, methyl ester	16.401	0.15
12.	Heptadecanoic acid, methyl ester	16.610	0.08
13.	6,9-Octadecadienoic acid, methyl ester	17.153	0.25
14.	8,11-Octadecadienoic acid, methyl ester	17.208	0.30
15.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.319	33.90
16.	9-Octadecenoic acid (Z)-, methyl ester	17.392	45.97
17.	Methyl stearate	17.551	4.47
18.	Eicosanoic acid, methyl ester	19.301	0.61
19.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.230	0.21
20.	Docosanoic acid, methyl ester	20.920	0.17
21.	Tetracosanoic acid, methyl ester	22.424	0.12
22.	Squalene	23.161	0.29
23.	Hexatriacontane	23.566	0.55

3.4.2-Antibacterial activity

Carum carvi oil was screened for antimicrobial activity against five standard pathogenic bacteria. The diameters of the growth of inhibition zones are shown in Table (3.9). Results of Table (3.9) were interpreted in conventional terms: (<9mm: inative;9-12mm: partially active;13-18mm: active; 13-18mm: active; very active). Tables (3.10) and (3.11) represents the antimicrobial activity of standard drugs.

Table 3.9: Antibacterial activity of *Carum carvi* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	50	18	-	16	16	17
	25	17	-	14	15	15
	12.5	16	-	-	14	13
	6.25	12	-	-	-	12

Table 3.10: Antibacterial activity of standard drug

Drug	Conc(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30		
	20	14	25		
	10	11	15		
Gentamycine	40	25	19	22	21
	20	22	18	18	15
	10	17	15	15	12

Table 3.11: Antifungal activity of standard chemotherapeutic agent

Drug	Conc.(mg/ml)	An	Ca
Clotramizole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

With the exception of *Bacillus subtilis, Carum carvi* oil showed activity against all test organisms at: 50-25mg/ml.The oil exhibited excellent activity against *Staphylococcus aureus* in the concentration range :50-25mg/ml. At: 50mg/ml it showed significant activity against the yeast: *Candida albicans.*

3.5- *Pimpinella anisum*

3.5.1- GC-MS analysis of *Pimpinella anisum* oil

GC-MS analysis of *Pimpinella anisum* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). The GC-MS analysis revealed the presence of 34 components (Table 3.12). The typical total ion chromatograms (TIC) is depicted in Fig 3-20.

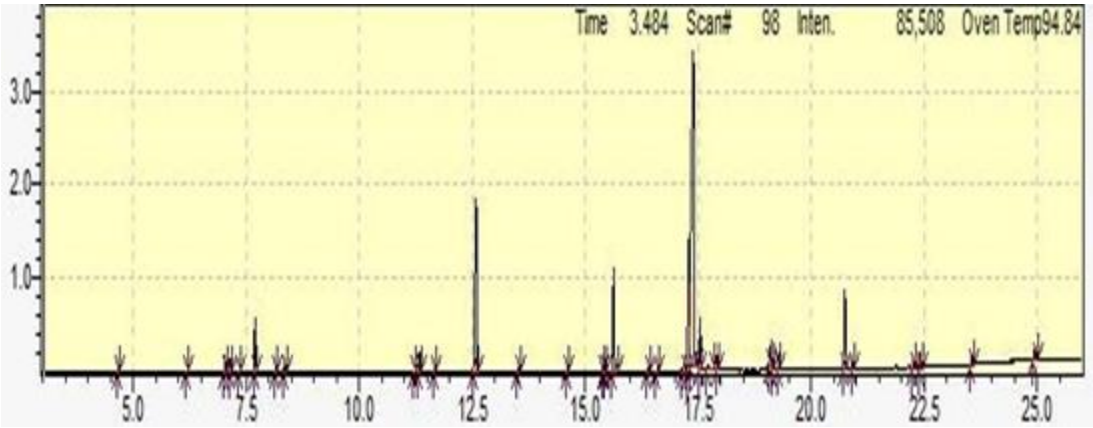


Fig 3-20: Total ions chromatograms

Mass spectra of the major constituents are briefly discussed below:

i)9-octadecenoic acid (Z)-, methyl ester (44.82%)

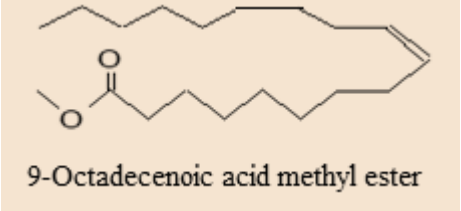


Fig 3-21 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester. The peak at m/z 296 which appeared at retention time (17.392) is due to M^+ $[C_{19}H_{36}O_2]^+$.The signal at m/z 264 is due to loss of a methoxyl .

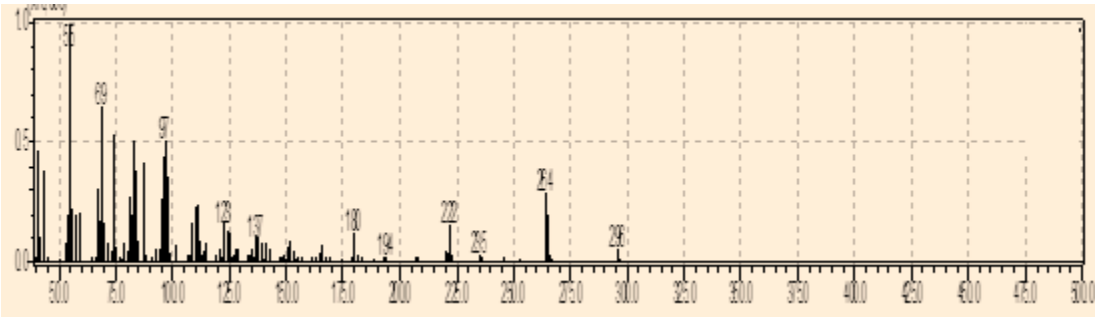
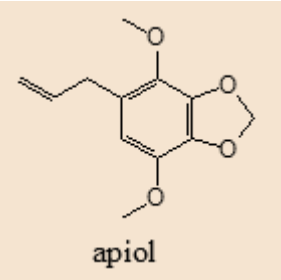


Fig 3-21: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

ii)apiol (15.13%)



apiol

Fig 3-22 shows the mass spectrum of apiol. The signal at m/z 222 (RT.12.589) is due to $M^+[C_{12}H_{14}O_4]^+$. Fig 3-23, illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z 294(RT.17.299) accounts for the molecular ion: $M^+[C_{19}H_{34}O_2]^+$.The signal at m/z 263 accounts for loss of a methoxyl.

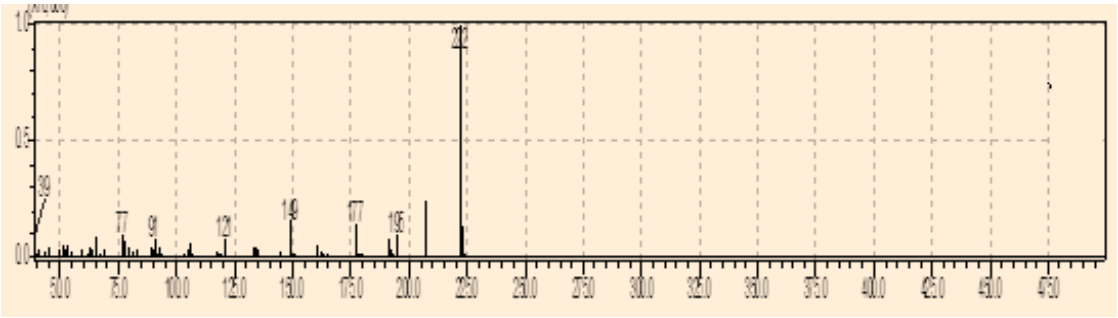


Fig 3-22: Mass spectrum of apiol

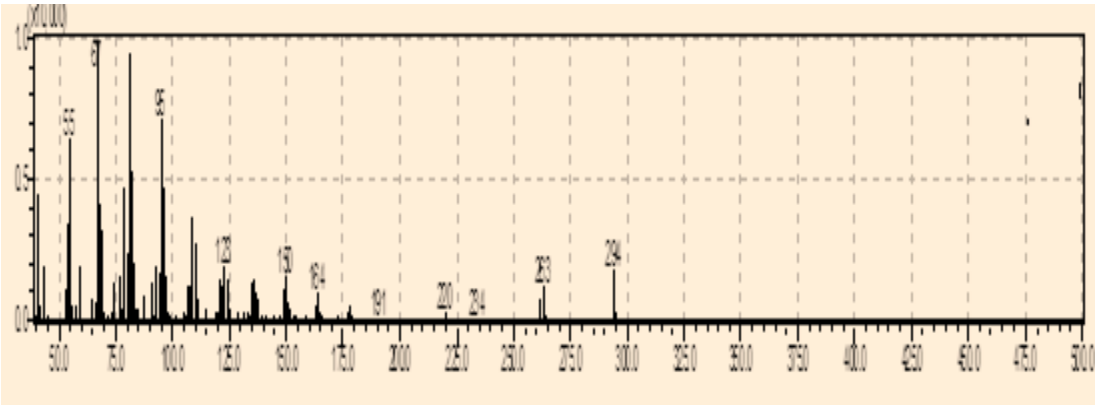
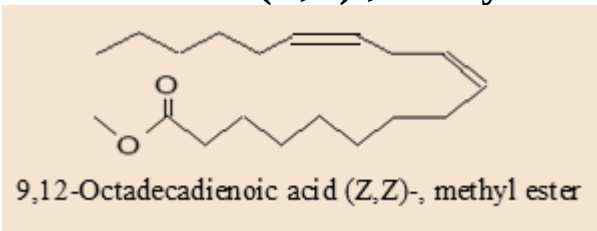


Fig 3-23: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

iii) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (11.22%)



The mass spectrum of hexadecanoic acid, methyl ester is shown in Fig 3-24. The peak at m/z 270, which appeared at retention time (15.635) accounts for the molecular ion: $M^+[C_{17}H_{34}O_2]^+$ .The signal at m/z 239 accounts for loss of a methoxyl. Fig 3-25 displays the mass spectrum of cis-10-nonadecenoic acid, methyl ester. The signal which appeared at m/z 310 (RT.20.748) coincides with the molecular ion: $M^+[C_{20}H_{38}O_2]^+$.The signal at m/z 278 accounts for loss of a methoxyl

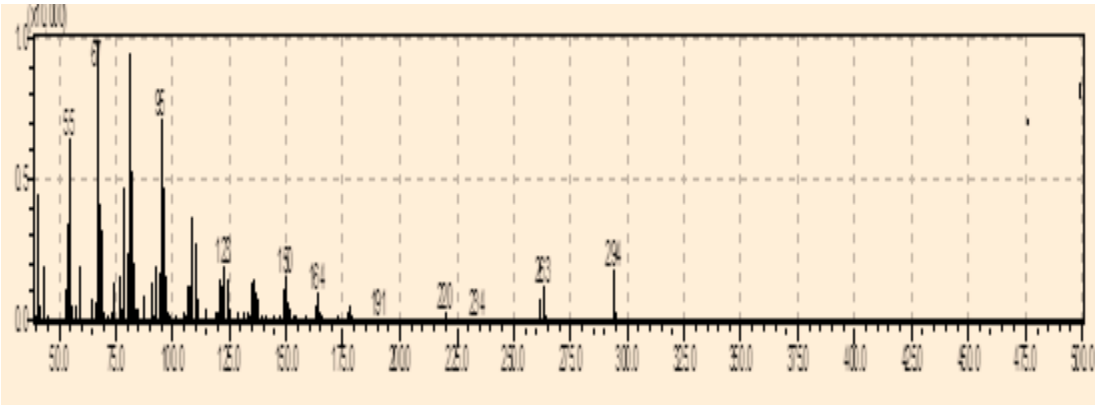


Fig 3-24: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

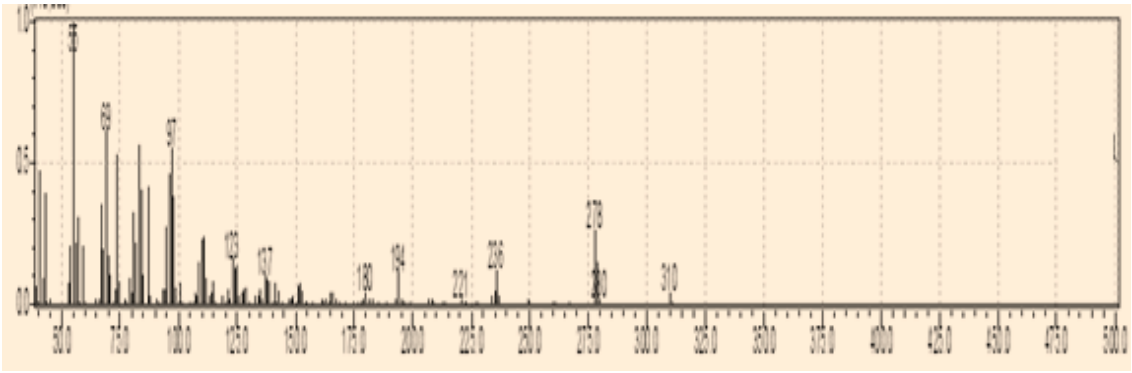


Fig 3-25: Mass spectrum of cis-10-nonadecenoic acid, methyl ester, methyl este

Table 3.12: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	D-Limonene	4.670	0.06
2.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	6.186	0.01
3.	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-	7.040	0.77
4.	3-Nonyne	7.156	0.13
5.	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	7.341	0.02
6.	(-)-Carvone	7.700	3.04
7.	1-methyl-4-(prop-1-en-2-yl)-7-oxabicyclo[4.1.0]heptan-2one	8.164	0.38
8.	Thymol	8.351	0.39
9.	Dodecanoic acid, methyl ester	11.216	0.06
10.	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	11.336	1.15
11.	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	11.671	0.11
12.	Apiol	12.589	15.33
13.	Methyl tetradecanoate	13.530	0.26
14.	Pentadecanoic acid, methyl ester	14.605	0.11
15.	7-Hexadecenoic acid, methyl ester, (Z)-	15.411	0.44
16.	9-Hexadecenoic acid, methyl ester, (Z)-	15.436	0.60
17.	Hexadecanoic acid, methyl ester	15.635	6.57
18.	cis-10-Heptadecenoic acid, methyl ester	16.399	0.11
19.	Heptadecanoic acid, methyl ester	16.610	0.09
20.	n-Nonadecanol-1	17.191	0.79
21.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.299	11.22
22.	9-Octadecenoic acid (Z)-, methyl ester	17.392	44.82
23.	Methyl stearate	17.552	2.91
24.	Oleic Acid	17.731	0.39
25.	Ethyl Oleate	17.927	0.17
26.	cis-11-Eicosenoic acid, methyl ester	19.104	1.41
27.	cis-13-Eicosenoic acid, methyl ester	19.153	0.29
28.	Eicosanoic acid, methyl ester	19.301	0.79
29.	cis-10-Nonadecenoic acid, methyl ester	20.748	5.22
30.	Docosanoic acid, methyl ester	20.921	0.35
31.	15-Tetracosenoic acid, methyl ester, (Z)-	22.270	0.30
32.	Tetracosanoic acid, methyl ester	22.424	0.23
33.	Hexatriacontane	23.567	0.45
34.	10-Nonadecanone	24.936	1.03

3.5.2-Antimicrobial activity

Pimpinella anisum oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard pathogenic microbes. The average of the diameters of the growth inhibition zones are displayed in Table (3.13). Results were interpreted as follows: less than 9 mm: considered inactive; 9-12mm: weak activity; 13-18mm: active and more than 18: very active. Ampicilin, gentamycin and clotrimazole have been used as positive controls.

Table 3.13: Antimicrobial activity of the oil

T	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa:*Staphylococcusaureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*

Bs.: *Bacillus subtilis*

Ca.:*Candida albicans*

At a concentration of 100mg/ml, the oil showed significant anticandidal activity. It also exhibited moderate activity against Gram Positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Escherichia*

coli. However, it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa*

3.6- *Foeniculum vulgare*

3.6.1-GC-MS analysis of *Foeniculum vulgare* oil

The essential oil of the medicinally important *Foeniculum vulgare* was investigated. GC-MS analysis showed 58 constituents. Major components are: 9-octadecenoic acid (Z)-, methyl ester (35.59%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (29.36%) ،hexadecanoic acid methyl ester (8.02%). The total ion chromatogram is presented in Figure 3.26, while the oil costituents are displayed in Table 3.14.

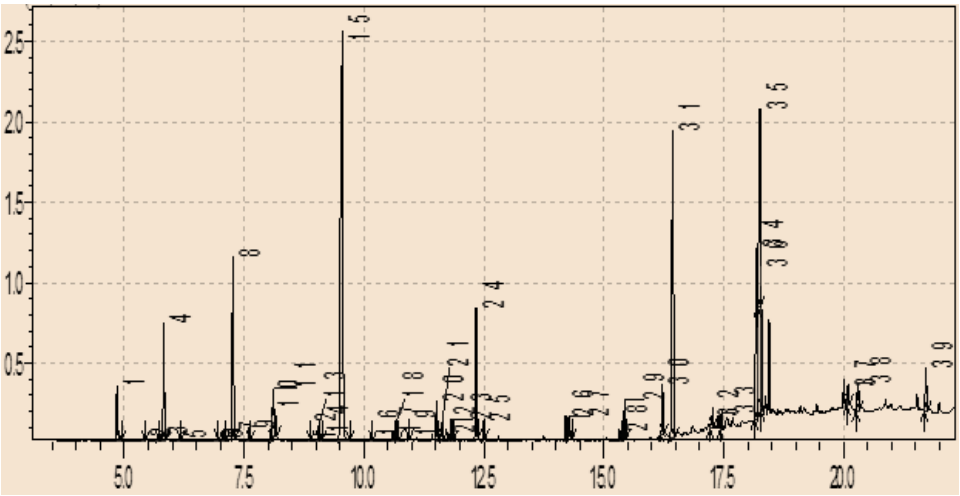
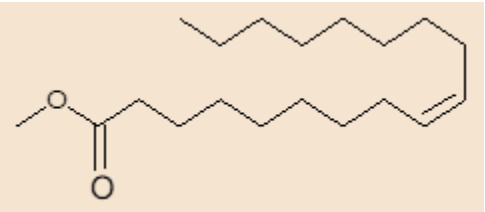


Fig 3-26: Total ions chromatograms

MS spectra of the major components are discussed below

i)- 9-octadecenoic acid (Z)-, methyl ester (35.59%)



9-Octadecenoic acid methyl ester

Fig 3-27 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester. The peak at m/z 296 which appeared at retention time (17.452) is due to M^+ $[C_{19}H_{36}O_2]^+$.The signal at m/z264 is due to loss of methoxyl.

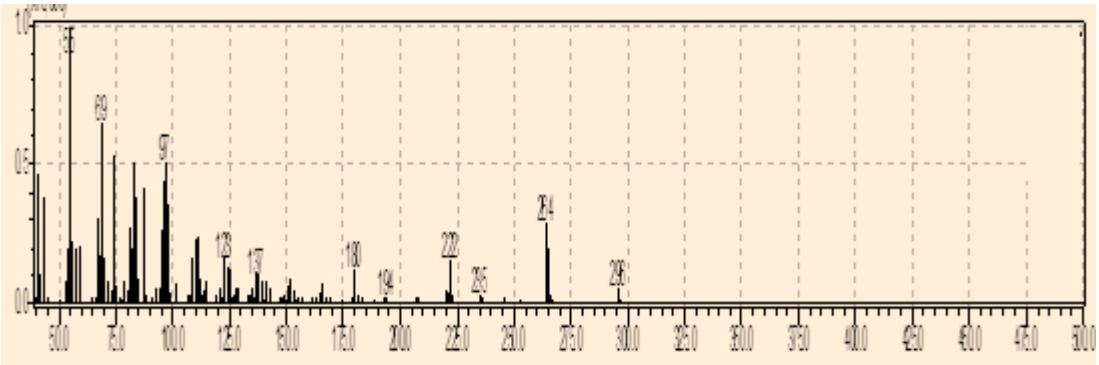
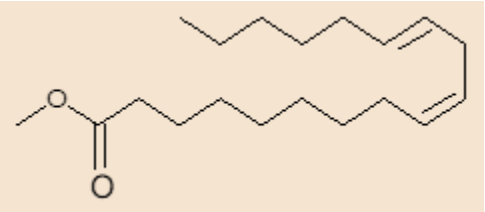


Fig 3-27: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

ii)-9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.36%)



9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is depicted in Fig 3-28.The signal at m/z 294 (R.T. 17.364) corresponds $M^+[C_{19}H_{34}O_2]^+$ while the signal at m/z263 is due to loss of methoxyl.

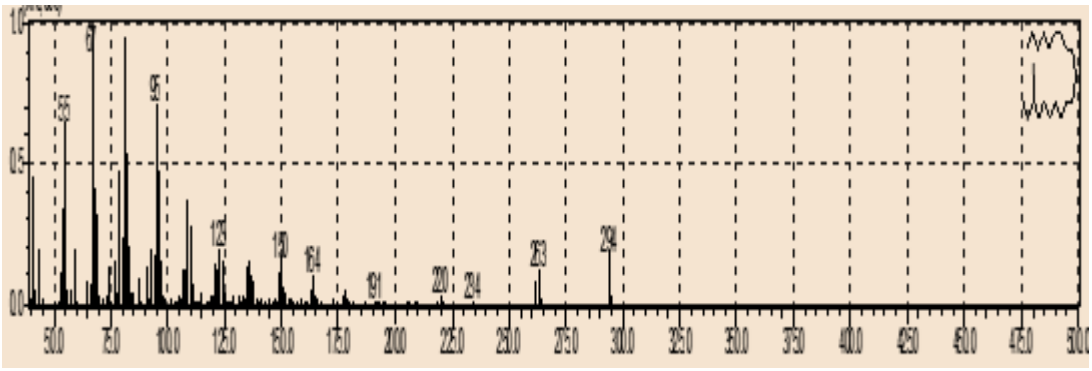
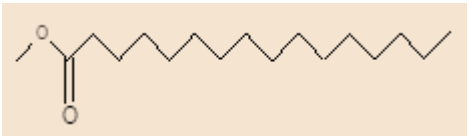


Fig 3-28: Mass spectrum of 9,12-octadecadienoic acid

iii)-Hexadecanoic acid, methyl ester (8.02%)



Hexadecanoic acid, methyl ester

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig 3-29. The peak at m/z 270 -which appeared at R.T. 5.663- accounts for the molecular ion: $M^{+}[C_{17}H_{34}O_{2}]^{+}$.The peak at m/z239 accounts for loss of methoxyl.

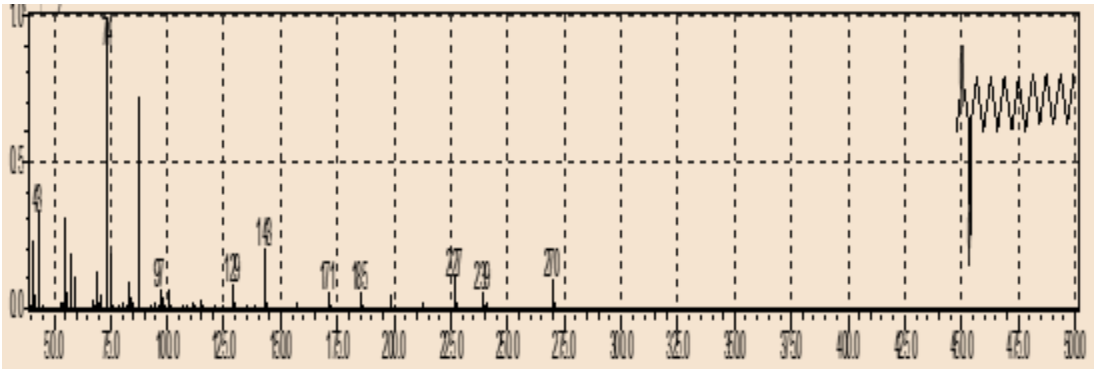


Fig 3-29: Mass spectrum of hexadecanoic acid, methyl ester

Table 3.14: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	.alpha.-Pinene	3.541	0.06
2.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	4.001	0.09
3.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	4.070	1.63
4.	.beta.-Myrcene	4.158	0.15
5.	.alpha.-Phellandrene	4.388	0.03
6.	o-Cymene	4.647	1.13
7.	D-Limonene	4.704	0.17
8.	.beta.-Phellandrene	4.725	0.04
9.	Eucalyptol	4.760	0.03
10.	.gamma.-Terpinene	5.100	4.32
11.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.630	0.03
12.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	5.678	0.02
13.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	6.801	0.01
14.	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	7.019	0.83
15.	Estragole	7.066	0.17
16.	Propanal, 2-methyl-3-phenyl-	7.691	4.97
17.	2-Caren-10-al	8.311	0.64
18.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	9.569	0.18
19.	Benzaldehyde dimethyl acetal	9.840	0.03
20.	Caryophyllene	10.138	0.10
21.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	10.253	0.05
22.	(E)-.beta.-Famesene	10.442	0.24
23.	.beta.-copaene	10.792	0.08
24.	Spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-	10.827	0.15
25.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	11.154	0.09
26.	Dodecanoic acid, methyl ester	11.254	0.02
27.	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	11.352	0.02
28.	11,11-Dimethyl-spiro[2,9]dodeca-3,7-dien	12.202	0.02
29.	Carotol	12.354	0.10
30.	Methyl tetradecanoate	13.565	0.15
31.	6-Octadecenoic acid, methyl ester, (Z)-	14.373	0.01
32.	cis-5-Dodecenoic acid, methyl ester	14.479	0.04
33.	Pentadecanoic acid, methyl ester	14.637	0.16
34.	1,4-Eicosadiene	14.769	0.08
35.	7,10-Hexadecadienoic acid, methyl ester	15.365	0.03
36.	7-Hexadecenoic acid, methyl ester, (Z)-	15.450	0.47
37.	9-Hexadecenoic acid, methyl ester, (Z)-	15.469	0.92

38.	Hexadecanoic acid, methyl ester	15.668	8.02
39.	cis-10-Heptadecenoic acid, methyl ester	16.430	0.35
40.	Heptadecanoic acid, methyl ester	16.641	0.12
41.	6,9-Octadecadienoic acid, methyl ester	17.192	0.51
42.	8,11-Octadecadienoic acid, methyl ester	17.245	0.45
43.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.364	29.36
44.	9-Octadecenoic acid (Z)-, methyl ester	17.452	35.59
45.	Methyl stearate	17.587	3.05
46.	8,11-Eicosadienoic acid, methyl ester	18.939	0.15
47.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.973	0.24
48.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	19.076	2.72
49.	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	19.261	0.96
50.	Eicosanoic acid, methyl ester	19.332	0.30
51.	Heneicosanoic acid, methyl ester	20.157	0.03
52.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.252	0.03
53.	Docosanoic acid, methyl ester	20.951	0.17
54.	1,5-Heptadien-4-one, 3,3,6-trimethyl-	21.249	0.05
55.	Tricosanoic acid, methyl ester	21.716	0.05
56.	Tetracontane	22.195	0.15
57.	Tetracosanoic acid, methyl ester	22.455	0.18
58.	Hexatriacontane	23.598	0.26

3.6.2-Antimicrobial activity

Foeniculum vulgar essential oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard human pathogens. The average of the diameters of the growth inhibition zones are displayed in Table (3.15).

Table 3.15: Inhibition zones (mm/mg sample)

] Conc.(mg/ml)		Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--

Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

Sa:*Staphylococcusaureus*
Ec.*: Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillusniger*
Ca.:*Candida albicans*
Bs.: Bacillus subtilis

At a concentration of 100mg/ml, the oil showed significant anticandidal activity. It also exhibited good activity against Gram Positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Escherichia coli.* However, it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa*

Conclusion

The oils from six medicinal plants collected from Jordon: *Foeniculum vulgare, Pimpinella anisum, Nigella sativa, Carum carvil, Petroselinum crispum and Lepidium sativum* have been studied. GC-MS analysis of the target oils, was conducted and the identification of the constituents was accomplished. In the antimicrobial assay the oils exhibited different antimicrobial responses.

Recommendations

- The isolated oils may be assessed for their antiviral antimalarial and other biological activities.
- *In vivo* antimicrobial potency of the isolated oil may be conducted.
- Other biologically interesting molecules in the targeted plants (like alkaloids and steroids) may be isolated and characterized and the biological activity could be evaluated.

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