Sudan University of Science and Technology

College of Graduate Studies

Constituents, Antimicrobial and Antioxidant Activity of Oils from Selected Plant Species

المكونات ومضادات الميكروبات ونشاط مضادات الاكسدة لزيوت بعض انواع النباتات المختارة

A Thesis Submitted in Fulfilment for the Requirements of the PhD. Degree in Chemistry

by

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February 2021

الآية

بسم الله الرحمن الرحيم

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ

صدق الله العظيم

سورة النور الآية 35

Dedication

To My

Mother's Soul

Dear Father

Brothers

Sisters

Husband

Sons

Acknowledgement

First and foremost praise is to Allah Almighty who provided me with strength to bring this work to completion.

I would like to express my gratitude and appreciation to my supervisor prof. Mohamed Abdelkarim Mohamed who did all him best to help, encourage and advice, without him valuable guidance this work would not have been finished.

My gratitude and thanks are extended to prof. Ibrahim Demirtas Dr.Serkan Koldas (Department of chemistry , faculty of Natural Sciences , Cankiri Karatekin University, Turkey) and Tevfik Ozen (Department of chemistry , faculty of Arts and Natural Sciences Ondokuz Mayis University, Turkey) for their care , support and helping me to carry the laboratory work.

Deep gratitude and thanks to my teachers' prof. Elshaikh Algilani and prof. Suad Abdelgader abdelrhaman (Alzaim Alazhari University) from their kind help.

I would also like to thank best friends and colleagues Enaam Khalil and Eman Salah for their efforts and standing with me to finish this thesis.

I would like to express my gratitude and appreciation to my father and family for their encouragement and patience for my own benefit and success.

My unlimited thanks to the faculty of graduate studies, Sudan University of Science and Technology for giving me the opportunity to do this research.

Abstract

The oils from targeted plant spies were extracted by n-hexane. Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied oils. The extracted oils have been screened for Antibacterial Activity against five standard human pathogens.

Citrullus colocynthis L. seed oil was characterized by GC-MS

Revealed the presence of (22) components. Major constituents are : 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (46.30 %), Hexadecanoic acid, methyl ester (16.35%) , 9-Octadecenoic acid (Z)-, methyl ester (15.58%) Methyl stearate (14.34%) .

Pimpinella anisum L. seeds oil was characterized by GC-MS

Revealed the presence of (24) components. Major constituents are

Anethole (82.75%) , Butanoicacid 2-methyl-, 2- methoxy-4-(2- propenyl) phenyl ester (6.25 %).

Melissa officinalis L. seeds oil was studied by GC-MS. The analysis revealed (26) constituents. Major constituents are:

9,12- Octadecadienoic acid (Z,Z)-, methyl ester (52.94%) - Octadecenoicacid (Z)-, methyl ester (12.01%) , Hexadecanoic acid, methyl ester (11.25%) and Methyl stearate (5.19%) .

`Origanum majarana L. seeds oil showed the presence of (35) constituents dominated by: 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (40.78%) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (33.61%) , Hexadecanoic acid, methyl ester (11.00 %) and Methyl stearate (5.72 %) .

Raphanus sativus L. seeds oil gave total of (25) components major components are:

13-docosenoic acid, methyl ester, (Z)-(28.02%),cis-13-eicosenoic acid, methyl ester(15.35%) , 9,12- octadecadienoic acid -(Z)-, methyl ester (12.75%) and hexadecanoic acid, methyl ester (8.63%).

Lepidium sativum oil showed total of (25) constituents .Major constituents are:

cis-13-eicosenoic acid methyl ester (16.48%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester(15.90 %) , hexadecanoic acid methyl ester(13.11%) and 9,12,15-octadecatrienoic acid methyl ester(Z,Z,Z)-(10.16%).

At a concentration of 100mg/ml*Citrullus colocynthis* L. seed

Oil was inactivate against *Bacillus subtilis*, partially active against *Staphylococcus aureus,* good against *Pseudomonas aeruginosa* and partially active against *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Candida albicans.* .

Pimpinella anisum L. seeds oil at a concentration of 100mg/ml the oil showed activate against *Bacillus subtilis*, partially active against *Staphylococcus aureus and Pseudomonas aeruginosa* and very activate against *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Candida albicans*.

Melissa officinalis L. seeds oil exhibited good activate against. *Staphylococcus aureus, Pseudomonas aeruginosa Candida albicans*and *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Bacillus subtilis.*

Origanum majarana L. seeds oil showed partially activate against, Staphylococcus *aureus and Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Bacillus subtilis.*

In the DPPH assay *Raphanus sativeus* oil showed IC50: 68.65±1.23 close to that of the standard antioxidant: butylated hydroxyl toluene (BHT) – (IC_{50} 61.11±1.78). In the superoxide anion scavenging assay , the oil

sample gave : IC_{50} 123.19±7.63 while the positive controls showed : BHT(IC_{50}, 60.5±0.22) and Trolox (IC_{50} 54.98±0.17).However , in the metal chelating assay the oil showed IC_{50}: 132.53±0.80) .

The antioxidant activity of *Lepidium sativum* oil was conducted. The oil showed a free radical scavenging capacity in the DPPH assay of: (IC_{50} 97.36±0.9). In the metal chelating assay, the studied oil showed $IC_{50:}$ 118.25±0.31. In the superoxide anion scavenging assay, the oil sample showed (IC_{50} 11.72±0.17) .

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المستخلص

تمت دراسة الزيوت المستخلصة من بعض النباتات بواسطة الهكسان .والتعرف على مكوناتها الكيميائية باستخدام كروماتوغرافياالغاز –طيف الكتلة , كما تم تحديد نشاطها الحيوي ضد خمسة انواع من الميكروبات المسببة للامراض للانسان .

زيت بذور نبات الحنظل وجد انه يحتوي على(22) مكون الاساسية منها : حمض 9, 12 – اوكتاديكاداييينويك ميثيل استر (46.30 %) , حمض هكساديكانويك ميثيل استر (16.35 %) حمض 9- اوكتاديكينويك ميثيل استر (15.58 %) ستيارات الميثيل (14.34 %).

زيت بذور نبات الينسون وجد انه يحتوي على (24) مكون : الاساسية منها انيثول (82.75 %) حمض بينتانويك -2 - ميثيل -2 - ميثوكسي – 4- (2 - برونيل) فينيل استر (6,25 %), سيس-(-) -2, a4, 5, 6, 9 a – هكساهيدرو3 ,5 , 5 ,9–(3,73 %).

زيت بذور نبات الترنجانة وجد انه يحتوى على (26) مكون : الاساسية منها حمض 9 , 12 – اوكتاديكاداييينويك ميثيل استر (52.94 %) , حمض 9- اوكتاديكينويك ميثيل استر (12.01%) حمض هكساديكانويك ميثيل استر (12.01 %) وستيارات الميثيل (5.19 %) .

زيت بذورنبات البردقوش وجد انه يحتوي على (35) مكون : الاساسية منها حمض 9, 12 , 15- اوكتا ديكاتريينويك ميثيل استر (40.78 %) ,حمص 9 , 12 -اوكتاديكاداييينويك ميثيل استر (33.0%) , حمض هكساديكانويك ميثيل استر (11.00 %) وستيارات الميثيل (5.72 %).

زيت بذور نبات الفجل وجد انه يحتوي على (25) مكون : الاساسية منها حمض دوكوسينويك ميثيل استر(28.02 %) , حمض سيس- 13- اكوسينويك ميثيل استر (15.35 %) , حمض9, 12- اوكتاديكاداييينويك ميثيل استر. 75. 12 , هكساديكانويك ميثيل استر(8,63)حمض 9- اوكتاديسنويك ميثيل استر (7.10 %).

زيت بذور نبات حب الرشاد وجد انه يحتوي على (25) مكون : الاساسية منها :حمض اكوسينويك ميثيل استر (16.48 %) , 9, 12 - اوكتاديكاداييينويك ميثيل استر (15.90 %) , حمض هكساديكانويك ميثيل استر (13.1 %) وحمض 9, 12 , 15- اوكتا ديكاتريينويك ميثيل استر(10.16 %).

تمت دراسة الفعالية البيولوجية للزيوت ضد خمسة من انواع مختارة من الاحياء المجهرية المرضية. عند تركيز (100ملجم / مل) اظهر نبات الحنظل نشاط جيد ضد بكتيريا الذائقة الزنجارية , نبات الينسون نشاط ضد العصوية الرقيقة ونشاط جيد جدا ضد الاريشية القولونية , نبات الترنجانة نشاط جيد ضد كل من العنقودية الذهبية , الذائفة الزنجارية , الاريشريشة القولونية , العصوية الرقيقة والخميرة , نبات البردقوش نشاط جزئي ضد كل من العنقودية الذهبية , الذائفة الزنجارية , الاريشريشة القولونية والخميرة.

أظهر نبات الفجل في دراسة مضادات الاكسدة بواسطة الجذر الحر (DPPH): (IC_{50}68.65±1.23) بينما اظهرت طريقة ربط المعادن نشاط :(IC_{50} 132.53±0.80) مقارنة مع ثنائى امين رباعي ايثيل حمض الخليك (EDTA) (IC_{50} 7.05±0.29) وطريقة سوبر اكسيد الهيدروجين (IC_{50} 123.19±7.63).

بينما اظهر نبات حب الرشاد نشاط مضاد للاكسدة بطريقة الجذر الحر (DPPH) عند (IC_{50} 97.36±0.96) اظهرت طريقة ربط المعادن نشاط :(IC_{50} 118.25±0.31) واظهرت طريقة سوبراكسيد الهيدروجين (IC_{50} 11.72±0.17).

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List of Abbreviations

MAP	Microwave Assisted Process
GC	Gas Chromatography
MS	Mass Spectrometer
HSV^{-1}	Herpes Simplex Virus type 1
HSV^{-2}	Herpes Simplex Virus type 2
EBV	Epstein-Barr Virus
POH	Perillyl alcohol
PA	Perillic Acid
Sa	*Staphylococcus aureus.*
Ec	*Escherichia coli.*
Bs	*Bacillus subtilis*
Ps	*Pseudomonas aeruginosa*
Ca	*Candida albicans*
DPPH	2,2-Diphenyl-1-picryldhydrazyl
BHT	butylated hydroxytoluene
BHA	butylated hydroxyanisole
NADH	Nicotamide adenine dinucleotide
NBT	Nitroblue tetrazolium
EDTA	Ethylene Diamine Tetra acetic Acid
IC_{50}	Inhibiting Concentration(half maximal)
PMS	Pantone Matching System
RT	Retention Time

CHAPTER ONE

1. Introduction

1.1Natural products

Natural products are chemical compounds or substances isolated from living organism.[1] The chemistry of the natural product include their biosynthesis, extraction, identification, quantification, structural elucidation, physical and chemical properties and reactions They are produced by the pathway of primary or secondary metabolism [2]. Metabolism is defined as series of enzyme-catalyzed biochemical reaction or transformation occurring within the cells of an organism which are mainly required for its growth, development and for proper response to its environment. [3] Metabolism can be in form of anabolism or catabolism. Metabolites are the intermediate or products of metabolism, the term metabolite is usually restricted to small molecules[4]. The secondary metabolites of plants have been mentioned as phytochemicals. Phytochemicals are the naturally occurring, biologically active compounds found in plants which have capabilities of disease inhibiting.[5.6] Phytochemicals are much effective in preventing disease because of antioxidant effect. Antioxidants defend molecules from oxidation if they are attacked by free radicals. They also prevent them from reactive oxygen. In this way prevention from many diseases and food spoilage is possible.[7.8] Before the introduction of orthodox medicines medicinal plants were used. Flowers, leaves, stems, seeds, roots, bark and fruit are constituents of the herbal medicines. Component of phytochemicals represent medicinal value of the natural plants.[9.10]. Phytochemicals perform physiological actions on human body. Important phytochemicals are tannins, alkaloids, phenolic compounds and flavonoids.[11]

1.2 Types of natural products

Several drug candidates are derived from various naturally occurring medicinal sources. These can be broadly divided into four categories: [12]

i. Natural products from microorganisms

Microorganisms as source of potential drug candidates were not explored until the discovery of penicillin in 1929[13] .Since then, a large number of terrestrial and marine microorganisms have been screened for discovery[14]

ii. Natural products from marine organisms

Two active compounds were isolated first time from the marine species. These were spongothymidine and spongouridine derived from Carribean sponge known as *Cryptotheca crypta* in 1950s. [15] The above compounds were nucleotides and represented great potential in the form of antiviral and anticancer agents. Discovery of these compounds led to the extensive research for the identification of novel drugs from the marine source. [16] Almost 70% of earth's surface is mainly covered by oceans. A number of marine organisms show sedentary lifestyle. So they synthesize complex and potent chemicals for the purpose of their defense from the predators. These chemicals may serve as remedies for ailments like cancer. Example is discodermolide isolated from marine sponge. [17] Discodermolide is a natural product having similar action to paclitaxel and possesses antitumor activity. It also has better solubility in water compared to paclitaxol. Combination of these two drugs led to reduce the tumor growth in various types of cancers. [15.18]

iv. Animals as a Source of Natural Products

Animals have been a major source of interesting compounds which were used as drugs. Epibatidine is obtained from skin of the Ecuadorian frog which is poisonous. It is 10 times more effective than the morphine. [19] Toxins and venoms obtained from animals played an important role in

the curing of several diseases. Example is Teprotide, extracted from the Brazilian viper. It led to development of the captopril and cilazapril which were effective against the hypertension. [20]

v. Natural Products from Plant Sources

The use of plant as medicines has a long history in the treatment of various diseases .The earliest known records for the use of plants as drugs are from Mesopotamia in 2600B.C. and these still are significant part of traditional medicine and herbal remedies[21] .To date ;35.000- 70.000 plant species have been screened for their medicinal use .[22]

1.3 Essential Oils

Essential oils are natural products that plants produce for their own needs other than nutrition (i.e. protection or attraction). In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. They are mainly made up by monoterpenes and sesquiterpenes whose main metabolic pathway is through mevalonate leading to sesquiterpenes and from methylerythritol leading to monoterpenes. They are located in different parts of the plant. They can be found in the root such as that of the vetiver grass (*Vetiveria zizanioides*), in stems like that of peteribi wood (*Cordia trichotoma*) and in cense, in leaves like in eucalyptus trees (*Eucalyptus citriodora*), citronella (*Cymbopogon nardus*), chinchilla (*Tagetes minuta*) and lemon grass (*Cymbopogon citratus*), in flowers like lavenders (*Lavandula officinalis*), in fruit like lemon, orange (*Citrus spp*.) and even in seeds as in the case of anise (*Pimpinella Anisum*), coriander (*Coriandrum sativum*) and pepper (*Piper nigrum*), among others.[23] They can work as internal messengers, like defense substances or plant volatiles aimed at natural enemies but also to attract pollinating insects to their host.[24]

Essential oil also can be defined as a "product obtained from natural raw material, either by distillation with water and steam, or from the epicarp of citrus fruits by mechanical processing. [25.26] Similarly, other names like essence, fragrant oil, volatile oil, etheric oil, aetheroleum or aromatic oil [27] have been used to describe essential oils. Essential oils can be obtained from various aromatic plants, most commonly grown in tropical and subtropical countries. They are obtained from various parts of the plants, such as seeds, buds, leaves, roots, fruits, rhizomes, barks and flowers. Oil cells, secretary ducts, cavities or in glandular hairs are some of the prominently explored cellular sources of essential oils in plants. Among many others, Apiaceae, Lauraceae, Rutaceae, Asteraceae, Pinaceae and Cup ressaceae are the well known and famous families rich in essential oil. Some of the essential oils can be found in animals sources such as musk, sperm whale, civet and can be produced by microorganism. Hydrodistillation, steam distillation, microwave-assisted distillation, solvent extraction, cold pressing and supercri tical fluid extraction. [28.29.30] are some the applied techniques used for extraction of oils.

Historically, the ancient Romans and Greeks in 1st century described the instrumental procedures for extraction. [31] Clear evidence which depicts the primitive form of distillation technology, which was in use in 400 BC is found in Taxila Museum, Pakistan [32]. While in late 12th or early 13th century (1235–1311 AD), Arnald de Villanova compiled detailed information about the conventional hydrodistillation method. [32]

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenous layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant. Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome

(galangal ginger), root (*Angelica archangelica*), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated factors, climatic, seasonal and geographical conditions, and harvest period and extraction techniques. [33] The yield of oils from the plants can also be affected by the stages of the plant growth.

Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators. The oxygenated molecules of essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers. [34.35, 36] These properties were established after the oils have been extracted from the plant materials.

1.3.1Extraction of essential oils

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and they are more or less modified during the preparation process.[37]

The oil droplets being stored in the oil glands or sacs can be removed by either accelerated diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are;

a. Hydrodistillation
b. Hydrodiffusion
c. Effleurage.
d. Cold pressing
e. Steam distillation
f. Solvent extraction
g. Microwave Assisted Process (MAP)
h. Carbondioxide extraction.

The technique of hydrodistillation involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapour and oil are removed through a water cool condenser. Hydrodiffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma. [38]

The process of **e**nfleurage is applicable to flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating

would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odourless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind only the essential oil; hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

Another method of extracting essential oil that has not found high application in scientific research is cold pressing. It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

Steam distillation is the most common method of extracting oils and is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel. Next steam is added and passed through the plant that contains the plants aromatic molecules or oils. The plant releases the aromatic molecules and the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapours. As they cool, they condense and transform into a liquid state.

Solvent extraction is also used to extract essential oils. This method involves the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used

for extraction. For instance, leaves, roots, fruits are extracted with benzene or with mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and colour. After the extraction, the solvent is removed by distillation under reduced pressure leaving behind the semisolid concentrate, this concentrate are extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil.

A Microwave assisted process is used to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants. [39] This technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction. [40. 41.42, 43.44] This technique has also been applied for the extraction of saponins from some medicinal plants. [45]

Carbon dioxide may also be used for extraction of essential oils**.** In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition of the oil.

1.3.2 Analysis of essential oils

The two main purposes of analyzing essential oils are:

i. To identify and quantify as many constituents as possible.

ii. To evaluate the quality of the oils and detect any possible adulteration that may affect their usage. Analysis of essential oils is generally performed using Gas chromatography (qualitative analysis) and Gas chromatography-mass spectroscopy (qualitative and quantitative analysis). [46] Gas chromatography analysis is a common confirmation test.

1.3.2.1Gas chromatography (GC)

Gas chromatography analysis is a process used for separating chemicals in a complex sample and provides a representative spectral output. The gas chromatography instrument vapourizes the sample and then separates and analyte components. Each component ideally produces a specific spectral peak. The time elapsed between injection and evaluation is called "Retention time".

The sample is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separate before eluting from the column end. The detector measure different compounds as they emerge from the column.

1.3.2.2 Gas chromatography-mass spectrometry (GC/MS)

Gas chromatography-mass spectrometry analysis is a method which combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. The gas chromatography-mass spectrometry instrument is made of two parts: The gas chromatography (GC) portion separates the chemical mixture into pulses of pure chemicals and mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed through the GC, the

chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions. This is important because the particles must be charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent, so that it can be put into various uses.

1.4 Medicinal and pharmacological uses of essential oils

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides. [38] Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure or mitigate or prevent diseases, infection and indisposition by means of inhalation. [47]

Inhalation of essential oils or their individual volatile terpenes has a significant role in controlling disorders of the central nervous system. For instance, aroma inhibit of storax pill essential oil and preinhalation of *Aconus gramineus* rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy.[48.49] The fragrance compounds, cisjasmonate, which characterized the aroma of *Jasminum grandiflorum*, have a tranquilizing effect on the brain upon inhalation. [50]They significantly increased the sleeping time of mice induced by pentobarbital. Cendrol, which is a major component of card wood essential oil, shows a sedative effect and prolonged pentobarbital induced sleeping time on rats upon inhalation .[51] The vapour of lavender essential oil or one of its main component linalool may also be applicable to the

treatment of menopausal disorder through inhalation. [52] Lavender essential oil demonstrated an analgesic activity. [53]

Medical professionals are more interested in the medicinal properties of essential oils. Many oils show antibacterial, fungicidal, relaxant, stimulating, antidepressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both by pathogenic and non-pathogenic diseases. Pathogenic diseases caused by bacterial, virus, and the fungi can be treated with essential oils.

Strong in vitro evidence indicates that essential oil can act as antibacterial agent against a wide spectrum of pathogenic bacteria strains including; *Listeria monocytogenes, Salmonella typhimurium, Shigella dysentria, Bacillus cerus,* and *Staphylococcus aureus*. [5455.56.57.58Thyme and oregano essential oils can inhibit some pathogenic bacteria strains such as *E.coli, Salmonella typhimurium, Salmonella entritidis* and *Salmonella choleraesuis* [59], with the inhibition directly correlated to the phenolic components carvacrol and thymol. Eugenol and carvocrol showed an inhibitory effect against the growth of four strains of *Escherichia coli* and *Listeria monocytogens*. [60]. Also, the presence of phenolic hydroxyl group in carvacrol particularly is credited with its activity against pathogens such as *Bacillus cereus.* [61]. Essential oil with high concentration of thymol and carvacrol e.g. oregano, savory and thyme, usually inhibit Gram positive more than Gram-negative pathogenic bacteria. [62] However, they show antibacterial activity against Gram- negative *Haemophilus influenza* and *Pseudomonas aeruginosa* respiratory pathogens, while Gram-positive *streptococcus pyrogens* was the most resistant to the oil [63]. Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural mouth

control,[64] general improvement of oral health [65], interdental hygiene [66] and to control oral mal odour [67]. Mouth rinses containing essential oils with chlorohexine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decreases chances of postoperative infections, decreases oral bacterial load and decrease aerolization of bacteria [68]. Mouth washes containing essential oils could also be used as part of plaque-control routine since they can penetrate the plaque biofilm kill pathogenic-wall and inhibiting their enzymatic activity. [69] In addition, essential oil in mouth washes prevent bacterial aggregation slows the multiplication and extract bacterial endotoxins.[70] *Croton cajucarabenth* essential oil was found to be toxic to some pathogenic bacteria and fungi associated with oral cavity diseases.[71]

Besides their antibacterial and antifungal activities, essential oils have also been reported to possess interesting antiviral activities alternative to synthetic antiviral drugs. They have demonstrated virucidal properties with the advantages of low toxicity [72]; *Herpes simplex* virus (type III) causes some of the most common viral infections in human and can be fatal. Synthetic antiviral drugs have been used to treat Herpes infection [73], but not all are efficacious in treatment of genital herpes infections. Incorporation of *Artemisia arboreseens* essential oils in Multilamella liposomes greatly improved its activity against intra cellular *Herpes simplex* virus type 1 (HSV-1) [74]. Due to the presence of citral and citronellal in *Melissa officinalis* L. essential oil, it also inhibits the replication of HSV-2 [75] and the ability to replicate of HSV-1 can be suppressed with different essential oils in vitro. [76] Also the effect of five essential oils on Epstein-Barr virus (EBV) (viridae) has been reported.which caused the infectious mononucleosis associated with Burkitt lymphoma and naso-pharynx carcinoma.

Essential oils can also be used for the treatment of non-pathogenic diseases. For instance, Garlic essential oil significantly lowered serum cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases [77]. The hypolipidemic action of garlic oil is primarily due to a decrease in hepatic cholestrogenesis [78]. Some essential oils also exert hypotensive activity when applied in vivo and they are used for treating hypertension. Oral administration of combination of oregano, cinnamon, cumin, and other essential oils decreases systolic blood pressure in rats and intravenous administration of the essential oil from the aerial parts of *Mentha villosa* induced a significant dose-dependent hypotension associated with decrease in heart rate. This activity was attributed to volatile component, piperitenone oxide which represents 55.4% of the oil. The hypotensive effect induced by the oil is probably due to its direct cardiodeplesant action and peripheral vasolidation, which can be attributed to both endothelium-dependent and endothelium-independent mechanism.

Intravenous administration of essential oil of basil (*Ocimum gratissium*) induced an immediate and significant hypotension ([79]. The hypotensive activity of the essential oil resulted from its vasodilator effect, acting directly upon vascular smooth muscles. This effect was attributed to eugenol; but from a safety point of view, care must be taken in dealing with eugenol due to its suspected carcinogenicity and hepatotoxicity.

Intravenous injection of the monoterpene alcohol terpinen-4-ol decrease main aortic blood pressure in a dose related manner, in a conscious DOCA-salt hypertensive rats. The mechanism of action was related to the induction of vascular smooth muscle relaxation rather than enhanced sympathetic nervous system activity. Terpinene-4ol is a major constituent of several essential oil, particularly tea tree [80] and sweet marjoram essential oils. Some essential oils may aggravate diabetes, for instance

rosemary essential oil showed hyperglycaemic and insulin release inhibitory effect in diabetic rabbits. [81] It has been emphasised that the lipophilic fraction of aromatic plants are not generally responsible for any anti-diabetic activity showed by these plants, but it was also indicated that an oral administration of a combination of essential oils including cinnamon, cumin, oregano, fennel, myrtle besides others was able to enhance insulin sensitivity in type II diabetes, in addition to lowering circulating glucose in diabetic rats. The essential oil of *Satureja khuzestanica* results in significant decreases in fasting blood glucose level in diabetic rats [82].

Essential oils and their individual aroma components showed cancer suppressive activity when tested on a number of human cancer cells lines including glioma, tumours, breast cancer, leukaemia and others. Glioma is one of the most malignant human tumours [83]. A significant effect on the treatment of glioma is by using the sesquiterpene hydrocarbon element which is found in small amounts in many essential oils, it prolonged quality survival time of patients with glioma [84].

Antiangiogenic therapy is one of the most promising approaches to control cancer. Perillyl alcohol (POH) which is the hydroxlated analogue of d-limonene has the ability to interfere with angiogenesis [85]. POH either alone or with PA (perillic acid, the major metabolite of POH in the body), has the potential use as an anticancer drug that stimulates different types of tumour to apoptosis inhibit their proliferation and overcomes their resistance to chemo/radiotherapy [86]. Treatment of human leukaemia cells with eucalyptus oil showed morphological changes (fragmentation of DNA) indicating an induction of apoptosis [87]. The essential oil of lemon balm (*Mellisa oficinalis* L) was found to be effective against a series of human cell lines(A549, MCF-7, Caco-2, HL-60, K562) and a mouse cell line (B16F10) [88] and that of *Artemisia annua* L. induced

apoptosis of cultured SMMC-7721 hepatocarcinoma cells.[89] The essential oils of Australian tea tree (*Melaleuca alternifolia*) and its major monoterpene alcohol, terpinen-4-ol, were able to induce apoptosis in human melanoma M14 WT cells.[90]

There is evidence to suggest that the effect of the total oil of terpinen-4-ol was meditated by their interaction within the plasma membrane and subsequent reorganisation of membrane lipids. Hepatic arterial infusion with Curcuma oil had a similar positive effect in treating primary liver cancer as that of the chemical drugs [91].

The essential oil of *Tetraclinis articulate*, (a conifer tree) showed the hallmarks of apoptosis when tested on a number of human cancer cell lines including melanoma, breast and ovarian cancer. [92]

1.5 Chemistry of essential oils

Essential oils are produced by various differentiated structures, especially the number and characteristics of which are highly variable. Essential oils are localized in the cytoplasm of certain plant cell secretions, which lies in one or more organs of the plant; namely, the secretory hairs or trichomes, epidermal cells, internal secretory cells, and the secretory pockets. These oils are complex mixtures that may contain over 300 different compounds [93]. They consist of organic volatile compounds, generally of low molecular weight below 300. Their vapor pressure at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in the vapor state. [94.95] These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohols, aldehydes, and ketones offer a wide variety of aromatic notes, such as fruity ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal (-seining).

Furthermore, essential oil components belong mainly to the vast majority of the terpene family(Figure 1) Many thousands of compounds belonging to the family of terpenes have so far been identified in essential oils [96], such as functionalized derivatives of alcohols(geraniol,bisabolol), ketones (menthone, p-vetivone) , aldehydes (citronellal, sinensal), esters (tepinyl acetate, cedryl acetate), and phenols (thymol). Essential oils also contain non-terpenic compounds biogenerated by the phenylpropanoids pathway, such as eugenol, cinnamaldehyde, and safrole.

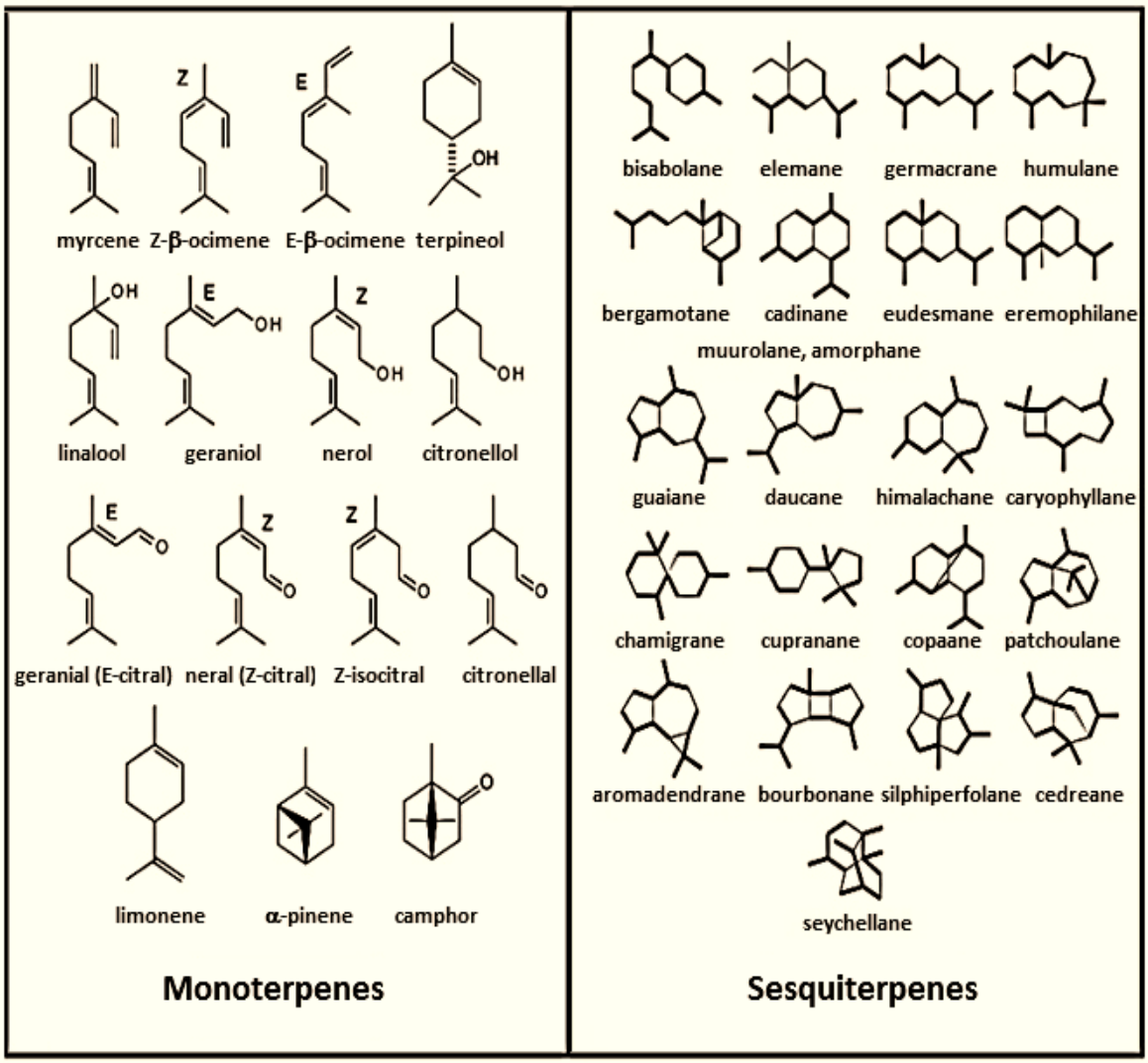


Figure 1.1 Structures of some terpenes

Essential oils have a very high variability of their composition, both in qualitative and quantitative terms. Various factors are responsible for this variability and can be grouped into two categories: (i) Intrinsic factors

related to the plant, and interaction with the environment (soil type and climate, etc.) and the maturity of the plant concerned, even at harvest time during the day (ii) extrinsic factors related to the extraction method and the environment.The factors that determine essential oil yield and composition are numerous. In some cases, it is difficult to isolate these factors from each other as they are interrelated and influence each other. These parameters include the seasonal variations, plant organ, and degree of maturity of the plant, geographic origin, and genetics. [97.98]

Several techniques are used for the trapping of volatiles from aromatic plants. The most often used device is the circulatory distillation apparatus described by Cocking and Middleton [99] introduced in the European Pharmacopoeia and several other pharmacopoeias. This device consists of a heated round-bottom flask into which the chopped plant material and water are placed and which is connected to a vertical condenser and a graduated tube, for the volumetric determination of the oil. At the end of the distillation process, the essential oil is separated from the water phase for further investigations. The length of distillation depends on the plant material to be investigated. It is usually fixed to 3–4 h.

A further improvement was the development of a simultaneous distillation–solvent extraction device by Likens and Nickerson in 1964 [100]. The device permits continuous concentration of volatiles during hydrodistillation in one step using a closed-circuit distillation system.

1.6 Antimicrobial activity of Essential Oils

The antimicrobial properties of essential oils and of their constituents have been considered [101.102] and the mechanism of action has been studied in detail [103]. An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable [104]. This can then cause leakage of ions and other cellular

molecules [105]. Although a certain amount of leakage is tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death. [106]

It has been reported that essential oils containing mainly aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol were characterized by the highest antibacterial activity, followed by those containing terpene alcohols. Others, containing ketones or esters, such as -myrcene, -thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive .[107]

Generally, essential oils are characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol which possess important antibacterial activities .[103.107]

These compounds are responsible for the disruption of the cytoplasmic membrane, the driving force of protons, electron flow, active transport, and also coagulation of cell contents. [104.106.108]

The chemical structure of essential oils affects their mode of action concerning their antibacterial activity. The importance of the presence of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, was confirmed. [105.109]

However, the relative position of the phenolic hydroxyl group on the ring does not appear to influence the intensity of the antibacterial activity.

The action of thymol against *Bacillus cereus, Staphylococcus aureus*, and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol, for example. [103.105] However, carvacrol and thymol act differently against Gram-positive and Gram-negative species. Thymol, eugenol, and carvacrol have an antimicrobial effect against a broad spectrum of bacteria: *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus*, and *Helicobacter pyroli* [110.111]

1.7 The target Plants

1.7.1 *Citrullus colocynthis*

Citrullus colocynthis **L.Schrad** is a valuable plant from Cucurbitaceae family, widely distributed in the barren region.It is a non-hardy, herbaceous perennial vine, branched from the base [112]. *Citrullus colocynthis* fruits are generally documented for its broad range of pharmaceutical uses as well as medicinal and nutraceuticals potential. It is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, antidiabetic, and insecticide [113].Cucurbitaceae family is one of the best genetically assorted accumulations of restorative plants in the plant kingdom. Most plants of this family are dry season tolerant, intolerant to wet, frost-sensitive and ineffectively drained soils [114]. In the course of the most recent two decades, India and China have been the biggest cucurbit makers took after by Russia, United States of America, Egypt and Republic of Iran. *Citrullus colocynthis* ***(*L.*)*** **Schrad**. Is a Cucurbitaceae family plant. [114] The plant is generally accessible in the Sahara and Arabian deserts, Sudan and a Southern piece of Asia including Pakistan, India and Southern Islands. The fruit is intense and globular with a smooth surface. It is hard and has a skin around it and contains 200–300 seeds/gourd. Seeds are small (6mm in length), ovoid, compressed, smooth and brownish when ripe. Seeds constitute about 75% of the weight of fruit of *Citrullus colocynthis* [115].

C. colocynthis is widely distributed around the world from Mediterranean Europe, Cyprus, the Syrian Arab Republic, Lebanon, and Jordan to Egypt, Kuwait, Saudi Arabia, Turkey, the Islamic Republic of Iran, Pakistan, Afghanistan, India, North Africa, and Sahel.

C. colocynthis is a perennial plant (in wild) or an annual herb [116] that can propagate both by vegetative and generative means.

- Scientific Classification:

Kingdom:	Plantae
Subkingdom :	Tracheobinta
Division :	Magnoliophyta
Class:	Magnoliopsida
Order :	Cucurbitale
Family :	Cucurbitaceae
Genus :	Citrullus Schrad.
Species:	Citrullus colocynthis (L.)

1.7. 2 *Pimpinella anisum*

Pimpinella anisum (anise) is a plant belonging to the Umbelliferae family. It is one of the oldest medicinal plants. It is an annual grassy herb with 30–50 cm high, white flowers, and small green to yellow seeds, which grows in the Eastern Mediterranean Region, West Asia, the Middle East, Mexico, Egypt, and Spain and also cultivated in Pakstan, Iran, Turkey India and many other African and Asin conuntries [117]. The plant is primarily grown for its fruits (aniseeds).

In the food industry, anise is used as flavoring and aromatic agent for fish products, ice cream, sweets, and gums [117.118]. Also it is popular used in medicine, perfumery and cooking.*Pimpinella anisum* seeds are used as an ingredient in cough medicines and reported to have diuretic and diaphoretic properties. Seeds are also used to treat dyspeptic complaints and catarrh of the respiratory tract. It was reported that anise seeds has several therapeutic effects [119.120] on several conditions such as digestive, neurologic, cough [121] and respiratory disorders. Anise seeds are used in Middle East as appetizer and are specially known for its digestive properties [122]. Anise seeds' extract of water when consumed after meals helps in the process of digestion. Among the reported pharmacological

effects we can find anise extracts active as anti-ulcer[123.124], antispasmodic[125],Antibiotic[125],performance enhancement (immunomodulation)[127] , Insecticidal[128]

Anise is belonging to the family of *Apiaceae* (Umbelliferae) which consists 300-455 genera and 3000-3750 species distributed in the northern hemisphere [129]. Members of this family have alternate leaves, widening at the base into a sheath that clasps the stem. The stems of these family members are often furrowed. The compound flowers are determined in umbels. The rays of the main umbel produced a secondary umbel with the flower bearing pedicels. The flowers of this family have 5 petals and 5 stamens. The fruits form below where the petals and stamen originate. Fruits or seeds are in pairs, commonly petals and stamen originate. Fruits or seeds are in pairs, commonly conspicuously ribbed, and sometime winged. The genus *Pimpinella* L. consist 150 species spread in Eurasia and Africa, more than16 of which present in Europe. The family *Apiaceae* can be familiar by certain characters that are generally found in the group including the herbaceous nature of the family; the frequent occurrence of compound leaves; small flowers, with a small number of floral parts arranged in whorls and grouped in shaped inflorescences. The genus includes herbaceous annual, biannual, or perennial plants, usually with a fine hair covering.

From medicinal and agricultural point of view, only few species are economically significance, these are including, *Pimpinella anisum* L., *P. major*, *P. saxifraga* L., *P. peregrina*L. And *P.diversifolia*L.[130].

Anise (*Pimpinella anisum* L.) is a slow growing annual herb which is cultivated throughout the world. For cultivating of anise plant a warm, sunny and dry autumn is ideal to meet economical yield and high quality of essential oil. So cultivation of anise in the northern part of the world does not pay, because the harvest is repeatedly poor. The anise plant

grows well in light to loose, humus soil. The field must be free from the weeds however rich in nutrients and not too dry. [131].

-Scientific classification:

Kingdom:	Plantae
Subkingdom :	Tracheobionta
Superdivision	Spermatophyta
Division :	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order :	Apiales
Family :	Apiaceae
Genus :	*Pimpinella* L.
Species:	*Pimpinella anisum* L.

1.7.3 *Melissa officinalis*

Melissa officinalis L (also called other common names like lemon balm, bee balm, Melissa, sweet balm) is a member of the mint (Labiatae) family. Melissa is the Greek word for bee, and the plant is a favorite honey bee. It is native of the southern Europe, western Asia, northern African and Iran. [132.133]

Melissa officinalis is a perennial plant growing wild in fields and gardens and a long road side. It is known for its lemony flavor and fragrance. Ancient Greeks and Romans used lemon balm in surgical dressings for wounds and in preparations to treat venomous or infectious bites and stings such as caused by dogs and scorpions. Today lemon balm's primary use involves the treatment of cold sores and teething. It is also commonly used as antibacterial and antifungal agent. Lemon balm combined with other calming herbs, such as valerian, helps to reduce anxiety and insomnia [134.135]. Lemon balm is recommended to induce sweating and relieve fever due to cold and flu and to ease menstrual cramps, insomnia, headaches and nervousness [136.137.138.139]. .The balm also relieves craps, dyspepsia, flatulence and colic [137.139]. Due to lemony smell and pretty white flowers, lemon balms are extensively cultivated in

gardens throughout the world and are commonly used today in perfumery; cosmetics and food industries Lemon balm in Turkish "ogulotu"or "kovanocu" is natively found in coastal part of Mediterranean. It is cultivated as a spice (flavouring salads), as a medicinal and as an ornamental plant. It is traditionally used as sedative, anxiolytic ,antispasmodic , carminative ,diaphoretic digestive and antiseptic in folk medicine[136.137.138].In traditional Austrian medicine *Melissa officinalis* leaves have been prescribed for internal use as tea or external application .As an essential oil it is used for treatment of the disorders of the gastrointestinal tract , nervous system , liver and bile[140] . In alternative medicine it is used as a sleep aid and digestive aid. *Mellissa officinal* (lemon balm) essential oils are popular in aromatherapy. [141]

Medicinal uses of this herb includes**:** **i**nsomnia and restless sleep, restlessness and hyperactivity in children, Nervousness, agitation and general anxiety disorder , improvement of cognitive function, concentration, memory, and focus. Improvement of cognitive function and reduction of agitation in Alzheimer's disease, antiviral activity against herpesn simplex 1 and 2. [142] The plant showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and several other Gram-positive strain of bacteria. It also exhibited antispasmodic activity on the smooth muscle of the digestive tract.The analgesic effects are connected to the active constituents; rosmarinic acids and eugenol which are known to reduce inflammation and pain. The herb is also antifungal inhibiting activity for yeasts and filamentous fungi [143] .It is traditionally used for gastrointestinal colic, spasm, pain, and nervous discomfort.

Lemon balm is widely cultivated in Europe and the United States, but also grows wild along paths and roadsides. The plants prefer sandy and

loamy fertile soils, well drained and at pH range 5 to 7. It grows well in full sun, but it also grows in partial shade [144].

-Scientific classification

Kingdom:	Plantae
Subkingdom	Angiosperms
Division :	Eudicots
Class:	Asterids
Order :	Lamiales
Family :	Lamiaceae
Genus :	Melissa
Species:	Melissa officinalis

1.7. 4 *Origanum majarana*

The *Origanum* genus belongs to the Lamiaceae family. [145] The species of this genus are important aromatic plants widely used in many countries in food industries [146]. They are also employed as powerful disinfectants, flavoring agents, in perfumes and in scenting soaps.[147] Origanum species which are well known for their essential oils, have been applied in the flavoring of various foods, soups, sauces, meat ,fish canned foods, liqueurs.[148)]. The ectivenessof this genus as a medicinal herb is attributed to the specific composition of essential oils, [149] flavonoids, [150] phenolic acids and other chemical constituents [151]. *Origanum majorana* is useful as a health complement and in food preservation. [152] Traditionally, *O. majorana* has been used as a Remedy against asthma, indigestion, headache and rheumatism. [153]

Many phytochemical studies have been conducted to investigate the chemical composition of the essential oil of *O.majorana*. [154.155]

Origanum majorana L. (MajoranahortensisMoench.), sweet marjoram, is a perennial, evergreen subshrub which grows in south Europe, North Africa and Turkey [156]. *O. majorana* has been used to treat colds and rhinitis [156]

The herb and essential oil is used against cramps, depression, gastrointestinal problems, headaches, and as a diuretic. The essential oil is used externally for chest congestion, muscle aches and arthritis. Warm olive oil infused with sweet marjoram is a reported remedy for ear infections. It can be prepared as an infusion, mouth wash, poultice; the oil is an ingredient in ointments and compound preparations. The oil of *O. majorana* is used commercially to scent soaps, lotions and perfumes [157.158].

-Scientific classification:

Kingdom: Plantae
Division : Magnoliophyta
Class: Magnoliopsida
Order : Lamiales
Family : Lamiaceae
Genus : Origanum
Species: Origanum majarana

1.7. 5 *Raphanus sativus*

Raphanus sativus L. (radish), a member of the Cruciferous family, is an annual herb consumed as vegetable throughout the world. Various varieties are available that differ mainly in the size, shape and color of their thick roots [159]. The roots are most valuable and edible part of radish, although the stem and leaves have been also used for food flavoring or preservation [160]. Radish is an excellent food remedy for stone, gravel, and scorbutic conditions. As similar to other cruciferous vegetables the nutritional value of radish is derived from its content of many essential minerals and vitamins, carbohydrates, high content of fiber and low content of fat [161]. The application of radish in traditional medicince to treat various infectious diseases has stimulated a great interest in investigating its antimicrobial activity [162] 10. Radish is also widely used in traditional medicine in various part of the world for treatment of different ailments and disorders affecting the respiratory

urinary and gastrointestinal system, anemia, female and male infertility and the skin infections [163,164]. Many of the pharmacological activites of radish are attributed to the occurrence of a wide range of secondary metabolites, including alkaloids, phenolics, flavonods, coumarins, carotenoids, antioxidant enzyme, terpenes, glucosinolates and other compounds [159.161.164.165].The seeds of the radish contain a high percentage of oil. Chromatographic anaylsis of these oils showed clearly their complete similarities to cotton seed oil.

-Scientific classification:

Kingdom:	Plantae
Subkingdom :	Tracheobinta
Superdivision	Sermatophyt
Division :	Magnoliophta
Class:	Magnoliopsida
Subclass:	Dillenidae
Order :	Capparales
Family :	Brassicaeae
Genus :	*Raphanus* L.
Species:	*Raphanus sativus* L.

1.7. 6 *Lepidium sativum*

Lepidium sativum **L.** is known as garden cress, garden pepper .It is a fast growing annual herb belonging to the Brassicaceae family that is native to Egypt and west Asia [166]. The seed yields up to 58% of edible oil that can be used for lighting [167] and traditional medicine. In traditional system of medicine various part of this plant have been used for the treatment of jaundice ,liver problems, spleen disease ,gastrointestinal disorders menstrual problem , fracture , arthritis and other inflammatory conditions [168.169.170].Seeds are antioxidant[171]. *Lepidium sativum* seeds are used in south Asia as traditional medicine to treat bronchitis, asthma and cough it is considered aborfacient, diuretic, expectorant, aphrodisiac, antibacterial, gastroprotective, laxative and stomadic [172].

-Scientific classification:

Kingdom:	Plantae
Subkingdom :	Tracheobinta
Superdivision:	Spermatophytes
Division :	Magnoliophta
Class:	Magnoliopsida
Subclass:	Dillenidae
Order :	Capparales
Family :	Brassicaea
Genus :	Lepidium
Species:	Lepidium sativum L.

Aim of this study

This study was aimed to:

-Extract oils from six potential plants: *Lepidium sativum, Raphanus sativus, Origanum majorana, Melissa officinalis, Pimpinella anisum, and Citrullus colocynthis.*

-Perform a GC/MS analysis to identify and quantify the constituents of the extracted oils.

-Evaluate the oils for their antimicrobial activity.

- Determination of antioxidant activity

CHAPTER TWO

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

Seed of *Lepidium sativum*, *Raphanus sativus Origanum majarana, Mellissa officinalis*, *Pimpinella anisum, Citrullus colocynthis* were purchased from the local market in Cankiri Kartekin city, Turkey. Plants were authenticated by direct comparison with reference herbarium samples.

2.1.2 Insteruments

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

2.1.3 Test organisms

The targeted oils were assessed for antibacterial activity using the standared microorganisms shown table (2.1).

Table 2.1 Test organisms

Ser.No	Micro organism	Type
1	*Bacillus subtilis*	G^{+}ve
2	*Staphylococcus aureus*	G^{+}ve
3	*Pseudomonas aeruginosa*	G^{-}ve
4	*Escherichia coli*	G^{-}ve
5	*Candida albicans*	Fungi

2.2 Methods

2.2.1 Extraction of oils

Powdered seeds of studies plant (350g) were macerated with n-hexane**.** The solvent was removed under reduced pressure and the oil kept in the fridge at 4^0 C for further work.

The oil (2ml) was placed in a test tube and 7 m*l* of alcoholic sodium hydroxide were added followed by 7m*l* of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. (2m*l*) of supersaturated sodium chl0ride were added, then (2m*l*) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5 µ*l*) of the hexane extract were mixed with (5m*l)* diethyl ether .The solution was filtered and the filtrate (1 µ*l*) was injected in the GC- MS vial.

2.2 .2 GC – MS analysis

The extracted oils were analysis by gas chromatography – mass spectrometry. A Shimadzo GC-MS –QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25m, thickness) was used. Helium(99%Pur) was used as carrier gas .Chromatographic conditions are as follows: column oven temperature : 150^0 C; Injection Temperature : 3oo.oo °C : Injection Mode : Split ; Flow Control Mode : Linear Velocity ; Pressure : 100.0 kPa ; total flow : 50.0m*l*/min ; column flow : 1.54m*l* /sec.; linear velocity: 47.2 cm/sec .;purge flow : 3.0 m*l* /min .; spilt ratio: -1.

2. 2. 3 Antibacterial assays

i. Preparation of 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 tine arid finally suspended in 100 ml normal saline to produce a Suspension containing about 10^8-10^9 colony 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 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 m*l*) 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. Preparation of 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 saline, and the suspension was stored in the refrigerator until used.

iii. Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil, (2ml) or the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45° in a water bath. (20ml) 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 corn borer (NO 4). Each one- of the halves was designed for one or 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 micro titer pipette and allowed to diffuse at room temperature for Two hours. The plates were then incubated upright position at 37°C for 24 hours.

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

2. 2 .4 Determination of antioxidant activity

The antioxidant activity of oil was conducted by different methods: metal chelating, free radical (DPPH$^{\cdot}$**)** and superoxide anion scavenging activity compared to positive standards. The metal chelating, free radical and superoxide anion scavenging activity were tested at different concentration to calculate IC_{50} (μg / ml) and r^2 values. All antioxidant assays were carried out in triplicate.

i. Free radical scavenging activity

In this assay, the bleaching rate of a stable free radical (DPPH$^{\cdot}$**)** is monitored at a characteristic wavelength (λ_{max} 517 nm) in the presence of sample. The test sample was mixed with DPPH$^{\cdot}$ **(**0.1 mM, 0.5 ml). The absorbance was recorded at λ_{max} 517 nm and compared with standards. During this assay there was a change in color from purple to yellow.

ii. Metal chelating activity

Ferrozine produces a violet complex with Fe^{2+}. In the presence of a chelating agent, complex formation is interrupted and as a result the

violet color of the complex is decreased[7]. The Fe^{2+}-chelating activity of the sample was recorded using the absorbance of the ferrozine-Fe^{2+}complex at λ_{max} 562 nm. The sample was added to $FeCl_2$ (2 mM, 0.05 mL). The test was initiated by the addition of ferrozine. The absorbance of the mixture was recorded at λ_{max} 562 nm after incubating at room temperature for 10 min.

iii. Superoxide anion scavenging activity

The activity of the sample was estimated according to the methods of Nishikimi et al[8] and Zhao et al.[9] with minor modification. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed by reduction of NBT. Briefly, a (1 ml) sample was thoroughly mixed separately with of (156 μM) NBT and (468 μM) NADH, respectively. The reaction started by adding (60 μM) PMS. After incubation, the absorbance of the mixture was measured at λ_{max} 532 nm. A decrease in absorbance of the mixture indicates an increase in superoxide anion-scavenging activity.

CHAPTER THREE

3. Results and Discussion

In this study six medicinal plants grown in Turkey has been analyzed by GC-MS and their biological activity have been investigated.

3.1 *Citrullus colocynthis*

The GC-MS analysis of *Citrullus colocynthis* L. seed oil was conducted. The constituents have been identified by retention times and interpretation of MS fragmentation pattern.

3.1.1 Constituents of oil

The GC –MS analysis of the studied oil revealed the presence of (22) components. Table (3.1) .The typical total ion chromatograms (TIC) are depicted in Fig. (3.1). They are (4) major constituents of the oil table (3.2).

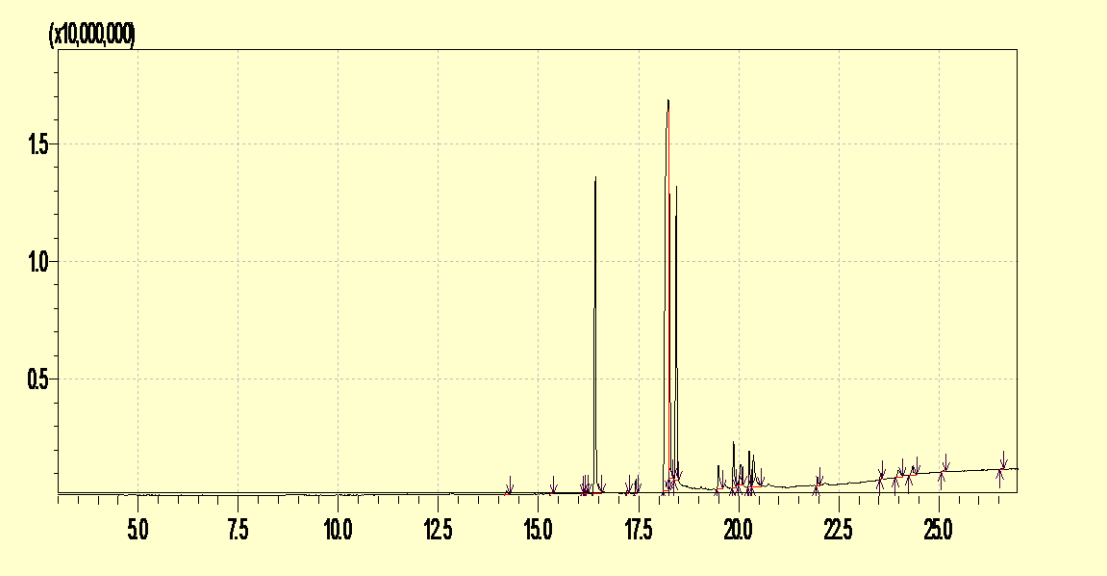


Fig. 3.1: Total ion chromatograms of *Citrullus colocynthis* L.

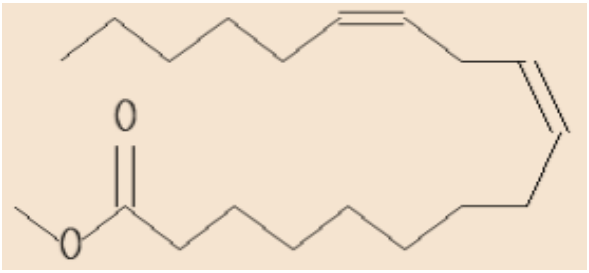
Table 3.1:constituent of Citrullus colocynthis L. seeds oil

ID#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.195	0.21
2.	Pentadecanoic acid, methyl ester	15.322	0.08
3.	7,10-Hexadecadienoicacid,methyl ester	16.098	0.02
4.	7-Hexadecenoicacid,methylester, (Z)-	16.155	0.03
5.	9-Hexadecenoicacidmethyl ester, (Z)-	16.201	0.11
6.	Hexadecanoic acid, methyl ester	16.416	16.35
7.	cis-10-Heptadecenoicacid,methyl ester	17.216	0.14
8.	Heptadecanoic acid, methyl ester	17.430	0.47
9.	9,12-Octadecadienoicacid(Z,Z)-, methyl ester	18.239	46.30
10.	9-Octadecenoicacid (Z)-, methyl ester	18.255	10.58
11.	Methyl stearate	18.442	14.34
12.	Methyl9.cis.,11.trans.t,13.trans.-octadecatrienoate	19.489	1.55
13.	Cyclopropaneoctanoic acid, 2methyl ester	19.868	2.24
14.	9-Octadecenoicacid,12-hydroxy-, methyl ester, [R-(Z)]-	20.035	1.43
15.	Eicosanoic acid, methyl ester	20.262	1.30
16.	PGH1, methyl ester	20.364	2.67
17.	Docosanoic acid, methyl ester	21.965	0.40
18.	Tetracosanoic acid, methyl ester	23.543	0.21
19.	.gamma.-Sitosterol	23.988	0.72
20.	Squalene	24.336	0.66
21.	Hexacosanoic acid, methyl ester	25.113	0.13
22.	.gamma.-Tocopherol	26.564	0.06

Table 3.2: Major constituent of Citrullus colocynthis L. seeds oil

No.	Name	R.T.	Formula	Mw	Area%
1	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.239	$C_{19}H_{34}O_2$	294	46.30
2	Hexadecanoic acid, methyl ester	16.416	$C_{17}H_{34}O_2$	270	16.35
3	Methyl stearate	18.442	$C_{19}H_{38}O_2$	298	14.34
4	9-Octadecenoic acid (Z)-, methyl ester	18.239	$C_{19}H_{36}O_2$	296	15.58

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.3.2. The peak at m/z294 with retention time 18.239 corresponds to the molecular ion M^+ [$C_{19}H_{34}O_2$] while the signal at m/z263 is due to loss of a methoxyl group. Fig.3.3 represents the mass spectrum of hexadecanoic acid, methyl ester,. The peak at m/z270 with retention time 16.416 is due to the molecular ion M^+ [$C_{17}H_{34}O_2$]. Fig.3.4 shows the mass spectrum of methyl stearate. The signal at m/z298 (retention time: 18.442) is due to the molecular ion M^+ [$C_{19}H_{38}O_2$].The peak at m/z267 is due to loss of a methoxyl .The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.3.5. The peak at m/z 296 with retention time 18.225 accounts for the molecular ion M^+ [$C_{19}H_{36}O_2$] .



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

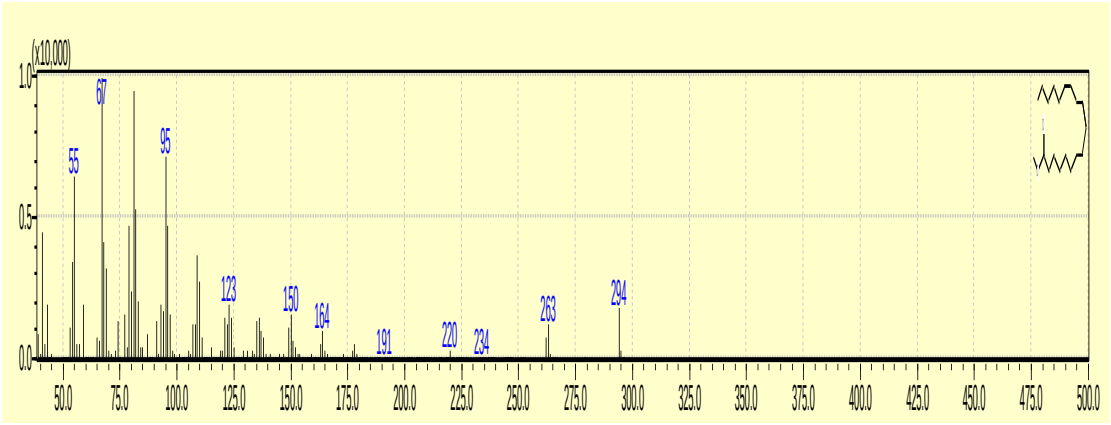
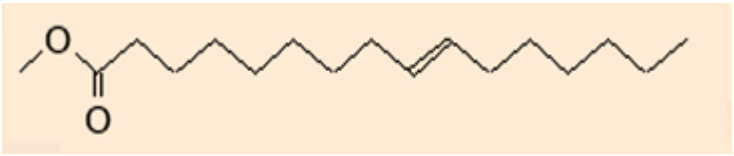


Fig. 3.2: mass spectrum of 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester



Hexadecanoic acid, methyl ester

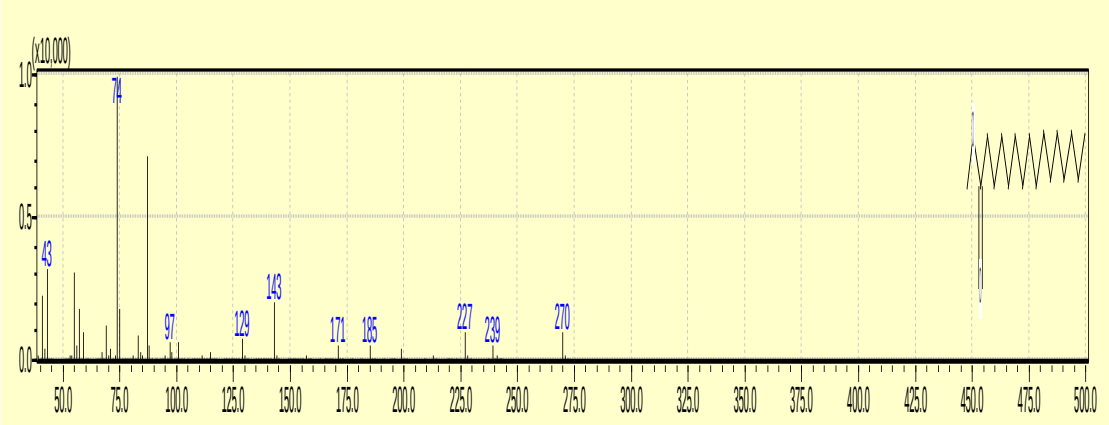
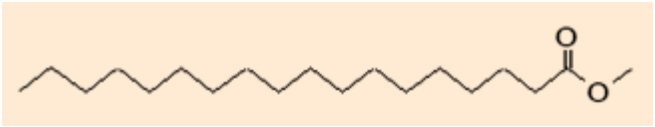


Fig.3.3: mass spectrum of Hexadecanoic acid, methyl ester



Methyl stearate

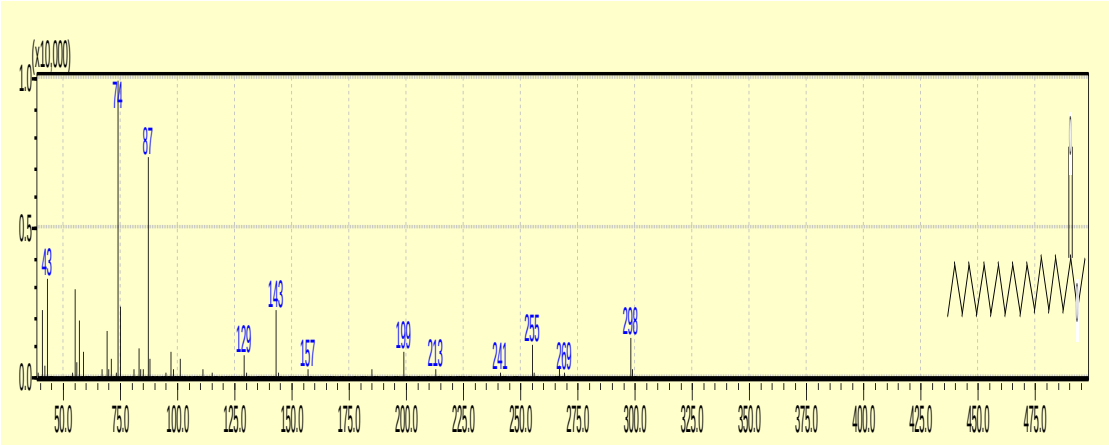
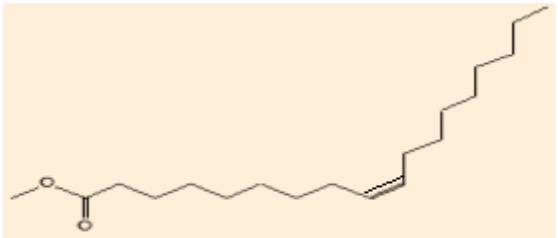


Fig.3.4: Methyl stearate



9-octadecenoic acid (Z)-, methyl ester

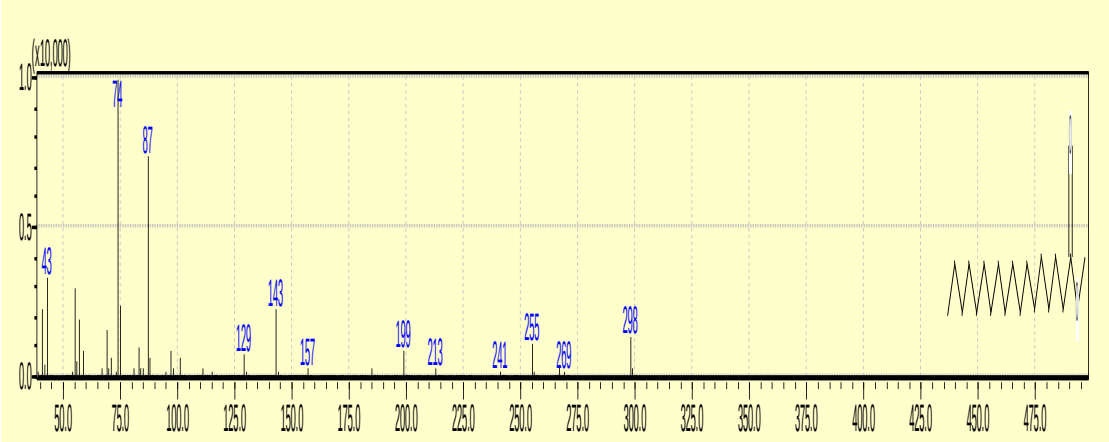


Fig.3.5: 9-Octadecenoic acid (Z)-, methyl ester

3.1.2-Antimicrobial activity

In cup plates agar diffusion assay the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (3.3).

At a concentration of 100mg/ml the oil showed inactivate against *Bacillus subtilis,* partially active against *Staphylococcus aureus,* good against *Pseudomonas aeruginosa* and partially active against *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Candida albicans.* Tables (3.4) and (3.5) represent the Antibacterial Activity of standard Antibacterial and antifungal drugs respectively.

Table3.3: Inhibition zones (mm) of *Citrullus colocynthis* oil

Sample	Conc	Ec.	Pa.	Sa.	Bs.	Ca.
Citrullus colocynthis	100	16	17.5	14	9	-

Table 3.4: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc mg/ml	Bs	Sa	Ec	Ps
Ampicillin	40 20 10	15 14 11	30 25 15	- - -	- - -
Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table 3.5 : Antifungal activity of standard chemotherapeutic agent

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

Sa: Staphylococcus aureus.

Ec: Escherichia coli.

Ps: Pseudomonas aeruginosa.

Bs: Bacillus subtilis

Ca: Candida albicans.

3.2-*Pimpinella anisum*

GC-MS analysis of *Pimpinella anisum* L. seeds oil was accomplished and the identification of the constituents was based on retention times and MS fragmentation pattern.

3.2.1-Constituents of oil

GS-MS analysis of the studied oil revealed the presence of (24) components - Table (3.6).The typical total ion chromatograms (TIC) is depicted in Fig (3.6). There are (3) major constituents of the oil table (3.7).

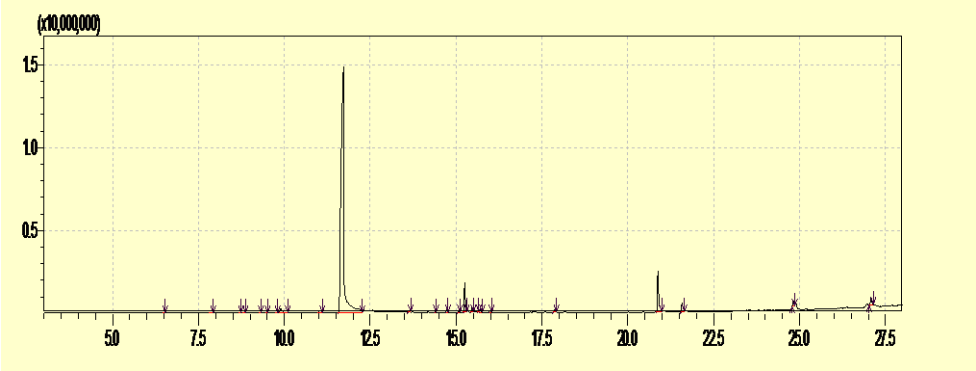


Fig. 3.6: total ion chromatograms of *Pimpinella anisum L*

Table 3.6: Constituents of *Pimpinella anisum L*. seed oil

ID#	Name	Ret.Time	Area%
1.	Eucalyptol	6.482	0.09
2.	1,6-Octadien-3-ol, 3,7-dimethyl-	7.850	0.07
3.	Cyclohexene, 5,6-diethenyl-1-methyl-	8.703	0.05
4.	(+)-2-Bornanone	8.808	0.04
5.	endo-Borneol	9.274	0.02
6.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	9.479	0.01
7.	.alpha.-Terpineol	9.761	0.03
8.	Phenol, 2-methyl-6-(2-propenyl)-	9.890	1.27
9.	Estragole	10.990	0.22
10.	Anethole	11.719	82.75
11.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	13.621	0.11
12.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	14.387	0.04
13.	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene	14.730	0.31
14.	Isolongifolene, 4,5-dehydro-	15.074	0.09
15.	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	15.248	3.73
16.	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	15.455	0.57
17.	1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-	15.638	0.12
18.	.beta.-Bisabolene	15.689	0.30
19.	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	15.985	0.25
20.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]	17.852	0.31
21.	Butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	20.874	6.25
22.	Butanoic acid, 2-methyl-, 4-methoxy-2-(3-methyloxiranyl)phenyl ester	21.576	1.40
23.	Tetracontane	24.816	0.75
24.	Hexatriacontane	27.080	1.22

Table 3.7: Major Constituents of *Pimpinella anisum L.* seeds oil

No.	Name	R.T	Formula	Mw	Area	Area%
1	Anethole	11.719	$C_{19}H_{36}O_2$	148	1919105	82.75
2	Butanoicacid 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	20.874	$C_{15}H_{20}O_3$	248	6939863	6.25
3	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	15.248	$C_{15}H_{24}$	204	41384 64	3.73

Fig.3.7 shows the mass spectrum of anisole. The peak at m/z148 which appeared at RT.11.719 in total ion chromatogram corresponds M^+ [$C_{19}H_{36}O_2$]. The mass spectrum of Butanoicacid 2-methyl-, 2-methoxy-4-(2- propenyl)phenyl ester(6.25%) is depicted in Fig.3.8.The peak at m/z248(RT. 20.874) is due to the molecular ion : to M^+ [$C_{15}H_{20}O_3$], while the peak at m/z 191) corresponds to loss of a methoxyl function .The mass spectrum of cis-(-)-2,4a,5,6,9a-Hexahydro- 35,5,9-tetramethyl (1H) benzocycloheptene is presented in Fig.3.9. The peak at m/z204 which appeared at RT. (15.248) in total ion chromatograms corresponds: M^+ [$C_{15}H_{24}$].

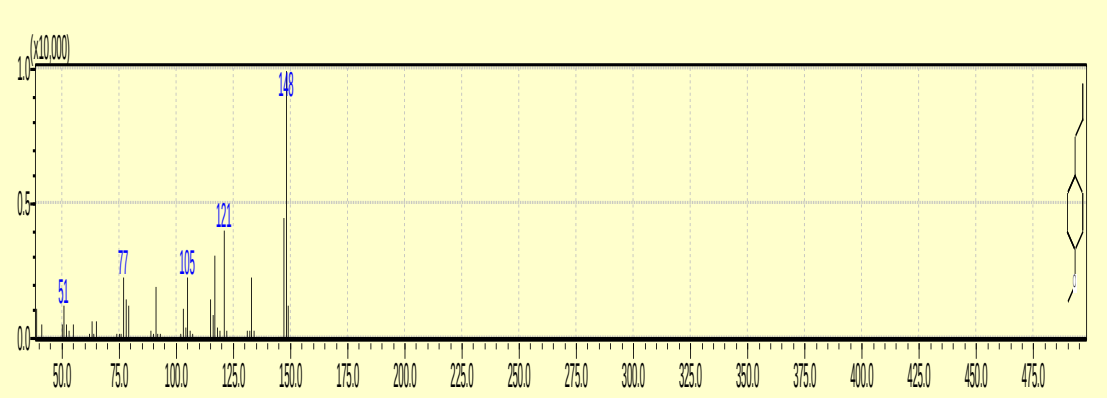


Fig.3.7: Mass spectrum of Anethole

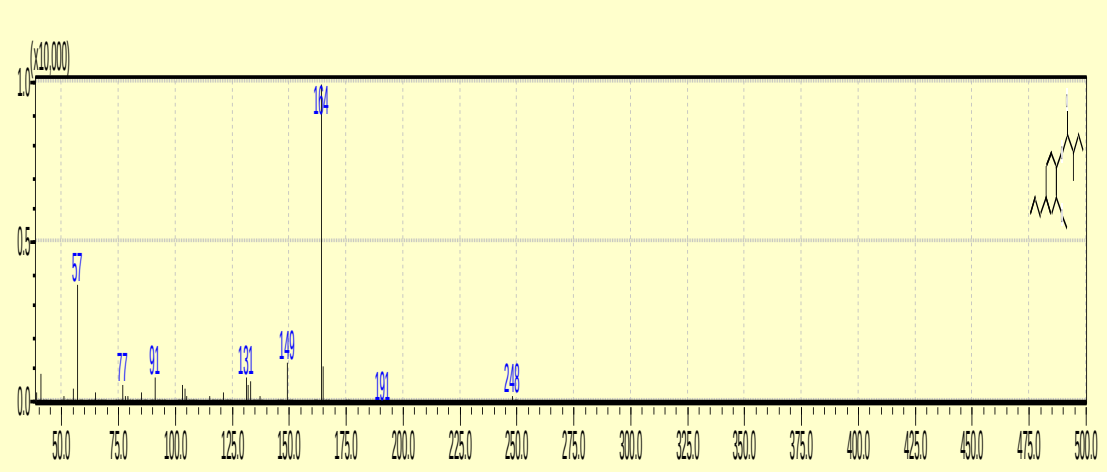


Fig.3.8: Butanoicacid 2-methyl-, 2-methoxy-4-(2-propenyl) phenyl ester

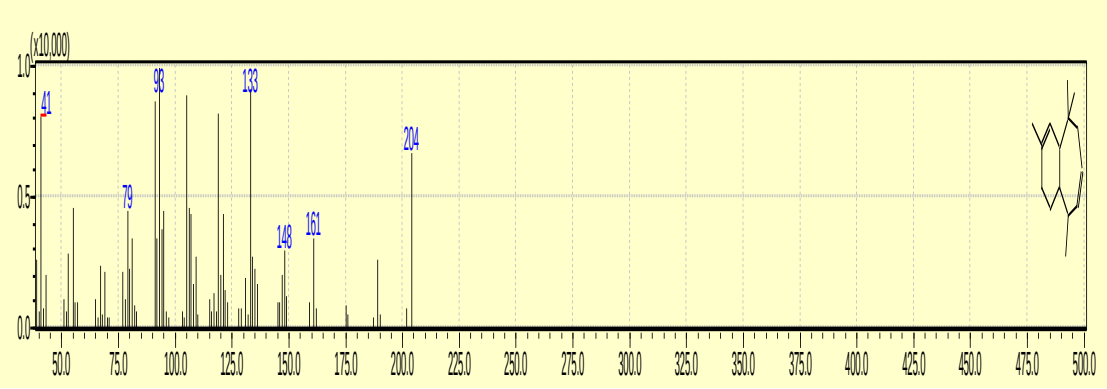


Fig.3.9: cis-(-)-2, 4a, 5, 6, 9a-Hexahydro-3, 5, 5, 9- tetramethyl (1H) benzocycloheptene

3.2. 2 Antibacterial Activity

Pimpinella anisum L seeds oil was screened for Antibacterial Activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (3.8). The results were interpreted in the following manner ;(< 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.).

At a concentration of 100mg/ml the oil showed activate against *Bacillus subtilis*, partially active against *Staphylococcus aureus and Pseudomonas aeruginosa* and very activate against *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Candida albicans.* Tables (3.9) and (3.10) represent the antibacterial activity of standard antibacterial and antifungal drugs respectively.

Table3.8: Inhibition zones (mm) of *Pimpinella anisum* seeds oil

Sample	Con	Ec	Pa	Sa	Bs	Ca
Pimpinella anisum oil	100	21.5	9	11.5	18	-

Table 3.9: Inhibition zones (mm) of standard chemotherapeutic agents

Drug	Conc mg/mL	Bs	Sa	Ec	Ps
Ampicillin	40 20 10	15 14 11	30 25 15	- - -	- - -
Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

3.3 - *Melissa officinalis*

Melissa officinalis L. seeds oil was studied by GC-MS. The analysis revealed (26) constituents Table-(3.10).The typical total ion chromatograms (TIC) is illustrated in Fig (3.10). They are (4) major constituents of the oil table (3.11).

Table 3.10: Constituents of *Melissa officinalis L.* seeds oil

ID#	Name	Ret.Time	Area%
1.	Hexanoic acid, methyl ester	3.353	0.03
2.	L-.alpha.-Terpineol	6.987	0.04
3.	Nonanoic acid, methyl ester	7.331	0.01
4.	Methyl tetradecanoate	13.569	0.09
5.	Pentadecanoicacid,14-methyl-, methyl ester	13.711	0.39
6.	Pentadecanoic acid, methyl ester	14.643	0.06
7.	7-Hexadecenoic acid, methyl ester, (Z)-	15.428	0.28
8.	9-Hexadecenoic acid, methyl ester, (Z)-	15.473	0.29
9.	Hexadecanoic acid, methyl ester	15.682	11.25
10.	cis-10-Heptadecenoic acid, methyl ester	16.439	0.39
11.	Heptadecanoic acid, methyl ester	16.647	0.22
12.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.418	52.94
13.	9-Octadecenoic acid (Z)-, methyl ester	17.441	12.01
14.	Methyl stearate	17.598	5.19
15.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	18.132	0.54
16.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.986	4.43
17.	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	19.105	0.87
18.	cis-11-Eicosenoic acid, methyl ester	19.141	1.55
19.	Eicosanoic acid, methyl ester	19.341	2.63
20.	7,10,13-Eicosatrienoic acid, methyl ester	19.396	0.89
21.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.507	1.08
22.	1H-Naphtho[2,1-b]pyran-8(4aH)-one, 3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-	19.748	0.34
23.	Heneicosanoic acid, methyl ester	20.164	0.30
24.	Docosanoic acid, methyl ester	20.959	2.69
25.	Tricosanoic acid, methyl ester	21.721	0.28
26.	Tetracosanoic acid, methyl ester	22.461	1.21

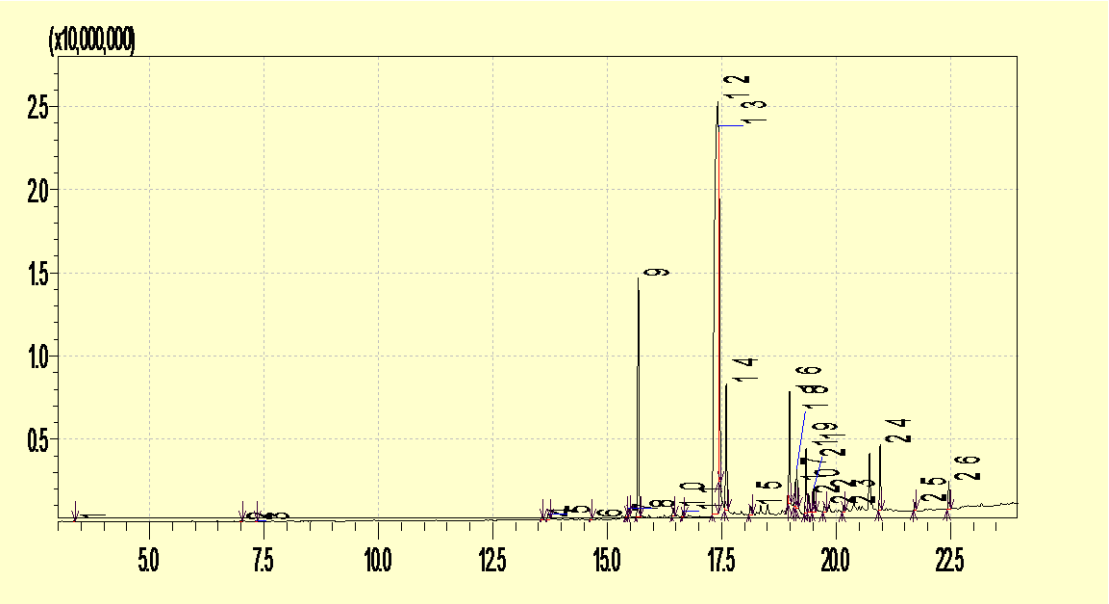


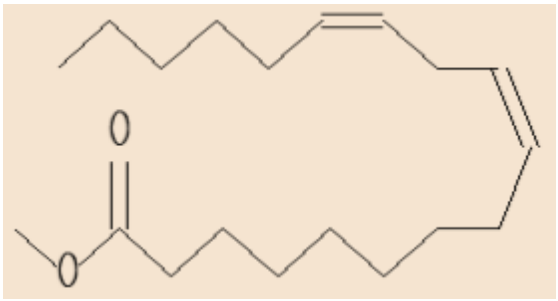
Fig. 3.10: Total ion chromatogram of *Melissa officinalis L.*

Table 3.11: Major Constituents of *Melissa officinalis L.* seeds oil

No.	Name	R.T	Formula	Mw	Area	Area%
1	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.418	$C_{19}H_{34}O_2$	294	145201315	52.94
2	9-Octadecenoic acid (Z)-, methyl ester	17.441	$C_{19}H_{36}O_2$	296	32948951	12.01
3	Hexadecanoic acid, , methyl ester	15.682	$C_{17}H_{34}O_2$	270	30851627	11.25
4	Methyl stearate	17.598	$C_{19}H_{38}O_2$	298	14237978	5.19

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.11. The peak at m/z294 with retention time 17.418 corresponds to the molecular ion M^+[$C_{19}H_{34}O_2$] while the signal at

m/z263 is due to loss of a methoxyl group. The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.12. The peak at m/z 296 with retention time 17.441 accounts for the molecular ion M^+ [C_{19} H_{36} O_2]. Fig.3.13 represents the mass spectrum of hexadecanoic acid, methyl ester,. The peak at m/z270 with retention time 15.682 is due to the molecular ion M+ [$C_{17}H_{34}O_2$]. Fig.14 shows the mass spectrum of methyl stearate. The signal at m/z298 (retention time: 17.598) is due to the molecular ion M+ [$C_{19}H_{38}O_2$] .The peak at m/z267 is due to loss of a methoxyl.



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

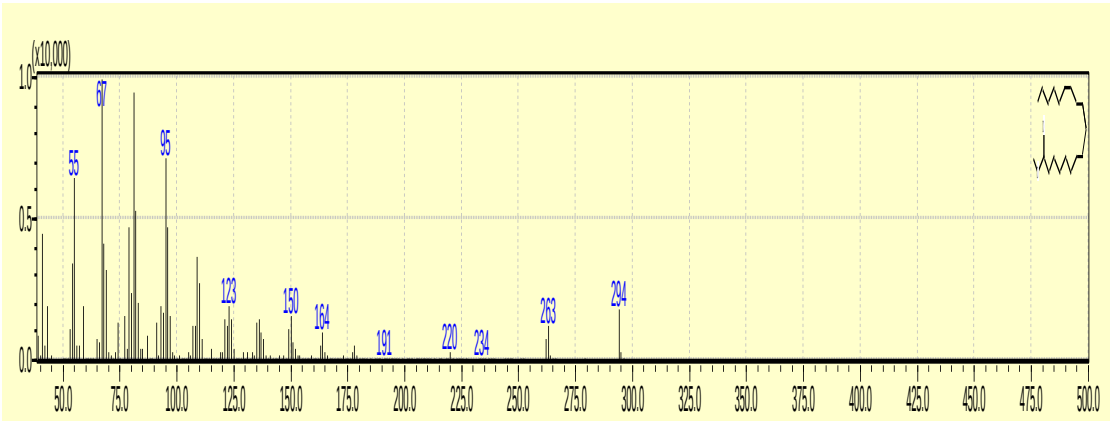
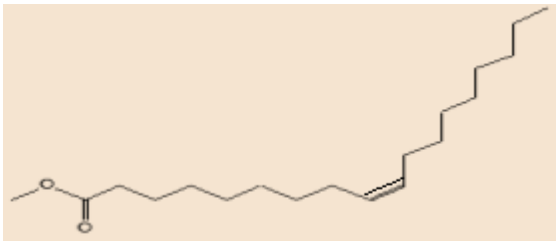


Fig.11: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester



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

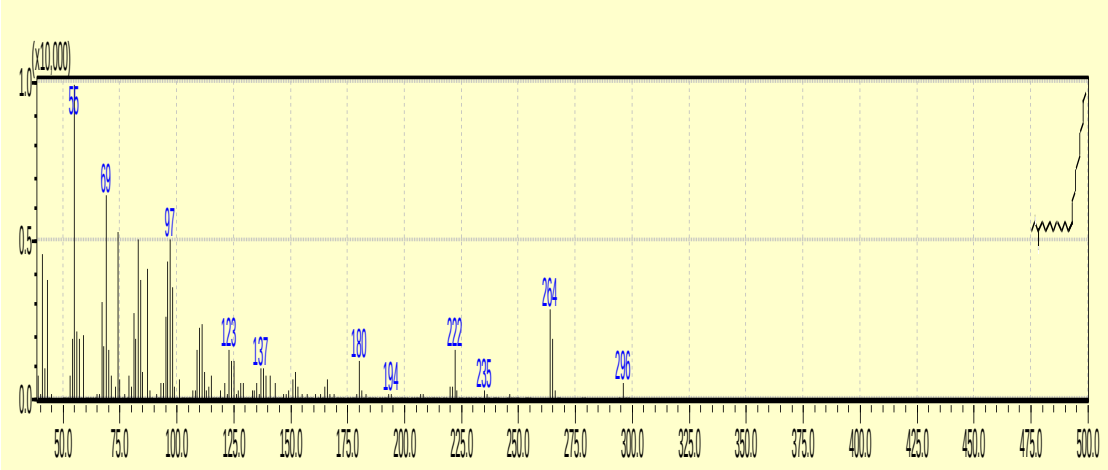


Fig: 12: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

Hexadecanoic acid, methyl ester

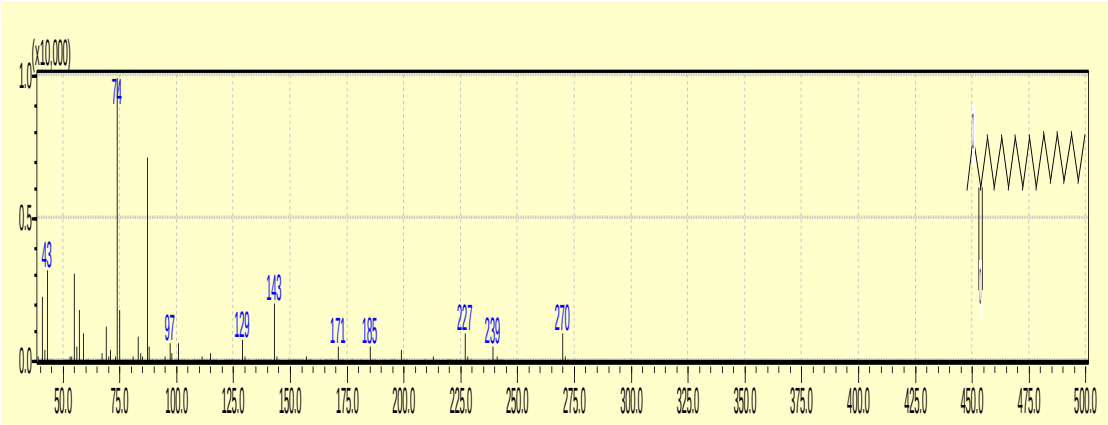


Fig.13: Mass spectrum of hexadecanoic acid, methyl ester

Methyl stearate

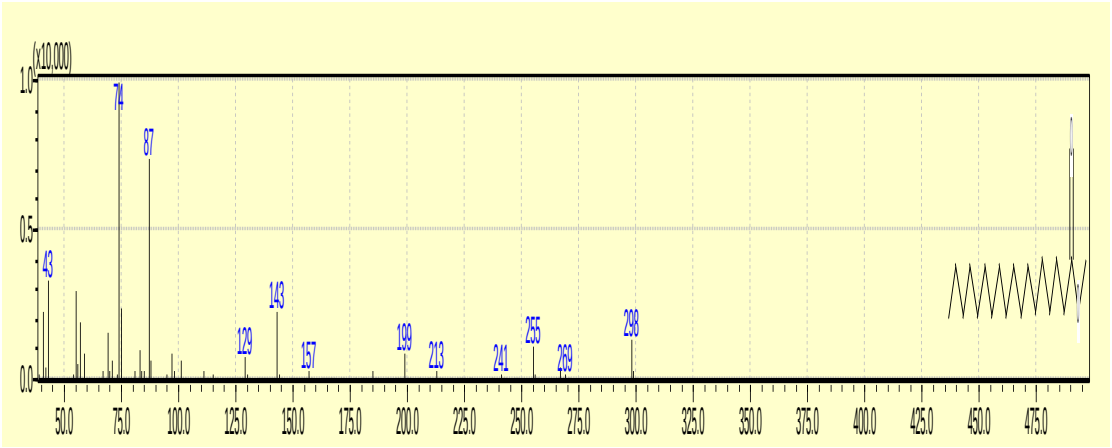


Fig.3.14: Mass spectrum of methyl stearate

3.3. 1. Antibacterial Activity

Melissa officinalis L. seeds oil was screened for Antibacterial Activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (3.12). The results were interpreted in the following manner ;(< 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.).

At a concentration of 100mg/ml the oil showed good activate against. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Candida *albicans*and *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Bacillus subtilis.*

Table3.12: Inhibition zones (mm) of *Melissa officinalis* oil

Sample	con	Ec	Pa	Sa	Bs	Ca
Melissa officinalis	100	17	15	18	-	16

3.4 -*Origanum majarana*

GC-MS analysis of *Origanum majarana* L. seeds oil was accomplished and the constituents of the oil have been identified and quantified

3.4.1-Constituents of oil

GS-MS analysis of the studied oil showed (35) constituents- Table (3.13).The typical total ion chromatograms (TIC) are illustrated in Fig (3.15). They are (4) major constituents table (3.14) .

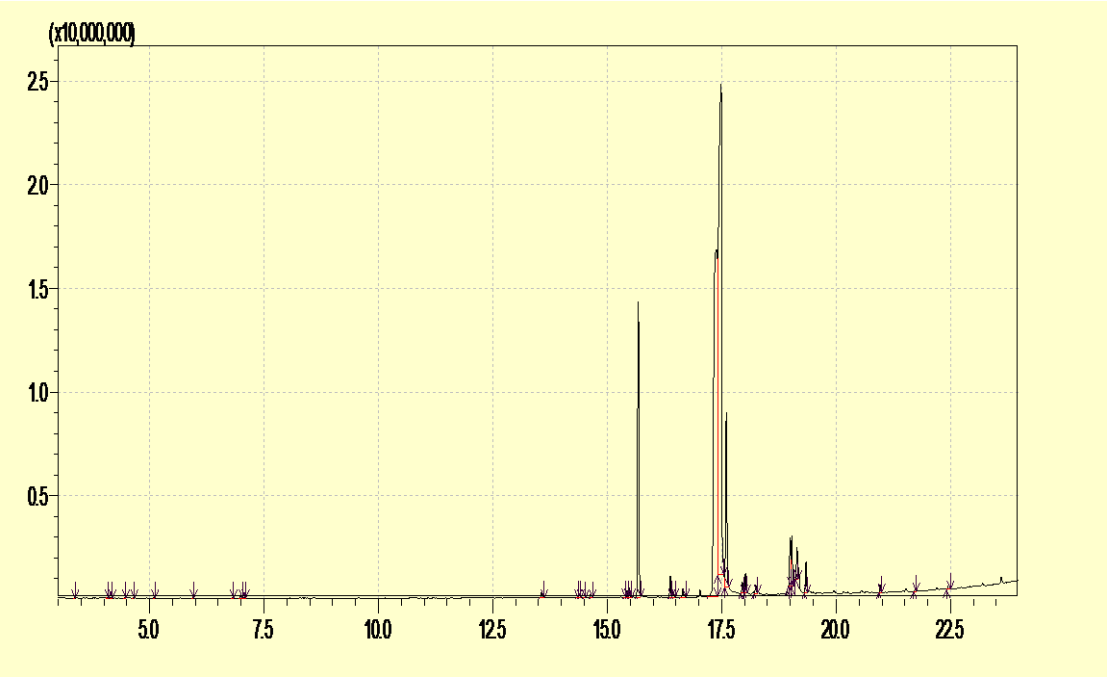


Fig .3.15: total ion chromatograms of *Origanum majarana* L.

Table 3.13: Constituents of *Origanum majarana* L. seed oil

ID#	Name	Ret.Time	Area%
1.	Hexanoic acid, methyl ester	3.353	0.00
2.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	4.065	0.00
3.	Heptane, 2,2,4,6,6-pentamethyl-	4.157	0.00
4.	6-Heptenoic acid, methyl ester	4.463	0.01
5.	Benzene, tert-butyl-	4.644	0.01
6.	.gamma.-Terpinene	5.094	0.01
7.	Octanoic acid, methyl ester	5.936	0.01
8.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	6.802	0.02
9.	.alpha.-Terpineol	6.985	0.07
10.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	7.076	0.01
11.	Methyl tetradecanoate	13.569	0.21
12.	Tridecanoic acid, 12-methyl-, methyl ester	14.343	0.02
13.	5-Octadecenoic acid, methyl ester	14.377	0.02
14.	4-Octadecenoic acid, methyl ester	14.482	0.01
15.	Pentadecanoic acid, methyl ester	14.644	0.14
16.	7,10-Hexadecadienoic acid, methyl ester	15.373	0.02
17.	7-Hexadecenoic acid, methyl ester, (Z)-	15.431	0.24
18.	9-Hexadecenoic acid, methyl ester, (Z)-	15.475	0.31
19.	Hexadecanoic acid, methyl ester	15.681	11.00
20.	Hexadecanoic acid, 14-methyl-, methyl ester	16.378	0.65
21.	cis-10-Heptadecenoic acid, methyl ester	16.437	0.18
22.	Heptadecanoic acid, methyl ester	16.648	0.31
23.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.388	33.61
24.	9,12,15-Octadecatrienoic acid, methyl ester,	17.488	40.78
25.	Methyl stearate	17.600	5.72
26.	cis-11,14-Eicosadienoic acid, methyl ester	17.953	0.32
27.	cis-10-Nonadecenoic acid, methyl ester	18.019	0.55
28.	Nonadecanoic acid, methyl ester	18.240	0.31
29.	.gamma.-Linolenic acid, methyl ester	18.995	1.47
30.	Octadecanoic acid, 9-oxo-, methyl ester	19.032	1.45
31.	cis-13-Eicosenoic acid, methyl ester	19.145	1.06
32.	Eicosanoic acid, methyl ester	19.339	0.95
33.	Docosanoic acid, methyl ester	20.960	0.26
34.	Tricosanoic acid, methyl ester	21.725	0.07
35.	Tetracosanoic acid, methyl ester	22.462	0.20

Table 3.14: Major Constituents of *Origanum majarana* L. seed oil

No.	Name	R.T	Formula	Mw	Area	Area%
1	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	17.488	$C_{19}H_{32}O_2$	292	112245070	40.78
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.388	$C_{19}H_{34}O_2$	294	92496802	33.61
3	Hexadecanoic acid	15.681	$C_{17}H_{34}O_2$	270	30270169	11.00
4	Methyl stearate	17600	$C_{19}H_{38}O_2$	298	15748926	5.72

The mass spectrum of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- is presented in Fig.3.16 .The peak at m/z292 which appeared at RT. (17.488) is due to M^+[$C_{19}H_{32}O_2$]; while the peak at m/z 261 corresponds to loss of a methoxy function . Fig.3.17 shows the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester .The peak at m/z294 which appeared at RT. (17.388) in total ion chromatogram fig.(3.15) corresponds to M^+[$C_{19}H_{34}O_2$]; while the peak at m/z 263 corresponds to loss of a methoxy function. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig.3.18 .The peak at m/z270 which appeared at RI (15.681) in total ion chromatogram fig.(3.15) corresponds to M^+[$C_{17}H_{34}O_2$]; while the peak at m/z 239 corresponds to loss of a methoxy function .Fig.3.19 illustrates the mass spectrum of methyl stearate. The peak at m/z298 which appeared at RT. (17.600) in total ion chromatogram is due to M^+[$C_{19}H_{38}O_2$]$^+$; while the peak at m/z 269 corresponds to loss of a methoxy function.

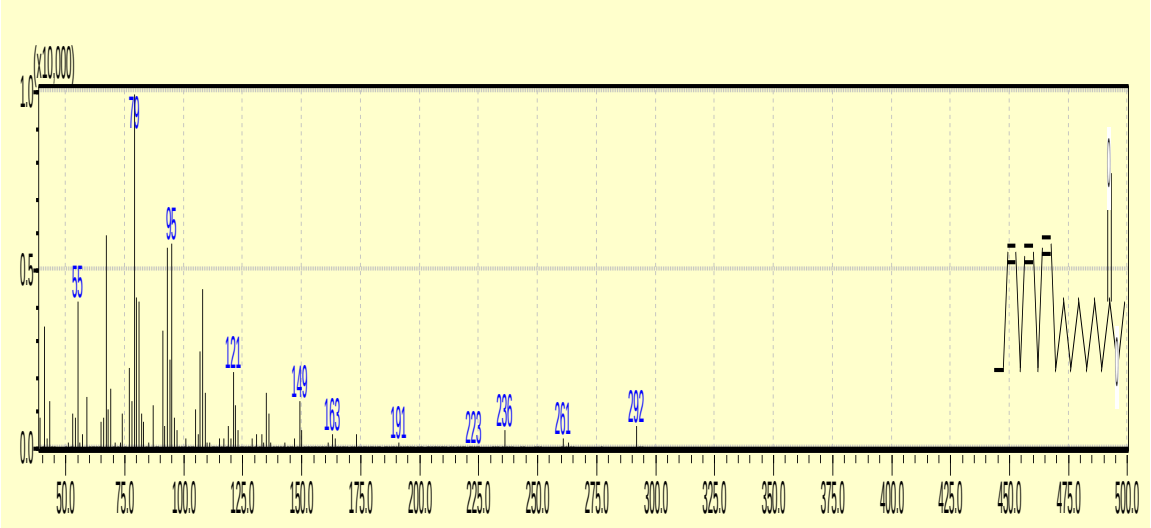


Fig.3.16: The mass spectrum of 9, 12, 15-Octadecatrienoic acid, methyl ester,

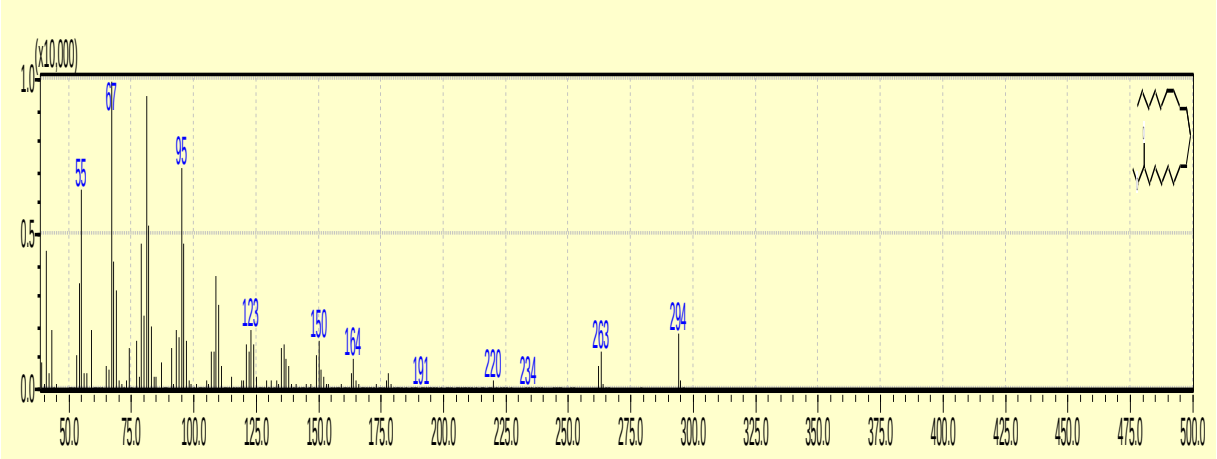


Fig.3.17: 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

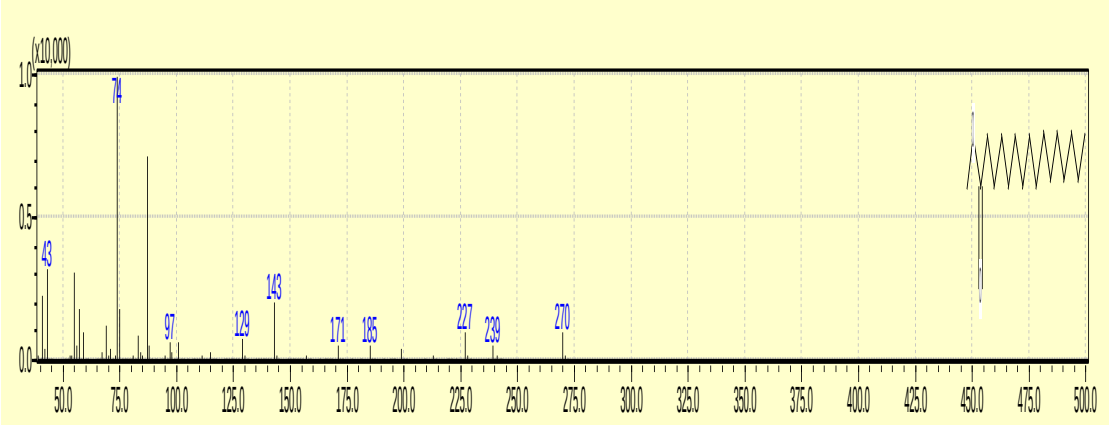


Fig.3.18: Mass spectrum of hexadecanoic acid methyl ester

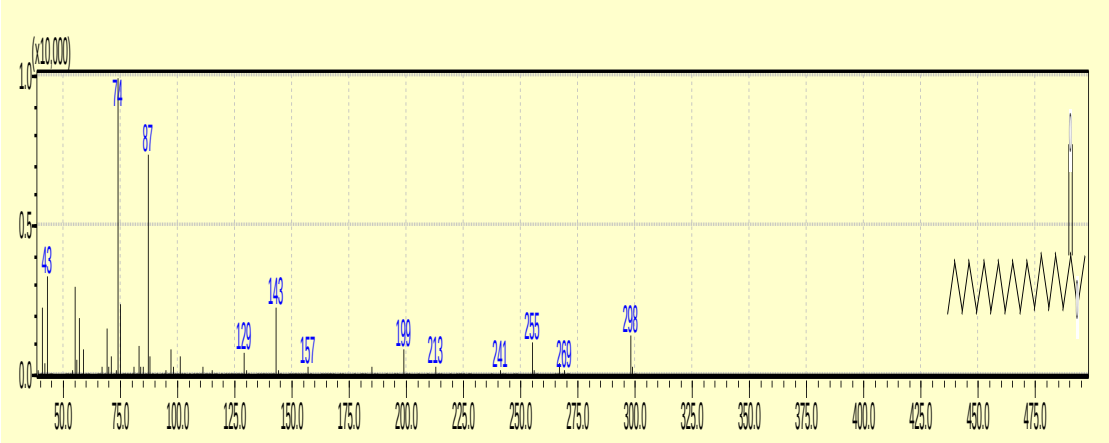


Fig.3.19: Mass spectrum of methyl stearate

3.4.2. Antibacterial Activity

Origanum majarana L. seeds oil was screened for Antibacterial Activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (3.15). The results were interpreted in the following manner ;(< 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active.).

At a concentration of 100mg/ml the oil showed partially activate against, Staphylococcus *aureus and Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli.* The oil failed to give any Antibacterial Activity against *Bacillus subtilis.*

Ta ble3.15: Inhibition zones (mm) of Origanum majarana oil

Sample	con	Ec	Pa	Sa	Bs	Ca
Origanum majarana oil	100	16	15	17	-	17

3.5- *Raphanus sativus*

3.5.1-GC-MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied oil. The analysis revealed the presence of 25 components - Table (3.16). Typical total ion chromatograms (TIC) are depicted in fig (3.20). They are (6) major constituents of the oil table (3.17)

Table 3.16: Constituent of *Raphanus sativus* L. seed oil

ID#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.198	0.18
2.	Pentadecanoic acid, methyl ester	15.326	0.05
3.	7,10-Hexadecadienoicacid,methyl ester	16.098	0.18
4.	7-Hexadecenoicacid,methylester, (Z)-	16.175	0.09
5.	9-Hexadecenoicacid,methylester, (Z)-	16.204	0.54
6.	Hexadecanoic acid, methyl ester	16.410	8.63
7.	cis-10-Heptadecenoicacid,methyl ester	17.221	0.10
8.	Heptadecanoic acid, methyl ester	17.433	0.10
9.	9,12-Octadecadienoicacid(Z,Z)-, methyl ester	18.174	12.75
10.	9-Octadecenoicacid (Z)-, methyl ester	18.253	7.10
11.	9,12,15-Octadecatrienoicacid, methyl ester, (Z,Z,Z)-	18.279	4.26
12.	Methyl stearate	18.424	4.33
13.	.gamma.-Linolenic acid, methyl ester	19.930	1.10
14.	cis-13-Eicosenoic acid, methyl ester	20.090	15.35
15.	cis-11-Eicosenoic acid, methyl ester	20.129	1.66
16.	Eicosanoic acid, methyl ester	20.267	3.33
17.	8,11-Eicosadienoic acid, methyl ester	20.551	0.07
18.	13-Docosenoicacid, methyl ester, (Z)-	21.852	28.02
19.	Docosanoic acid, methyl ester	21.971	2.59
20.	cis-10-Nonadecenoicacid,methyl ester	22.602	0.14
21.	Tricosanoic acid, methyl ester	22.769	0.08
22.	15-Tetracosenoicacid,methylester, (Z)-	23.391	5.55
23.	Tetracosanoic acid, methyl ester	23.544	2.46
24.	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-	23.875	1.17
25.	Hexacosanoic acid, methyl ester	25.112	0.17

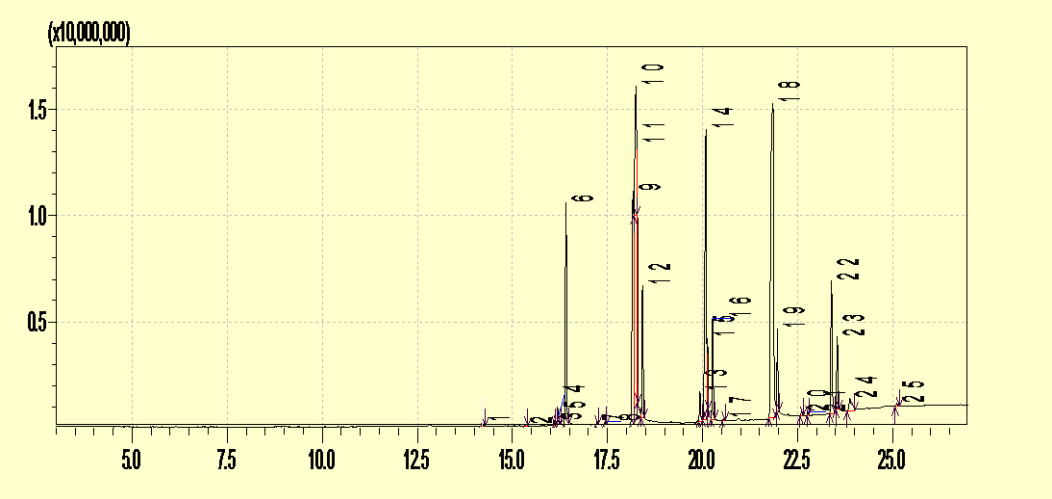


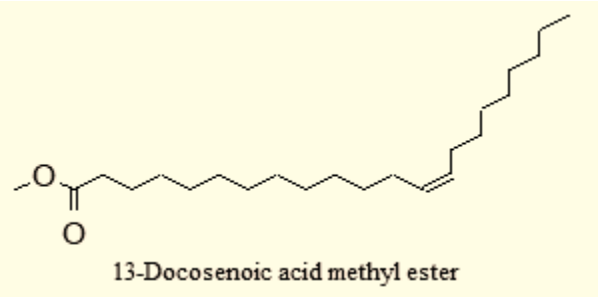
Fig .3.20: total ion chromatograms of Raphanus sativus L

Table 3.17: Major constituent of Raphanus sativus L. seeds oil

No.	Name	R.T	Formula	Mw	Area	Area%
1	13-Docosenoic acid, methyl ester,	21.852	$C_{23}H_{44}O_2$	352	77907421	28.02
2	cis-13-Eicosenoic acid, methyl ester	20.090	$C_{21}H_{40}O_2$	324	42697666	15.35
3	9,12 Octadecadienoic acid, -, methyl ester	18.174	$C_{19}H_{34}O_2$	294	35458395	12.75
4	Hexadecanoic acid, methyl ester	16.415	$C_{17}H_{34}O_2$	270	24000986	8.63
5	9-Octadcenoic acid (Z)-, methyl ester	18.253	$C_{19}H_{36}O_2$	296	19653654	7.10

Fig.3.20 shows the mass spectrum of 13-docosenoic acid, methyl ester, .The peak at m/z 352 which appeared at RT. 21.852 - in total ion chromatogram- corresponds M^+ [$C_{23}H_{44}O_2$], while the peak at m/z 320 corresponds to loss of a methoxy. The mass spectrum of cis-13-eicosenoic acid, methyl ester is presented in Fig.21.The peak at m/z 324 which appeared at (RT.20.090) is due to M^+ [$C_{21}H_{40}O_2$], while the peak

at m/z 292 corresponds to loss of a methoxy .Fig.3.22 illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The signal at m/z 294 which appeared at RT.18.174 accounts for the molecular ion: M^+ [$C_{19}H_{34}O_2$] . The peak at m/z 263 is attributed to loss of a methoxyl .Fig.3.23 shows the mass spectrum of hexadecanoic acid methyl ester.The signal at m/z 270 (RT.16.410) is due to M^+ [$C_{17}H_{34}O_2$], while the peak at m/z 239 is attributed to loss of a methoxyl function. The mass spectrum of 9-octadecenoic acid methyl ester is illustrated in Fig. 3.24. The signal at m/z296 accounts for the molecular ion: M^+ [$C_{19}H_{36}O_2$].



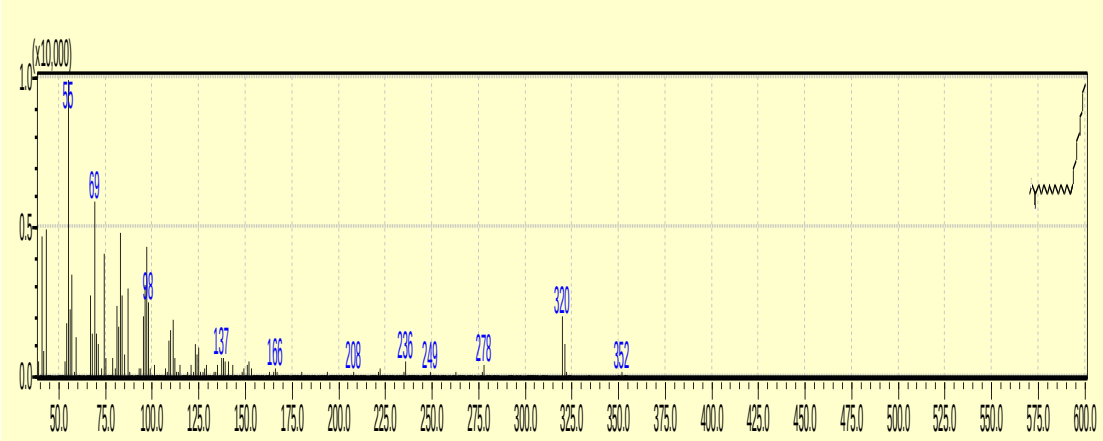
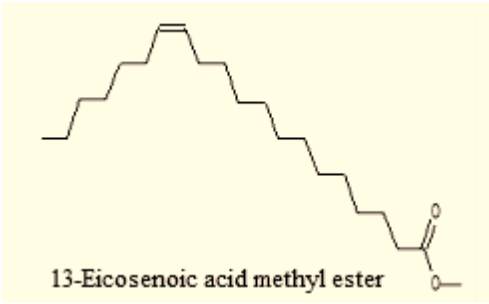


Fig. 3.20: mass spectrum of 13-docosenoic acid, methyl ester



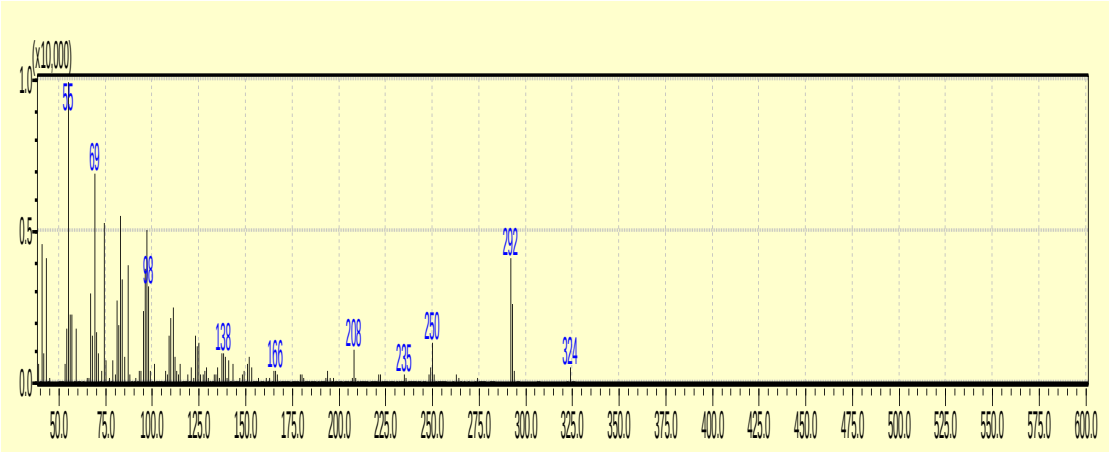
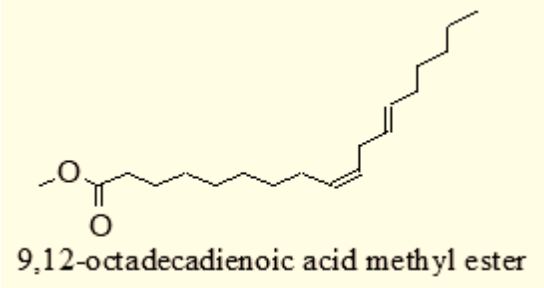


Fig. 3.21: mass spectrum of cis-13-eicosenoic acid, methyl ester



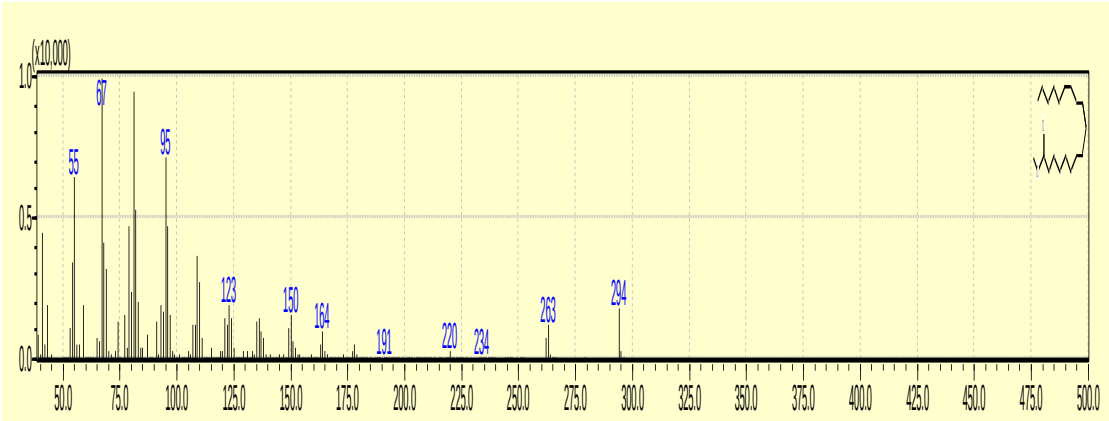
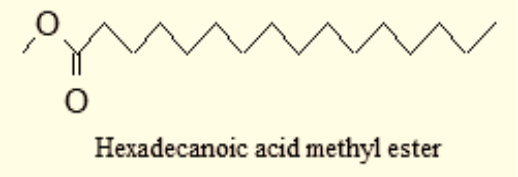


Fig. 3.22: mass spectrum of 9, 12-octadecadienoic acid methyl ester



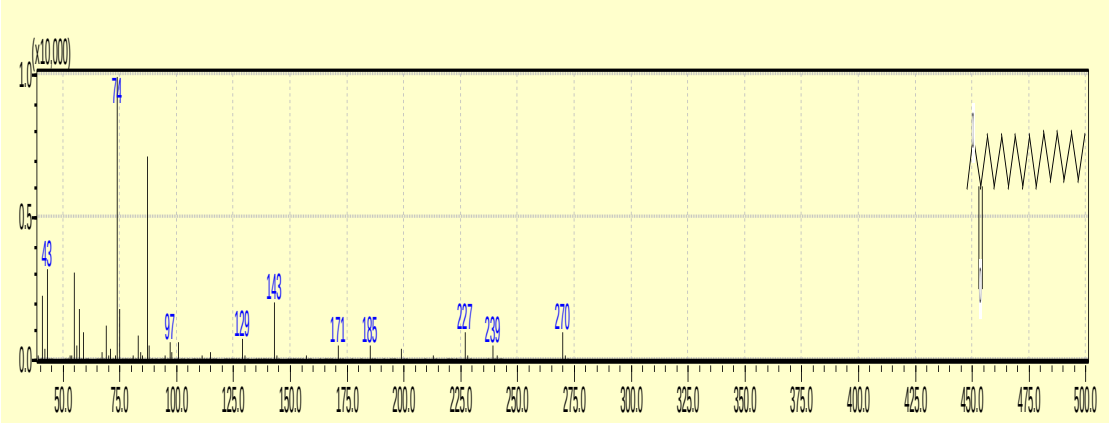
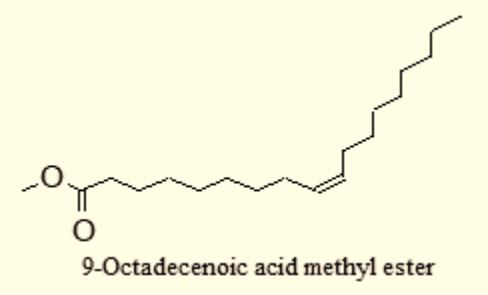


Fig. 3.23: mass spectrum of hexadecanoic acid, methyl ester



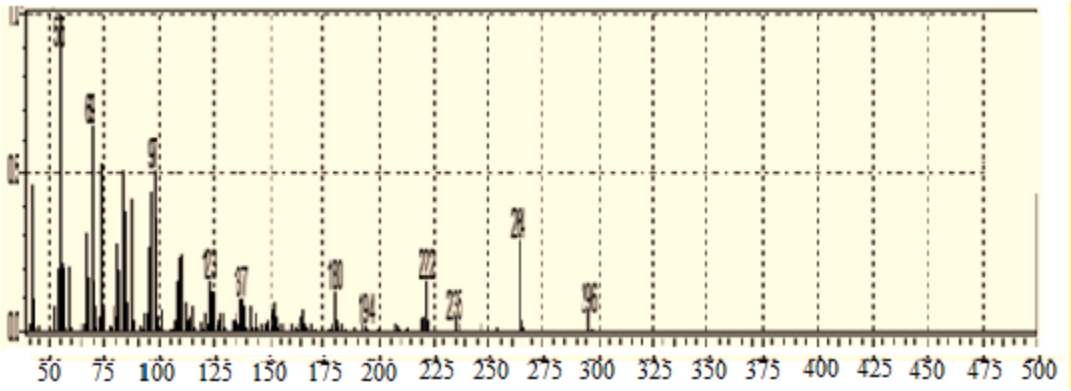


Fig.3.24: mass spectrum of 9-octadecenoic acid methyl ester

3.5.2-Antioxidant assay

The DPPH –scavenging model is a widely used method of evaluating the antioxidant activity within a relatively short time compared with other methods. The effect of antioxidant potential in the DPPH bioassay is explained in terms of the hydrogen donating ability of the sample. DPPH .is a stable free radical and accepts one electron or hydrogen radical to form a stable diamagnetic molecule. The studied oil showed (Table 3.18) significant free radical scavenging capacity in the DPPH assay (IC_{50} 68.65±1.23) .The antioxidant activity of the oil was close to that of the standard antioxidant: butylated hydroxyl toluene (BHT) (IC_{50} 61.11±1.78).

The metal chelating method is based on the ability of the sample to inhibit the formation of Ferrozine – Fe^{2+} chelate. Measurement of the rate of red color reduction allows evaluation of the level of chelating capacity. The results of Table (3.18) indicates that the studied oil has weak metal chelating properties (IC_{50} 132.53±0.80) compared to EDTA (IC_{50} 7.05±0.29).

In the superoxide anion scavenging assay, the oil sample showed moderate antioxidant activity (IC_{50} 123.19±7.63) compared with the positive controls BHT (IC_{50}, 60.5±0.22) and Trolox (IC_{50} 54.98±0.17) - Table 3.18 .All assays showed excellent reproducibility as shown in Table (3.18).

Table 3 .18: IC_{50} and r^2 values of for antioxidant activity

Sample	Free radical (DPPH·) scavenging activity		Metal chelating activity		Superoxide anion scavenging activity	
	IC_{50}, mg/mL	r^2	IC_{50}, mg/mL	r^2	IC_{50}, mg/mL	r^2
Oil	68.65±1.23	0.87	132.53±0.80	0.66	123.19±7.63	0.98
BHA	80.14±1.01	0.79	-	-	18.22±0.17	0.99
BHT	61.11±1.78	0.96	-	-	60.5±0.22	0.94
Trolox	31.57±2.07	0.95	-	-	54.98±0.17	0.89
EDTA	-	-	7.05±0.29	0.95	-	-

3.6- *Lepidium sativum*

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

Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied essential oil. The analysis revealed the presence of (25) components - Table (3.19). Typical total ion chromatograms (TIC) are depicted in fig (3.25). They are (4) major constituents of the oil table (3.20).

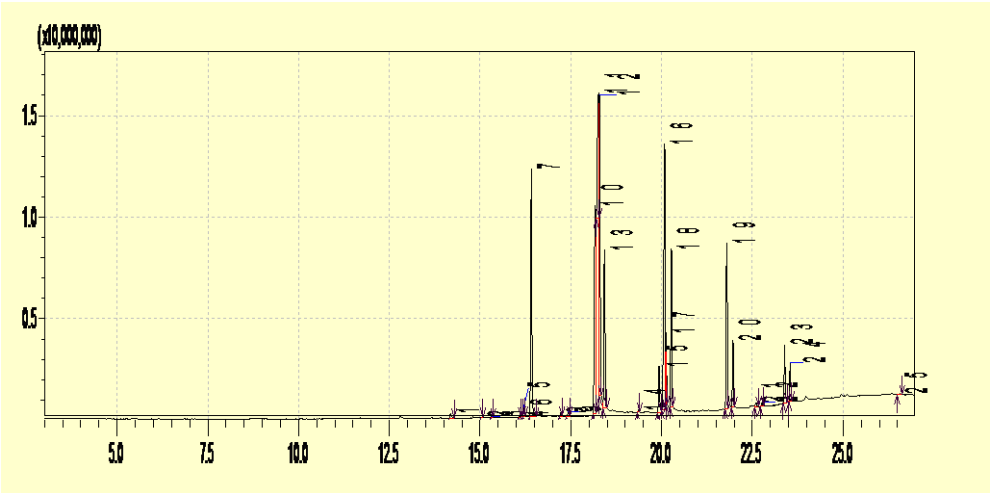


Fig .3.25: total ion chromatograms *Lepidium sativum* L.

Table 3.19: Constituent of *Lepidium sativum* L. seed oil

No	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.189	0.26
2.	cis-5-Dodecenoic acid, methyl ester	15.047	0.03
3.	Pentadecanoic acid, methyl ester	15.319	0.06
4.	7,10-Hexadecadienoicacid,methyl ester	16.095	0.05
5.	7-Hexadecenoicacid,methylester,(Z)-	16.157	0.16
6.	9-Hexadecenoicacid,methylester,(Z)-	16.200	0.42
7.	Hexadecanoic acid, methyl ester	16.413	13.11
8.	cis-10-Heptadecenoicacid,methyl ester	17.217	0.14
9.	Heptadecanoic acid, methyl ester	17.430	0.21
10.	9,12-Octadecadienoicacid(Z,Z)-, methyl ester	18.172	15.90
11.	9-Octadecenoicacid(Z)-, methyl ester	18.264	8.73
12.	9,12,15-Octadecatrienoicacid,methyl ester, (Z,Z,Z)-	18.293	10.16
13.	Methyl stearate	18.427	7.16
14.	Nonadecanoic acid, methyl ester	19.359	0.08
15.	.gamma.-Linolenic acid, methyl ester	19.929	2.18
16.	cis-13-Eicosenoic acid, methyl ester	20.085	16.48
17.	11,14,17-Eicosatrienoicacid,methyl ester	20.125	1.92
18.	Eicosanoic acid, methyl ester	20.270	7.09
19.	cis-10-Nonadecenoic acid, methyl ester	21.795	8.34
20.	Docosanoic acid, methyl ester	21.966	2.69
21.	13-Docosenoic acid, methyl ester, (Z)-	22.600	0.23
22.	Tricosanoic acid, methyl ester	22.767	0.19
23.	15-Tetracosenoic acid, methyl ester, (Z	23.389	2.72
24.	Tetracosanoic acid, methyl ester	23.542	1.61
25.	.gamma.-Tocopherol	26.552	0.08

Table3.20. Major Constituent of *Lepidium sativum* L. seeds oil

No.	Name	R.T	Formula	Mw	Area%
1	Cis 1.3 Eicosenoic acid methyl ester	20.085	$C_{21}H_{40}O_2$	324	16.48
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.172	$C_{19}H_{34}O_2$	294	15.90
3	Hexadecanoic acid, methyl ester	18.413	$C_{17}H_{34}O_2$	270	13.11
4	9,*12*,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.293	$C_{19}H_{32}O_2$	292	

The mass spectrum of Cis-13-eicosenoic acid, methyl ester is presented in Fig.3.25.The peak at m/z 324 which appeared at (RT.20.090) is due to M^+ [$C_{21}H_{40}O_2$], while the peak at m/z 292 corresponds to loss of a methoxyl. Fig. 3.26 illustrates the mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester. The signal at m/z 294 which appeared at RT18.174 accounts for the molecular ion: M^+ [$C_{19}H_{34}O_2$]. The peak at m/z 263 is attributed to loss of a methoxyl. .Fig.3.27 shows the mass spectrum of hexadecanoic acid methyl ester.The signal at m/z 270 (RT.16.410) is due to M^+ [$C_{17}H_{34}O_2$], while the peak at m/z 239 is attributed to loss of a methoxyl function. Fig. 3.28 illustrates the mass spectrum of 9, 12, 15-octadecatrienoic acid methyl ester. The signal at m/z 292 which appeared at RT18.293 accounts for the molecular ion: M^+ [$C_{19}H_{32}O_2$].

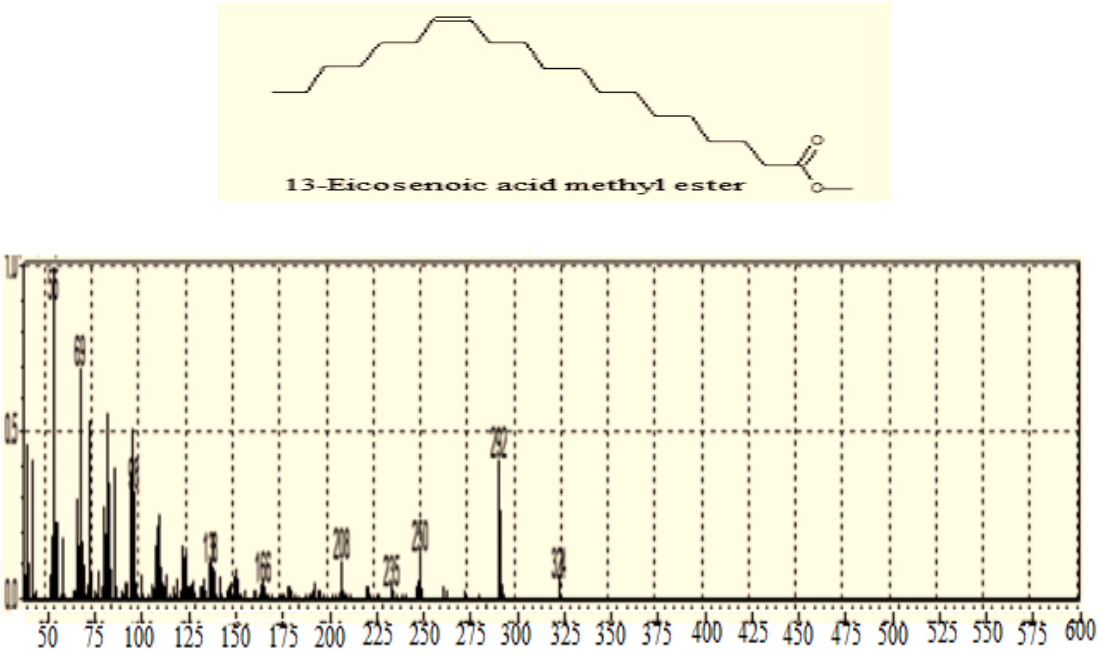


Fig.3.25: mass spectrum of cis-13-eicosenoic acid, methyl ester

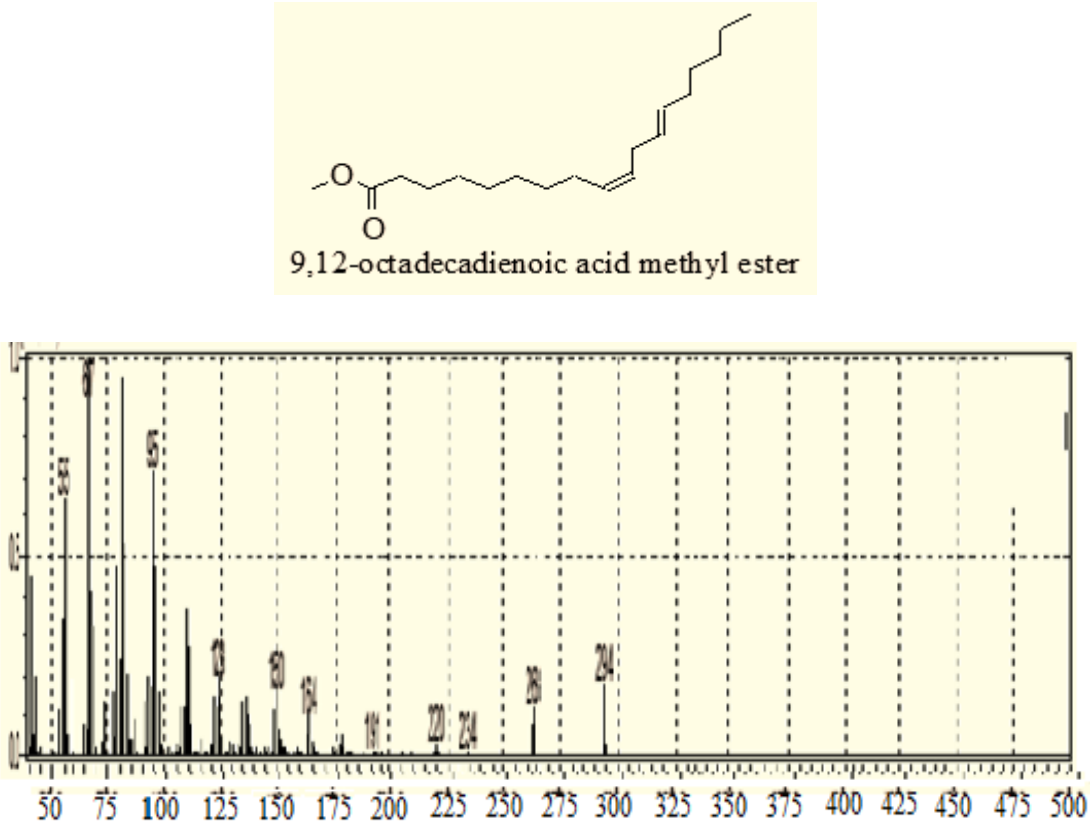
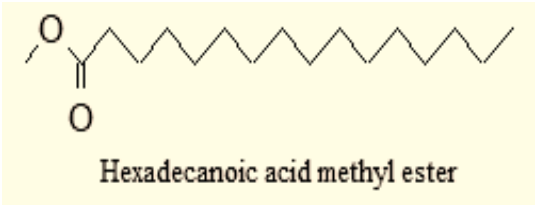


Fig. 3.26: mass spectrum of 9, 12-octadecadienoic acid methyl ester



.

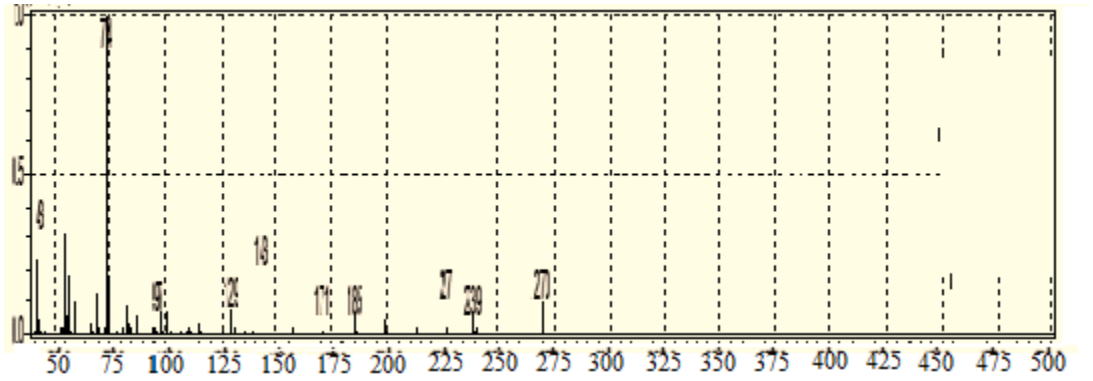


Fig. 3.27: mass spectrum of hexadecanoic acid, methyl ester

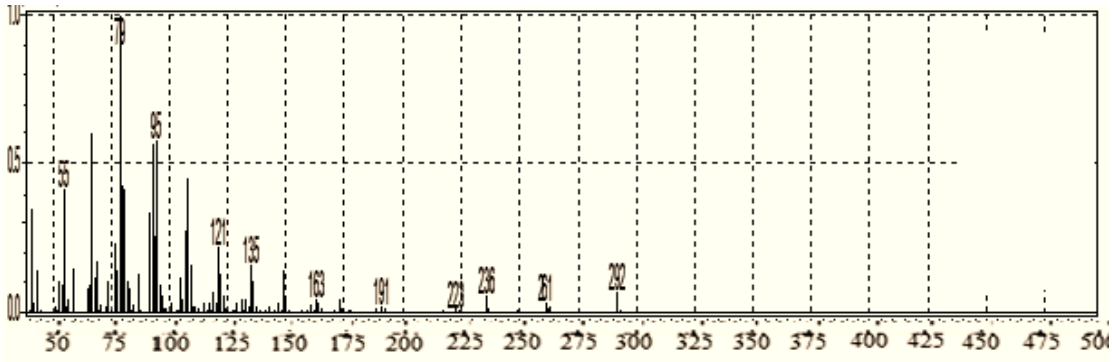


Fig.3.28: mass spectrum of *9, 12, 15*-octadecatrienoic acid, methyl ester,

3.6.2-Antioxidant assay

The DPPH –scavenging model is a widely used method of evaluating the antioxidant activity within a relatively short time compared with other methods .The effect of antioxidant potential in the DPPH bioassay is explained in terms of the hydrogen donating ability of the sample . DPPH .is a stable free radical and accepts one electron or hydrogen radical to form a stable diamagnetic molecule. The studied oil showed significant (Table 3.21) free radical scavenging capacity in the DPPH assay (IC50 97.36±0.9) .In this assay, the antioxidant activity of the oil was higher than the activity of the positive controls: butylated hydroxyl

anisole (BHA) – (IC_{50} 80.14±1.02).; butylated hydroxyl toluene(BHT) - (IC_{50} 61.11±1.78) and Trolox(IC_{50} 31.57±2.07).

The metal chelating method is based on the ability of the sample to inhibit the formation of Ferrozine – Fe^{2+} chelate. Measurement of the rate of red color reduction allows evaluation of the level of chelating capacity10. The results of Table (3.2) indicates that the studied oil has weak metal chelating properties (IC_{50} 118.25±0.31) compared to EDTA (IC_{50} 7.05±0.29).

In the superoxide anion scavenging assay, the oil sample showed significant antioxidant activity (IC_{50} 11.72±0.17) compared with the positive controls: BHA (18.22±0.17); Trolox (IC50 54.98±0.17) and BHT (IC50, 60.5±0.22) .All assays showed excellent reproducibility as shown in Table (3.21).

Table 3.21: IC_{50} and r^2 values of oil and standards

Sample	Free radical (DPPH·) scavenging activity		Metal chelating activity		Superoxide anion scavenging activity	
	IC_{50}, mg/ml	r^2	IC_{50}, mg/ml	r^2	IC_{50}, mg/ml	r^2
Oil	97.36±0.96	0.87	118.25±0.31	0.75	11.72±0.17	0.89
BHA	80.14±1.01	0.79	-	-	18.22±0.17	0.99
BHT	61.11±1.78	0.96	-	-	60.5±0.22	0.94
Trolox	31.57±2.07	0.95	-	-	54.98±0.17	0.89
EDTA	-	-	7.05±0.29	0.95	-	-

Conclusion

Pimpinellanisum.Origanum majarana,Raphanus sativus,Melissa officinalis ,Lepidium sativum) were extracted by n-hexane . Gas chromatoghy –mass spectrometry has been used for the identification and quantifiction of the constituents of the studied oils . Extractted oils have been screened for antimicrobil activity against five standared human pathogens.The antioxidant activity of oils has been evaluated . The studied oils showed differents antimicrobial and antioxidant responses.

Recommendations

The following Recommendations may be considered as a future work:

- The isolated oils may be investigated for other biological activites like antimicrobial and cytotxicity .
- Other phytochemicals of the eargeted plants my be isolated and their structures being characterized by a combination of spectral tools.
- Other phytochemcals occurring in the studied plantsmay be assessed for their biological activity.

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