Sudan University of Science and Technology

College of Graduate Studies

Constituents of Oils from Some Medicinal Plants and their Antimicrobial Activity

مكونات زيوت بعض النباتات الطبيعية وفعاليتها كمضاد للميكروبات

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

by

Osman Abdalla Elhassan Abdalla

B.Sc. Chemistry
M.Sc. Chemistry

Supervisor

Prof. Mohammed Abdelkarim

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قال تعالى:

(وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ)

صدق الله العظيم

سورة التوبة الاية (105)

Dedication

To

may Parents

my wife

children

brothers and *sisters*

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I acknowledge your infinite help **Almighty Allah**, without your help and love this work would not have been possible and I hope that you give me more strength and good health to continue even further in research work.

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Abstract

In this study, six Sudanese key species in folk medicine (*Allium cepa Subsp. cepa, Cucurbita pepo, Eruca sativa, Mentha virdis, Anethum graveolens and Artemisia herpa-alba*) have been investigated. These plant species are of medicinal attributes and potentials. They have been studies by GC-MS. The target plants have also been assessed for their antimicrobial activity.

The GC-MS analysis of *Allium cepa* Subsp. Cepa oil showed the presence of 27 components. Major constituents of the oil are:

i) 9, 12- octadecadienoic acid methyl ester (42.80%)

ii) 9, octadecenoic acid methyl ester (16.43 %)

iii) Hexsdecanoic acid methyl ester (12.80%)

iv) Methyl stearate (5.90%)

Cucurbita pepo oil was analyzed by GC-MS. The analysis showed 19 components. Major constituents are:

i) 9, 12- octadecadienoic acid methyl ester (35.69%)

ii) 9, octadecenoic acid methyl ester (27.24 %)

iii) Hexsdecanoic acid methyl ester (17.28%)

GC-MS analysis of Eruca sativa oil gave twenty seven components. Major constituents are:

i) 13- Docosenoic acid methyl ester (32.41%)

ii) Cis- 11 – Eicosenoic acid methyl ester (12.66%)

iii) 9, 12- octadecadienoic acid methyl ester (12.87%)

iv) Hexsdecanoic acid methyl ester (7.87%)

v) 9, octadecenoic acid methyl ester (6.22 %)

GC-MS analysis of *Mentha virdis* oil gave fifty two constituents. Major components are:

i) D-Carvone (39.84%)

ii) D-Limonene (22.36%)

GC-MS analysis of *Anethum graveolens* volatile oil showed the presence of 30 components:

i) D-Carvone (37.83%)

ii) D-Limonene (18.13%)

iii) Apiol (16.16%)

Artemisia herpa-alba oil was analyzed by GC-MS. Forty nine constituents were identified. Major components are:

i) n-Propl cinnamate (11.89%)s

ii) 2,6-Octadienal, 3,7-dimethyl, (E)-bornyl aceatate (7.60%).

The target oils exhibited different responses in the antimicrobial assay. At a concentration of 100mg/ml *Allium cepa* oil showed good activity against *Escherichia coli*. However, at the same concentration, it exhibited partial

activity against *Staphylococcus aureus*. The oil failed to give any anticadidal activity.

Cucurbita pepo oil showed significant antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, *Eruca sativa* oil failed to give any response in the antimicrobial assay.

Mentha virdis oil showed significant activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* and moderate activity against *Staphylococcus aureus*. On the other hand *Anethum graveolens* oil showed significant activity against *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis*, while it showed very good activity against *Staphylococcus aureus*.

Artemisia herpa-alba oil showed significant antibacterial activity against *Pseudomonas aeruginosa* and very good activity against *Staphylococcus aureus.*

المستخلص

في هذه الدراسة أخذت ستة عينات من النباتات الطبية (البصل، الكوسه، الجرجير، النعناع، الشبت، الشيح) وتم تحليليها وهذه النباتات ذات فاعلية طبية وتم دراستها بواسطة كورموتفرافيا وتم التعرف على نشاطها الحيوي تحليل كورموتفرافيا الغاز لزيت البصل وضح أن هناك 27 مكوناً من أهمها:

1. 9,12 – حامض ثماني ديكادايونيك مثيل استر (42,80%) .
2. 9– حامض ثماني ديكادايونيك مثيل استر (16,43%).
3. حامض سداسي ديكادايونيك مثيل استر (12,80 %).
4. ستيارات المثيل (5,90%).

زيت الكوسه تم تحليله بواسطة جهاز كورموتفرافيا الغاز، التحليل أوضح أنا هناك 19 مكوناً من أهمها:

1. 9,12 – حامض ثماني ديكادايونيك مثيل استر (35,69%) .
2. 9– حامض ثماني ديكادايونيك مثيل استر (27,24%).
3. حامض سداسي ديكادايونيك مثيل استر (17,28 %).

تحليل كورموتفرافيا الغاز بزيت الجرجير نتج عنه (27) مكوناً أهمها:

1. 13 حامض دوكوسينويك مثيل استر (32,41%).
2. سيس – 11 – حامض إيكو دوكوسينويك مثيل استر (12,66%)
3. 9,12 – حامض ثماني ديكادايونيك مثيل استر (12,87%).
4. سداسي ديكادايونيك مثيل استر (7,87%).
5. 9– حامض ثماني ديكادايونيك مثيل استر (6,22%).

تحليل الغاز كورموتفرافيا لزيت النعناع أعطى 52 مكوناً أهمها:

1. D كارفون (39,84%) .
2. D ليمونين (22,36%) .
3. أبيول (16,16%).

زيت الشيح تم تحليل بواسطة غاز كورموتفرافيا. 42 مكوناً تم إستخلاصها من أهمها:

1. n – بروبايل سناميت (11,89%).
2. 2,6 – ثماني دانيال 3,7 – ثنائي مثيل (E) برونايل اسياتيت (7,60 %).

هذه الزيوت أعطت نتائج مختلفة في سلوكها الحيوي.

عند تركيز 100 ملجم / مل زيت البصل أظهر نشاطاً جيداً ضد Escherichia Coli عموماً عند نفس التركيز أظهرت نشاطاً ضعيفا ضد Staphlococcus والزيت فشل في إعطاء اي نشاط حيوي أخر. زيت الكوسه أظهر نشاطاً ضعيفاً ضد Pseudomonas aeruginosa و Staphylococcus وعموماً زيت الجرجير فشل في أعطاء اي نشاط حيوي. زيت النعناع أظهر نشاطاً ضعيفاً ضد Pseudomonas aeruginosa و Bacillus Subtilis ونشاطاً متوسطاً ضد Staphylococcus ومن ناحية أخرى زيت الشبت أظهر نشاطاً ضعيفاً ضد Escherichia و Pseudomonas aeruginosa بينما أظهر نشاطاً جيداً ضد Staphylococcus وزيت الشيح أظهر نشاطاً بكتيرياً ضد Pseudomonas aeruginosa و نشاطاً جيداً ضد Staphylococcus aureus.

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1. Introduction

1.1 – Essential oils

Essential oils are complex mixtures of volatile chemicals found in natural matrices of living organisms. They are responsible for the odour and sometimes taste properties of aromatic materials which maybe of plant, animal or microbial origin occurring in terrestrial and marine realms.

Aromatic plants are the major source of essential oils which may be found in almost all parts of a plant such as leaves, flowers, bark, seeds, fruits, wood, rhizome, root, root bark, etc. Essential oils, their fractions or aromachemicals isolated from them are ingredients of flavors and fragrances.

Essential oils are "low volume – high value" products. They are either used as such, or pure aroma chemical or an oil fraction can be added to flavor or fragrance compositions. However, flavor and fragrance industries prefer to use their synthetic derivatives in compounding due to their lower cost. Essential oils are also used in food industries especially in flavoring sauces and package food [1].

Essential oils are frequently associated with gums and/or resins.

They are freed from such products by distillation. The Council of Europe describes essential oil as a product obtained from vegetable raw material.

In some plant species, volatiles are in glycosidical form bound with sugars and some type of hydrolysis is needed to free them to become fragrant[2]. Therefore, rose petals, for instance, are subjected to a short wilting period to allow enzymes to free the volatiles prior to distillation in order to increase the yield of essential oil.

Due to their liquid nature at room temperature, essential oils are called as oil. However, they should not be confused with fixed or fatty oils, which comprise naturally occurring mixtures of lipids which may not necessarily be volatile. Therefore, essential oils differ entirely from fixed oils both in chemical and in physical properties.

A simple test can verify the occurrence of an essential oil. When dropped on a filter paper, essential oil evaporates completely; however, fixed oil leaves a permanent stain which does not evaporate even when heated[3].

The function of essential oil in plants is not fully understood. Microscopic examination of plant parts that contain the oil sacs

readily shows their presence. The odours of flowers are said to act as attractants for insects involved in pollination and thus may aid in preservation and natural selection. Essential oils are almost always bacteriostats, often bacteriocides and antimicrobials having a wide range activity spectrum. Many chemicals of essential oils are active and thus could participate readily in metabolic reactions. They are sources of plant metabolic energy, although some chemists have referred to them as waste products of plant metabolism[4].

Composition depends on place of origin. The habitat where the plant grows (normally warm climates have more essential oils), the moment of harvesting, extraction methods, etc… are also important.

Among the main therapeutic properties of essential oils antiseptics stands out (for many years these spices have been added to foodstuffs not just for flavoring but to help preserve them). Other properties are: antispasmodic, expectorant, carminative, eupeptic… We should bear in mind that certain essential oils, especially in high doses, may be toxic to the central nervous system in particular. Others, such as rue or juniper have abortive properties. Others may cause skin problems, rashes or allergies [5].

In addition to having therapeutic properties, essential oils are widely used in the pharmaceutical, food, and perfume industries.

1.2 - Physical properties of essential oils:

Essential oils are volatile and become liquid at room temperature. When distilled they are at first colourless or slightly yellowish. They are less dense than water (sassafras essence and clove essence being exceptions). They are nearly always rotational and have a high refractory index. They are soluble in alcohol and in the usual organic solvents, such as ether or chloroform, and also in high grade alcohol. They are lipo-soluble and not very soluble in water, but can be dragged using steam[5].

1.3 - Chemical properties of essential oils

Essential oil components are divided into terpenoids and non-terpenoids[5].

(i) Non-terpenoids: This group contains short-chain aliphatic substances, aromatic substances, nitrogenated substances, and substances with sulphur. They are less important than terpenoids in terms of uses and applications.

(ii) Terpenoids: These are more important commercially and in terms of their properties.

Terpenes are derive from isoprene units (C5) bonded in a chain. Terpenes are a type of chemical substance found in essential oils, resins, and other aromatic plant substances, (pines, citrus fruits…). They are usually found in monoterpene oils (C15) and diterpenes (C20).They may be aliphatic, cyclic, or aromatic [6].

According to their function group they can be [6]:

- Alcohols (menthol, bisabolol) and phenols (timol, carvacrol)
- Aldehydes (geranial, citral) and cetones (camphor, thuyone)
- Esthers (bornile acetate, linalile acetate, methyl salicilate, anti-inflammatory compound similar to aspirin)
- Ethers (1.8 - cineol) and peroxides (ascaridol)
- Hydrocarbons (limonene, pinene α and β).

1.4 - Uses of essential oils

1.4.1 Food industry:

They are used to season or condiment meats, dried and cured meats, soups, ice-cream, cheese… the most commonly used essential oils are cilantrum, orange, and mint. They are also used in the elaboration of alcoholic and soft drinks, especially the latter.

1.4.2 Pharmaceutical industry:

They are used in toothpastes (mint and fennel essences), analgesics, and decongestant inhalers (eucalyptus). Eucalyptol is also widely used in dentistry. They are used in many medicines to neutralise unpleasant tastes (essence of orange or mint, for example) [6].

1.4.3 Cosmetic industry:

This industry uses essential oils to make cosmetics, soaps, scents, perfumes, and make-up. We should mention geranium, lavender, roses and patchouli essences as common examples[6].

1.4.4 Veterinary product industry:

This industry uses the essential oil of the Chenopodium ambrosoides, which is highly prized for its ascaridol (worm-killer) content. Limonene and menthol are also used to make insecticides[6].

1.4.5 Industrial deodorants:

At present, the use of essences to disguise the unpleasant smell of industrial products like rubber, plastic and paint is being developed. Paint manufacturers use limonene as a biodegradable solvent. Toys are also scented. In the textile industry they are used to mask unpleasant smells before and after dyeing. In paper manufacture,

products such as notebooks, toilet paper, and face wipes are scented[7].

1.5 - Methods of Application

1.5.1 Massage:

Massage is one of the most beneficial methods of using essential oils because it combines the therapeutic aspects of touch with the potent restorative powers of essential oils. It is an especially beneficial way to relieve stress and tension caused by anxiety. Essential oils must be diluted in carrier oil. Add 1-5% dilution of essential oil to a carrier oil of choice[6].

1.5.2 Bath:

The ritual of bathing is the easiest, most therapeutic way of using essential oils in the home. A pleasurable blend of steam and hot water combine the benefits of inhalation with the powers of absorption through the skin and mucous membranes. Bathing with essential oils provides an experience that is both cleansing for the skin and balancing for the emotions. In such bath a maximum of 1-5% dilution of pure essential oils is used in 25 mls of carrier oil. Ome oils are irritants to the skin on baths like Citrus and Peppermint oils[6].

1.5.3 Inhalation:

Vapour inhalation is one of the quickest methods in which a few drops of essential oil can enter the body. Inhalation is highly beneficial for helping respiratory ailments or sinus congestion. Oils such as eucalyptus, peppermint, cajeput, and frankincense may be used[7].

1.5.4 Diffuser:

A diffuser is an indispensable tool in the art of aromatherapy. By heating the oil, a diffuser effectively introduces the fragrance and beneficial properties of an essential oil to any environment. When essential oils are vaporized, their specific properties can have positive effects on the mind, body, and spirit[7].

1.5.5 Creams and Lotions:

Creams and lotions are a very convenient method of applying essential oils for beauty and skin care. However, one should understand that the level of therapeutic value is lowered due to the emulsifying agents and the short life span of a cream-blended oil. One should also take into consideration that these natural creams and lotions with pure essential oils are a better alternative than a synthetic form of product.

1.5.6 Ointments:

Ointments are highly recommended for cuts, grazes, insect bites and stings, athlete's foot, cold sores and chilblains. An ointment is more of a spot treatment that can be used locally to combat infections and inflammations[7].

1.5.7 Gels:

Gels are recommended for use as a carrier of essential oils without an oil base. Gels are highly recommended for those with oily skin or in areas that do not need an oil-based product[7].

1.5.8 Compresses:

Compresses are valuable when it comes to muscular pain, sprains, strains, and bruises. They are also effective in reducing pain and congestion in internal organs[7].

1.6 – Method of extraction of essential oils

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential

oils are obtained from plant raw material by several extraction methods[8.9].

1.6.1 – Classical and conventional methods

There are several methods of extraction behavior of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused in copious parts of the globe. Hydrodistillation (HD), steam distillation (SD), solvent extraction, enfleurage, cohobation, and maceration are the roughly traditional and generally used methods.

1.6.1.1 – Hydrodistillation

Hydrodistillation is a traditional method for removal of essential oils. Water or Hydrodistillation is one of the oldest and easiest methods[10] being used for extraction of essential oils.

Hydrodistillation normally used to isolation of essential oils from the aromatic and medicinal plant. The conventional method for the extractions of essential oils is Hydrodistillation (HD), in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser. The setup comprises also a condenser and decanter to collect the condensate and to separate essential oils from water, respectively. The principle of extraction is based on the isotropic

distillation. In fact, at atmospheric pressure and during extraction process (heating), water or other solvent and oils molecules. Hydrodistillation (HD) is a variant of steam distillation, which is bespoke by the French Pharmacopoeia for the extraction of essential oils from dried plants. There are three types of Hydrodistillation: with water immersion, with direct vapor injection and with water immersion and vapor injection. It is a multilateral process that can be utilized for large or small industries. The distillation time depends on the plant material being processed. Prolonged distillation produces only a small amount of essential oil, but does add unwanted high boiling point compounds and oxidation products.

1.6.1.2 – Steam distillation

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plants such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation is still important in certain industrial sectors[11]. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top.

Steam distillation is a method where steam flows through the material. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. The vapor is then condensed further and the essential oil is collected[12]. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 ºC so that the volatile components with the boiling points ranging from 150 to 300 ºC can be evaporated at a temperature close to that of water. Furthermore, this technique can be also carried out under pressure depending on the essential oils extraction difficultly.

1.6.1.3 – Solvent extraction

Solvent extraction, also known as liquid-liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example, water and an organic solvent. In the solvent-extraction method of essential oils recovery, an extraction unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost[13] . The most frequently applied sample preparation procedure in plant material analysis. The

quality and quantity of extracted mixture are determined by the type of extra heat applied because of the method is limited by the compound solubility in the specific solvent used. Although the method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction tine, relatively high solvent consumption and often unsatisfactory reproducibility[14].

1.6.1.4 – Soxhlet Extraction

A soxhlet extractor is a piece of laboratory apparatus[15] invented in 1897 by Franz von Soxhlet[16] . It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation

while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extraction liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a present level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analysis into the bulk

liquid[17]. This procedure is repeated until virtually complete extraction is achieved. There are several advantages of the Soxhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extractable material and enhances the removal of the analyte from the matrix. Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed[14].

1.6.1.5 – Cold Pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperature and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process insures that the resulting oil is 100% pure and retains all the properties of the plant. It is a method of mechanical extraction

where heat is reduced and minimized throughout the batching of the raw material. The cold pressed method is also known as scarification method[15].

Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils[18]. In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation[15].

1.6.2 – Innovative techniques of essential oils extraction

One of the disadvantages of conventional techniques is related with the thermolability of essential oils components which undergo chemical alteration (hydrolysis, isomerization, oxidation) due to high applied temperatures. The quality of extracted essential oils is therefore extremely damaged particularly if the extraction time is long. It is important that extraction methods could maintain essential oils chemical composition and natural proportion at its original state. Since Economy, competitiveness, eco-friendly, sustainability, high efficiency and goof quality become keywords of the modern industrial production, the development of essential oils extraction techniques has never been interpreted. Strictly speaking, conventional techniques are not the only way for the extraction of essential oils. Novel techniques, for example,

abide by green extraction concept and principles have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of official methods. New extraction techniques must also reduce extraction times, energy consumption, solvent and emissions[14,15].

Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on a commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be wildly used for commercial productions of essential oils but are considered valuable in certain situations, such as the production of costly essential oil in natural state without any alteration of their thermo-sensitive components or the extraction of essential oils for micro-analysis[14,15].

1.6.2.1 – Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is

Usually from a solid matrix, but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO2) for several practice reasons. A part from having relatively low critical

pressure (74 bars) and temperature (32ºC), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from extract[19]. The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes[20]. These essential oils can include limonene and others. Carbon dioxide is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods [21]. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that can not be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of superior quality with better functional and biological activities[22]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

1.6.2.2 – Microwave-assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizing microwave oven in the extraction process. Golmakani et al[23]. Reported some recently published studies

have successfully utilized a microwave oven for the extraction of active components from plants. The efficiency of Microwave-assisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample[24]. Conventional technique for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step [25]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique. Application of microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [26,27] and consuming only a fraction of the energy used in conventional extraction methods[28]. The use of microwave-assisted hydrodistillationin industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions.

Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvent, selectively,

volumetric heating and controllable heating process. The principle of heating using microwave-assisted hydrodistillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously[29].

1.6.2.2 – Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could involve the increase in the estimate of some food by-products when used as sources of natural compounds or plants material[30]. The major importance will be more effective extraction, so saving energy, and also the use of mean temperature, which is beneficial for heat-sensitive components. This technique was developed in 1950 at laboratory apparatus[31]. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation.

Ultrasound technology has been featured as a valuable method in food engineering processes and plants[30]. In these applications the power ultrasound increases the surface wetness

Evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material affecting cluster transfer[31,32]. The plants raw material is immersed in water or another solvent (methanol or ethanol or anyone other solvent) and at the same time, it is subjected to the work of

ultrasound[33]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds[34].

1.6.2.4 – Solvent-free microwave extraction (SFME)

Solvent-free microwave extraction (SFME) is in the extraction procedure of essential oil which is achieved by the in site water of the plant material without added many solvent[35]. It has been developed by Cheat and co-workers[26] . And is based on the integration of dry distillation and microwave heating energy. It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent --. In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature and extraction time were controlled by the panel in the instrument. The separated essential oil is dried over

anhydrous sodium sulfate and stored at 4ºC in the dark. The extraction yield of essential oil was calculated as follows[36]:

Extraction yield (ml/kg) = V/M

Where V is the volume of essential oil in herb samples (ml), and M is the mass of the herb sample (kg).

1.6.2.5 – Microwave hydrodiffusion and gravity (MHG)

Microwave hydrodiffusion and gravity is a new green technique for the extraction of essential oils. This green extraction technique is an original microwave blend microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants[37]. Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not requiring solvent or water and as it does require less energy[38]. The performances and advantages of this technique are

A reduction of extraction time (in the case of hydro-distillation it takes 90 min or more but in this technique only 20 min) and reducing environmental impact and power savings[39].

1.7 – Essential oils as antimicrobial

Herbs, and their essential oils (EOs), have been used since the beginning of human history for flavored foods and beverages; they have been empirically used to disguise unpleasant odors, attract other individuals and control health problems, contributing to the

welfare humans and animals, thus demonstrating the cultural and economic importance use of these products [40].The EOs are typically liquid, clear and unusually colored, complex and the present compounds are volatile, characterized by a strong odor and synthesized by aromatic plants during secondary metabolites, which act to protect the plant against microorganisms and insects. They can be synthesized in several plant organs such as buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or bark, being stored in secretory cells, cavities, channels, epidermal cells or trichomes [41]. Temporal and spatial variations in the total content of secondary metabolites products from plants occur at different levels and, despite the existence of a genetic control, the expression may undergo changes resulting from biochemical, physiological, ecological and evolutionary interactions that represent an important interface between chemistry and the environment surrounding the plants [42]. As for industrial production, EOs are obtained by steam distillation, which is on the rise in food and pharmaceutical applications, and pressurized supercritical fluid, especially carbon dioxide [43]. Eos

have several biological properties, such as larvicidal action [44], antioxidant [45], analgesic and antiinflammatory [46], fungicide [47] and antitumor activity [48]. The in vitro antimicrobial activity of EO has been researched extensively against a variety of microorganisms [49].Nevertheless, the emergence of multidrugresistant bacteria poses a challenge to treating infections, so the need to find new substances with antimicrobial properties for use in the fight against these microorganisms is evident [50] [51].Historically, most antibiotics come from a small set of functional molecular structures whose lives were extended by generations of synthetic

reorganizations and arrangements [52] .Moreover, the food, pharmaceutical and cosmetic industries have shown great interest in the antimicrobial properties of EOs, as the use of natural additives has received importance as a trend in the replacement of synthetic preservatives [53] .

Essential oils are distillates of the volatile compounds of a plant's secondary metabolism and may act as phyto-protective agents [54] .Their curative effect has been known since antiquity. It is based on a variety of pharmacological properties which are specific for each plant species. Therefore, essential oils are used in the pharmaceutical industry as active ingredients or constituents of drugs, soaps, shampoos, perfumes and cosmetics[55,57].They are also applied as food conservatives in the food industry[58].Therefore, interest in essential oils and other extracts of plants as sources of natural products has increased during the last years. They have been screened for their potential uses as alternative remedies for treatment of many infectious diseases [59] .Since essential oils possess complex chemical constituents, which vary depending on the amount of rainfall and daylight to which plants are exposed, and the soil conditions, humidity, elevation, even the time of day at which the plants are harvested [60, 61] .resistance among bacteria is not yet detected [62] .

A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics and chemotherapeutics, which leads to the insufficiency of antimicrobial treatment [63] .The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes [64, 65] .There is a strong necessity for the development of new drugs for the cure of infections provoked by resistant and multiresistant bacterial species.

It has been recognized that some essential oils have different antimicrobial activities against individual strains of microorganisms [66, 67] .Besides antibacterial properties, essential oils also have insecticidal [68] , antiparasitic [69] , antiviral and antifungal activities [70 71] , which are important both for food preservation and the control of human and plant diseases that are of microbial origin [72] .Therefore, our interest was directed toward antimicrobial activity of some essential oils and possibilities for their application in veterinary medicine.

1.8 – The target plant species

1.8.1- MENTHA VIRIDIS

Scientific classification:

Kingdom: Plantae.

Order: lamiales

Family: Lamiaceae.

Subfamily: Nepetoideae
Tribe: Mentheae
Genus: Mentha
Arabic name:Nanaa.
Botanical name: Mentha Viridis

Mentha is a genus of plants in the family Lamiaceae (mint family).[73, 74] It is estimated that 13 to 18 species exist, and the exact distinction between species is still unclear. [75] Hybridization between some of the species occurs naturally. Many other hybrids, as well as numerous cultivars, are known.

The genus has a sub cosmopolitan distribution across Europe, Africa, Asia, Australia, and North America. [76] Mints are aromatic, almost exclusively perennial, rarely annual herbs. They have wide-spreading underground and overground stolons[77], and erect, square, [78] branched stems. The leaves are arranged in opposite pairs, from oblong to lanceolate, often downy, and with a serrated margin. Leaf colors range from dark green and gray-green to purple, blue, and sometimes pale yellow.[76] The flowers are white to purple and produced in false whorls called verticillasters. The corolla is two-lipped with four subequal lobes, the upper lobe usually the largest. The fruit is a nutlet, containing one to four seeds.

Mentha virids

While the species that make up the genus Mentha are widely distributed and can be found in many environments, most grow best in wet environments and moist soils. Mints will grow 10–120 cm tall and can spread over an indeterminate area. Due to their tendency to spread unchecked, some mints are considered invasive[79]. Mentha virids has many important applications including:

1- Culinary:

The leaf, fresh or dried, is the culinary source of mint. Fresh mint is usually preferred over dried mint when storage of the mint is not a problem. The leaves have a warm, fresh, aromatic, sweet flavor with a cool aftertaste, and are used in teas, beverages, jellies, syrups, candies, and ice creams. In Middle Eastern cuisine, mint is used on lamb dishes, while in British cuisine and American cuisine, mint sauce and mint jelly are used, respectively. Mint is a necessary ingredient in Touareg tea, a popular tea in northern African and Arab countries[79].

Mint essential oil and menthol are extensively used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gum, desserts, and candies, such as mint (candy) and mint chocolate. The substances that give the mints their characteristic aromas and flavors are menthol (the main aroma of peppermint and Japanese peppermint) and pulegone (in pennyroyal and Corsican mint). The compound primarily responsible for the aroma and flavor of spearmint is L-carvone.

Mints are used as food plants by the larvae of some Lepidoptera species, including buff ermine moths[79].

2-Traditional medicine and cosmetics**:**

Mint was originally used as a medicinal herb to treat stomach ache and chest pains[80] There are several uses in traditional medicine[81] and preliminary research for possible use in treating irritable bowel syndrome. [81]

Menthol from mint essential oil (40–90%) is an ingredient of many cosmetics and some perfumes. Menthol and mint essential oil are also used in aromatherapy which may have clinical use to alleviate post-surgery nausea[81, 82].

1.10 - Faidherbia Albida

Scientific classification:

Kingdom: plantae

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpiniodeae

Genus: Faidherbia

Species: F.Albida
Arabic Name: Khorame
Bonatical name: Faidherbia Albida (Del) A.Chev

-Description:

Faidherbia albida is one of the fastest growing indigenous trees. It is deciduous and can grow up to 30 m tall. It has branching stems and an erect to roundish crown. Greenish grey to whitish grey colour and smoothness is evident on the young stems, but grey and smooth to rough on older branches and stems. The straight, whitish thorns, which are in pairs, are up to 40 mm long.

Pale grey-green leaves which are twice-compound, have a conspicuous gland at the base of each pair of pinnae (leaflets).

Scented, pale cream-coloured flowers form an elongated spike up to 35-160 x 20 mm. The flowers show from March to September, followed by fruit from September to December. The fruit is orange to red-brown in colour, non-splitting and curved to twisted pod. The size of the pods fruit ranges from 100-350 x 20-50 mm. The seeds are mostly eaten by butterfly larvae[83].

Faidherbia alba seeds Faidherbia albida

Faidherbia albida is a widely used tree well documented for increasing the yields of crops grown under it. This plant is highly valued in conservation efforts. It is the only species which loses its leaves during the rainy season. In Nigeria, the pod is used as camel food. The gum that exudes spontaneously from the trunk is sometimes collected like gum arabic. The timber, though straight grained, close, and weighty, is soft, fibrous, and unsuitable for agricultural implements [84]. Wood is used for canoes, mortars, and pestles. The bark is pounded in Nigeria and used as a packing material on pack animals. Ashes of the wood are used in making soap and as a depilatory and tanning agent for hides. The wood is used for carving; the thorny branches useful for a natural barbed fence[85]. Pods and foliage are highly regarded as livestock fodder.

Rhodesians use the pods to stupefy fish. Humans eat the boiled seeds in times of scarcity in Rhodesia.

1.8.3 - Allium Cepa subsp. Cepa

Scientific classification:

Kigdom : plantae .

Order: Asparagales

Family: Lilaceae (Amaryllidaceae).

Subfamily: Allioideae

Genus: Allium.

Species: A.cepa

Description:

The onion (*Allium* cepa subsp. cepa), also known as the bulb onion or common onion, is a vegetable that is the most widely cultivated species of the genus *Allium*. Its close relatives include the garlic, shallot, leek, chive, [86] and Chinese onion.[87]

This genus also contains several other species variously referred to as onions and cultivated for food, such as the Japanese bunching onion (Allium fistulosum), the tree onion (A. ×proliferum), and the Canada onion (Allium canadense). The name "wild onion" is applied to a number of Allium species, but A. cepa is exclusively known from cultivation. Its ancestral wild original form is not

known, although escapes from cultivation have become established in some regions. [88] The onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season.

The onion plant has a fan of hollow, bluish-green leaves and its bulb at the base of the plant begins to swell when a certain day-length is reached. The bulbs are composed of shortened, compressed, underground stems surrounded by fleshy modified scale (leaves) that envelop a central bud at the tip of the stem. In the autumn (or in spring, in the case of overwintering onions), the foliage dies down and the outer layers of the bulb become dry and brittle. The crop is harvested and dried and the onions are ready for use or storage. The crop is prone to attack by a number of pests and diseases, particularly the onion fly, the onion eelworm, and various fungi cause rotting. Some varieties of A. cepa, such as shallots and potato onions, produce multiple bulbs.

Onions are cultivated and used around the world. As a food item, they are usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles or

chutneys. They are pungent when chopped and contain certain chemical substances which irritate the eyes[88].

Onion

1- Origin and history

Because the wild onion is extinct and ancient records of using onions span western and eastern Asia, the geographic origin of the onion is uncertain,[89,90] with likely domestication worldwide.[81] Food uses of onions date back thousands of years in China, Egypt and Persia[89, 91].

Traces of onions recovered from Bronze Age settlements in China suggest that onions were used as far back as 5000 BCE.

1.8.4 – Brassica eruca

Scientific classification:

Kingdom: Plantae.

Order: Brassicales

Family: brassicaceaea

Genus: brassica.

Species: *Brassica eruca*

-Description:

Brassica eruca is an edible annual plant in the Brassicaceae family used as a leaf vegetable for its fresh peppery flavor. Other common names include garden rocket[92] or simply rocket (British, Australian, South African, Irish and New Zealand English)[93] Brassica eruca, which is widely popular as a salad vegetable, is a species to the Mediterranean region, from Morocco and Portugal in the west to Syria, Lebanon, and Turkey in the east[94].

Brassica eruca grows 20–100 centimeters (8–39 in) in height. The pinnate leaves have four to ten small, deep, lateral lobes and a large terminal lobe. The flowers are 2–4 cm (0.8–1.6 in) in diameter, arranged in a corymb in typical Brassicaceae fashion, with creamy white petals veined in purple, and having yellow stamens; the sepals are shed soon after the flower opens. The fruit is a siliqua (pod) 12–35 millimeters (0.5–1.4 in) long with an apical beak, and containing several seeds[93, 94]

Eruca sativa

1.8.5 - Anethum graveolens

Scientific classification:

Kingdom: plantae

Order: Apiales

Family: apiaceae

Subfamily: Apioideae

Tribe: Apieae

Genus: Anethum

Species: A.graveolens

Botanical name: Anethum graveoles L

Arabic name: Shabt

-Description:

Dill (Anethum graveolens) is an annual herb in the celery family Apiaceae. It is the only species in the genus Anethum. Dill is widely grown in Eurasia where its leaves and seeds are used as a herb or spice for flavouring food.

Dill

Dill grows up to 40–60 cm (16–24 in), with slender hollow stems and alternate, finely divided, softly delicate leaves 10–20 cm (4–8 in) long. The ultimate leaf divisions are 1–2 mm (0.04–0.08 in) broad, slightly broader than the similar leaves of fennel, which are threadlike, less than 1 mm (0.04 in) broad, but harder in texture. The flowers are white to yellow, in small umbels 2–9 cm (0.8–3.5 in) diameter. The seeds are 4–5 mm (0.16–0.20 in) long and

1 mm (0.04 in) thick, and straight to slightly curved with a longitudinally ridged surface[95].

Fresh and dried dill leaves (sometimes called "dill weed" to distinguish it from dill seed) are widely used as herbs in Europe and central Asia.

Like caraway, the fernlike leaves of dill are aromatic and are used to flavor many foods. Dill oil is extracted from the leaves, stems and seeds of the plant. The oil from the seeds is distilled and used in the manufacturing of soaps[95].

1.8.6 - Cucurbita pepo

Scientific classification:

Kingdom: plantae

Order: Cucurbitales

Family: Cucurbitaceae.

Genus: Cucurbita.

Species: C. pepo

Botanical name: Cucurbita pepo L.

Arabic name: Kosaa

Cucurbita pepo is a cultivated plant of the genus Cucurbita. It yields varieties of winter squash and pumpkin, but the most widespread

varieties belong to Cucurbita pepo subsp. pepo, called summer squash.[96]

Due to their varied genetic background, members of C. pepo vary widely in appearance, primarily in regards to their fruits. The plants are typically 1.0-2.5 feet high, 2–3 feet wide, and have yellow flowers.[97] Within C. pepo, the pumpkins, scallops, and possibly crooknecks are ancient and were domesticated separately. The domesticated species have larger fruits and larger yet fewer seeds[98].

Cucurbita pepo

It is an ingredient in "schumaakwe cakes" and is used externally for rheumatism and swelling. A poultice of seeds and blossoms is applied to cactus scratches[99] Fresh squash is cut into spiral strips, folded into hanks and hung up to dry for winter use. The blossoms

are cooked in grease and used as a delicacy in combination with other foods[99].

1.8.6 - *Artemisia herba-alba*

Artemisia herba-alba grows to 20–40 cm (8–16 in). Leaves are strongly aromatic and covered with fine glandular hairs. The leaves of sterile shoots are grey, petiolate, ovate to orbicular in outline; whereas, the leaves of flowering stems, more abundant in winter, are much smaller[100].

Artemisia herba-alba

- The parts that grow above the ground are used as medicine. This species is widely used in herbal medicine for its antiseptic, vermifuge and antispasmodic properties[100].

1.9 - Aim of this work

This work was aimed to:

- Extraction of essential oils from target plants.
- Conducting GC-MS analysis.
- Screening the isolated oils for their antimicrobial activity.

2- Materials and Methods

2.1 – Materials

2.1.1 – Plant Material

Seeds of *Allium cepa* Subsp. *Cepa, Cucurbita pepo, Eruca sativa, Mentha virids, Anethum praveolensvand Artemisia herpa*-alba were purchased from the local market at Oswan (Egypt) and authenticated by direct comparison with reference herbarium samples.

2.1.2 – Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010C Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25µm, thickness).

2.1.3 – Test organisms

The studied oil were screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table1: Test organisms

Ser.NO	Micro organism	Type
1	Bacillus subtilis	G+ve
2	Staphylococcus aureus	G+ve

3	Pseudomonas aeroginosa	G-ve
4	Esherichia coli	G-ve
5	Candida albicans	fungi

2.2 – Methods

2.2.1 – Extraction of oils

Powered seeds of studied plants (400g) were exhaustively extracted with n-hexane by maceration. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4ºC for further manipulation.

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

2.2.2 – GC-MS analysis

The studied oils were analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25µm,

thickness) was used. Helium (purity;99.99%) was used as carrier gas. Oven temperature program is presented in Table 2, with other chromatographic conditions are depicted in Table3.

Table 2: Oven temperature program

Rate	Temperature (ºC)	Hold time (min. $^{-1}$)
4.00	150.0	1.00
	300.0	0.00

Table 3: Chromatographic conditions

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

2.2.3 – Antimicrobial assay

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 37ºC for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100mL of 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 4ºC until used. The average number of viable organism per mL of the stock suspension was determined by means of surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02mL) 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 37ºC for 24 hours.

ii) – Preparation of fungal suspensions

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

iii) – Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess antibacterial activity of the oil. (2mL|) of the standardized bacterial stock suspension were mixed with 200 mL of sterile Molten nutrient agar which was maintained at 45ºC in a water path. (20 mL) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes,

the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

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

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

3- Results and Discussion

3.1 – *Alliym cepa Subsp. Cepa oil*

GC-MS analysis of Allium cepa Subsp. Cepa oil was conducted and the identification of the constituents was accomplished by retention times and MS fragmentation pattern. A 90-95% match was observed when comparing the mass spectra with the database on MS library.

3.1.1 – Constituents of oil

The GC-MS analysis of the Allium cepa subsp. Cepa oil revealed the presence of 27 constituents (Table 3.1). The typical total ion chromatograms (TIC) is illustrated in Fig (3.1)

Major constituents of the oil are:

i) 9, 12- octadecadienoic acid methyl ester (42.80%)

ii) 9, octadecenoic acid methyl ester (16.43 %)

iii) Hexsdecanoic acid methyl ester (12.80%)

iv) Methyl stearate (5.90%)

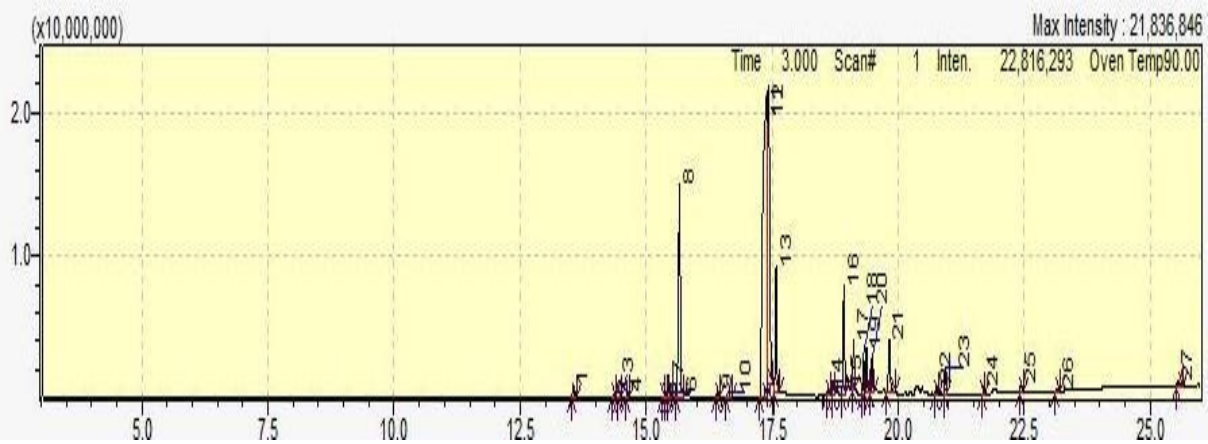


Fig (3.1): Total ions Chromatograms

Table 3.1: Constituents of Allium cepa oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	13.550	1812908	0.59
2.	5-Octadecenoic acid, methyl ester	14.359	279916	0.09
3.	cis-5-Dodecenoic acid, methyl ester	14.467	37252	0.01
4.	Pentadecanoic acid, methyl ester	14.623	773579	0.25
5.	7,10-Hexadecadienoic acid, methyl ester	15.351	47981	0.02
6.	7-Hexadecenoic acid, methyl ester, (Z)-	15.409	632284	0.21
7.	9-Hexadecenoic acid, methyl ester, (Z)-	15.453	2849357	0.93
8.	Hexadecanoic acid, methyl ester	15.664	39396632	12.80
9.	cis-10-Heptadecenoic acid, methyl ester	16.417	990583	0.32
10	Heptadecanoic acid, methyl ester	16.626	733076	0.24
11	9,12-Octadecadienoic acid, methyl ester	17.395	131742272	42.80
12	9-Octadecenoic acid (Z)-, methyl ester	17.433	50570373	16.43
13	Methyl stearate	17.578	18152074	5.90
14	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	18.609	852725	0.28
15	9-Octadecynoic acid, methyl ester	18.718	1227870	0.40
16	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.921	16362209	5.32
17	cis-13-Eicosenoic acid, methyl ester	19.115	6027115	1.96
18	Eicosanoic acid, methyl ester	19.313	4950622	1.61
19	PGH1, methyl ester	19.368	6587977	2.14
20	1-Naphthalenol, decahydro-4a-methyl-	19.479	5058301	1.64
21	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	19.820	9838604	3.20
22	cis-10-Nonadecenoic acid, methyl ester	20.755	1219007	0.40
23	Docosanoic acid, methyl ester	20.930	3219580	1.05
24	Tricosanoic acid, methyl ester	21.695	1047386	0.34
25	Tetracosanoic acid, methyl ester	22.433	1384230	0.45
26	Squalene	23.171	414714	0.13
27	Vitamin E	25.547	1509205	0.49

Major constituents are discussed below:

i) 9,12-Octadecadienoic acid methyl ester(42.80%)

The mass spectrum of 9, 12-octadecadienonic acid methyl ester is depicted in Fig (3.2). The signal which was observed at m/z294 (R.T. 17.395) is due to M^+ $[C_{19}H_{34}O_2]^+$, while the signal at m/z263 corresponds to loss of a methoxyl.

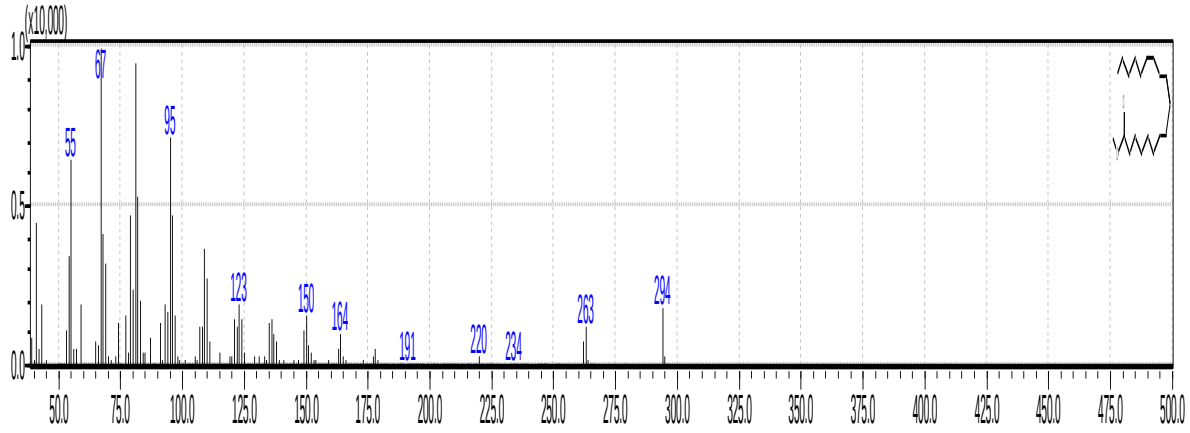


Fig (3.2): Mass spectrum of 9, 12 –octadecadienoic acid methyl ester

ii) 9- octadecadienoic acid methyl ester (16.43%)

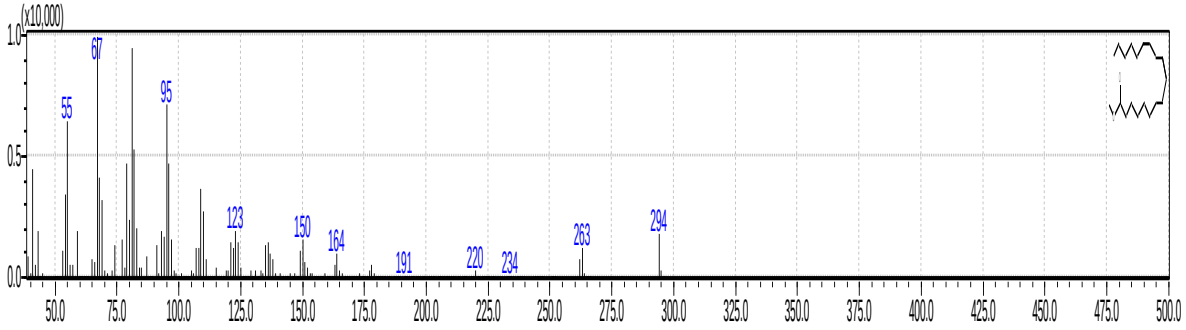


Fig (3.3): Mass spectrum of 9–octadecadienoic acid methyl ester

The mass spectrum of 9-octadecadienonic acid methyl ester is shown in Fig (3.3). The peak at m/z 296, which appeared at R.T.

17.433 in total ion chromatogram, corresponds the molecular ion: $M^{+}[C_{19}H_{34}O_{2}\ ]^{+}$. While the signal at m/z266 is due to loss of a methoxyl.

iii) Hexadecanoic acid methyl ester (12.80%)

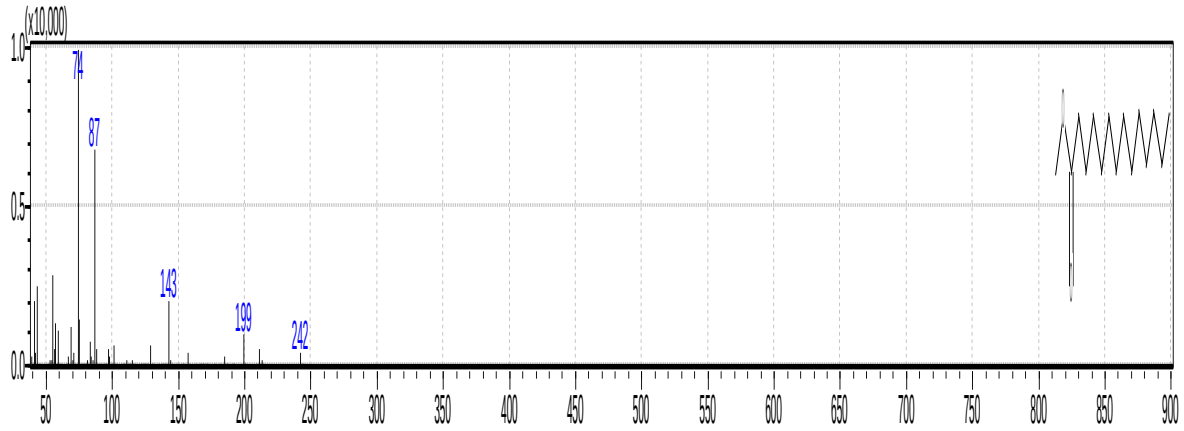


Fig (3.4): Mass spectrum of hexadecanoic acid methyl ester

Fig (3.4) shows the mass spectrum of hexadecanoic acid methyl ester. The peak m/z 270 (R.T. 15.664) was detected in the spectrum. It corresponds $M^{+}[C_{17}H_{34}O_{2}\ ]^{+}$. The peak at m/z239 is due to loss of methoxyl.

IV) Methyl stearate (5.90%)

The EI mass spectrum of methyl stearate is displayed in Fig (3.5). The peak at m/z 298 (R.T. 17.578) is due to $M^{+}[C_{19}H_{38}O_{2}\ ]^{+}$, while the signal at m/z267 corresponds to loss of a methoxyl group.

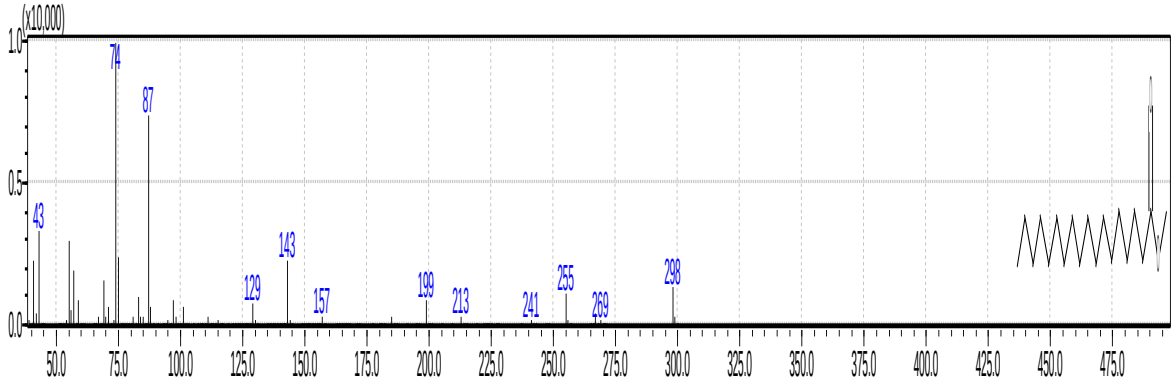


Fig (3.5): Mass spectrum of methyl stearate

3.1.2- Antimicrobial activity

Allium cepa seed 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 (3.2). The result were interpreted in the following manner: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active ;> 18mm: very active). Table (3.3) and (3.4) represent the antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed good activity against Escherichia coli. However, at the same concentration, it exhibited partial activity against Staphylococcus aureus. The oil failed to give any anticandidal activity.

Table (3.2): Antimicrobial Activity of the Allium cepa seed oil

oil	Antimicrobial Activity				
	Gram positive		Gram negative		
Mg/ml	Bs.	Sa.	Ec.	Pa.	Ca.
100	--	10	14	--	--

Table (3.3): Antimicrobial Activity of standard chemotherapeutic

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

Table 3.4: Antimicrobial Activity of standard chemotherapeutic agent

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

3.2- Cucurbita pepo

3.2.1- GC-MS analysis

GC-MS analysis of Cucurbita pepo oil was conducted. The GC-MS analysis revealed the presence of 19 components (Table 3.5). The typical total ion chromatograms (TIC) is depicted in Fig (3.6).

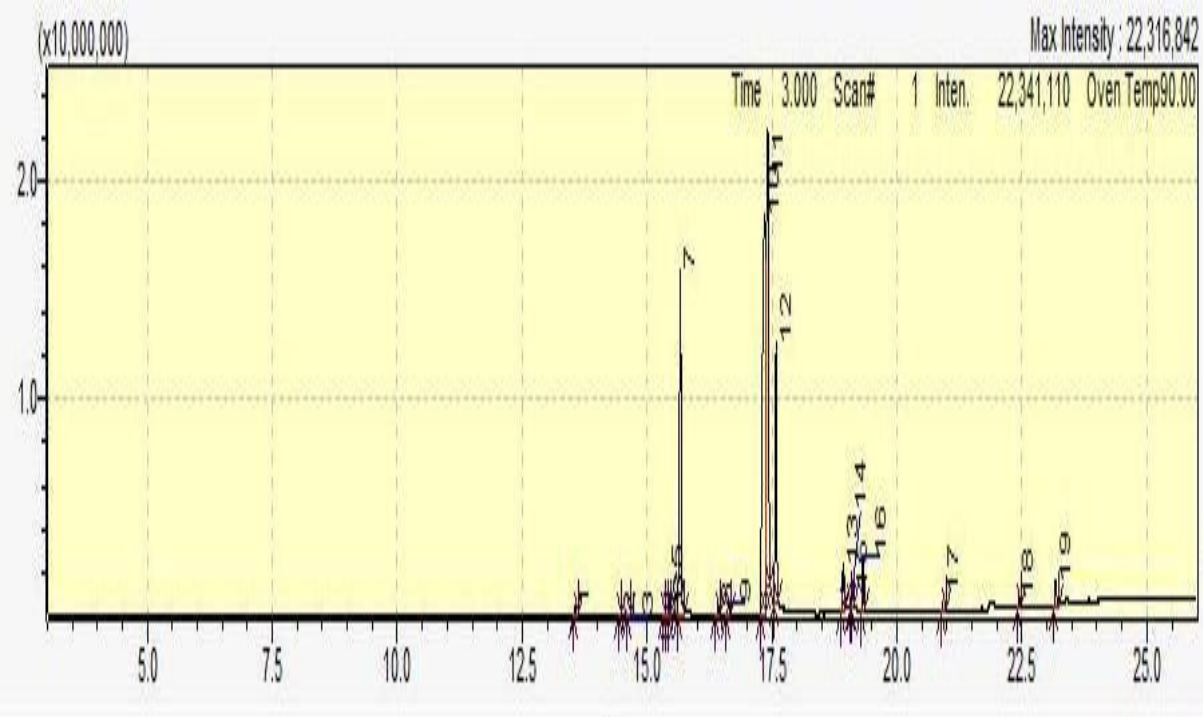


Fig (3.6): Chromatograms of Cucurbita pepo oil

Table (3.5): Constituents of Cucurbita pepo oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	13.554	1009677	0.44
2.	cis-5-Dodecenoic acid, methyl ester	14.469	31922	0.01
3.	Pentadecanoic acid, methyl ester	14.626	107090	0.05
4.	7,10-Hexadecadienoic acid, methyl ester	15.353	40873	0.02
5.	7-Hexadecenoic acid, methyl ester, (Z)-	15.410	101811	0.04
6.	9-Hexadecenoic acid, methyl ester, (Z)-	15.454	1115189	0.48
7.	Hexadecanoic acid, methyl ester	15.668	40096778	17.28
8.	cis-10-Heptadecenoic acid, methyl ester	16.419	483147	0.21
9.	Heptadecanoic acid, methyl ester	16.626	950456	0.41
10.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.354	82820988	35.69
11.	9-Octadecenoic acid (Z)-, methyl ester	17.416	63213794	27.24
12.	Methyl stearate	17.581	25130245	10.83

13	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.919	4666147	2.01
14	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	19.083	888078	0.38
15	cis-13-Eicosenoic acid, methyl ester	19.115	1358584	0.59
16	Eicosanoic acid, methyl ester	19.313	4997129	2.15
17	Docosanoic acid, methyl ester	20.936	1635446	0.70
18	Tetracosanoic acid, methyl ester	22.440	822825	0.35
19	Squalene	23.175	2588620	1.12

Major constituents are discussed below:

i) 9, 12-Octadecadienoic acid methyl ester (35.69%)

Fig. 3.7 shows the mass spectrum of 9, 12-octadecadienonic acid methyl ester the peak at m/z294 (R.T. 17.354) coincides with M^+ $[C_{19}H_{34}O_2]^+$, while the peak at m/z263 corresponds to loss of a methoxyl.

9, 12- Octadecadienoic exists in lipids and cell membrane. It belongs to one of the two families of essential fatty acids. Such acids cannot be synthesized by human body and are available through diet.

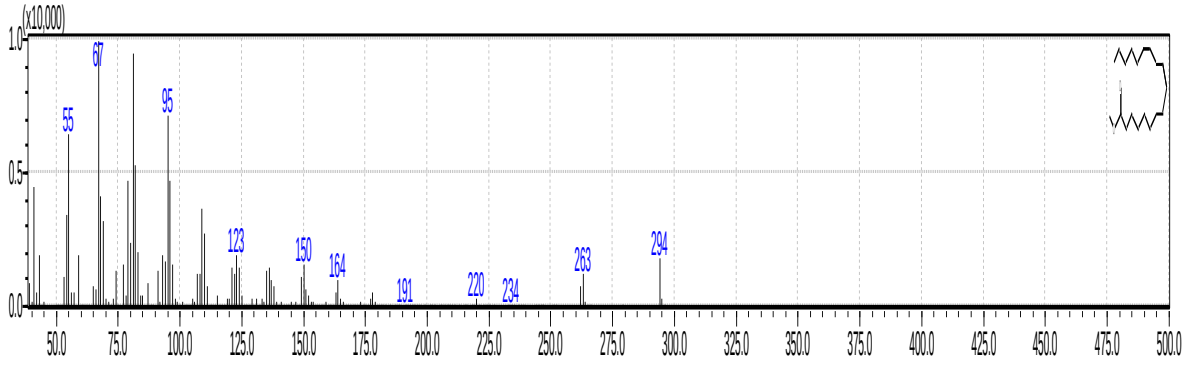


Fig (3.7): Mass spectrum of Octadecadienoic acid methyl ester

ii) 9, 12-Octadecadienoic acid methyl ester (27.24%)

The mass spectrum of 9, 12-octadecenoic acid methyl ester is displayed in Fig (3.8). The peak at m/z 296 (R.T. 17.416) corresponds M^+ $[C_{19}H_{36}O_2\ ]^+$, while the signal at m/z266 is attributed to loss of a methoxyl.

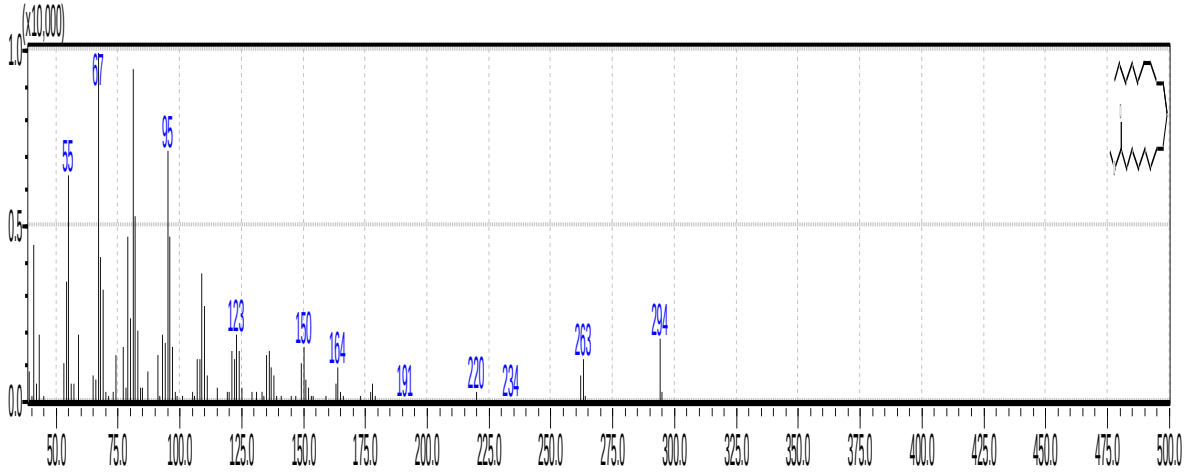


Fig (3.8): Mass spectrum of Octadecadienoic acid methyl ester

9-Octadecenoic acid (oleic acid) is included in animal's fats and vegetables, hence it is included in the normal human died. Oleic acid is used as emollient. It is used in a small amount as excipient in pharmaceutical industries. 9-Octadecenoic acid could be responsible for the hypotensive effect of olive oil. It has been claimed that the presence of oleate in olive oil is associated with decreased risk of breast cancer.

iii) Hexadecanoic acid methyl ester (17.28%)

Figure (3.9) presents the mass spectrum of Hexadecanoic acid methyl ester. The molecular ion: M^+ $[C_{17}H_{34}O_2\ ]^+$ appeared at m/z 270 R.T. 15.668 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl function.

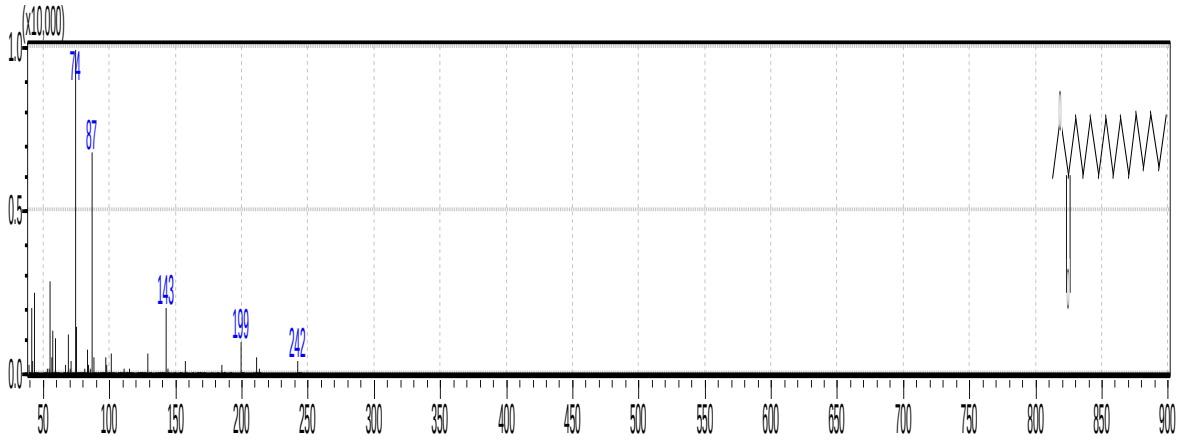


Fig (3.9): The mass spectrum of Hexadecanoic acid methyl ester

Hexadecanoic acid (palmitic acid) is saturated fatty acid and it is considered as the most common fatty acid in animals and humans. Palmitic acid is the precursor of long-chain fatty acids. This acid is a major lipid component of human breast milk. Palmitic acid, beside being used in soap industry, is wildly used in food industry.

Iv) Methyl stearate (10.83%)

Figure (3.10) displays the EI mass spectrum of methyl stearate. The molecular ion: $M^+[C_{19}H_{38}O_2\ ]^+$ appeared as expected at m/z 298

(R.T. 17.581). the peak at m/z 267 corresponds to loss of a methoxyl.

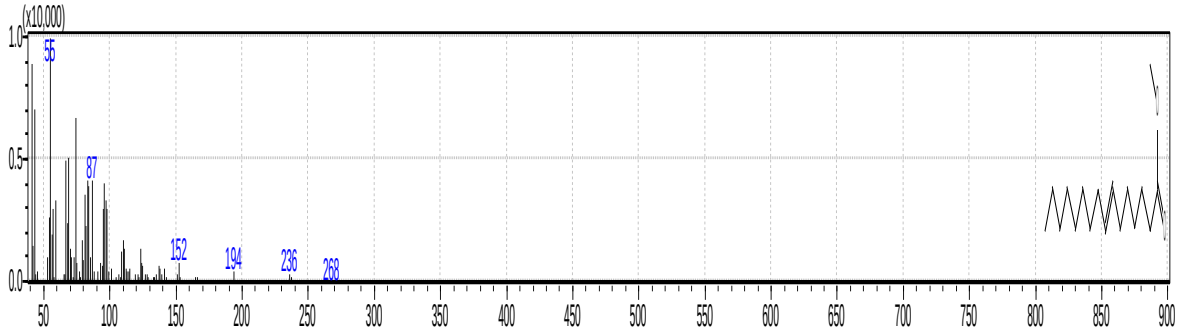


Fig (3.10) Mass spectrum of methyl stearate

3.2.2 – Antimicrobial Activity

In cup plate agar diffusion bioassay, the oil was evaluated for antimicrobial activity. The averages of diameters of the growth inhibition zones are shown in Table (3.6).

Table (3.6): Antimicrobial Activity of the Cucurbita pepo oil

Sample	Ec	Pa	Sa	Bs	Ca
Gerwia tenax Oil (100mg/ml)	--	20	17	-	--

The oil showed significant antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus.

3.3 – Eruca sativa

3.3.1 – The GC-MS analysis

Eruca sativa oil was studied by GC-MS. The GC-MS analysis revealed the presence of 27 constituents (Table 3.7). The typical total ion chromatograms (TIC) is depicted in Fig (3.11).

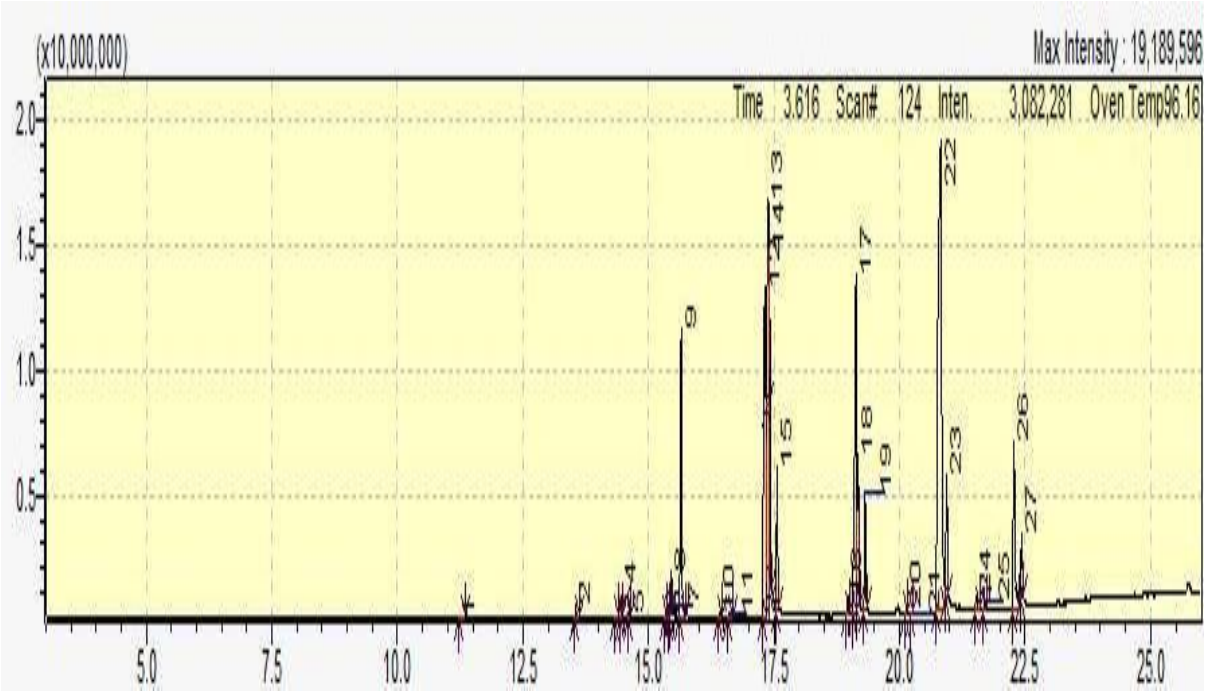


Fig (3.11): Total ions chromatograms

Table (3.7): Constituents of Eruca sativa oil

ID#	Name	Ret.Time	Area	Area%
1.	Dodecanoic acid, methyl ester	11.254	63536	0.02
2.	Methyl tetradecanoate	13.550	862597	0.28
3.	5-Octadecenoic acid, methyl ester	14.357	56541	0.02
4.	cis-5-Dodecenoic acid, methyl ester	14.463	25305	0.01
5.	Pentadecanoic acid, methyl ester	14.621	211047	0.07
6.	7,10-Hexadecadienoic acid, methyl ester	15.348	359985	0.12
7.	cis,cis,cis-7,10,13-Hexadecatrienal	15.413	790298	0.26
8.	9-Hexadecenoic acid, methyl ester, (Z)-	15.451	2534715	0.82
9.	Hexadecanoic acid, methyl ester	15.651	24298872	7.87

10	cis-10-Heptadecenoic acid, methyl ester	16.416	338603	0.11
11	Heptadecanoic acid, methyl ester	16.622	348387	0.11
12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.316	39726768	12.87
13	9-Octadecenoic acid (Z)-, methyl ester	17.387	19216324	6.22
14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.408	16870138	5.46
15	Methyl stearate	17.561	10205239	3.31
16	.gamma.-Linolenic acid, methyl ester	18.967	1331937	0.43
17	cis-11-Eicosenoic acid, methyl ester	19.133	39097785	12.66
18	cis-13-Eicosenoic acid, methyl ester	19.174	10434954	3.38
19	Eicosanoic acid, methyl ester	19.313	8815217	2.86
20	Heneicosanoic acid, methyl ester	20.137	276841	0.09
21	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.231	144692	0.05
22	13-Docosenoic acid, methyl ester, (Z)-	20.823	100121453	32.41
23	Docosanoic acid, methyl ester	20.938	9248908	3.00
24	cis-10-Nonadecenoic acid, methyl ester	21.529	1196767	0.39
25	Tricosanoic acid, methyl ester	21.694	599459	0.19
26	15-Tetracosenoic acid, methyl ester, (Z)-	22.280	16957144	5.49
27	Tetracosanoic acid, methyl ester	22.431	4622334	1.50

Major constituents are briefly discussed below:

i) – 13-Docosenoic acid methyl ester (32.41%)

13-Docosenoic acid, methyl ester (6.31%) The mass spectrum of 13-Docosenoic acid, methyl ester is shown in Fig 12. The peak at m/z 352, which appeared at R.T. 20.780 in total ion chromatogram, corresponds M^+ $[C_{23}H_{44}O_2\ ]^+$. The peak at m/z 322 corresponds to loss of methoxyl function.

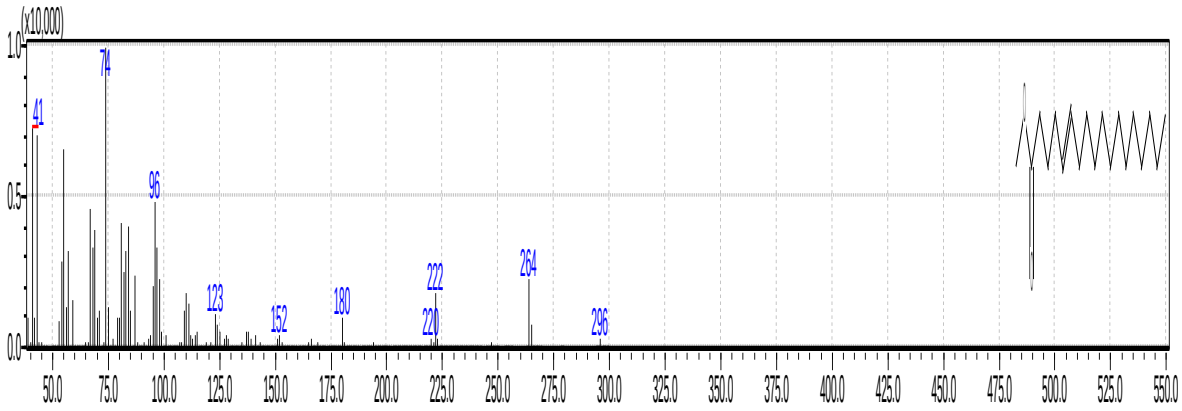


Fig. 3.12 mass spectrum of 13-Docosenoic acid, methyl ester

ii) Cis-11-Eicosenoic acid methyl ester (12.66%)

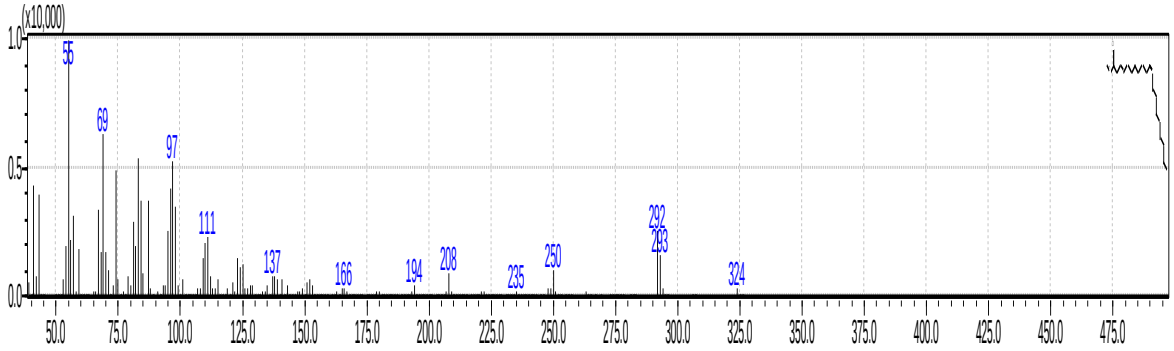


Fig. 3.13: mass spectrum of Cis-11-Eicosenoic acid methyl ester

The EI mass spectrum of cis- 11 Eicosenoic acid methyl ester is shown in Fig.3.13. The peak at m/z324, which appeared at R.T.19.133 in total ion chromatogram, corresponds to M^+ $[C_{21}H_{40}O_2\ ]^+$. The peak at m/z293 corresponds to loss of a methoxyl function.

iii) 9, 12- Octadecadienoic acid methyl ester (12.87%)

The EI mass spectrum of 9, 12- Octadecadienoic acid methyl ester is shown in Fig(3.14). The peak at m/z294 (R.T. 17.316) coincide

with M^+ $[C_{19}H_{34}O_2\ ]^+$, while the peak at m/z263 is due to loss of a methoxyl.

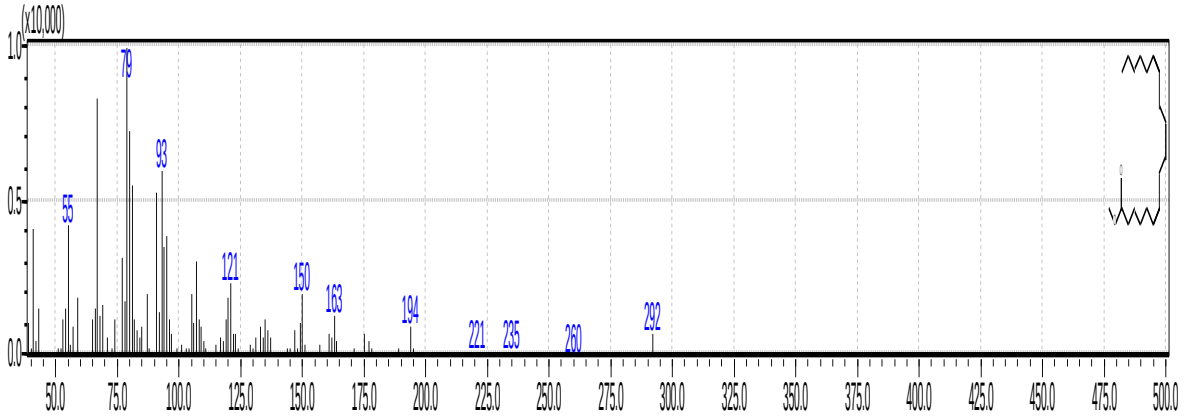


Fig. (3.14: mass spectrum of 9, 12- Octadecadienoic acid methyl ester

iv)Hexadecanoic acid methyl ester (7.87%)

Figure (3.15) shows the mass spectrum of Hexadecanoic acid methyl ester. The molecular ion: M^+ $[C_{17}H_{34}O_2\ ]^+$ appeared at m/z270 at (R.T.15.651) in total ion chromatogram. The fragment at m/z239 is due to loss of methoxyl function.

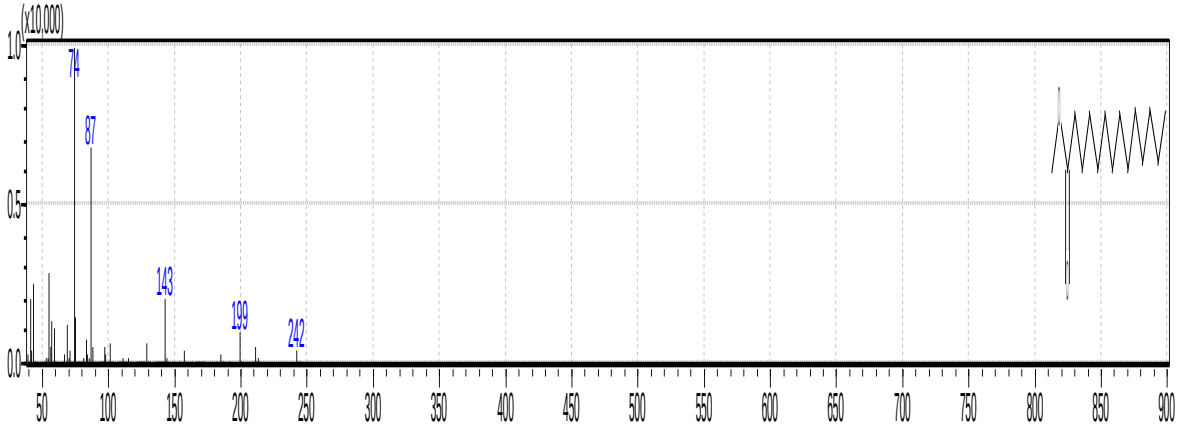


Fig. (3.15): Mass spectrum of Hexadecanoic acid methyl ester

v) 9- Octadecadienoic acid methyl ester (6.22%)

The mass spectrum of 9, 12- Octadecadienoic acid methyl ester is displayed in Fig. (3.16).The peak at m/z 296 (R.T. 17.387) corresponds M^+ $[C_{19}H_{36}O_2]^+$, while the signal at m/z266 is attributed to loss of a methoxyl.

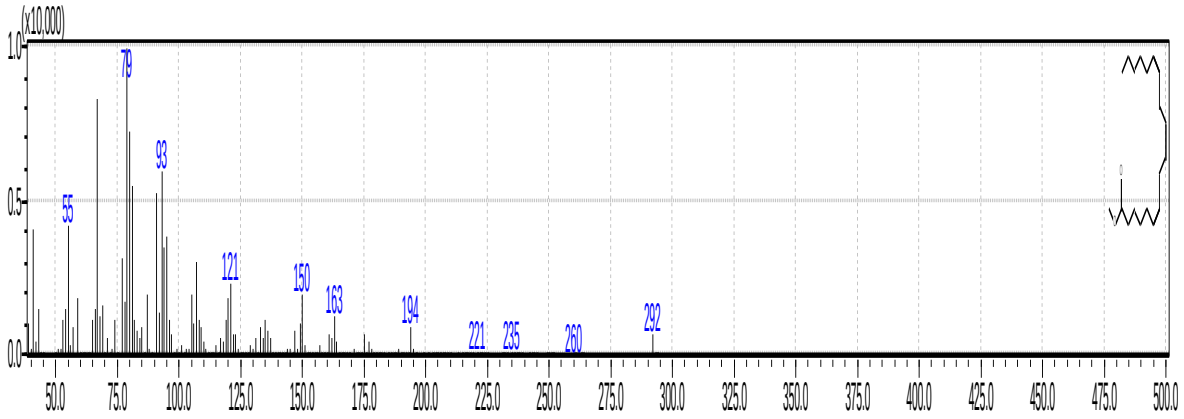


Fig (3.16): Mass spectrum of 9, 12- Octadecadienoic acid methyl ester

3.3.2 – Antimicrobial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of diameters of the growth inhibition zones are shown in Table (3.8).

Table (3.8): Antimicrobial activity of the Eruca sativa oil

oil	Antimicrobial Activity				
	Gram positive		Gram negative		
Mg/ml	Bs.	Sa.	Ec.	Pa.	Ca.
100	--	--	--	--	--

The oil failed to give any response in the antimicrobial assay.

3.4 – Mentha virids

GC-MS analysis of the studied oil showed the presence of 52 components (Table 3.9) the typical total chromatograms (TIC) is shown in Fig 3.17.

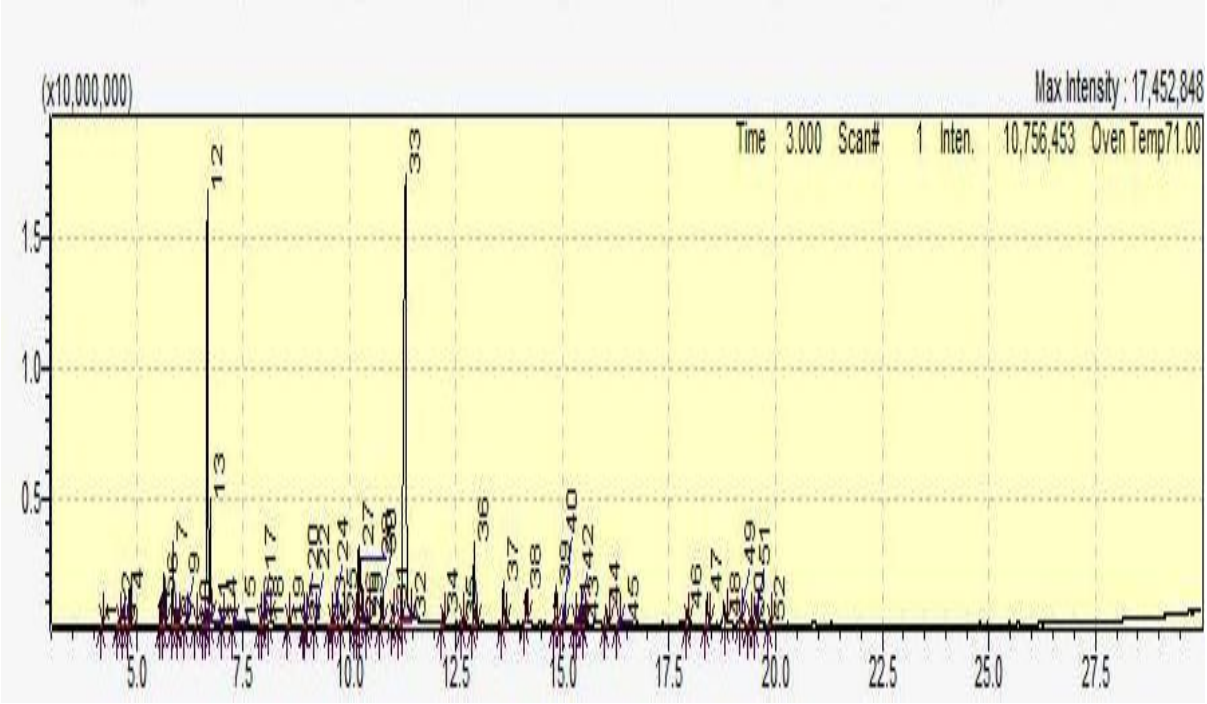
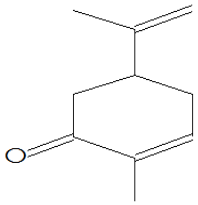


Fig 3.17: The typical total ion chromatograms (TIC)

Major components are discussed below:

(i) – D-Carvone (39.84%)



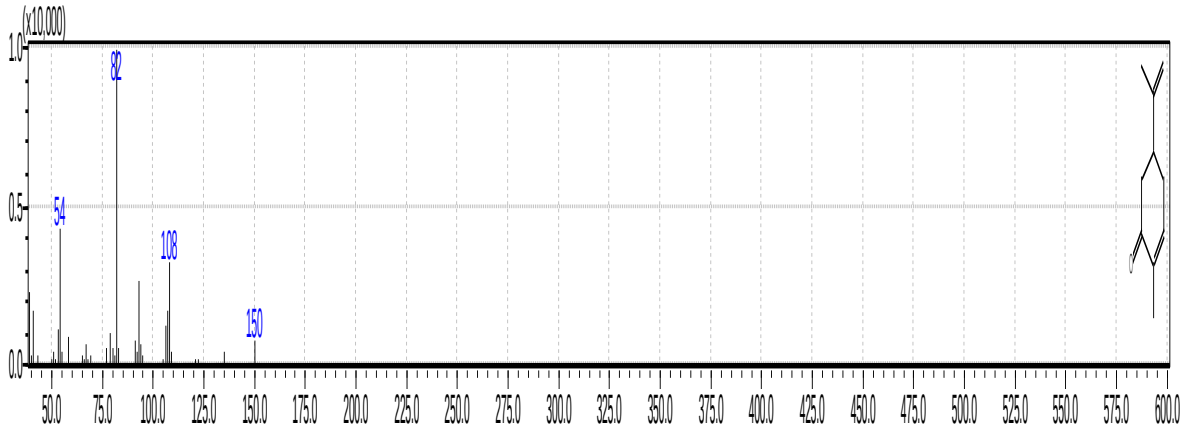
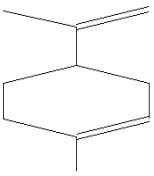


Fig.3.18: Mass spectrum of D-Carvone

The Mass spectrum of D-Carvone is presented in Fig.3.18. The peak at m/z150 (R.T.11.366) is due to the molecular ion M^+ $[C_{10}H_{14}O_2\ ]^+$.

Carvone is a terpenoids. Found naturally in many essential oils. However, it is most abundant in the oils from seeds of Carum carvi and Mentha spicata. Carvone is used in aromatherapy and food industry. It is also used in air fresheners for it pleasant smell. Carvone has several therapeutic effects including the treatment of coughs, bronchitis, and bronchial asthma.

D-Limonene (22.36%)



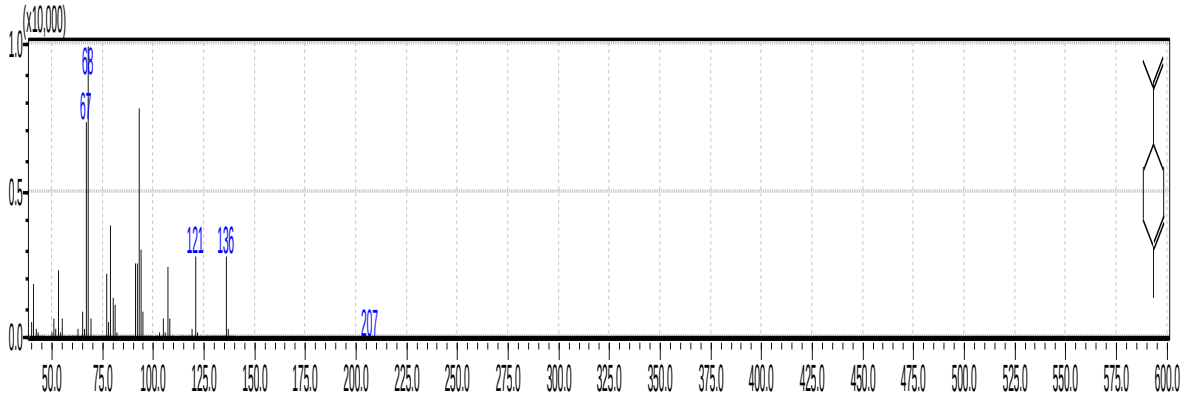


Fig.3.19: Mass spectrum of D-Limonene

In Fig. 3.19, the signal at m/z136 corresponds the molecular ion: M^+ $[C_{10}H_{16}\ ]^+$. Limonene (1-methyl-4-prop-en-2- ylcyclohexene). Is a cyclic monoterpene. The D-Limonene is used a flavoring agent in food manufacturing. [6][7] It is also used in chemical synthesis as aprecursor to carvone. It is used as a fragrance ingredient for cosmetics products. [7]. D-Limonene is also used in food manufacturing and pharmaceutical industry.

Table 3.9: constitutes of Mentha virids volatile oil

ID#	Name	Ret.Time	Area	Area%
1.	Furan, 2,5-diethyltetrahydro-	4.190	132454	0.07
2.	.beta.-Pinene	4.580	275248	0.14
3.	.alpha.-Phellandrene	4.700	72386	0.04
4.	.alpha.-Pinene	4.837	2489602	1.28
5.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.567	1916975	0.99
6.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.648	3585222	1.85
7.	.beta.-Myrcene	5.857	5640396	2.91
8.	3-Octanol	5.966	371943	0.19
9.	.beta.-Ocimene	6.158	300227	0.15
10	(+)-2-Carene	6.406	82952	0.04
11	o-Cymene	6.574	184932	0.10
12	D-Limonene	6.670	43396884	22.36

13	Eucalyptol	6.733	7916910	4.08
14	trans-.beta.-Ocimene	7.014	109144	0.06
15	.gamma.-Terpinene	7.272	186008	0.10
16	2-Carene	7.902	204522	0.11
17	Benzene, 1-methyl-4-(1-methylethenyl)-	7.940	127381	0.07
18	1,6-Octadien-3-ol, 3,7-dimethyl-	8.121	173792	0.09
19	3-Octanol, acetate	8.579	379914	0.20
20	p-Mentha-1(7),8-dien-2-ol	8.931	58408	0.03
21	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	8.977	147651	0.08
22	Spiro[2.4]heptane, 4-methylene-	9.186	83334	0.04
23	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-	9.571	270967	0.14
24	Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl-	9.633	512431	0.26
25	Terpinen-4-ol	9.849	1081749	0.56
26	.alpha.-Terpineol	10.136	567541	0.29
27	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	10.217	6459917	3.33
28	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	10.263	5064308	2.61
29	3-Hexadecyne	10.436	670221	0.35
30	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	10.747	2503891	1.29
31	Carveol	11.014	1408263	0.73
32	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	11.172	711498	0.37
33	D-Carvone	11.322	77366609	39.84
34	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2.alpha.,4a.alpha.,8a.alpha.)-	12.192	662202	0.34
35	Isopulegol acetate	12.638	239888	0.12
36	trans-Shisool	12.924	7006717	3.61
37	trans-Carveyl acetate	13.618	4127201	2.13
38	(-)-.beta.-Bourbonene	14.148	2999717	1.55
39	Caryophyllene	14.845	3661868	1.89
40	.gamma.-Muurolene	15.011	489835	0.25
41	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	15.307	334260	0.17
42	(E)-.beta.-Famesene	15.373	1017417	0.52
43	Humulene	15.500	275736	0.14
44	.beta.-ylangene	16.010	1708767	0.88
45	Bicyclogermacrene	16.305	244628	0.13
46	Cyclohexane, 1,2-diethenyl-4-(1-methylethylidene)-, cis-	17.928	1031847	0.53
47	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde	18.391	2791641	1.44
48	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	18.849	386227	0.20
49	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	19.180	820080	0.42

50	Andrographolide	19.398	355238	0.18
51	Longifolene-(V4)	19.535	1067450	0.55
52	2H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)	19.862	385695	0.20

3.4.2 – Antimicrobial activity

In cup plate agar diffusion assay, the oils 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 (3.10). The result were interpreted in terms of the commonly used terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active ;> 18mm: very active.)

Table.10: inhibition zones of Mentha virids volatile oil

Type	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Oil	100	-	22	11	20	-

Ec. Escherichs coli.

Ps. Pseudomonas aeruginosa.

Sa. Staphylococus aureus.

Bs. Bacillus subtitis.

Ca. Candida albicans.

The oils showed significant activity against Pseudomonasa aeruginosa and Bacillus subtilis and moderate activity against staphylococcus aureus.

3.5 – Anethum graveolens

3.5.1 – GC-MS analysis of Anethum graveolens volatile oil

GC-MS analysis of Anethum graveolens volatile oil was conducted. The analysis showed the presence of 30 components (Table 3.11).

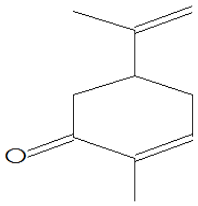
Table 3.11: Constituents of Anethum graveolens oil

ID#	Name	Ret.Time	Area	Area%
1.	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	4.727	41547	0.02
2.	.alpha.-Pinene	4.866	620308	0.25
3.	Camphene	5.152	69638	0.03
4.	3-Carene	5.425	140933	0.06
5.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.598	614349	0.25
6.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.680	119985	0.05
7.	.beta.-Myrcene	5.890	1556132	0.64
8.	(+)-2-Carene	6.121	443621	0.18
9.	.alpha.-Phellandrene	6.198	4641066	1.89
10.	p-Cymene	6.606	2711653	1.11
11.	D-Limonene	6.703	44418650	18.13
12.	.gamma.-Terpinene	7.307	191612	0.08
13.	o-Isopropenyltoluene	7.979	502353	0.21
14.	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	8.529	414931	0.17
15.	trans-p-Mentha-2,8-dienol	8.668	274790	0.11
16.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	8.919	154398	0.06
17.	(+)-2-Bornanone	9.197	5850944	2.39
18.	Santolina alcohol	9.584	157868	0.06
19.	1-(1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone	9.743	410595	0.17
20.	Terpinen-4-ol	9.878	559105	0.23
21.	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	10.057	1387040	0.57

22	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	10.294	5398128	2.20
23	Cyclodecene, 1-methyl-	10.464	12268672	5.01
24	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	10.793	424815	0.17
25	1-Isopropenyl-3-propenylcyclopentane	10.996	591357	0.24
26	Carveol	11.063	245862	0.10
27	D-Carvone	11.366	92690764	37.80
28	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	11.551	27727606	11.32
29	Apiol	18.605	39592766	16.16
30	Tolclofos-methyl	22.646	828727	0.34

Some important constituents are discussed below:

(i)- D-Carvone (37.83%)



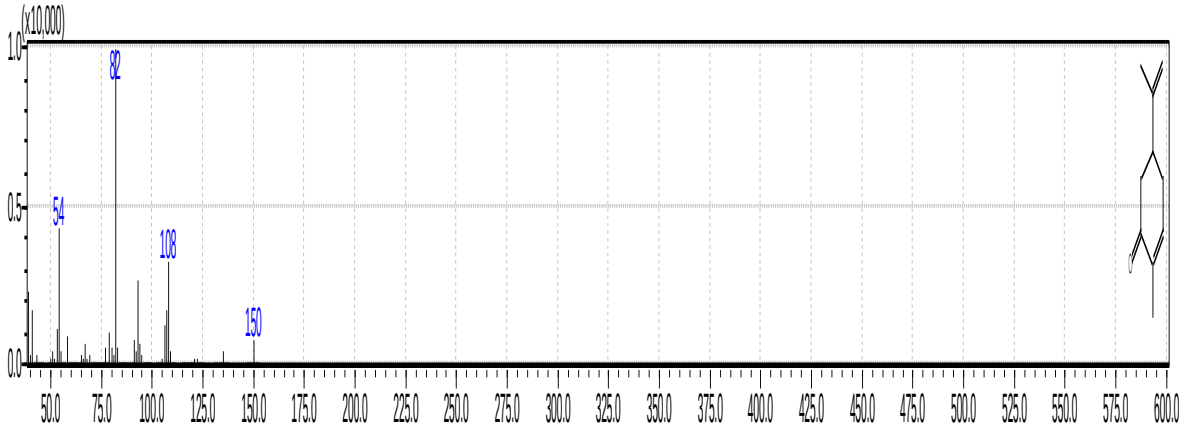


Fig. 3.21: Mass spectrum of D-Carvone

The Mass spectrum of D-Carvone is shown in Fig. 3.21. The molecular ion M^+ $[C_{10}H_{14}O\ ]^+$appeared at m/z150 (R.T.11.366).

D-Limonene (18.13%)

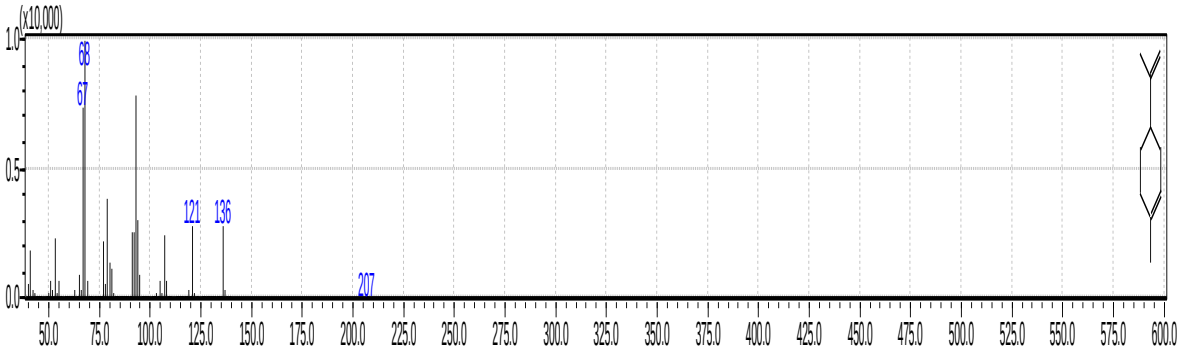


Fig. 3.22: Mass spectrum of D-Limonene

The mass spectrum of D-Limonene is displayed in Fig. 3.22. The signal at m/z136 corresponds the molecular ion M^+ $[C_{10}H_{16}]^+$.

Apiol (16.16%)

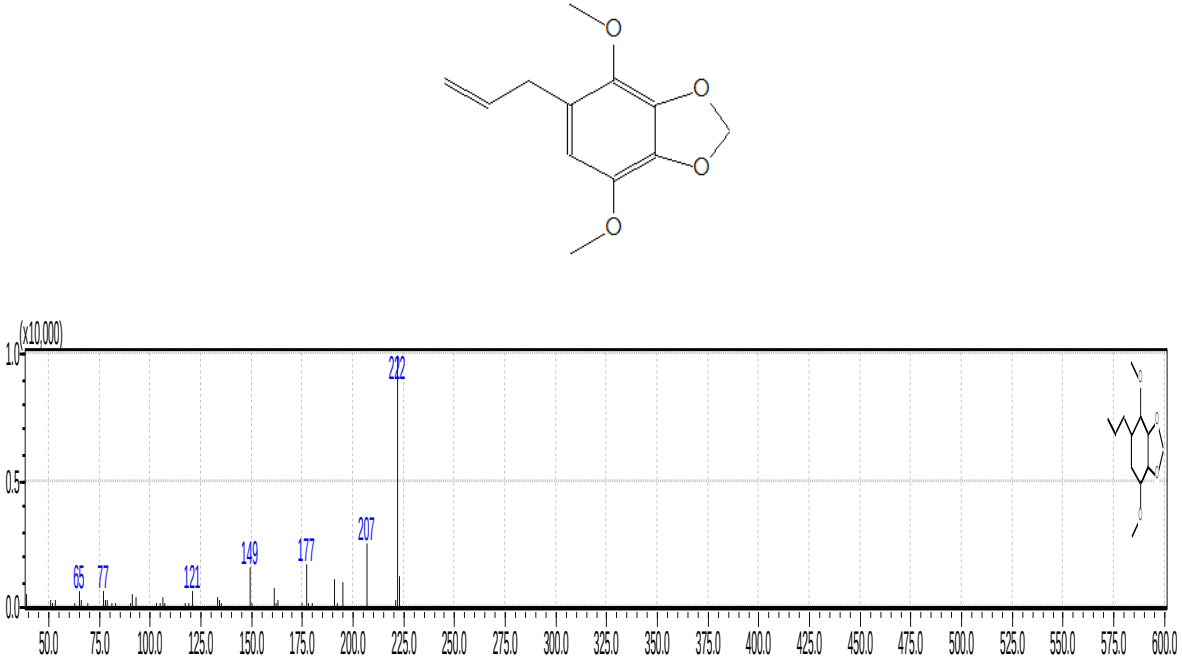


Fig. 3.23: Mass spectrum of Apiol

Fig. 3.23 shows the Mass spectrum of Apiol. The signal which appeared at retention time 18.605(m/z 222) corresponds the molecular ion M^+ $[C_{12}H_{14}O_4\ ]^+$.

Apiol, also known as 'liquid apiol' or 'green oil of parsley' is the extracted oleoresin of parsley, rather than the distilled oil. Due to its similarity to the term apiole, care should be taken to avoid confusion. Apiol is an irritant and, in high doses, it can cause liver and kidney damage. Cases of death due to attempted abortion using apiol have been reported.

3.5.2 – Antimicrobial activity

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 (3.12). The result were interpreted in terms of commonly used terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active ;> 18mm: very active).

Table 3.12: for Antimicrobial activity of Anethum graveolens oil

Type	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Oil	100	20	20	17	20	-

Ec. Escherichs coli.

Ps. Pseudomonas aeruginosa.

Sa. Staphylococus aureus.

Bs. Bacillus subtitis.

Ca. Candida albicans.

The oil showed significant activity against Escherichia coli, Pseudomonasa aeruginosa and Bacillus subtilis, while it showed very good activity against staphylococcus aureus.

3.6 – Artemisia herpa-alba

3.6.1 – GC-MS analysis

GC-MS analysis of Artemisia herpa-alba was accomplished. The GC-MS analysis of the studied oil revealed the presence of 49 components (Table 3.13). The typical total ion chromatograms (TIC) is depicted in Fig (3.24).

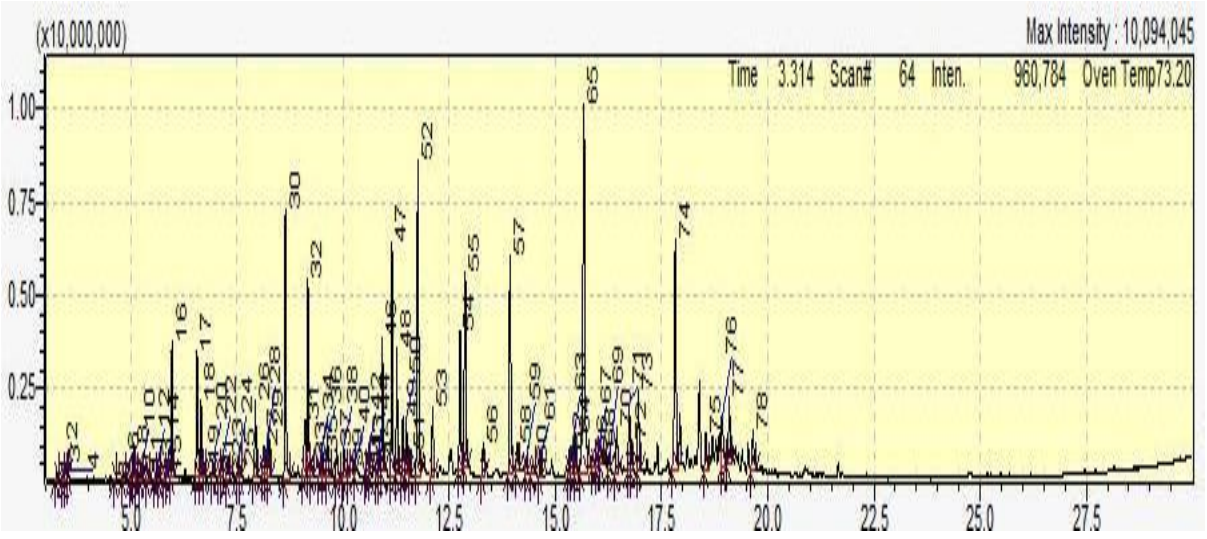


Fig. 3.24: Chromatograms of Artemisia herpa-alba. Oil

Table 13: constituents of Artemisia herpa-alba oil

ID#	Name	Ret.Time	Area	Area%
1.	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	3.262	231049	0.08
2.	1,3-Cyclopentadiene, 5-(1,1-dimethylethyl)-	3.396	210064	0.07
3.	Butanoic acid, 2-methyl-, ethyl ester	3.472	626451	0.22
4.	Pentanoic acid, ethyl ester	3.515	280467	0.10
5.	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	4.642	74446	0.03
6.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	4.841	373661	0.13
7.	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	5.013	232798	0.08
8.	1-Octadecyne	5.069	1367784	0.49
9.	Camphene	5.126	291557	0.10
10.	2(5H)-Furanone, 5,5-dimethyl-	5.228	318989	0.11
11.	Benzaldehyde	5.389	779285	0.28
12.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.570	54527	0.02
13.	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	5.656	215684	0.08
14.	5-Hepten-2-one, 6-methyl-	5.811	221336	0.08
15.	.beta.-Pinene	5.862	90632	0.03
16.	Benzene, 1,2,3-trimethyl-	5.971	6752813	2.41
17.	Benzene, 1,2,4-trimethyl-	6.564	7359635	2.62
18.	D-Limonene	6.659	3468269	1.24
19.	Eucalyptol	6.735	774803	0.28
20.	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	6.936	1124027	0.40
21.	Cyclopentene, 1-pentyl-	7.087	229684	0.08
22.	Lilac alcohol C	7.142	1062385	0.38
23.	1,5-Heptadien-4-one, 3,3,6-trimethyl-	7.295	1387798	0.49
24.	Lilac alcohol D	7.534	1233783	0.44
25.	Spiro[2.4]heptane-5-methanol, 5-hydroxy-	7.586	434613	0.15
26.	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	7.936	4211579	1.50
27.	Grandisol	8.125	1349634	0.48
28.	Cyclopentadiene, 2,5,5-trimethyl-	8.180	851074	0.30
29.	Bicyclo(3.3.1)non-2-ene	8.254	2600069	0.93
30.	Isophorone	8.644	17387115	6.20
31.	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	9.111	4888601	1.74
32.	(+)-2-Bornanone	9.175	10786067	3.84
33.	2,5-Furandione, 3-(1,1-dimethylethyl)-	9.256	878126	0.31
34.	Ethinamate	9.454	550412	0.20
35.	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	9.522	1514318	0.54
36.	endo-Borneol	9.637	2515430	0.90
37.	Terpinen-4-ol	9.856	2245276	0.80
38.	Benzenemethanol, .alpha.,.alpha.,4-trimethyl-	10.022	1738097	0.62
39.	.alpha.-Terpineol	10.138	389670	0.14
40.	1,7-Octadiene, 2,7-dimethyl-3,6-bis(methylene)-	10.276	612376	0.22

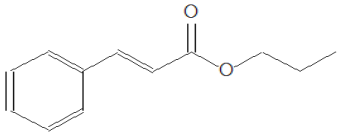
41	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	10.533	379508	0.14
42	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	10.571	628141	0.22
43	1,7-Heptanediol	10.607	1049547	0.37
44	2-Furanacetaldehyde, .alpha.-propyl-	10.774	437658	0.16
45	Citronellol	10.852	1107887	0.39
46	Lilac aldehyde B	10.929	7196454	2.56
47	2,6-Octadienal, 3,7-dimethyl-, (Z)-	11.154	14379269	5.12
48	(-)-Carvone	11.268	7121666	2.54
49	Geraniol	11.410	3335994	1.19
50	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	11.493	2730990	0.97
51	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	11.568	1335781	0.48
52	2,6-Octadienal, 3,7-dimethyl-, (E)-	11.764	21322866	7.60
53	Bornyl acetate	12.110	4422048	1.58
54	1,6-Dimethylhepta-1,3,5-triene	12.757	8718545	3.11
55	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	12.878	13653502	4.87
56	2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-, (Z)-	13.301	1971128	0.70
57	2-Propenoic acid, 3-phenyl-, ethyl ester	13.933	15534857	5.54
58	Benzoic acid, 4-ethenyl-, methyl ester	14.104	1818504	0.65
59	2-Naphthalenamine, 1,2,4a,5,6,7,8,8a-octahydro-4a-methyl-	14.307	935133	0.33
60	.alpha.-Bisabolol	14.477	896993	0.32
61	(1,2,3-Trimethyl-cyclopent-2-enyl)-methanol	14.644	1297388	0.46
62	1-(2-Isopropyl-5-methylcyclopentyl)ethanone	15.330	1215083	0.43
63	4-(1-Hydroperoxy-2,2-dimethyl-6-methylene-cyclohexyl)-pent-3-en-2-one	15.378	1317519	0.47
64	3(2H)-Isoquinolinone, octahydro-, (4ar-trans)-	15.462	2059418	0.73
65	n-Propyl cinnamate	15.675	33298515	11.89
66	Globulol	15.884	895260	0.32
67	2-Pentanone, 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	15.976	564204	0.20
68	Davana ether	16.080	1372352	0.49
69	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	16.255	963612	0.34
70	Longipinane, (E)-	16.445	3414418	1.22
71	1,6,6-Trimethyl-8-oxabicyclo[3.2.1]octan-2-one	16.737	4121909	1.47
72	(+)-(Z)-Longipinane	16.790	1336729	0.48
73	Cyclohexanone, 2-acetyl-	16.953	4696575	1.67
74	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	17.824	17379343	6.19
75	Selina-6-en-4-ol	18.544	2806402	1.00
76	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1.alpha.,2.alpha.(Z)]-	18.920	4934911	1.76

77	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	19.093	5131641	1.83
78	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	19.654	3080805	1.10

Major constituents are:

i) – Propyl cinnamate (11.89%)

Fig. 3.25 shows the mass spectrum of n-Propyl cinnamate. The signal which appeared at retention time 15.675(m.z190) corresponds the molecular ion M^+ $[C_{12}H_{14}O_2\ ]^+$.



n-Propyl cinnamate

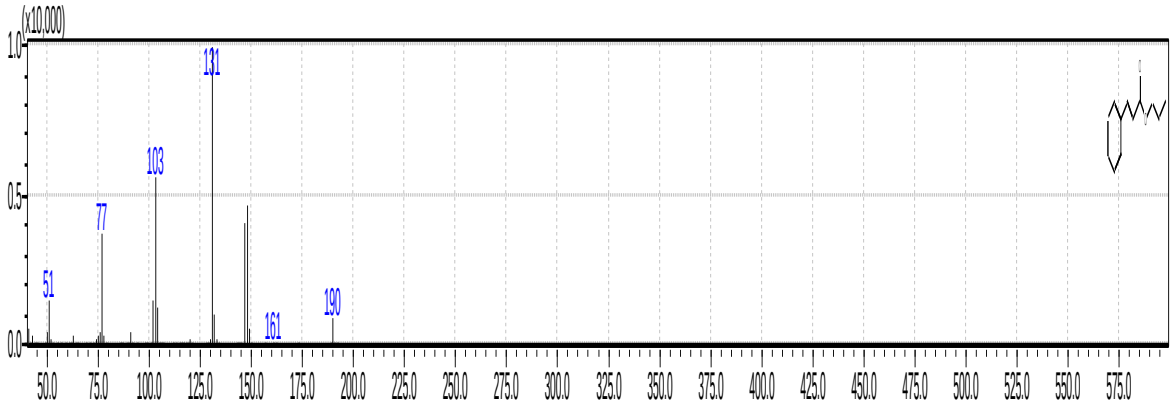
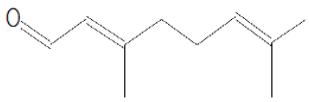


Fig. 3.25: Mass spectrum of n-Propyl cinnamat

ii) – 2, 6-Octadienal, 3, 7-dimethy, (E)-bornyl aceatate (7.60%)

Fig. 3.26 shows the mass spectrum of 2, 6-Octadienal, 3, 7-dimethy, (E)-bornyl aceatate. The signal which appeared at

retention time 11.764(m.z152) corresponds the molecular ion M^+ $[C_{10}H_{16}O_2\ ]^+$.



2, 6-Octadienal, 3, 7-dimethy, (E)-bornyl aceatate

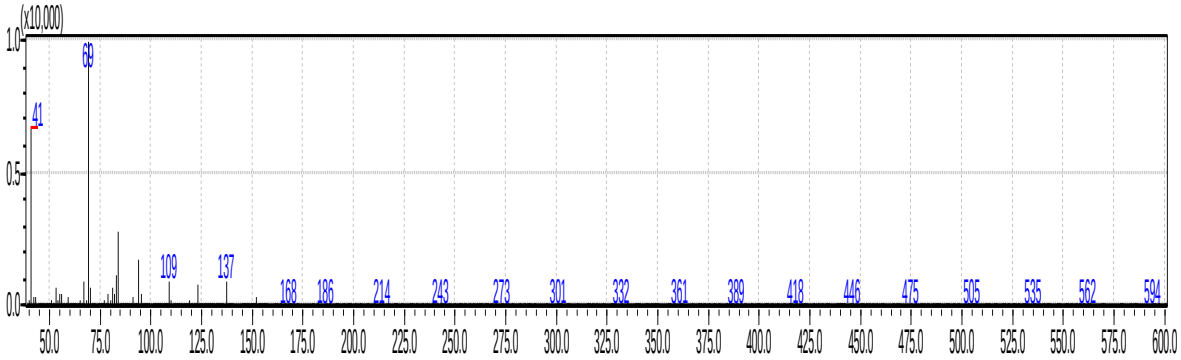


Fig. 2.26: Mass spectrum of 2, 6-Octadienal, 3, 7-dimethy, (E)-bornyl aceatate

3.6.2 – Antimicrobial activity

In cup plate agar diffusion assay, the oils was evaluated for Antimicrobial activity. The average of the diameters of the growth of inhibition zones are depicted in Table (3.14).

Table (3.14): Inhibition zones of the oil

Sample	Ec	Pa	Sa	Bs	Ca
Lawsonia mermus Oil (100mg/ml)	--	30	16	-	-

The oil significant antibacterial activity against Pseudomonasa aeruginosa and very good activity against Staphylococcus aureus.

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