Sudan University of Science and Technology

College of Postgraduate Studies

Constituents and Antimicrobial Potential of Oils from Some Medicinal Plants

مكونات وفعاليه مضاد الميكروبات لزيوت بعض النباتات الطبيه

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

By

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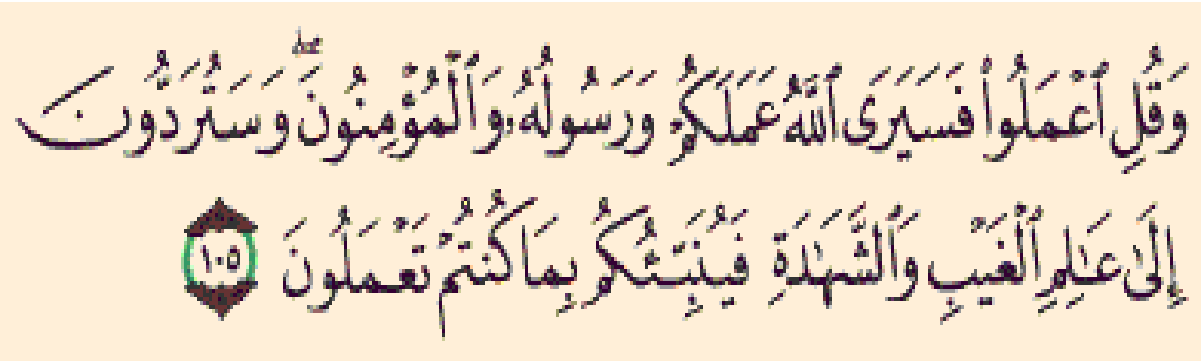

(التوبة-105)

Dedication

To….

my parents

husband

sons

brothers and sisters

Acknowledgement

First of all, I would like to thank **Allah Almighty** for giving me the ability and strength to accomplish this work.

I would like to express my thanks , gratitude and respect to my supervisor Prof. Mohamed Abdel Kareem for his interest ,close supervision and continuous advice.

Thanks for the technical staff, Dept. of chemistry, Sudan University of Science and Technology for all facilities.

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Deep thanks to my family for their support.

Abstract

In this study five medicinal plants(*Lycopersicum esculentum*, *Ocimum basilicum* ,*Sterculia setigera* , *Carthamus tinclorius* and *Prunus mahaleb*) have been studied by GC-MS and the antimicrobial activity has been evaluated. *Lycopersicum esculentum* seed oil gave 17 constituents dominated by: i)9,12-octadecadienoic (Z,Z)- (35.16%) ii)9,12-octadecadienoic (Z,Z),acid methyl ester(19.79%) iii)9-octadecenoic acid (Z)- methyl ester (10.84%).The oil showed significant activity against *Bacillus subtilis.*

Sterculia setigera oil showed 20 components.Major constituents are: i)-cis-9-hexadecenal (32.66%) ii)-oleic acid (20.29%). *Sterculia speciesare* oil showed partial activity against *Staphylococcus aureus* and *Escherichia coli*.

The GC-MS analysis of *Carthamus tinclorius* oil exhibited 21 constituents dominated by: i)9,12-octadecadienoic acid methyl ester(55.82%) ii) hexadecanoic acid methyl ester (14.04%) iii) 9-octadecenoic acid methyl ester (9.32%).This oil exhibited good activity against *Pseudomonas aeruginosa.*

Fifty six components were detected in *Ocimum basilicum* oil dominated by: i-9,12-octadecadienoic acid methyl ester(40.77%) ii-9,12,15-octadecatrienoic acid methyl ester(26.56%) iii-hexdecanoic

acid methyl ester(14.74%) iv-methyl stearate (9.83%).However this oil failed to show any antimicrobial activity.

Prunus mahaleb oil has been investigated by GC-MS. The analysis showed 40 constituents dominated by: i) 9-octadecenoic acid methyl ester (36.80%)ii) 9, 12-octadecenoic acid methyl ester (25.87%) ii) hexdecanoic acid (7.91%) iv)6a,14a-methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-(4.21%).

المستخلص

فى هذا البحث تمت دراسة خمسة نباتات لها استخدامات طبية(الريحان, القرطم, الطماطم ,المحلب ,الورطاب) حيث تم تحديد المك ونات بتقنية الكروموتوغرافيا الغازية –طيف الكتلة كما واجرى اختبار مضاد الميكروبات. اعطى نبات الطماطم 17 مكونا اهمها:

i)9,12-Octadecadienoic (Z,Z)- (35.16%).

ii)9,12-Octadecadienoic (Z,Z), acid methyl ester (19.79%).

iii)9-Octadecenoic acid (Z)- methyl ester (10.84%)ز

فى اختبار مضاد الميكروبات ابدى الزيت فعاليه ضعيفه ضد:*Bacillus subtilis* .

اما نبات الورطاب فقد اعطى 20 مكونا اهمها:

i)-cis-9-hexadecenal (32.66%) ii)-oleic acid (20 i)-cis-9-hexadecenal (32.66%) ii)-oleic acid (20..29%).

فى اختبار مضاد الميكروبات ابدى الزيت فعاليه ضعيفه ضد:

Staphylococcus aureus and *Escherichia coli*.

اعطى تحليل الكروموتوغرافيا الغازية –طيف الكتلة لزيت نبات القرطم 21 مكونا اهمها:

i)9,12-octadecadienoic acid methyl ester(55.82%) ii) hexadecanoic acid methyl ester (14.04%) iii) 9-octadecenoic acid methyl ester (9.32%).

فى اختبار مضاد الميكروبات ابدى الزيت فعاليه جيده ضد: *Pseudomonas aeruginosa*

احتوى زيت الريحان الطيار على 56 مكونا اهمها:

i-9,12-octadecadienoic acid methyl ester(40.77%)ii-9,12,15-octadecatrienoic acid methyl ester(26.56%) iii-hexdecanoic acid methyl ester(14.74%) iv-methyl stearate (9.83%).

فى اختبار مضاد الميكر وبات لم يكن ازيت ذ و فعاليه ضد الميكر وبات قيد الاختبار. اما زيت المحلب فقد احتوى على 40 مركبا اهمها:

i) 9-Octadecenoic acid methyl ester (36.80%)

ii) 9 12-Octadecenoic acid methyl ester (25.87%)
ii) Hexdecanoic acid (7.91 %)
iv)6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-(4.21%).

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1-Introduction

1.1-General approach

Nature is a huge and renewed source of potential medicinal plants. Medicinal plants are plants that are commonly used in treating and preventing specific ailments and diseases that are generally considered to be harmful to humans[1] .These plants are either wild plant species growing spontaneously in self-maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or domestic. Domesticated plants species are those that have arisen through human actions such as selection or breeding and depend on management for their existence, for example *Aloe barbadensis*[2]. People of all continents have long applied poultice and imbibed infusions of indigenous plants dating back to prehistory for health purposes and is still in use today[3] .

Phytomedicine has been used in healthcare delivery in many parts of Africa and the rest of the world . Effective health cannot be achieved in Africa, unless orthodox medicine is complemented with traditional medicine . At least 80% of Africans depend on plant medicine for their healthcare[3]. Fruits and vegetables have been recognized as natural sources of various bioactive compounds which could be attributed to their

phyto constituents such as flavonoids, anthocyanins, vitamins C and E, phenolic compounds, dietary fiber, and carotenoids present in fruits and vegetables[4].

The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Traditional methods such as maceration and Soxhlet extraction are commonly used at the small research setting or at Small Manufacturing Enterprise (SME) level. Significance advances have been made in the processing of medicinal plants such as the modern extraction methods; microwave-assisted (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), in which these advances are aimed to increase yield at lower cost. Moreover, modifications on the methods are continuously developed. With such variety of methods present, selection of proper extraction method needs meticulous evaluation. The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drugs constitute a major part in the entire traditional system of medicines[5] . Higher plants is their capacity to produce a large number of organic chemicals of high structural diversity, the so called secondary metabolites[6] .

Phytochemical screening of plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic

agents representing molecular diversity engineered by nature. It is therefore necessary and urgent to fight against emerging and re-emerging infectious diseases with a view to discover and invent new agents of greater therapeutic profile to mitigate frequent outbreaks of diseases which have posed a new threat to global health security.

Essential oils are fragrant essences of plants, usually volatile oils obtained from an odoriferous, single species of plant. Most essential oils are primarily composed of terpenes and their oxygenated derivatives and are obtained by steam distillation or solvent extraction of different parts of the aromatic plants including the buds, flowers, leaves, seeds, roots, stems, bark, wood, and rhizomes etc.

Compounds formed via shikimic acid phenyl propanoid route. Essential oils are used in perfumery, aromatherapy, cosmetics, medicine, incense, household cleaning products, and for flavoring food and drinks etc. Variations in climatic conditions, type of soil in which the plant was grown etc. will produce natural variations in the relative distribution of components in essential oils. For example, the same oil extracted from plants grown at different locations can manifest quantitative change in the oil composition. Essential oils are very complex; hundreds of components can be present, and most of the components which confer aroma or flavor may be present only at ppm levels[7].

Fixed oils extracted from plants have an important functional and sensory role in food products, because of their fatty acids composition and the fat-soluble vitamins (A, D, E, and K). They are also sources of energy and essential fatty acids like linoleic and linolenic that are responsible for growth and the health of organisms[8].

The technique of gas chromatography-mass spectroscopy has become an efficient and precise method for qualitative, as well as quantitative estimation, for almost all combinations of components in such mixtures down to minute traces. The most commonly used capillary column for the analysis of essential oils is Polyethylene Glycol (PEG). Most of the components of essential oils are identified using capillary GC with Mass Detector. The data can be compared to an established profile or fingerprint for that particular essential oil to finally determine the purity of that oil. Certain key components which are valuable to the particular essential oil are often quantified using suitable standards to determine the quality or grade and the value of the essential oil. Complex essential oils such as peppermint oil, turpentine oil, eucalyptus oil, cinnamon oil, citronella oil, lemon grass oil, thymol, camphor, menthol, and methyl salicylate, etc. are well analyzed using GC-MS. Conventional analytical methods based on GC and GC/MS operate with 30-60 m long columns.

High chromatographic efficiencies are required to achieve baseline separation and quantitative determination of the important groups of components. Such methods generally require 30-60 minutes to perform an overall analytical cycle.

The phytochemical screening of diverse medicinal plans for antimicrobial agents has gained much importance because lately World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in these countries[9] .

1.2 Plant oils

Plants may contain fixed and /or essential oils. Essential oils are liquid products of steam or water distillation of plant parts (leaves, stems, bark, seeds, fruits, roots and plant exudates). Expression is used exclusively for the extraction of citrus oil from the fruit peel, because the chemical components of the oil are easily damaged by heat. Citrus oil production is now a major by-product process of the juice industry. An essential oil may contain up to several hundred chemical compounds and this complex mixture of compounds gives the oil its characteristic fragrance and flavour. An essential oil may also be fractioned and sold as individual natural components. Other processing options can also produce further products that can be sold

alongside essential oils. The plant parts can be extracted with organic solvents to produce oleoresins, concretes and absolutes or extracted with a near or supercritical solvent such as carbon dioxide to produce very high quality extracts. These oleoresins and extracts contain not only the volatile essential oil but also the concentrated non-volatile flavor components and these have wide application in the food and pharmaceutical industries. The solvent extraction processes are more difficult and complex than steam distillation and will normally be beyond the financial resources of most small scale processors, but supplying the raw materials to these extraction plants can be a market option. Essential oils have been traditionally used for treatment of infections diseases all over the world for centuries[10] .

Recently the applications and uses of essential oils is a growing market and there are a considerable range of applications. The oils are used, for example, in the food and beverages industry and as fragrances in perfumes and cosmetics, but the oils also cover a broad spectrum of biological activity which has lead to an increased interest among researchers. In recent years there has been extensive research to explore and determine the antimicrobial activity of essential oils. All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others. Thymol, carvacrol, linalool and eugenol are main constituents of some plant

essential oils that have been shown to have a wide spectrum of activity against microbes . Members of this class are known to be either bactericidal or bacteriostatic, depending upon the concentration used. The mechanism of action is still unclear but some studies suggest that compounds penetrate the cell, where they interfere with cellular metabolism. Other studies suggest that phenols such as carvacrol and eugenol disturb the cellular membrane and react with active sites of enzymes.

As far as the classification of essential oils is concerned essential oils may be classified according to different parameters including : consistency, origin, and chemical nature of the main components.

Depending on their consistency, essential oils are classified as: essences, balsams, resins

a) Fluid essences are liquids which are volatile at room temperature.

(b) Balsams are natural extracts obtained from a bush or tree. They usually have a high benzoic and cynamic acid content with their corresponding ethers. They are thicker, not very volatile, and less likely to react by polymerising. Examples of balsams are copaiba balsam, Peruvian balsam, Banguy balsam, Tolu balsam, Liquid amber. (c) Resins are amorphous solid or semi-solid products of a complex chemical nature. They are physiological or physio-pathological in origin. Colophony, for example, is

obtained by separating trementine an oleoresin. It contains abietic acid and derivatives.

A Homogeneous mixtures of resins and essential oils is termed oleoresins. Trementine, for example, is obtained by making incisions in the trunk of different pine species. It contains resin (colophony) and essential oil (trementine essence) which are separated by steam drag distillation.

Oleoresin is also used to refer to vegetable extracts obtained using solvents. They are frequently used instead of spices in foodstuffs and pharmacy because of their advantages (stability, microbiotic and chemical uniformity, and easy to add). They have the aroma of the plant in concentrated form and are highly viscous liquids or semi-solid substances (black pepper, paprika oleoresin, cloves etc).

Depending on their origin, essential oils are classified into (i) natural, (ii)artificial and (iii) synthetic.

Natural oils are obtained straight from the plant and are not modified physically or chemically afterwards. However, they are expensive because of their limited yield.

Artificial oils are obtained using processes of enriching the essence with one or several of its components. For example, essences of rose, geranium, and jasmine are enriched with linalool, and aniseed essence with ethanol.

Synthetic oils, as the name suggests, are usually produced by combining their chemically synthesized components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences).

Most of essential oils are highly complex chemical compounds. The proportion of these substances varies depending on the oil, but also on season, time of day, growing conditions, and genetics.

Chemo-type is a term used to describe the variation in chemical composition of an essential oil, even of the same species. A chemo-type is a distinct chemical entity, different from secondary metabolites. Certain small variations in the environment, geographical location, genes, which have little or no effect on a morphological level can, however, produce big changes in chemical phenotypes. Thyme (*Thymus vulgaris*) is a typical example. It has 6 different chemo-types depending on which are the main component of its essence (timol, carvacrol, linalool, geraniol, tuyanol -4, or terpineol). When this is the case, the plant is named using the name of the species followed by the main component of its chemo-type. For example, *Thymus vulgaris* linalool, *Thymus vulgaris* timol.

1.3 Constituents of essential oils

Essential oil components are divided into terpenoids and non-terpenoids.Non-terpenoids essential oils 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. Terpenoids essential oils are more important commercially and in terms of their properties. Terpenes, derived from isoprene units (C_5) bonded in a chain, are type of chemical substance found in essential oils, resins, and other aromatic plant substances, (pines, citrus fruits etc). They are usually found in monoterpene oils (C_{15}) and diterpenes (C_{20}). They may be aliphatic, cyclic, or aromatic. According to their functional group they can be:

-Alcohols (menthol, bisabolol) and phenols (timol, carvacrol)

-Aldehydes (geranial, citral) and ketones (camphor, thuyone)

-Esters (bornile acetate, linalile acetate, methyl salicylate; anti-inflammatory compounds similar to aspirin)

-Ethers (1.8 - cineol) and peroxides (ascaridol)

-Hydrocarbons (limonene, pinene α and β).

a. Monoterpenic hydrocarbons

Monoterpenic hydrocarbons are the dominant components in essential oils, and precursors of the more complex oxidised terpenes. Their names end in –ene. Limonene, for example, is the precursor to the main components of mint essences (*Mentha* spp,

Lamiaceae Family) such as carvone and menthol. Limonene is also found in citric plants and in dill (*Anethum graveolens*, Apiaceae family). Pinene α and β are also widely present in nature, especially in trementine essence of the *Pinus* genre (Pinaceae family).

b. Alcohols

Alcohols are highly sought after for their aroma. Linalool, for example, has two forms. R-linalool is found in roses and lavender and is the main component of *Mentha arvensis*. S-linalool found in lavender oil at > 5% indicates adulteration. Linalool gives tea, thyme, and cardamom leaves their taste. Menthol, another compound found in this group, is responsible for the smell and taste of mint. Mint essence may contain up to 50% of this component: Geraniol from scented geraniums (*Pelargonium* spp**),** citronelol from roses (*Rosa gallica*), borneol from rosemary and santalol from sandalwood (*Santalum album*, Santalaceae family).

c. Aldehydes

Aldehydes are characterized by high reactivity. Examples are : geraniol – geranial, and citronelol – citronelal. They are found in abundance in citrus plants, and are responsible for their characteristic smell, particularly the isomers geranial (α citral) and neral (β citral) known as citral in combination (see graphic). In addition to its characteristic aroma, citral has anti-viral,

antimicrobiotic, and sedative properties. But many aldehydes, including citral, cause irritation to the skin and cannot be used externally. Other important groups are the aromatic aldehydes, such as benzaldehyde**,** main ingredient of bitter almond oil and cause of their typical aroma.

d. Phenols

Phenolic compounds are only found in few plant species . The most important are thimol and carvacrol, which are found in thyme (*Thymus*) and oregano (*Origanus*), both of the Labiatae family. Another important phenol is eugenol, which is found in many species, for example, clove essence. It is both a powerful bactericide and also anaesthetic, and is used in dentistry.

e. Phenolic ethers

Phenolic ether are dominant constituents of celery and parsley (apiol), aniseed (anetol), basil (metilchavicol), and estragon (estragol). Safrol is a component which is used extensively in the perfume industry and is found in the bark of the sassafras tree (*Sassafras albidum* Lauraceae family).

f. Ketones

Ketones of essential oils are produced by the oxidation of alcohols and they are very stable molecules. The ketone carvone is found in *Mentha spicata*. Tuyone -first isolated in Tuya- (*Thuja occidentalis* Cupressaceae family) and pulegone are fairly toxic and should never be used during pregnancy. Tuyone is

found in plants of the Artemisia genus (*Artemisia absinthium* with which absinthe and vermouth are made), and in salvia (*Salvia officinalis*). Pulegona was first isolated in *Mentha pulegium*.

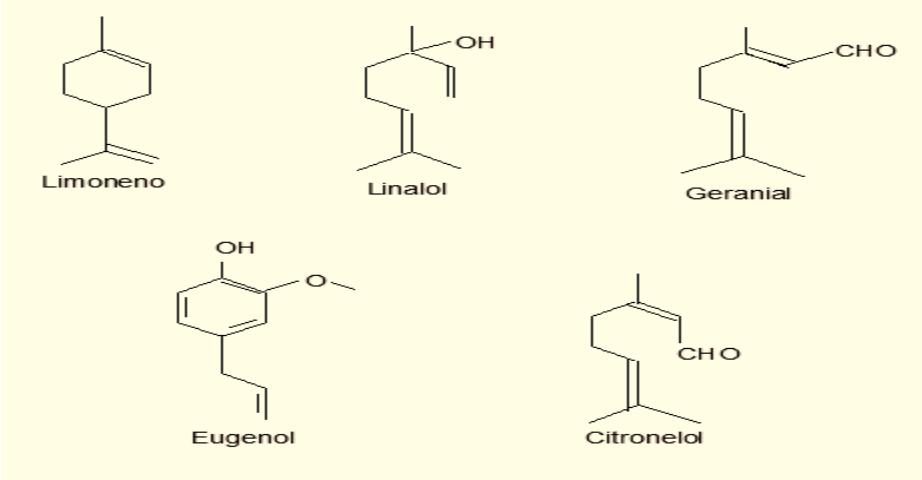
g. Ethers

Ethers or monoterpenic oxides are relatively unstable molecules. An example is bisabolol oxide found in camomile (*Matricaria chamomilla*). Another common ether is 1.8 –cineol (also known as eucaliptol), which is the main component of eucalyptus oil. It is an expectorant and mucolitic, and the main component of cough medicines. The aroma of eucalyptus oil varies depending on 1.8 –cineol content: the oil with a high content (*Eucalptus globulus*) is used for medicinal purposes, whereas that with a lower content (*Eucaliptus radiata*) is used in aromatherapy.

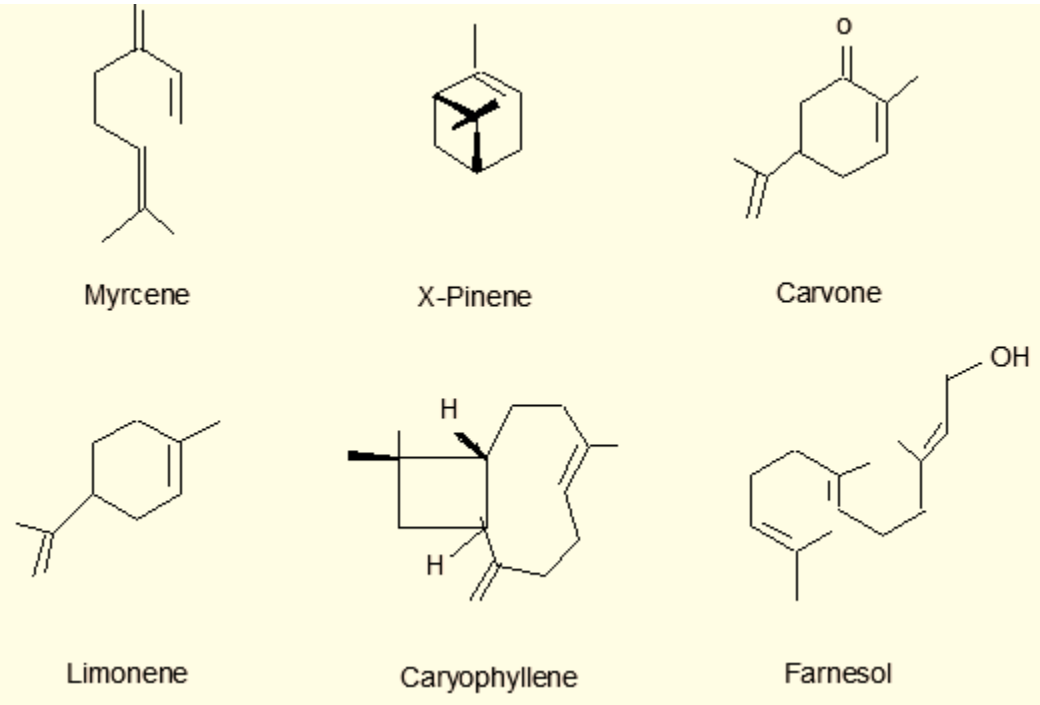
h. Esters

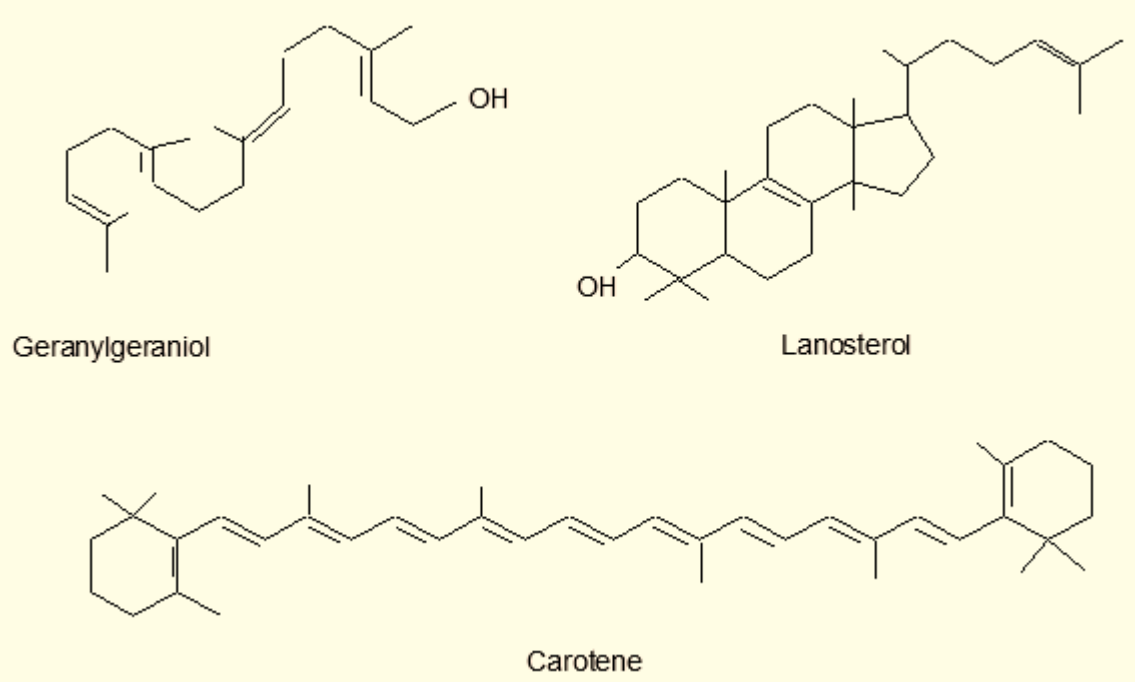
When a monoterpenic alcohol reacts with acid - such as acetic acid- the result is an ester. The aroma of such esters is characteristic of the oils in which they are found. Lavender oil, for example, contains linalool in its ester, linalool acetate. The relative abundance of both these components is a sign of good quality. Methyl salicylate, a derivate of salicylic acid and methanol, is an anti-inflammatory compound similar to aspirin and is found in a certain type of heather (*Gaultheria procumbens* Ericaceae family). It is used externally in liniments.

The structures of some monoterpene esters are presented below:



The structures of some typical terpenes are shown below:





1.4 Vegetable or fixed oils

Fixed oils are known to be natural products with vegetable origin that contained mixtures of esters derived from glycerol that have chains of fatty acid with 14 to 20 carbon atoms that have different degrees of unsaturation[11] .

Fixed oils play a key and sensory role in food products, because of their fatty acids composition and the fat-soluble vitamins (A, D, E, and K). They are also sources of energy and essential fatty acids like linoleic and linolenic that are responsible for growth and the health of organisms[8].

The physico-chemical properties of triglyceride and its applications depend on fatty acid constituents in molecule.

However, the differences appear due the chain length, unsaturation degree and position of unsaturation.

Those oils which consist of fatty acids with short chain have lower melting point and are more soluble in water. Whereas, the oils that contain fatty acids with longer chain have higher melting points. Unsaturated acids will have a lower melting point compared to saturated fatty acids of similar chain length[12] .

1.4.1 Lipids

Lipids are compounds which are insoluble in water but soluble in common organic solvents such as benzene, ether and chloroform .

There are two types of plant lipids namely : the structural and the storage. The former are present as constituents of various membranes and protective surface layers and make up about 7% of leaves of higher plants , while the latter occur in fruits and seeds and are predominantly oils[13] .

On the basis of their backbone structures lipids may be classified into:

a) Glycerol based lipids

Glycerol based lipids may be in turn classified into:

i) Simple lipids (fats and oils)

ii) Compound or complex lipids (glycolipids and phosphoglycerides)

b) Non-glycerol based or derived lipids (splingomyelin, cerebrosides, waxes, steroids, terpenes, phostaglandis etc)[14].

Natural fats and oils are the trimesters of glycerol with long chain carboxylic acids (12 to 20 Carbons).

Fats (solid trimesters of glycerol) and oils (liquid trimesters of glycerol) differ in melting point at room temperature.

1.4.2 Fatty Acids

In natural triglycerides (oils or fats) the carboxylic acids ester chains may be saturated. But most naturally occurring fatty acids are unbranched and have an even number of carbon atoms ranging from 4 to 24 carbon atoms per molecule[14].

Unsaturated fatty acids are usually classifed into monoenoic (those with 1, 2 or fewer double bonds per molecule) and polyenoic or poly unsaturated fatty acids (PUFA). The double bonds in PUFA exist in trans and cis configurations, with cis configuration as a form in which most natural fatty acids occur[13] .

Some fatty acids are termed essential fatty acids. These acids are indispensable in the diet and include arachidonic acid. Such acids have long been considered part of the lipid supply necessary for energy, growth, cellular metabolism and muscle activity[14] .

Some essential fatty acids act as indispensable dietary precursors for eicosanoid (postagalandins, thromboxames and prostacyclins) formation hence they provide greater significance to the study of their role in health and disease[15].

However the primary sources of essential fatty acids are terrestrial and marine plants (especially oil seeds) and phytoplankton . Essential fatty acids are found in marine animals (especially fish) and liver [16].

Lipid deterioration may occur in fats and oils leading to the formation of off-flavour, colour defects and potentially harmful products. These changes are generally referred to as rancidity . Hydrogenation is one of the treatments done to fats and oils to alter their physical state. Hydrogenated fat becomes rancid much less readily than does a non-hydrogenated fat[17].

Rancidity may occur due to two types of reactions one of them is hydrolysis and oxidation. In the hydrolytic rancidification, the ester linkages of a glyceride are hydrolysed to give the original fatty acid while in the oxidative rancidification; atmospheric oxygen attacks the carbon-carbon double bounds in the unsaturated side-chains of gylceride. Thus short-chain volatile acids and aldehyde form. These have extremely bad odours[17] .

The role of essential fatty acids in human diet and health cannot be overemphasized. The human brain is estimated to be nearly

60 percent fat. More so, essential fatty acids (EFAs) usually of plant origin play vital roles in the maintenance of optimal health and brain functions. FAs and EFAs have been demonstrated to regulate lymphocyte proliferation and metabolism, enhance the immune system induce increased granulocyte macrophage-colony, induced cell death in T cells, macrophage cells and also enhance autoimmunity[18].

1.5 Oils as antimicrobials

Since time immemorial volatile oils from plants have been known to possess biological activity, notably antibacterial, antifungal, and antioxidant properties[19].

Biological potential of such essential oils on their chemical compositions, which is determined by the plant genotype and is greatly influenced by several factors such as geographical origin and environmental and agronomic conditions . However, plants possess antioxidants, which have certain degree of resistance to oxidation. Plant oils are used for the prevention of some human diseases such as atherosclerotic cardiovascular diseases, cancer and degenerative eye diseases [20].

Essential oils are composed of terpenoids, specially monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also be present, and of a variety a low molecular- weight aliphatic hydrocarbons, acids, alcohol, aldehydes, phenolic compounds, acyclic esters, or lactones.

Plants may exert antimicrobial activity due to their essential oil fractions. Some scientists reported the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, and onion against both bacteria and molds. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial[20].

Those oils containing phenolic groups are usually most effective as antimicrobial agents. The components present in essential oils have been known to possess antimicrobial activity and some can be used to prevent post-harvest growth of native and contaminant bacteria. The essential oil fractions sensitize the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, as well as the impairment of bacterial enzyme system and cell respiration[21].

Recently there has also been an increased interest in essential oils and their antimicrobial activity due to the spread of antibiotic resistance. Since the discovery of penicillin by Alexander Fleming in 1929 many new classes of antibiotics have become available for treatment of bacterial infections, but due to excessive and often unnecessary use of antibiotics in humans and animals, bacterial resistance has now been reported against every currently available antibiotic[22]. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and resistant strains of *Pseudomonas* are

examples of multiresistant bacteria that are becoming an alarming problem within the healthcare system. MRSA is probably the most common antibiotic resistant bacterium found in hospitals throughout the world and it naturally colonises skin and infects wounds. Today the prevalence of MRSA is between 25-50 percent in parts of the world, including the USA, Australia, South America and central parts of Europe. Even in Scandinavian countries, where MRSA rates have been low, the frequency is beginning to rise[22]. VRE has also spread throughout the world since it was first discovered and isolated in the late 80's and can now be found in every continent. *Enterococci* can cause bacteremia, wound infection and urinary tract infection, but serious infections of VRE usually occurs in patients with significantly compromised host defenses. *Candida* and *Pseudomonas* are other opportunistic pathogens that usually lead to serious infections in immune compromised individuals.

For *Candida species* therapies have been difficult because of the limited number of antifungal agents, and for *Pseudomonas* even drug-susceptible strains have considerable defenses against antibiotics[23]. Essential oils are known for their biological activities. These attributes are reflected in their antimicrobial, antifeedants, insecticidal, larvicidal and molluscidal properties[24].

It has been established that the antimicrobial potential of essential oils could be due to separate and synergetic actions of

the constituents on the organisms[25]. Such constituents include monoterpenoids, sesquiterpenoids and aromatic compounds. However, monoterpenoids and sesquiterpenoids constituted large proportion of essential oils. Inuoye, et al.[26] reported that bacteria are more susceptible to oxygenated compounds than hydrocarbons. In this report, cinnamaldehyde of Cinnamon bark oil had significant activities against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogen*, *Staphylococcus pneumonia* and *H. influenzae*. This was followed by citral and then penalladehyde, octanol and nonanol. Thymol, a phenolic constituent of thyme oil, showed activity comparable to that of cinnamaldehyde, terpenols exhibit moderate activity while terpenes such as D – limonene and α- pinene were lowest in activity. Meanwhile the activities of the oil major constituents were close to the parent oils. It has been shown that[27] the antibacterial properties of camphor is due to the presence of 1, 8 - cineole found in the essential oils of *Achillae frasil* and *Achillae toygetea*.

It has been established that essential oils exhibit antifeedants and cytotoxic properties, these properties was reported for volatile oil of *Echiochilon fruiticosum* against adult of *Tribolium confusum* by Zardi - Bergaoui, et al[28]. Similarly, the cytotoxic activities of the essential oils of *Aeollanthus pubescens* and *Ocimum gratissimum* on the human epidermic cell line, have been

evaluated and it was found that the essential oils of *A. pubescens* was more active than those of the *O. gratissimum*[29]. Essential oils of *Cryptomeria japonica* was also reported to show significant lethality against brine shrimp larvae.Essential oils from *cyperus* species are generally constituted by sesquiterpenoids and traces of monoterpenoids. The main hydrocarbon is always cyperene. Many other sesquiterpene of caryophyllane, eudesmane, patchoulane and rotundane types are also present as hydrocarbon and oxygenated compounds. Mustakone and cryophyllene oxide have been identified in rhizome essential oil of Brazilian grown *Cyperus articulatus* by Zohgbi et al[30]. Sönwa, and Konig[31] had also isolated (-) - norotundane, (-) - isorotundone, cypera - 2, 4(15) - diene and (+) - cyperadone from *Cyperus rotundus*. The rhizome essential oils of red and black types of *C. articulatus* growing in Nigeria were studied[32] . Like other cyperus species both oils were richer in sesquiterpenoids than monoterpenoids. However, the oils showed substantial quantitative and qualitative variations in their constituents. Some of the identified constituents were present in both oils while others were characteristics of one of the oil. Oil of the red type was characterized by the abundance of cypertundone, piperitone, α - maaline, germacrone, α - epicubenol, α - pinene amd cyperene oxide. For the oil of the black type, the main constituents were; cedrol, guai - 5 - en - 11 -

ol, cyperotundone, sabinene, α - pinene, trans - pinocarveol, cis - carveol, trans - carveol and α - cardinol. With the abundance of oxygenated sesquiterpenoids in both oils, the oils are expected to be biologically active[33]..

1.6 Health benefits of some medicinal plants

The beneficial uses of seed oil plants have been known since time immemorial. Apart from their uses as food items, oils extracted from seeds are also used for different purposes ranging from medicinal to biofuels. Their chemical compositions, physical and chemical properties generally determine their applications for different purposes. Many microbial diseases worldwide have become a serious threat to human health because of the emergence of drug resistant or multi-drug resistant (MDR) microbial strains[34].

The challenge of multidrug resistance led the scientists to search for the new alternatives including seed oil producing plants and their oil, which are known for their antimicrobial properties. Several *in vitro* studies have been published confirming the effect of seed oils and their major compounds on pathogenic microbes (Burt, 2004). However, there are only limited data available on the antifungal activity of these seed oils against fungal pathogens[35].

1.7 Extraction of the oils

Oils are generally extracted via two major techniques: Distillation (includes hydrodistillation) and Expression. Also, can be extracted via solvent extraction or enfleurage, although enfleurage is rarely performed in nowadays.

1.7.1 The Distillation Technique

In the distillation process the plant material is placed upon a grid inside the still. Once inside, the still is sealed, and, depending upon the above methods, steam or water/steam slowly breaks through the plant material to remove its volatile constituents. These volatile constituents rise upward through a connecting pipe that leads them into a condenser. The condenser cools the rising vapor back into liquid form. The liquid is then collected in a vehicle below the condenser. Since water and essential oil do not mix, the essential oil will be found on the surface of the water where it is siphoned off. Occasionally an essential oil is heavier than water and is found on the bottom rather than the top[36].

Generally three are three types of distillation techniques including:

a)Water Distillation

During water distillation the plant material comes into direct contact with the water. This method is most often employed with flowers (rose and orange blossoms), as direct steam causes these flowers to clump together making it difficult for steam to pass through.

b)Water and Steam

Water – steam distillation is used for herbs and leaf material. During this process, the water remains below the plant material, which has been placed on a grate while the steam is introduced from outside the main still (indirect steam)[36].

c)Steam Distillation

A commonly used estraction technique is steam distillation. During this process, steam is injected into the still, usually at slightly higher pressures and temperatures than the above two methods[36].

Other methods used for extraction of oils include the following techniques:

i)Percolation or Hydrodiffusion

This is a relatively recent method and is very similar to steam distillation except that the steam comes in through the top rather than the bottom, and there is a shorter distillation time. It is

useful in extracting essential oils from woody or tough material or seeds.

Hydrosols, also known as hydrolats, are the by-product or product (depending on the distiller purpose) of the distillation process. Hydrosols contain the water-soluble constituents of the aromatic plant and retain a small amount of essential oil. Every liter of hydrosol contains between 0.05 and 0.2 milliliter of dissolved essential oil, depending on the water solubility of the plant's components and the distillation parameters[37].

ii)Expression extraction

Cold pressing, or expression is a process of extraction specific to citrus essential oils, such as tangerine, lemon, bergamot, sweet orange, and lime. In older times, expression was done in the form of sponge pressing, which was literally accomplished by hand. The zest or rind of the citrus would first be soaked in warm water to make the rind more receptive to the pressing process. A sponge would then be used to press the rind, thus breaking the essential oil cavities, and absorb the essential oil. Once the sponge was filled with the extraction, it would then be pressed over a collecting container, and there it would stand to allow for the separation of the essential oil and water/juice. The essential oil would finally be siphoned off[37].

iii)Solvent Extraction

When the plant material is so fragile to be distilled an alternative technique must be employed. Solvent extraction is the use of solvents, such as petroleum ether, methanol, ethanol, or hexane, to extract the odoriferous lipophilic material from the plant. The solvent will also pull out the chlorophyll and other plant tissue, resulting in a highly colored or thick/viscous extract. The first product made via solvent extraction is known as a concrete. A concrete is the concentrated extract that contains the waxes and/or fats as well as the odoriferous material from the plant. The concrete is then mixed with alcohol, which serves to extract the aromatic principle of the material. The final product is known as an absolute[37].After the solvent extraction process has been completed, the resulting absolute will have an extremely low concentration of solvent residue, approximately 5 to 10ppm (parts per million). The current European Union standards are for less than 10 parts per million solvent residues in a finished absolute. However, even with such a potentially small residue (less than .0001%), many aromatherapists disagree with the use of absolutes for individuals with a compromised immune system due to the potential effect of the residual pesticide[38].

1.8 Chromatography

Chromatography is a term used to describe a set of laboratory techniques for the separation of mixtures. The analyte in this technique is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation[38].

The chromatographic process may be preparative or analytical. Preparative chromatography is to separate the components of a matrix for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture[38].

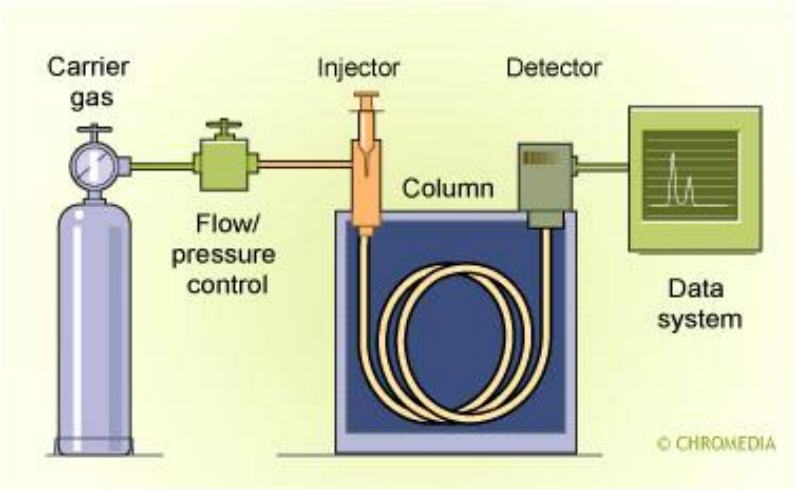
Chromatographic techniques have been employed for the separation of amino acids, proteins and carbohydrates. It is also used for the analysis of drugs, hormones, vitamins.

Chromatographic fractionation is helpful for the qualitative and quantitative analysis of complex mixtures. The technique is also

useful for the determination of molecular weight of proteins. Types of Chromatography include; Paper Chromatography ,Thin Layer Chromatography(TLC) , Gel Chromatography, Column Chromatography, Ion Exchange Chromatography, Gel Filtration Chromatography, Gas Liquid Chromatography, Affinity Chromatography[39].

1.8 .1 Gas chromatography (GC)

(GC) or gas chromatography is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound in preparative chromatography; GC can be used to prepare pure compounds from a mixture[40].



Schematic of the GC system

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex matrix. A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature[40].

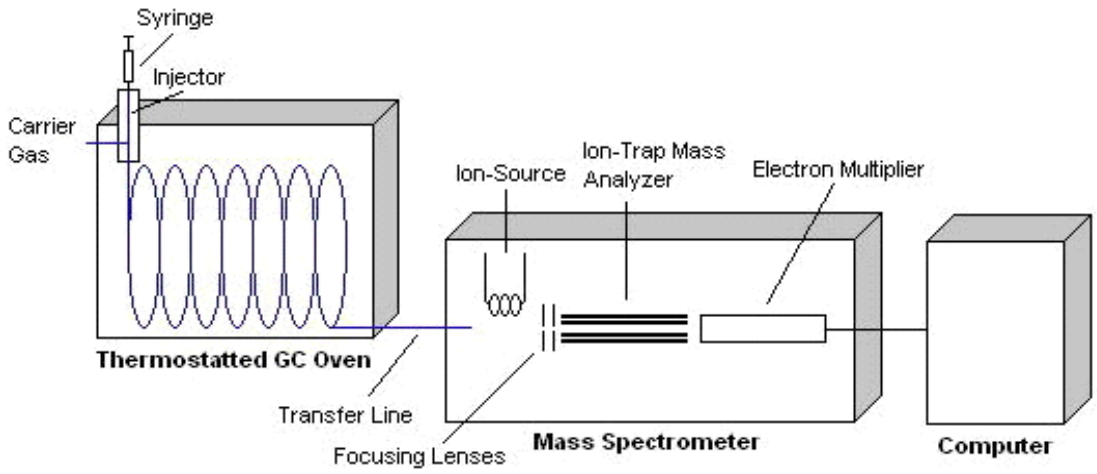
Generally speaking, substances that vaporize below 300 °C (and therefore are stable up to that temperature) can be measured quantitatively by this technique. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

Gas chromatographs are sometimes connected to a mass spectrometer which acts as the detector. This hyphenated technique is known as GC-MS. Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR is connected to an infrared spectrophotometer which acts as a backup detector. This combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS[35].

1.8.2 The technique of gas chromatography–mass spectrometry

The hyphenated technique - gas chromatography–mass spectrometry (GC-MS)- is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation,

environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification[40].



Schematic of the GC/MS system

The use of a mass spectrometer as the detector in gas chromatography dates back to the 1950s after being innovated by James and Martin in 1952.

Gas chromatography in combination of mass spectrometry allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate

identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample. For the analysis of volatile compounds, a purge and trap (PT) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an airtight chamber. An inert gas such as nitrogen (N_2) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line

onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. PT/ GC-MS is particularly suited to volatile organic compounds (VOCs) and aromatic compounds associated with petroleum)[40].

1.9 The studied plant species

1.9.1 *Lycopersicum esculentum*

Lycopersicum esculentum is a warm –season plant reaching 1-3m in height in the family Solanaceae. The fruit is red and edible. The plant is worldwide cultivated for its economic value and about 130 million tons of tomato are produced annually[41].

Lycopersicum esculentum

In its native habitat , the plant is perennial. The fruit is rich in lycopene which is a carotenoid with beneficial health effects[42] . Lycopene has antioxidant activity[43]. Lycopersicum esculentum is rich in vitamins and contains many minerals including iron, calcium and phosphorus[44]. Some observational studies indicated that intake of lycopene-rich tomato may reduce the risk of prostate[45] and pancreatic cancers[46;47]. Tomato juice is used traditionally to stop wound bleading. It is used against scorpion bite and edema. The juice is also used by local healers against liver and kidney disorders[48]. Intake of tomato- which is rich in potassium- may reduce the risk of heart diseases[49].

1.9.2 *Carthamus tinctorius*

Carthamus tinctorius is an annual highly branched herb in the family Compositeae[50]. This plant is cultivated mainly for seed oil.The plant is also used for coloring foods and as a flavoring agent.

Carthamus tinctorius

Seed oil is used in cooking and cosmetics. *Carthamus tinctorius* thrives in arid climates like Sudan, Egypt and Southern Asia.The flower petals ,which produce shades of colors, have previously been used as dyes[51].Seed oil contains large amount of linoleic acid(70%)[52,53].The palnt has been used traditionally as purgative,analgesic and antipyretic[54]. *Carthamus tinctorius* is also used by traditional healers against rheumatism, bronchitis ,menstrual cramps and whooping cough[55].Some local healers use *Carthamus tinctorius* against phlegmatic fever, melancholia ,diabetes and dropsy[56-58]. Flowers of *Carthamus tinctorius* have been used against cardiovascular ,cerebrovascular and gynecological complications[12].Some research shed light on the therapeutic potential of the aqueous extract of *Carthamus tinctorius* for cardiovascular disease[59]. It has been reported that the aqueous extract has antihypertensive, antioxidant,

anticoagulant ,anticancer properties beside immunosuppressive and neuroprotective properties[59].

1.9.3 *Ocimum basilicum*

Ocimum basilicum which has been used for centuries in traditional medicine, is a herb of many attributes. It is a medium size herb in the family Lamiaceae. The plant contains bioactive constituents like tannins, saponins and cardiac glycosides[60].

Ocimum basilicum

Ocimum basilicum essential oils has been used traditionally against , nervous disorders and digestive troubles. The plant is claimed to be cardioprotective, stomachic , antipyretic and anthelmintic[61]. The antifungal, antiviral , antinociceptic and larvicidal properties of Ocimum basilicum oil has been documented[62-64].The oil has also been used in ethnomedicine against fever, achne, snake bite, nausea, migraine, abdominal cramps, gonorrhea, inflammation, dysentery, headache, piles,

cough , colic pain , paralysis and nervous temperament[65,66]. The immunomodulatory properties of leave extract has been reported[67]. The ethanol extract and the essential oil of Ocimum basilicum exhibited free radical scavenging capacity[68-70].The in vivo antihyperglycemic and hypolipidemic properties of Ocimum basilicum extracts has been reported[71,72].In the paw edema model, Ocimum basilicum seeds showed antiinflammatory activity[73,74]. The in vivo hepatoprotective potential of leave extract has also been demonstrated[75].

1.9.4 *Prunus mahaleb*

Prunus mahaleb is adeciduous tree or large shrub up to 2-10m in height. It is native to the Mediterranean region and central Asia.The plant is cultivated as spice[76]. Seeds have a fragrant smell.Wood and seeds are used traditionally as anti-inflammatory and sedative. They also possess vasodilating effect [77].

Prunus mahaleb

1.9.5 *Sterculia setigera*

Sterculia setigera is a multifunctional forest woody tree species in sub-Saharan Africa, and the plant is known in the African contenant for its economic value.

Sterculia setigera

The gum is an important cash product since several decades[78-82]. Boiled leaves of Sterculia setigera are used traditionally to treat malaria, and the stem bark decoction is used for the treatment of asthma, bronchitis, wound, fever, toothache, gingivitis sore, abscess, and diarrhea[80 ,83-85] . A supportive evidence of the use of the plant in folkloric medicine was provided by the study of[86] .

Aim of this study

This study was set to:

-Extract oils from five selected potential medicinal plants.

-Study of the constituents of the oil by GC-MS.

-Screening the oils for their antimicrobial activity.

2-Materials and Methods

2.1-Materials

2.1.--Plant material

Seeds of *Lycopersicum esculentum Sterculia setigera Carthamus tinclorius* and *Prunus mahaleb* were purchased from the local market-Khartoum-Sudan. Seeds of *Ocimum basilicum* were obtained from the local Riyadh-Saudi Arabia. The plant materials were authenticated by direct comparison with a reference herbarium samples.

2.1.2-Instruments

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

2.1.3-Test organisms

The studied oils were screened for antimicrobial activity using the standard microorganisms shown in 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 aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

2.2-Methods

2.2.1-Extraction of oil

Powdered seeds (300g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil.

The oil was esterified as follows :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 supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was 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 vial.

2.2.2-GC/MS analysis

The studied oils were analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument.Helium was used as carrier gas. Chromatographic conditions are presented below:

- *Oven temperature program*

Rate : --- ; Tempt. , 150.0^{0}C ; Hold time(min.$^{-1}$) ,1.00

Rate : 4.00 ; Tempt. , 300.0^{0}C ; Hold time(min.$^{-1}$) ,0.00

Column oven temperature	150.0°C
Injection temperature	300.0°C
Rate	4/min
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.
Spilt ratio	- 1.0

2.2.3-Antimicrobial assay

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to manufacture instructions.

Broth cultures($5.0x10^7$cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs(Oxid,6mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria the plates were incubated at $37^oC$for 24h., while for fingi the plates were incubated at 25^oC for 3days.The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamycin and clotrimazole were used as positive control while DMSO was used as negative control.

3-Results and Discussion

In this study six medicinal plants(*Lycopersicum esculentum Ocimum basilicum Sterculia setigera Carthamus tinclorius* and *Prunus mahaleb*) have been studied by GC-MS and the antimicrobial activity has been evaluated.

3.1- *Lycopersicum esculentum*

3.1.1-GC-MS analysis of *Lycopersicum esculentum* oil

Lycopersicum esculentum seed oil was analyzed by GC-MS. Figure 3.1 presents the total ions chromatograms, while Table 3.1 displays the different constituents of the oil.

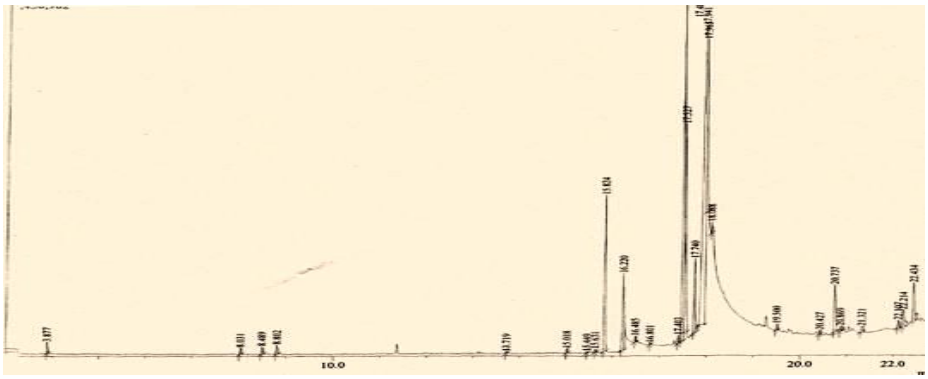


Figure 3. 1: Total ions chromatograms

The following major constituents have been detected by GC-MS analysis:

i)9,12-Octadecadienoic (Z,Z)- (35.16%).

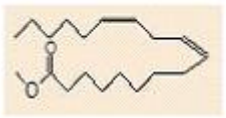
ii)9,12-Octadecadienoic (Z,Z), acid methyl ester (19.79%).

iii)9-Octadecenoic acid (Z)- methyl ester (10.84%)

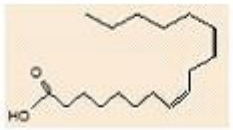
The mass spectrum of 9,12-octadecadienoic acid is shown in Fig. 3.2. The peak at m/z 280 (R.T. 17.941) is due to the molecular ion : M+[C18H32O2]+ . The mass spectrum of 9,12,-octadecadienoic acid(Z,Z) methyl ester is shown in Fig. 3.3. The peak at m/z 294, which appeared at R.T. 17.486 in total ion chromatogram, corresponds M+[C19H34O2]+. The signal at m/z263 is due to loss of a methoxyl function. The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.3.4.The peak at m/z 296, which appeared at R.T. 17.527 accounts for: M+[C19H36O2]+ .The signal at m/z265 is due to loss of a methoxyl.

Table 3.1: Constituents of the oil

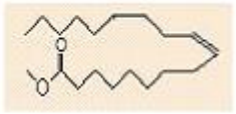
Peak#	R.Time	Area	Area%	Name
1	3.877	353457	0.46	2-Heptenal, (E)-
2	8.031	235517	0.30	2-Undecenal
3	8.489	306988	0.40	2,4-Decadienal, (E,E)-
4	8.802	362228	0.47	2,4-Decadienal
5	13.719	49253	0.06	Methyl tetradecanoate
6	15.018	154974	0.20	2-Pentadecanone, 6,10,14-trimethyl-
7	15.449	43442	0.06	Pentadecanoic acid, 14-methyl-, methyl ester
8	15.631	100855	0.13	9-Hexadecenoic acid, methyl ester, (Z)-
9	15.824	5494398	7.10	Hexadecanoic acid, methyl ester
10	16.220	3581091	4.63	Pentadecanoic acid
11	16.485	194747	0.25	Hexadecanoic acid, ethyl ester
12	16.801	54968	0.07	Hexadecanoic acid, 15-methyl-, methyl ester
13	17.403	304280	0.39	Heptadecanoic acid, 16-methyl-, methyl ester
14	17.486	15317690	19.79	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
15	17.527	8390651	10.84	9-Octadecenoic acid (Z)-, methyl ester
16	17.740	2568410	3.32	Methyl stearate
17	17.941	27219215	35.16	9,12-Octadecadienoic acid (Z,Z)-
18	17.965	5874065	7.59	Oleic Acid
19	18.088	357686	0.46	n-Propyl 9,12-octadecadienoate
20	19.500	220957	0.29	Eicosanoic acid, methyl ester
21	20.427	146256	0.19	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
22	20.737	2324942	3.00	9,12-Octadecadienoyl chloride, (Z,Z)-
23	20.869	204142	0.26	2-Ethylbutyric acid, eicosyl ester
24	21.321	333286	0.43	Butyl 9,12-octadecadienoate
25	22.107	339186	0.44	3-n-Butylthiophene-1,1-dioxide
26	22.214	1019723	1.32	9,12,15-Octadecatrienoic acid, methyl ester
27	22.434	1852378	2.39	Isopropyl linoleate
		77404785	100.00	



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



9,12-Octadecadienoic acid



9-Octadecenoic acid methyl ester

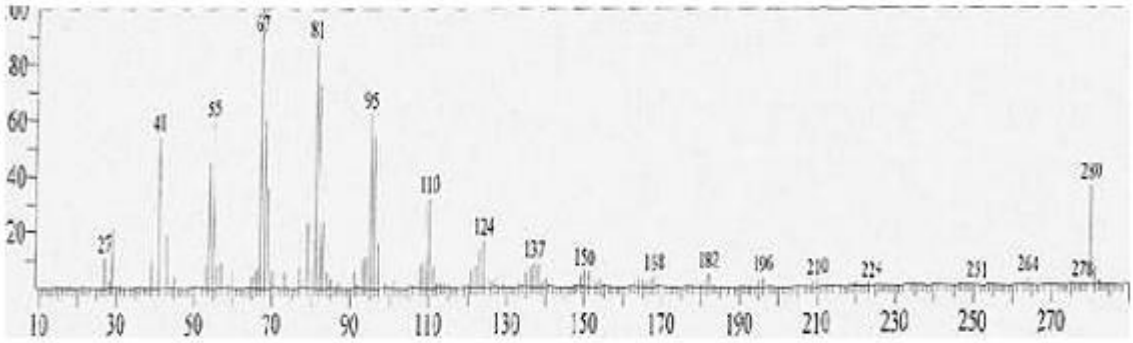


Fig. 3.2: Mass spectrum of 9,12-octadecadienoic acid

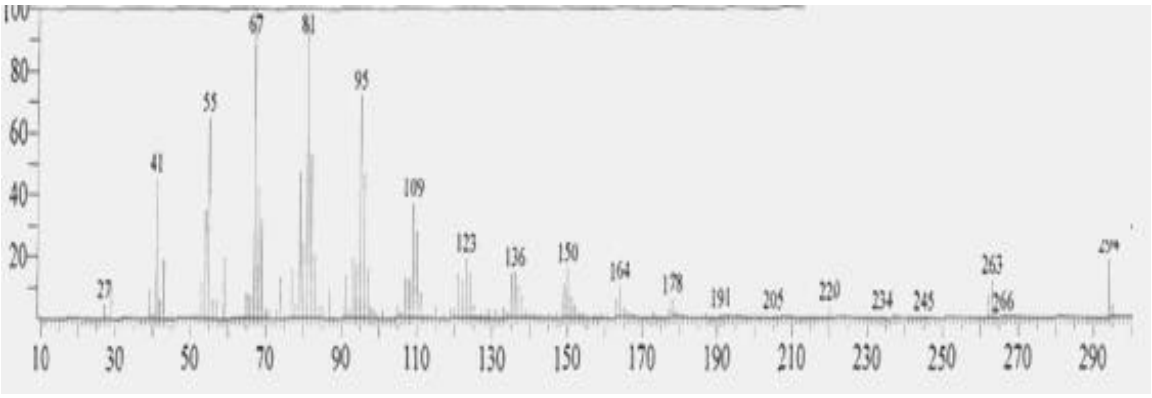


Fig. 3.3: Mass spectrum of 9,12-octadecanoic acid(z,z), methyl ester

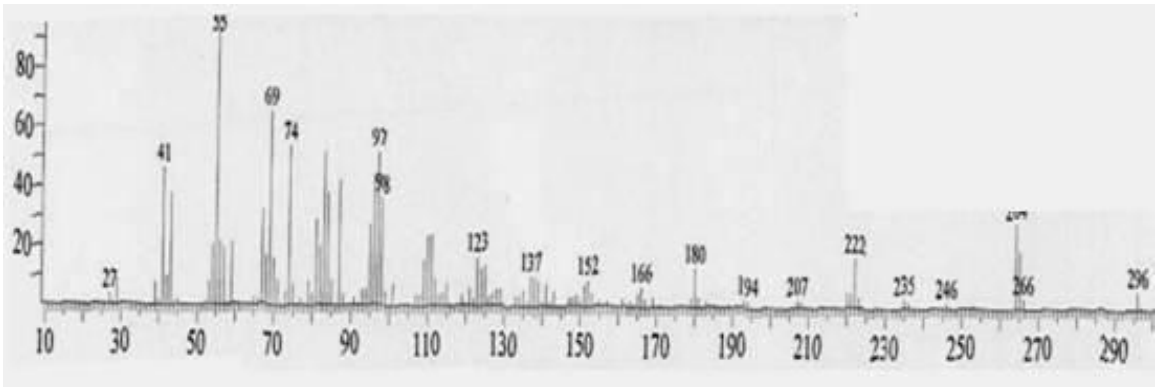


Fig. 3. 4: Mass spectrum of 9-octadecenoic acid methyl ester

3.1.2-Antimicrobial activity of *Lycopersicum esculentum* oil

The studied oil was screened for antimicrobial activity against five standard microbial strains. The inhibition zones are displayed in Table 3.2.The oil showed significant activity against *Bacillus subtilis*. It also exhibited moderate activity against *Pseudomonas aeroginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans.*

Table 3.2: Inhibition zones of *Lycopersicum esculentum* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	20	16	15	16
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

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

3.2- *Sterculia setigera*

3.2.1-Constituents of *Sterculia setigera* oil

The total ion chromatograms of *Sterculia setigera* oil is shown in Fig. 3.5 and the constituents of the oil are depicted in Table 3.3. The GC-MS analysis revealed the presence of 20 components.

Major components of the oil are:

i)-cis-9-Hexadecenal (32.66%)

ii)-Oleic acid (20.29%)

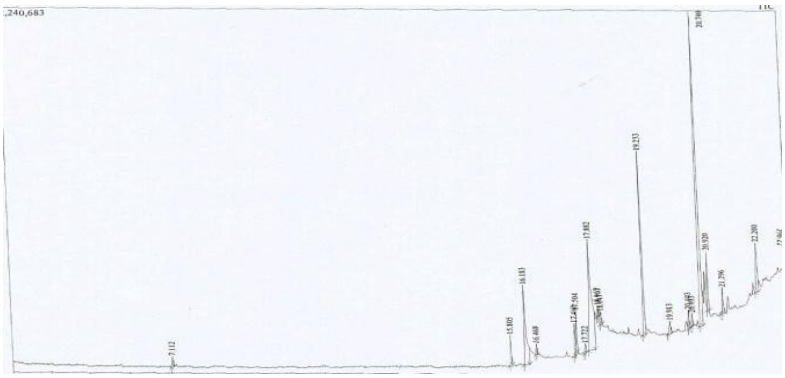


Fig. 3.5: Total ion chromatograms

Table 3.3: Constituents of *Sterculia setigera* oil

No.	R. Time	Area %	Name
1	7.112	0.66	Alpha-Terpineol
2	15.805	1.72	Hexdecanoic acid methyl ester
3	16.183	10.07	Pentadecanoic acid methyl ester
4	16.468	0.59	Hexadecanoic acid ethyl ester
5	17.460	1.96	9,12-Octadecadienoic acid (Z,Z)-methyl ester
6	17.504	2.17	9-Octadecenoic acid (Z) methyl ester
7	17.722	0.59	Methyl stearate
8	17.882	20.29	Oleic acid
9	18.067	0.71	9,12-Octadecadienoic acid(Z)-methyl ester
10	18.107	0.52	Ethyl oleate
11	18.147	0.81	Methyl 2-octylcyclopropene-1-octanoate
12	19.253	12.39	1-(+)-Ascorbic acid 2,6-dihexadecanoate
13	19.913	1.28	9-Octadecenoic acid, 1,2,3-propanetriyl ester
14	20.403	1.08	Phenol , 2,2`-methylene -bis 6-(1,1-dimethyl)-4-ethyl-
15	20.493	0.71	Methyl 10-trans, 12-cis-octadecadienoate
16	20.740	32.66	Cis-9-Hexadecenal
17	20.920	3.49	Tristearin
18	21.296	1.78	E-11(12-Cyclopropyl)dodecen-1-ol acetate
19	22.200	4.70	Cis-6-Octadecenoic acid, trimethyl silyl ester
20	22.961	1.80	Decyl sulfide
		100.00	

Fig. 3.6 represents the mass spectrum of cis-9-hexadecenal. The peak at m/z 236 (RT, 20.740) is attributed to: M^+ $[C_{16}H_{30}O]^+$ - 2H. The mass spectrum of oleic acid is presented in Fig. 3.7. The signal at m/z 282 (RT.17.882) is due to the molecular ion: $M^+[C18H34\ O2]^+$, while the peak at m/z 265 accounts for loss of a hydroxyl function.

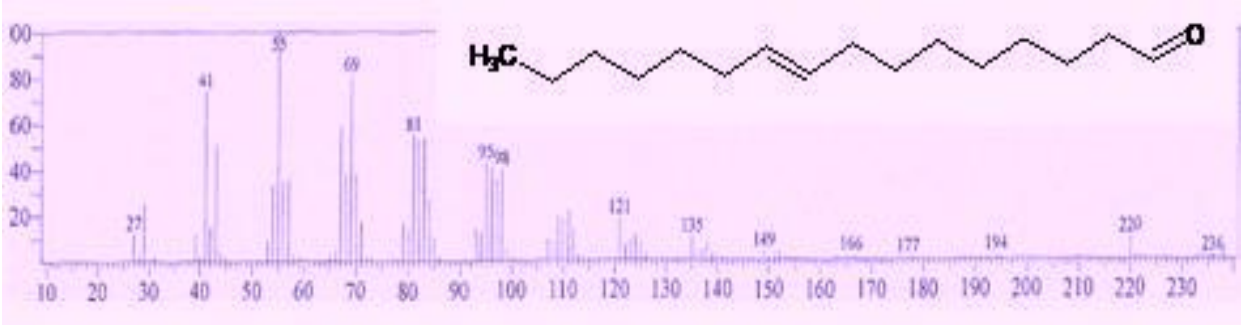


Fig. 3.6: Mass spectrum of cis-9-hexadecenal

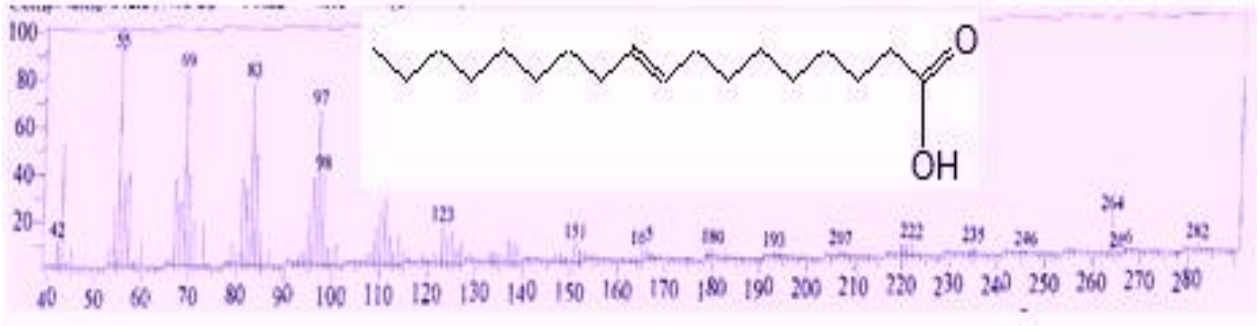


Fig. 3.7: Mass spectrum of oleic acid

3.2.2-Antimicrobial activity of *Sterculia setigera* oil

The oil was screened for antimicrobial activity against five standard microorganisms (Table 3.4). The results are depicted in

Table 3.5. Results were interpreted in the following conventional terms: (>9mm: inative; 9-12mm: partially active; 13-18 mm: active; <18mm: very active). *Sterculia speciesare* oil showed partial activity against *Staphylococcus aureus* and *Escherichia coli*.

Table 3.4: Test organisms

No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale, England
*ATCC. American type culture collection, Maryland, USA

Table 3.5: Inhibition zones(mm)

Sample	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml	10	--	9	7	--

Sa.: *Staphylococcus aureus*, Ec.: *Escherichia coli*, Pa.: *Pseudomonas aeruginosa*, Bs.: *Bacillus subtilis;*

Ca.: *Candida albicans*

Table 3.6: Inhibition zones of standard drugs

Drug	Sa	Bs	Ec	Ps	Ca
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

3.3- *Carthamus tinclorius*

3.3.1-GC-MS analysis of *Carthamus tinclorius* oil

GC-MS analysis of *Carthamus tinclorius* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library.The GC-MS analysis of the studied oil revealed the presence of 21components (Table 3.7).The typical total ion chromatograms (TIC) is shown in Fig.3.8.

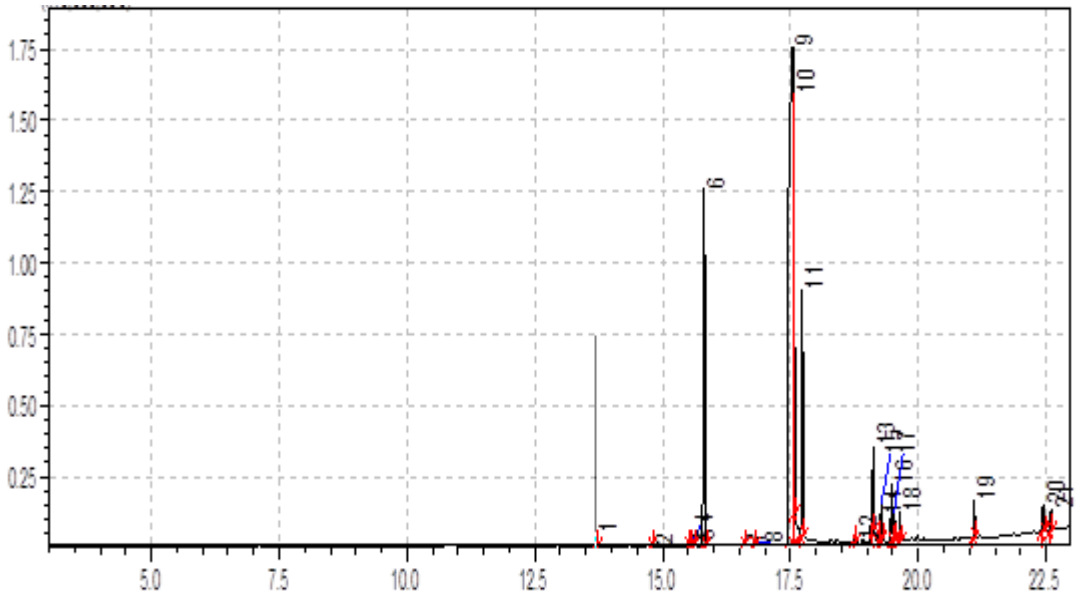


Fig.3.8: Chromatograms of *Carthamus tinclorius* oil

Table 3.7: Constituents of *Carthamus tinclorius* oil

No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	13.701	0.40
2	Pentadecanoic acid, methyl ester	14.777	0.06
3	7,10-Hexadecadienoic acid, methyl ester	15.506	0.02
4	7-Hexadecenoic acid, methyl ester, (Z)-	15.566	0.08
5	9-Hexadecenoic acid, methyl ester, (Z)-	15.611	0.13
6	Hexadecanoic acid, methyl ester	15.814	14.04
7	cis-11,14-Eicosadienoic acid, methyl ester	16.573	0.11
8	Heptadecanoic acid, methyl ester	16.783	0.11
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.543	55.83
10	9-Octadecenoic acid (Z)-, methyl ester	17.565	9.32
11	Methyl stearate	17.733	8.82
12	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.762	0.12
13	Tridecanedial	19.119	2.48
14	Oxiraneoctanoic acid, 3-octyl-, methyl ester	19.245	0.85
15	cis-13-Eicosenoic acid, methyl ester	19.279	1.07
16	Eicosanoic acid, methyl ester	19.477	1.79
17	PGH1, methyl ester	19.531	0.92
18	1-Naphthalenol, decahydro-4a-methyl-	19.642	1.01
19	Docosanoic acid, methyl ester	21.098	1.15
20	15-Tetracosenoic acid, methyl ester, (Z)-	22.449	0.97
21	Tetracosanoic acid, methyl ester	22.602	0.72

The GC-MS analysis revealed the following major constituents **:**

i) 9,12-Octadecadienoic acid methyl ester(55.82%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.9.The peak at m/z294 (R.T. 17.543) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl .

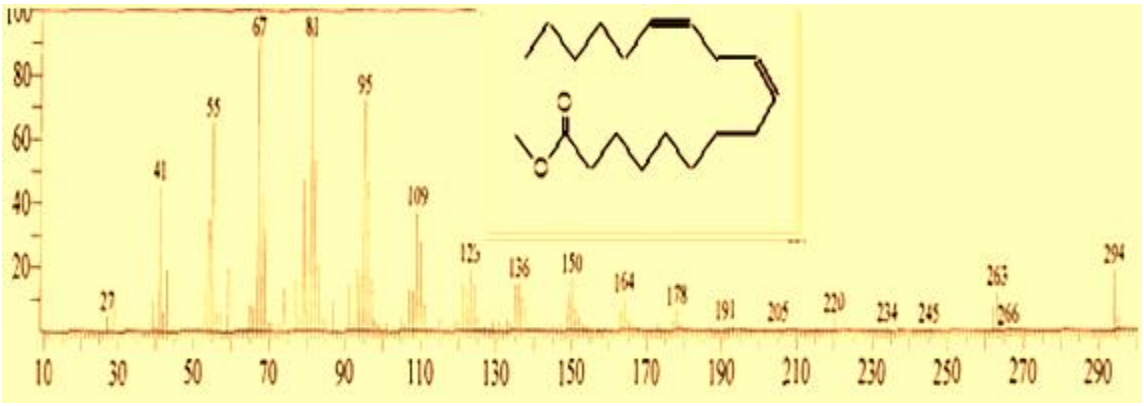


Fig.3.9: Mass spectrum of 9,12-octadecadienoic acid methyl ester

ii) Hexadecanoic acid methyl ester (14.04%)

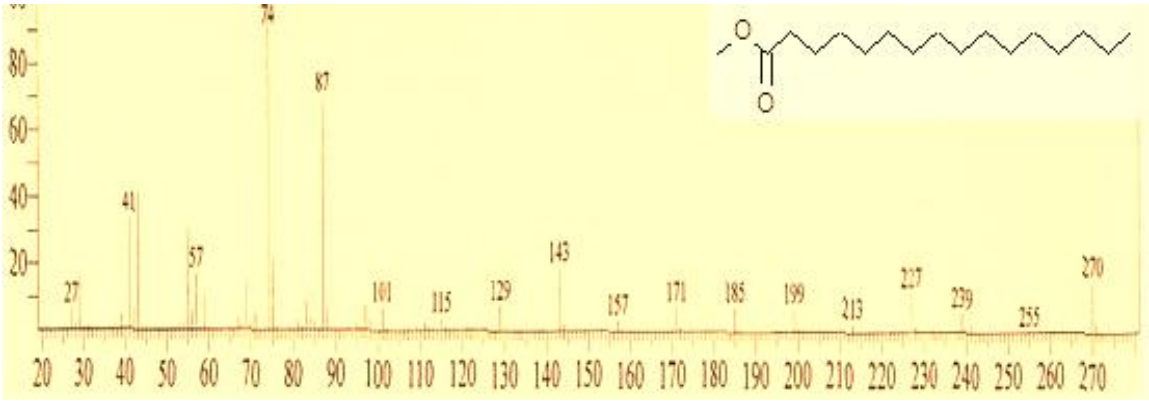


Fig.3.10: Mass spectrum of hexadecanoic acid methyl ester

Fig.3.10 shows the mass spectrum of hexadecanoic acid methyl.The peak m/z 270(R.T.,15.814) was detected in the spectrum.It corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$ .The peak at m/z239 is due to loss of a methoxyl .

iii) 9-Octadecenoic acid methyl ester (9.32%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.3.11.The peak at m/z 296 (R.T., 17.565) corresponds M^{+} $[C_{19}H_{36}O_2]^{+}$, while the signal at m/z266 is attributed to loss of a methoxyl .

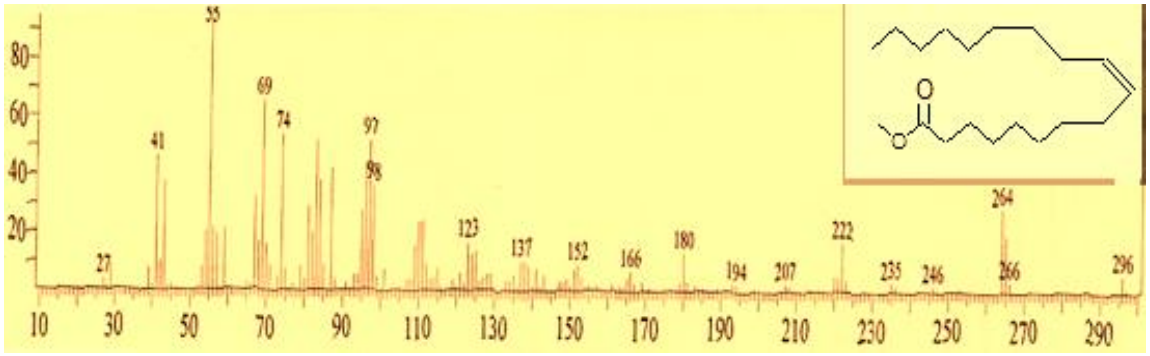


Fig.3.11: Mass spectrum of 9-octadecenoic acid methyl ester

iv) Methyl stearate(8.82%)

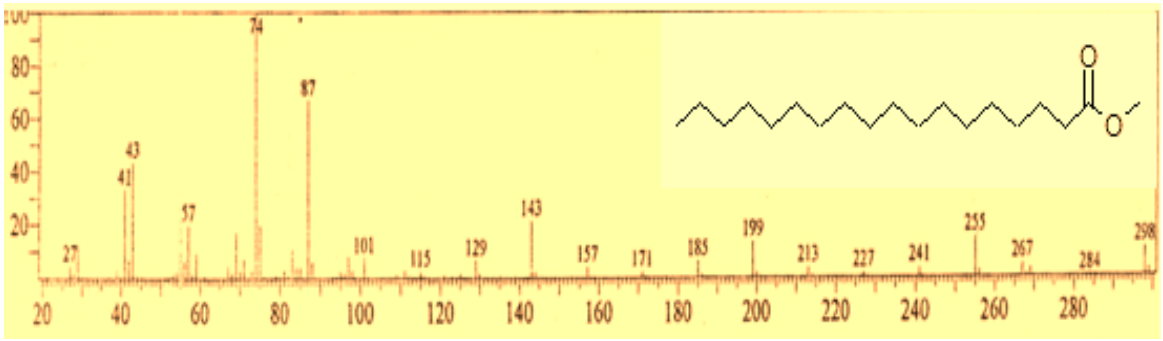


Fig.3.12 : Mass spectrum of methyl stearate

The EI mass spectrum of methyl stearate is displayed in Fig.3.12.The peak at m/z 298 with R.T. 17.733 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl group.

3.3.2-Antimicrobial activity of *Carthamus tinclorius* oil

Carthamus tinclorius 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 3.8.The results were interpreted in commonly used terms (<9mm: inative;9-12mm:partially active; 13-18mm: active; >18mm:very active)

Table 3.8: Antimicrobial activity of *Carthamus tinclorius* oil

Sample	Ec	Pa	Sa	Bs	Ca
Carthamus tinclorius (100mg/ml)	-	15	12	12	13

3.4- *Ocimum basilicum*

3.4.1-GC-MS analysis of *Ocimum basilicum* oil

The oil extracted from *Ocimum basilicum* was investigated by GC-MS analysis. Identification of oil constituents was based on retention

times and the observed fragmentation pattern. Fifty six components were detected in total ion chromatogram. The typical total ion chromatogram (TIC) is presented in Fig. 3.13 . The constituents of the oil are outlined in Table 3.9 .The GC-MS analysis revealed the following major constituents:

i-9,12-Octadecadienoic acid methyl ester(40.77%)

ii-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

iii-Hexdecanoic acid methyl ester(14.74%)

iv-Methyl stearate (9.83%).

Fig. 3.13: Total ion chromatograms

Table 3.9: Constituents of the oil

No.	Name	Ret.Time	Area%
1.	.beta.-Pinene	4.162	0.01
2.	o-Cymene	4.772	0.01
3.	D-Limonene	4.829	0.22
4.	Eucalyptol	4.885	0.10
5.	.gamma.-Terpinene	5.244	0.02
6.	.alpha.-Methyl-.alpha.-[4-methyl-3-	5.451	0.00

	pentenyl]oxiranemethanol		
7.	3,3,6-Trimethyl-1,4-heptadien-6-ol	5.524	0.00
8.	Spiro[4.5]decane	5.583	0.01
9.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.817	0.04
10.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	6.593	0.03
11.	Benzene, pentyl-	6.696	0.01
12.	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	6.823	0.00
13.	.alpha.-Terpineol	7.255	0.01
14.	4-Isopropyl-5-methylhexa-2,4-dien-1-ol	7.384	0.01
15.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	7.590	0.01
16.	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	7.861	0.08
17.	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	8.058	0.03
18.	2-Cyclohexen-1-one, 5,5-dimethyl-3-(1-methylethyl)-	8.746	0.01
19.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	9.486	0.17
20.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	10.122	0.03
21.	Caryophyllene	10.576	0.01
22.	4,5-di-epi-aristolochene	11.242	0.04
23.	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	11.289	0.02
24.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.344	0.07
25.	Naphthalene, decahydro-4a-methyl-1-	11.487	0.83

	methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-		
26.	Ledol	12.044	0.19
27.	Methyl tetradecanoate	14.189	0.09
28.	Pentadecanoic acid, methyl ester	15.320	0.04
29.	7-Hexadecenoic acid, methyl ester, (Z)-	16.156	0.06
30.	9-Hexadecenoic acid, methyl ester, (Z)-	16.201	0.35
31.	Hexadecanoic acid, methyl ester	16.435	14.74
32.	Hexadecanoic acid, 14-methyl-, methyl ester	17.147	0.54
33.	cis-10-Heptadecenoic acid, methyl ester	17.217	0.09

Table 2: Contd.

34	Heptadecanoic acid, methyl ester	17.432	0.19
35	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.305	40.77
36	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.387	26.56
37	Methyl stearate	18.473	9.83
38	cis-10-Nonadecenoic acid, methyl ester	18.889	0.11
39	Octadecanoic acid, 17-methyl-, methyl ester	19.112	0.36
40	Nonadecanoic acid, methyl ester	19.362	0.03
41	.gamma.-Linolenic acid, methyl ester	19.933	0.41
42	7-Tetradecenal, (Z)-	19.961	0.31
43	cis-11-Eicosenoic acid, methyl ester	20.062	0.43
44	Eicosanoic acid, methyl ester	20.265	1.18
45	Methyl 18-methylicosanoate	20.903	0.17
46	Heneicosanoic acid, methyl ester	21.132	0.04
47	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.291	0.05
48	13-Docosenoic acid, methyl ester, (Z)-	21.790	0.03
49	Docosanoic acid, methyl ester	21.966	0.25

50	Methyl 20-methyl-docosanoate	22.563	0.08
51	Tricosanoic acid, methyl ester	22.771	0.12
52	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	23.322	0.29
53	Tetracosanoic acid, methyl ester	23.543	0.16
54	.gamma.-Sitosterol	23.985	0.55
55	Squalene	24.336	0.14
56	.gamma.-Tocopherol	26.545	0.07

The characterization of the major components of the oil is briefly discussed below:

i-9,12-Octadecadienoic acid methyl ester(40.77%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.3.14. The signal which was observed at m/z294(R.T. 18.305) is due to M+[$C_{19}H_{34}O_2$]+, while the signal at m/z263 corresponds to loss of a methoxyl.

ii-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

Mass spectrum of 9,12,15-octadecatrienoic acid,methyl ester is depicted in Fig. 3.15.The peak at m/z292,which appeared at R.T.18.387 corresponds to M+ [C_{19} $H_{44}O_2$]$^+$, while the peak at m\z261 is attributed to loss of methoxyl.

iii-Hexdecanoic acid methyl ester(14.74%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.3.16. The peak at m/z 270 (R.T. 16.435) accounts for M^+ [$C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of methoxyl .

iv-Methyl stearate (9.83%)

The mass spectrum of methyl stearate is shown in Fig.3.17. The peak at m/z 298 (R.T.18.473) is due to M^+ [$C_{19}H_{38}O_2$]$^+$, while the signal at m/z 267 correspond to loss of a methoxyl .

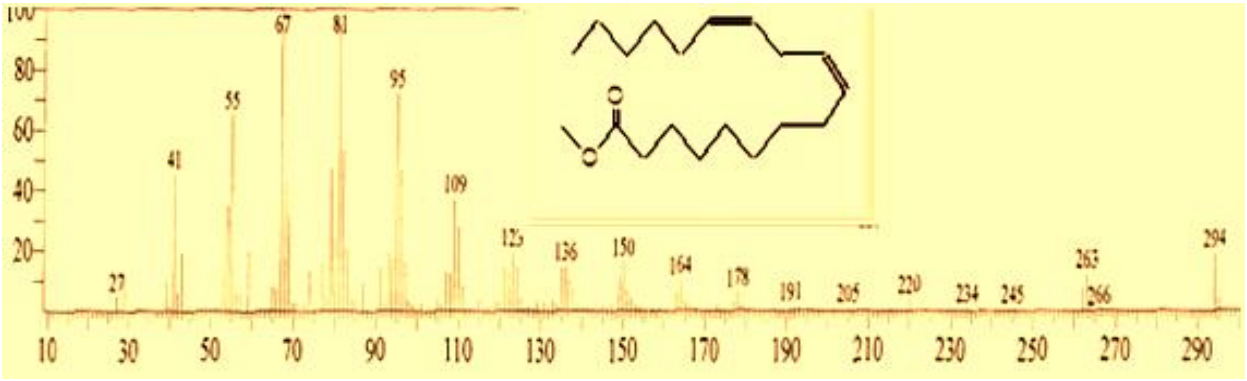


Fig.3.14: mass spectrum of 9,12-octadecadienoic acid (Z,Z)-methyl ester

Fig.3.15: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

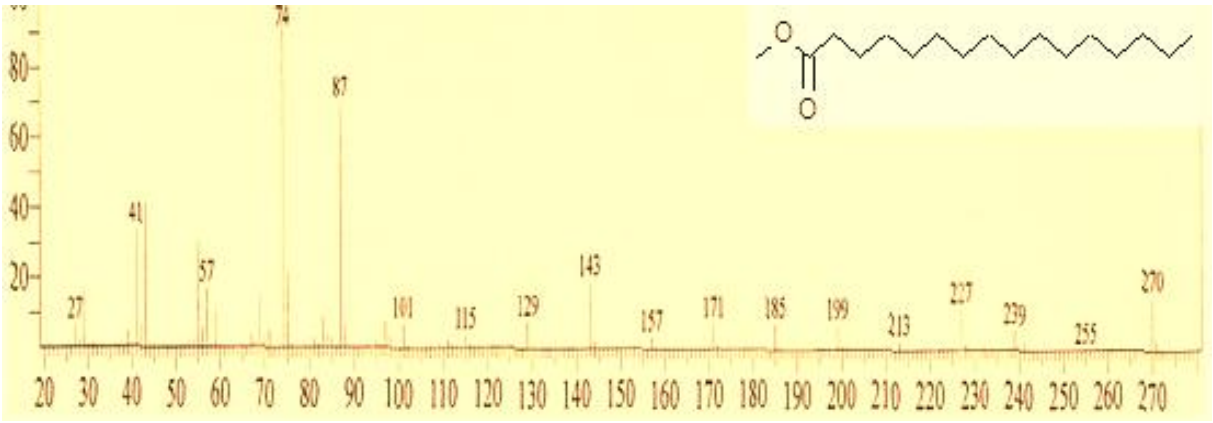


Fig.3.16: mass spectrum of hexadecanoic acid, methyl ester

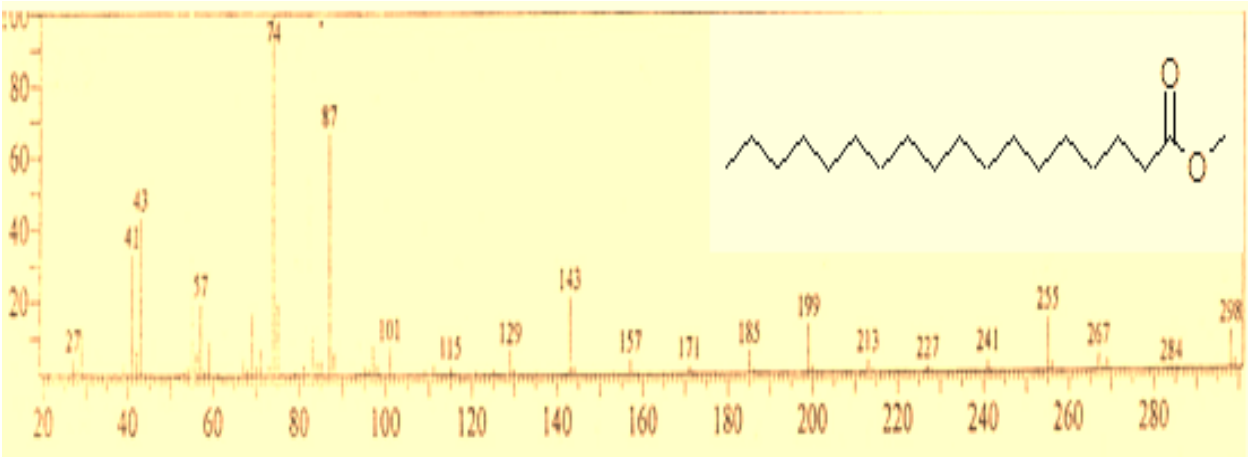


Fig.3.17: mass spectrum of methyl stearate

3.4.2Antimicrobial assay of *Ocimum basilicum* oil

Ocimum basilicum oil was investigated for antimicrobial activity against five standard microbial isolates. However, the oil failed to give antimicrobial potential against the following microbial isolates : *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans.*

3.5- *Prunus mahaleb*

3.5.1-GC- MS analysis

Prunus mahaleb oil has been investigated by GC-MS. The analysis showed 40 constituents - see Table 3.10.The total ion chromatogram is depicted in Fig.3.18.

Major constituents are:

i) 9-Octadecenoic acid methyl ester (36.80%)

ii) 9 12-Octadecenoic acid methyl ester (25.87%)

ii) Hexdecanoic acid (7.91 %)

iv)6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-(4.21%).

v) Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-(4.11%)

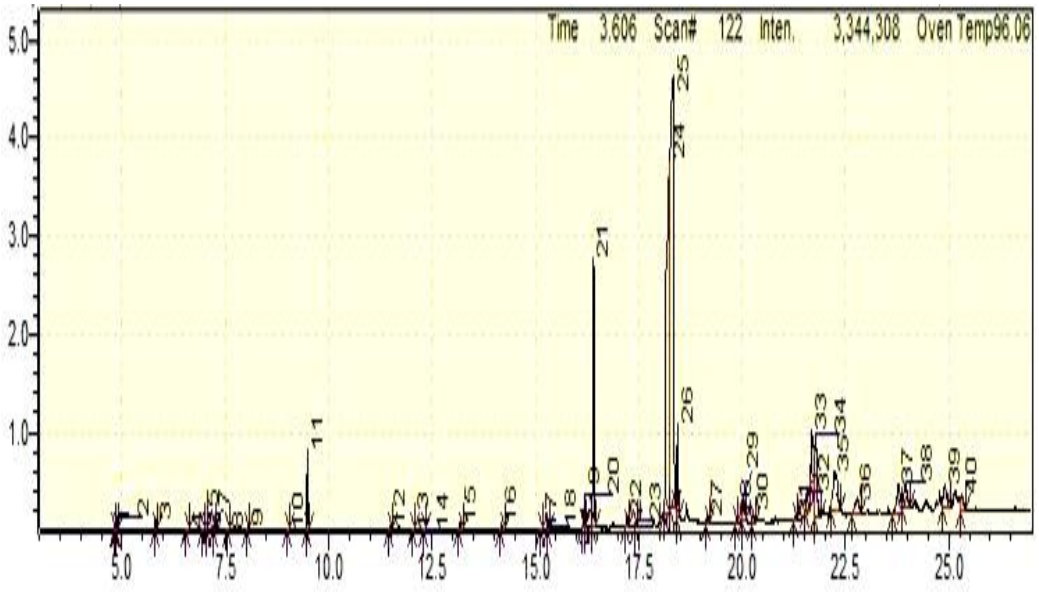


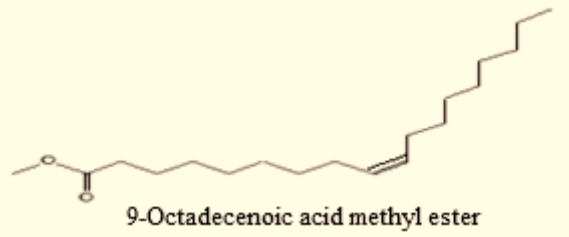
Fig. 3.18: Total ion chromatograms

Table 3.10: Constituents of *Prunus mahaleb* essential oil

No.	Name	R.Time	Area%
1.	D-Limonene	4.827	0.08
2.	Eucalyptol	4.883	0.23
3.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.813	0.17
4.	(+)-2-Bornanone	6.590	0.03
5.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	6.970	0.02
6.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	7.052	0.01
7.	.alpha.-Terpineol	7.250	0.15
8.	6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-	7.588	0.03
9.	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	8.057	0.07
10.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	9.029	0.01
11.	trans-.beta.-Terpinyl butanoate	9.487	1.96
12.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	11.486	0.09
13.	Ledol	12.042	0.02
14.	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	12.315	0.02
15.	Apiol	13.183	0.20
16.	Methyl tetradecanoate	14.182	0.17
17.	cis-5-Dodecenoic acid, methyl ester	15.150	0.02
18.	Pentadecanoic acid, methyl ester	15.315	0.09
19.	7-Hexadecenoic acid, methyl ester, (Z)-	16.180	0.43
20.	9-Hexadecenoic acid, methyl ester, (Z)-	16.198	0.77
21.	Hexadecanoic acid, methyl ester	16.410	7.91
22.	cis-10-Heptadecenoic acid, methyl ester	17.214	0.31
23.	Heptadecanoic acid, methyl ester	17.430	0.17
24.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.245	25.87
25.	9-Octadecenoic acid (Z)-, methyl ester	18.335	36.80
26.	Methyl stearate	18.440	2.25
27.	cis-10-Nonadecenoic acid, methyl ester	19.156	0.24
28.	8,11-Eicosadienoic acid, methyl ester	19.861	0.15
29.	cis-11-Eicosenoic acid, methyl ester	20.037	0.73
30.	Eicosanoic acid, methyl ester	20.261	0.38
31.	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.286	0.11
32.	.gamma.-Sitosterol	21.599	1.36
33.	.beta.-Sitosterol	21.686	2.54
34.	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	21.785	4.11

35.	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-	22.233	4.21
36.	.beta.-Amyrin	22.766	1.54
37.	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	23.776	2.37
38.	.alpha.-Amyrin	23.955	1.79
39.	Vitamin E	24.905	1.80
40.	Pregn-4-ene-3,20-dione, 14,17-dihydroxy-	25.319	0.79

Fig.3.19 shows the mass spectrum of 9-ctadecenoic acid methyl ester . The signal which appeared at m/z 296 (RT.18.335) accounts for the molecular ion: $M^+[C_{19}H_{36}O_2]^+$. The mass spectrum of 9,12-octadecadienoic acid methyl esteris depicted in Fig.3.20 . The signal at m/z 294(RT. 18.245) is due to the molecular ion : M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 3.21.The peak at m/z 270 which appeared at (RT.16.410) is due to M^+ $[C_{17}H_{32}O_2]^+$. The mass spectrum of 6a,14a-methanopicene,perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-is shown in Fig.3.22. The peak at m/z 426 which appeared at (RT.22.233) is accounting for the molecular ionM^+ $[C_{30}H_{50}O]^+$: Fig.3.23 shows the mass spectrum of stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-. The peak at m/z 414 which appeared at (RT.21.785) is due to M^+ $[C_{29}H_{50}O]^+$.



9-Octadecenoic acid methyl ester

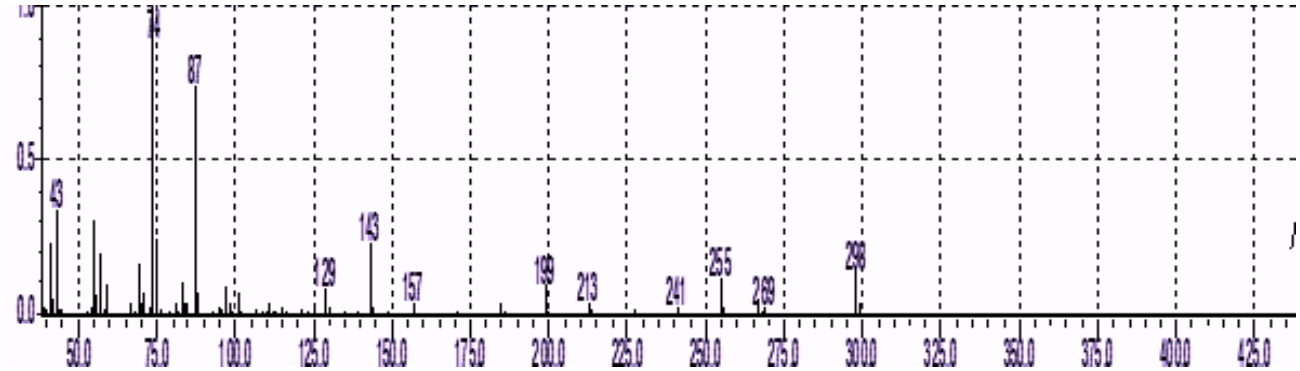
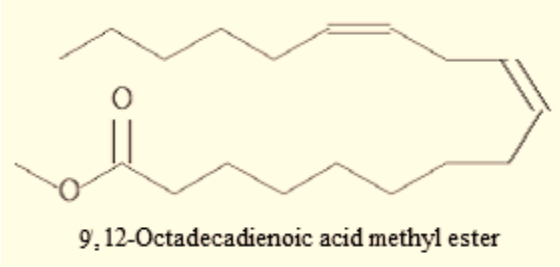


Fig: 3.19: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester



9, 12-Octadecadienoic acid methyl ester

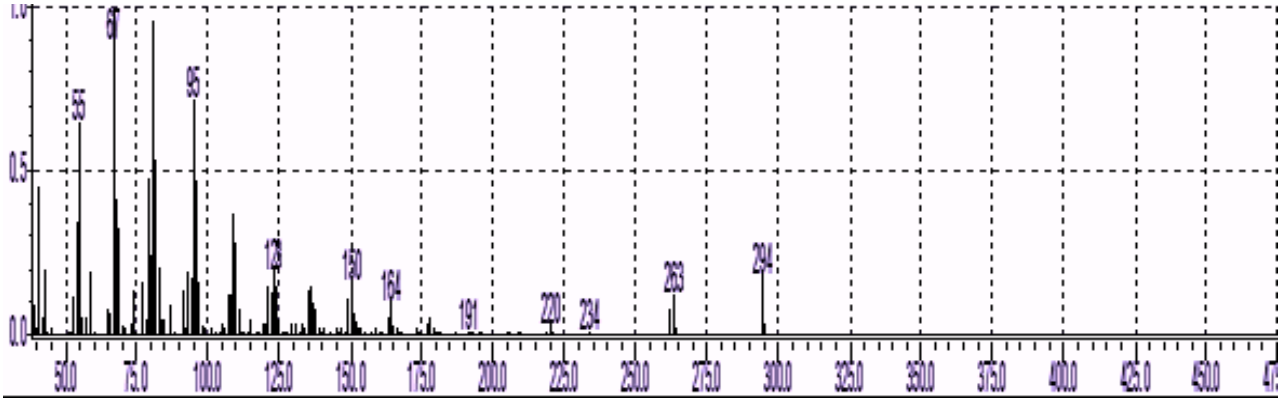
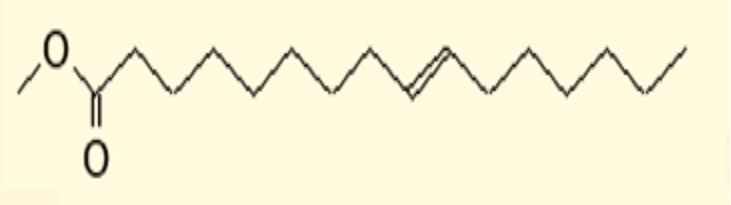


Fig.3.20: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester



hexadecanoic acid, methyl ester

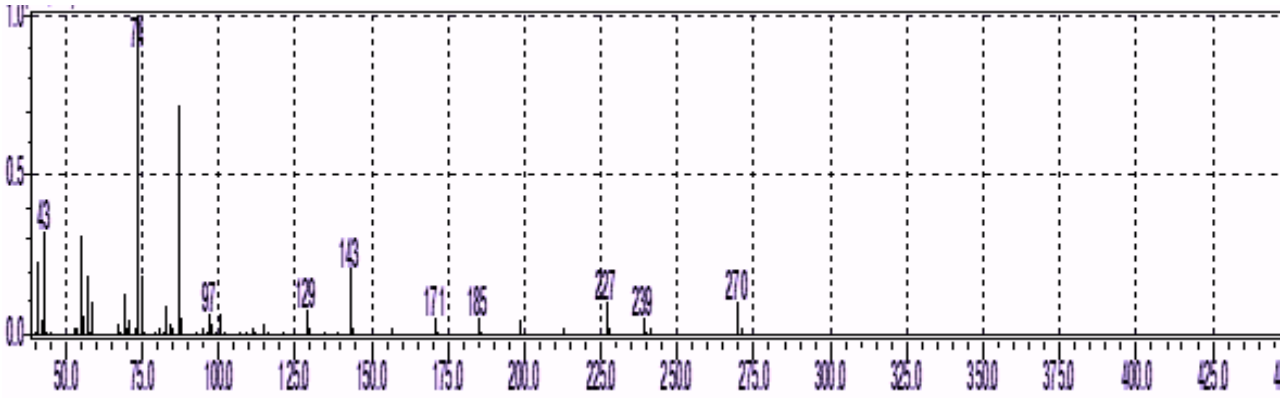
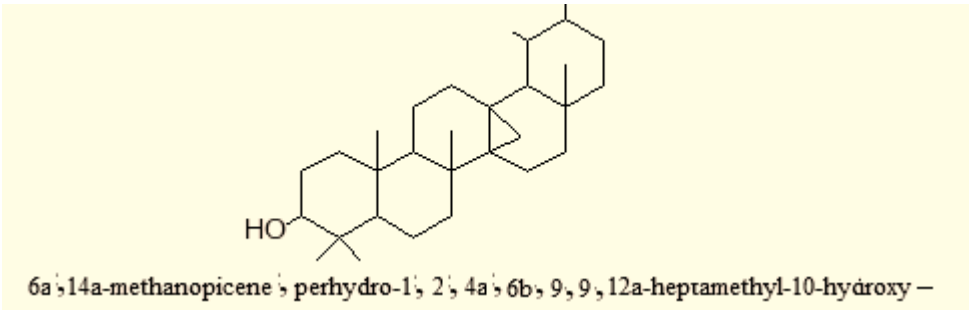


Fig.3.21 :Mass spectrum of hexadecanoic acid, methyl ester



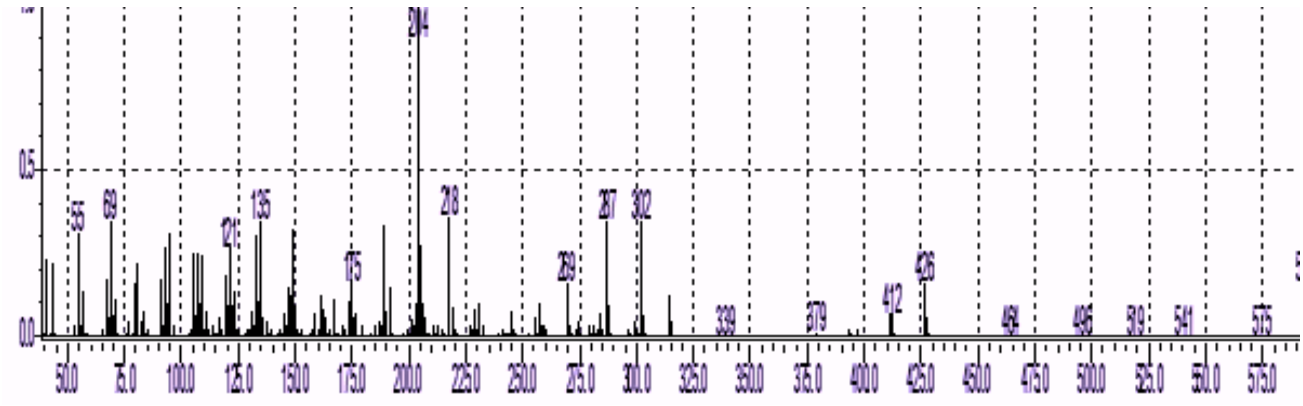
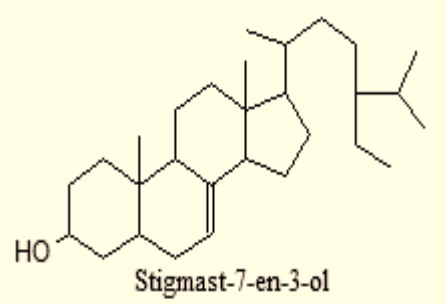


Fig.3.22: Mass spectrum of 6a,14a-methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-



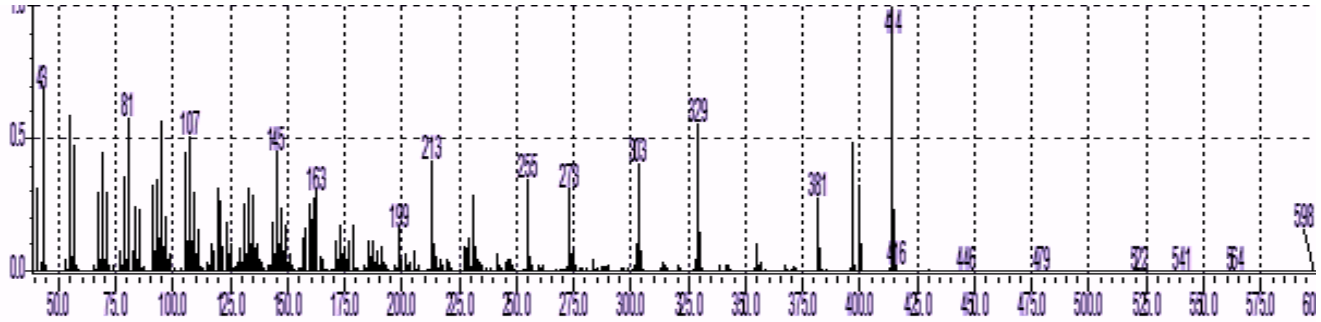


Fig. 3.23: Mass spectrum of Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-

Conclusion

In this study five medicinal plants(*Lycopersicum esculentum*, *Ocimum basilicum* ,*Sterculia setigera* , *Carthamus tinclorius* and *Prunus mahaleb*) have been studied. The constituents of the oils extracted from these plant species have been characterized by GC-MS and the antimicrobial activity has been evaluated and different antimicrobial responses hve been observed.

Recommendations

1-It would be challenging to evaluate the extracted oils for their antispasmodic, antimalarial and anti-inflammatory activities.

2- The secondary metabolites of the targeted plants may also be isolated , characterized and their biological activity may be studied.

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