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Chemical Characterization of Oils from Some Sudanese Medicinal Plants and their Biological Activity

By

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استهلال

بسم الله الرحمن الرحيم

قال تعالى:

(تُولِجُ ٱلَّيْلَ فِي النَّهَارَ وتُولِجُ النَّهَارَ فِي اللَّيلِ وَتُخْرِجُ الْحيَّ

مِنَ الْمَيِّتِ وَتُخْرِجُ ٱلْمَيِتَ مِنَ ٱلحَيِّ وَتَرْزُقُ مَن تَشَآءُ بِغَيْرِ حِسَابِ ﴿27﴾)

صدق الله العظيم

سورة ال عمران

DEDICATION

To:

The memory of my Parents, Brothers and Sisters

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Abstract

The oils from *Prosopis juliflora* was analyzed by GC-MS. The GC-MS spectrum of the studied oil revealed the presence of 24components dominated by methyl-10-trans,12-cis-octadecadienoate (32.83%). *Prosopis juliflora* oil showed significant activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, it also showed very good anti candida potential. GC-MS analysis of *Acacia seyal* has revealed the presence of 41 components dominated by 9,12-octadecadienoic acid (Z, Z), methyl ester (31.18%). *Acacia seyal* oil showed significant activity against all test bacteria, it also gave very good anti-candida potency. GC-MS analysis of *Solenostemma argel* oil was conducted, it revealed the presence of components dominated by 7-hexadecenal, (Z)-(18.23%). *Solenostemma argle* oil which showed excellent activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The oil exhibited very good activity against *Staphylococcus aureus*. It also showed good anti candida potency. The GC-MS analysis of *Coriandrumm Sativum* oil revealed the presence of 52 components dominated by 9-octadecenoic acid (Z)-, methyl ester (61.60%).

GC-MS analysis of *Medicago sativa* oil was conducted. The GC-MS analysis revealed the presence of 26 components dominated by methyl 10-trans,12-cis-octadecadienoate (43.46%). *Medicago sativa* oil showed moderate activity against *Bacillus subtilis*. However, it exhibited partial activity against other test organisms. GC-MS analysis of *Corchorus*

olitorius showed the presence of 28 components dominated by 9,12-octadecadienoic acid (z,z)-methyl ester (39.13%). GC-MS analysis of *Pimpinella ansium* oil was conducted. The GC-MS analysis revealed the presence of 49 dominated by 9-octadecenoic acid(Z)-, methyl ester (41.47%). *Pimpinella anisum* oil showed excellent activity against *Staphyococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. It was inactive against *Escherichia coli* and the fungus *Candida albicans*.

مستخلص

الكروموتوغرافيا الغازية-طيف الكتلة بينت أن زيت نبات المسكيت يحوي 24 مركب اهمها methyl 10-trans,12-cis-octadecadienoate (32.83%), قد اظهر الزيت المستخلص فاعلية ضد كل من *Escherichia coli* and *Staphylococcus aureus*. الكروموتوغرافيا الغازية-طيف الكتلة بينت ان زيت نبات الطلح يتكون من 41 مركب اهمها 9,12-octadecadienoic acid (Z,Z), methyl ester (31.18%), اظهر الزيت فاعلية ممتازة ضد البكتريا قيد الاختبار وفطر *Candida albicans*. تحليل زيت نبات الحرجل بواسطة الكروموتوغرافيا الغازية-طيف الكتلة بين وجود 31 مركب اهمها . 7-hexadecenal, (Z)-(18.23%) اظهر زيت نبات الحرجل فاعلية ممتازة ضد كل من *Escherichia coli* and *Pseudomonas aeruginosa* وفاعلية معتدلة ضد*Staphylococcus aeruginosa*. الكروموتوغرافيا الغازية –طيف الكتلة للزيت المستخلص من نبات الكسبرة بين وجود 52 مركب اهمها 9-octadecenoic acid(Z)-, methyl ester (61.60%). تحليل الزيت المستخلص من نبات البرسيم بواسطة الكروموتوغرافيا الغازية اظهر وجود26 مكون اهمها -10 methyl trans,12-cis-octadecadienoate (43.46%) اظهر المستخلص فاعلية متوسطة ضد Bacillus subtilis وفاعلية ضعيفة ضد المكروبات الاخري.التحليل بواسطة الكروموتوغرافيا – طيف الكتلة للزيت المستخلص من نبات الملوخية اظهر 28 مركب منها -9,12 octadecadienoic acid(Z,Z)-,methyl ester(39.13%) وقد ابدي نبات الملوخية فاعلية ضد *Staphylococcus aureus*. تحليل الزيت المستخلص من نبات اليانسون بين وجود 49مركب اهمها 9-octadecenoic acid(Z)-, methyl ester (41.48%), 9- اظهر المستخلص فاعلية ممتازة ضد كل من *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas* ولم يظهر فاعلية مع *Escherichia coli* وفطر*Candida albicans*

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1. Introduction

1.1-General overview

In developing countries, where modern medicine is beyond affordability, medicinal plants play an important role in treating a wide array of human disorders. A considerable number of modern drugs have been isolated or derived from plant material[1,3] examples include: atropine, morphin, cocain, etc. More than 80% of the world population now depend on medicinal plants which contribute to the primary healthcare of different communities[4, 6]. This is mainly due to the side effects of several synthetic drugs and the unaffordable cost of modern drugs. Medicinal plants include bioactive constituents (steroids, alkaloids, flavonoids. etc) which are very helpful in treating various ailments[7]and may serve as leads for drug discovery and drug development.

1.2. Natural products

A natural product is a chemical compound or substance produced by a living organism that is, found in nature. In the broadest sense, natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients[1].

1.2.1. Tannins

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of un ripened fruit or tea. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruits. Tannins have molecular weights ranging from 500 to over 3,000 (garllic acid esters) and up to 20,000 (proanthocyanidins)[1].

1.2.2. Saponins

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative[1].

The aglycone (glycoside-free) portions of the saponins are termed sapogenins. The number of saccharide chains attached to the sapogenin/aglycone core can vary - giving rise to another dimension of nomenclature (monodesmosidic, bidesmosidic, etc.) - as can the length of each chain. A somewhat dated compilation has the range of saccharide chain lengths being 1-11, with the numbers 2-5 being the most frequent, and with both linear and branched chain saccharides being represented. Dietary monosaccharides such as D-glucose

and D-galactose are among the most common components of the attached chains[1].

1.2.3. Steroids

Steroids comprise a group of cyclic organic compounds whose most common characteristic is an arrangement of seventeen carbon atoms in a four-ring structure, where the rings are three composed of 6-carbons (rings A, B, and C) followed by one with 5-carbons (ring D). Further common features are an 8-carbon side chain attached to a carbon on ring D, and two or more methyl groups at the points where adjacent rings are "fused". Hundreds of distinct steroids are found in animals, fungi, plants, and elsewhere, and specific steroids underlie proper structure and function in many biological processes. Their core tetracyclic ring structure is synthesized in each organism by biochemical pathways that involve cyclization of a thirty-carbon chain, squalene, into an intermediate, either lanosterol or cycloartenol. From such intermediates, organisms then derive critical steroids such as cholesterol, the sex hormones estradiol and testosterone and bile acids. Based on such structures, synthetic and medicinal chemists synthesize novel steroids for use as drugs such as the anti-inflammatory agent dexamethasone[1].

1.2.4. Glycoside

In chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the

sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body[2].

1.2.5. Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus[1].

1.2.6. Flavonoids

Flavonoids are the low molecular weight polyphenolic secondary metabolic compounds, universally distributed in green plant kingdom, located in cell vacuoles. Flavonoids play a variety of biological activities in plants, animals, and bacteria. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for color and aroma of flowers, fruit to attract pollinators consequently fruit dispersion; help in seed germination, growth and development of seedling. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, Function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimation and freezing tolerance [1].

Flavonoids form a family of known natural products present in most of the plant families. More than 8000 different flavonoids have been isolated from their natural source to date. The structural variations of these flavonoids are associated with many different biological and pharmacological activities, including anticancer activity, protection against cancer formation (chemo-protection), antioxidant activity, cardiovascular and hepatic protection, antibacterial, antifungal and antiviral activity. Flavonoids have also been reported to play an important role in hormone-related female diseases, such as breast cancer and menopausal syndrome. Natural flavonoids have therefore been subjected to many chemical modifications in order to improve their activity [2].

1.2.7. Essential oil

The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes from the epicarp of Citrus, or ‘’dry’’ distillation. The essential oil is usually separated from the aqueous phase by physical means[8]. This definition encompasses products obtained always from a vegetable raw material, but using other extraction methods, such as using non-aqueous solvents or cold absorption, thus we can define four type of product[9].

Essential oils are soluble in alcohol, ether, and fixed oils but insoluble in water. The volatile oils are generally liquid and color less at room temperature. They have a characteristic odor and are usually liquid at room

temperature and have a density less than unity, with the exception of a few cases (cinnamon, sassafras and vetiver)

Essential oils possess a refractive index and a very high optical activity. Those volatile oils contained in herbs are responsible for different scents that plants emit. They are widely used in the cosmetics industry, perfumery, and also aromatherapy. The latter is intended as a therapeutic technique including massage, inhalation, or baths using these volatile oils, last key will serve as a chemical signal allowing the plant to control to regulates environment attraction of pollinating insect, repellent to predator inhibition of seeds germination or communications between plants moreover essential oils possesses anti fungi or insecticide and deterrent activities. All parts of aromatic plants may contain essential oils. They exist in flowers including: orange, pink, lavender, and the clove buds. They also occur in leaves including: eucalyputs, mint, thyme, bay leaf, savory, sage, pine needle, and tree underground organs root vetiver. Plant rhizomes like ginger contain essential oils. The seed of many plant species like carvi are known to contain essential oils. Fruits like fruits of fenel and anise do possess essential oils. Essential oils may also be found in wood and bark examples include cinnamon, and sandal wood.

1.3. Chemistry of essential oils

Essential oils are produced by various differentiated structure, especially the number and characteristics of which are highly variable. Essential oils are localized in the cytoplasm of certain plant cells secretions which lies in one or

more organs of the plant, namely, the secretly hair or tri chomes, epidermal cells, internal secretly, and the secretly pockets. These oils are complex mixture that may contain over 300 different compounds [8].

They consist of organic volatile compounds, generally of low molecular weight below 300.The vapor pressure of essential oils at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in vapor state[10,11]. These volatile compounds belong to various chemical classes like alcohols, ethers, oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohol, eldehyds and ketones offer a wide variety of aromatic notes such as fruity (nerolidol) floral, (linalool) citrus (limonene) herbal (selinene). Furthermore, essential oils components belong mainly to the terpene family. Many thousands of compound belonging to the family terpenes have so far identified in essential oils [12].

It is known that terpenoids and phenyl propanoids have different primary metabolic precursors and are generated through different biosynthetic routes. The pathways involved in terpenoids are the mevalonate and mevalonate _independent (deoxyxylulose phosphate) pathways, where phenyl propanoids originate through the Shikmate pathway. Some authors have reviewed the biosynthetic pathways of terperoidss and phenylpropanoids[13,14]. The essential oils have a diverse composition, both in qualitative and quantitative terms and various factors are responsible for this variability and can be grouped into two categories:

- Intrinsic factors related to interaction with environment (soil type and climate) and the maturity of the plant concerned.
- Extrinsic factors such as the extraction method and the environment.

The factors that determine essential oil composition are numerous. In some case it is difficult to isolate these factors from each other as they are interrelated and influence each other. Such parameters include the seasonal variations, plant organ, degree of maturity of the plant, geographic origin and genetics[15,16].

1.4. Extraction of essential oils

1.4.1. Steam distillation

Steam distillation is a special type of distillation for temperature- sensitive material like natural aromatic compounds. Once it has been a popular laboratory method for purification of organic compounds, but has become obsolete after emergence of vacuum distillation. However, steam distillation remains important in certain industrial sectors[17].

In steam distillation, water or steam is introduced into the distillation apparatus. The water vapor carries small amounts of the vaporized compounds to the condensation flask, where the condensed liquid phase separate, allowing for easy collection. This process effectively allows for distillation at lower temperature, reducing the deterioration of the desire product, if the substances to be distilled are very sensitive to heat, steam distillation may be applied under reducing the operating temperature further. After distillation, the vapors are condensed. Usually the immediate product is two phase system of water

and organic distillate allowing for separation of the compounds by decantation, partitioning or other suitable methods[18].

Steam distillation is also widely used in petroleum refineries and petrochemical plant where it is commonly referred to as steam stripping[19,20]. Also steam distillation is an important process for the separating fatty acids from a matrix and for treating crude products such as tall oils to extract and separate soaps and other commercially important organic compounds[21].

1.4.1.1. Hydrodistillation

Hydrodistillation is the most common at it is simple and temperature in the body is raised by direct firing often using spent residues as fuel. Here oil quality is directly related to the skill of the operator, not only in managing the still but in selecting or preparing the raw material.

1.4.1.2. Vacuum distillation

The technique of vacuum distillation allows very accurate control of distillate since it can be adjusted according to the boiling points of various oil constituents.

1.4.1.3. Molecular distillation

This technique processes material under a high vacuum and can be used on raw material or to reprocess crude or other oils. Products obtained by this technique are of vary high quality and odor.

1.4.1.4. Enfleurage

This method of extraction is suitable for extracting flower oils by absorption on a matrix of wax or fat and then recovering the oil by solvent extraction,

Layers of flowers are laid on trays of specially prepared fat and the flower layers removed and renewed until fat is saturated. However, this process is highly labor intensive, but products are of extremely high quality.

1.4.2. Solvent extraction

Extraction via this technique involves passing a solvent through the raw material and then evaporating the solvent. It can take place under normal atmospheric condition, in a partial vacuum or in the presence of gas. Commercial plants used batch, battery or continuous flow system, single or multi-solvent techniques, and include solvent recovery and oil refining equipment. These plants are generally expensive to construct and operate and are frequently located in developed countries using dried material. Since solvent extraction removes volatile and non-volatile constituents, composition of the oil obtained can differ significantly from distilled oil, and may contain undesirable components requiring removal. The solvent used frequently influences the oil obtained as a residue or odor moderate, but solvent extracted oils are generally considered to reflect a plants natural odor more accurately than distilled oils. Commonly used is petroleum ether, hexane, toluene or other binary solvents.

1.4.3. Gaseous extraction

Liquid carbon dioxide, which is under pressure and regulated temperature, is passed through the material, then via a separator to recover oil and gas. However, gaseous extraction is considered superior to liquid solvent, since it preserves important heat -sensitive components and requires less energy.

Beside that, carbon dioxide is safe, non-combustible, odorless, tasteless, inexpensive and readily available which are ideal properties for an extraction solvent, while its low viscosity anable to penetrate the material being extracted and its latent heat of evaporation allows it to be removed without residue [22].

1.5. Biological activities of essential oils

1.5.1. Antibacterial activity

The antimicrobial properties of many essential oils have been reviewed and the mechanism of action has been studied[25.]An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable[26,27].This can then cause leakage of ions and other cellular molecules[28,29] .Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death[30].

Essential oils can have a single target or multiple targets of their activity for instance, *trans*-cinnamaldehyde can inhibit the growth of *Escherichia coli* and *Salmonella typhimirium* without isintegrating the OM or depleting intracellular ATP (Adinoizine triple phosphate). Thymol and Carvacrol oils gain access to the periplasm and deeper portions of the cell[31]. Carvone oil can also be inter affective against the OM and does not affect the cellular ATP pool[32].

Also it has been shown that essential oils containing mainly aldehydes or phenols, such as citral, carvacrol, eugenol, or thymol do possess the highest antibacterial activity, followed by essential oils containing terpene alcohol. Other essential oils containing ketones or esters such as geranyl acetate show much weaker activity, while volatile oil containing terpenes hydrocarbons are usually inactive[33,34]. Essential oils characterized by a high level of phenolic compound, such as carvacrol, eugenol, and thymol, have important antibacterial activities[35,36]. Such compounds are responsible for the disruption of the cytoplasm membrane [26,36,40]. It has been shown that the chemical structure of essential oils effects their mode of action concerning their antibacterial activity [35]. The crucial presence of hydroxyl group in the phenolic compounds, such as carvarol and thymol, was confirmed[28,34]. However the relative position of the phenolic hydroxyl group on the ring does not appear influence the antibacterial activity.

The action of thymol against *Bacillus cereus, Staphylococcus aureus,* and *Pesudomons aeruginosa* appears to be comparable to that of carvarol for example[24,29]. It has been reported that carvarol and thymol act differently against Gram positive and Gram negative species[29]. Thymol, carvarol have an antimicrobial effect against a broad spectrum of bacterial strains including: *Escherichia coli, Bacillus cereus, Liserimano cytogenes, Samonella enteric, Clostridium jejuni, Lactobacillus sake, Staphylococcus* and *Helicobacter pyroli* [39,40].

Other essential oils also have valuable antibacterial properties like those containing certain alcohols, aldehydes, ketones and monoterpenes. Among these compounds, carvarol is the most active. Carvarol is used as a presevative and food flavoring in drink, sweats, and other preparation. It has been shown that essentials are more active against Gram positive than Gram negative bacteria [40,45]. The latter are less susceptible to the action of essential oils with the outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through the lipopolysaccharids film [42]. Furthermore, the antibacterial activity of essential oils is related to their chemical composition, the properties of volatile molecules, and their interactions [35,40,44]. An additive effect is observed when the combination is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less important when they are tasted together than when used individually [40.]

A synergistic effect is observed when the combination of substance is greater than the sum of the individual effects [41]. Some studies have shown that the use of the whole essential oils provides an effect which is greater than that of the major components used together [42.] This suggests that minor components are essential for activity and may have synergistic effect. The additive and synergistic effects of the combination of 1,8-cineole and aromadendrene against methicillin - resistant *Staphylococcus aureus (*MRSA) and thancomycin - resistant enterococci (VRE) has been demonstrated [43.] In addition, essential oils have also revealed to be effective on the inhibition of

growth and reduction in numbers of the more serious food borne pathogens, such as *Salmonella spp.* and *E. coli* [44].

1.5.2. Antioxidant activity

Some studies have demonstrated that the antioxidant potential of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties[47]. Regarding the antioxidant properties, thymol and carvacrol are potentially active phytochemicals. The activity of this natural products is directly related to their phenolic compounds having redox properties and thus play an important role in neutralizing harmful free radicals[42].

The antioxidant activity of essential oils is also due to certain alcohol, esters, ketones, aldehyde, and monoterpenes[45]. Essential oils with important scavenging capacity of free radicals may play an important role in some diseases prevention, such as, brain dysfunction, cancer, heart disease, and immune system decline. In facts the disease may result from cellular damage caused by free radicals[41,46]. Essential oils have shown their action as hepatoproective as well as against ageing. Also it has been proved that they possess a beneficial impact upon the poly unsaturated fatty acids (PUFAs), in particular the long chain C_{20} and C_{22} acids[48].

1.5.3 Cancer chemoprotective activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researcher for their potential activity against cancer. Essential oils

and their constituents target the discovery of new anticancer natural products[42].

Essential oils would act in the prevention of cancer as well as, at its removal. It is well known that certain food, such as garlic and turmeric, are good sources of anticancer agents[49]. Garlic essential oil is source of sulfur compounds recongnized for their preventive effect against cancer. Daily sulfide, disulfide, and tri sulfide are examples[32].

These compounds activity in rates, the enzymes involved in the detoxification process of hepatic phase1(disintegraction of chemical bonds that link carcinorganictoxins to each other) and phase 2 (bonds to toxins released detoxifying enzymes, such as glutathione s- transferase). Metabolism happens mainly in the liver - the body largest internal organ. The portal vein carries blood from the small intestine directly to the liver. Sixty percent of liver tissue is made up of hepatic cells. More chemical processes happen in these than in any other group of cells in the body. Phase 1 metabolism involves chemical reactions, such as oxidation (most common) reduction and hydrolysis.

There are three possible results of phase 1 metabolism:

- The drug becomes completely inactive i.e. the metabolites are pharmacologically in active.
- The metabolites are pharmacologically active, but less so than the original drug.
- The original substance is not pharmacologically active, but one of its metabolites.

Phase 2 metabolism involves reaction that chemically changes the drug or phase1 metabolites into compounds that are soluble enough to be excreted via urine. In the reaction, the metabolites which are attached to an ion is capable of grouping. This is called conjugation and the products called a conjugate[53].

It has been demonstrated that many essential oils have a cytotoxin activity namely *Melissa fficinalis*[54]. *Melaleuca alternifolia. Artemisia annua* and *comptonia peregrine*[55].

1.6. Quality control of essential oils

For quality control of any volatile oils pharmacopoeias require different tests physical measurements (refractive index etc), determination of various indexes (esters, fatty acids and carbonyl) and analysis the essential oils by chromatographic techniques.

1.7. Medicinal and commercial uses of essential oils

Many plants containing essential oils have been long used either medicinally or industrially.

1.7.1. Pharmaceuticals

In this respect, the drugs containing essential oils are used in their crude form for their therapeutic effect particularly as external antiseptics, but the major uses of the essential oils are the aromatization of other pharmaceutical, and as carminatives as well.

1.7.2- Perfumery:

Perfumery materials such as volatile oils are used directly. Also they are used in cosmetology and the products are of higher cost. The other route for

volatile oils in this category is the usage in manufacturing of soaps, toiletries, deodorants, household cleaners, polishers and insecticides. In perfume industry, volatile oils are classified into three categories(i) those with high volatility leaving the skin rapidly like lemon, lavender, odorant (ii) with intermediate volatility as thyme, neroli and the last categories include (iii) those constituents of low volatility which are also described as fixatives like vanillin and musk.

1.7.3 Food industry

A large number essential oils are used widely as flavor for foods, confections and in spice. As spice drugs are used raw (herb and spices) others are used as essential oils, resinoids, oleoresins, dispersed, encapsulated, or complexed. Different sectors of food technology are using volatile oils including non coholic beverages, dairy products, meat products.

1.8- Storage of essential oils

Essential oils are relatively un stable, this makes their storage difficult. Examples are oxidative cleavage of phenyl propanoids, peroxidation of hydrocarbons, and decomposition to Ketones and alcohols (limonene) thermo isomerization (citrals) and many others.

One way to overcome this problem is by using small vials made of amber glass, aluminum or stainless steel, completely filled, and tightly closed. Low temperature storage, storage under an inert nitrogen atmosphere or by addition of antioxidants are possible choices[56].

1.9- Analysis of essential oils

Chromatography is a very specific and selective separation technique, utilizing the small differences in the distribution of each component between two phases: the stationary and the mobile phase. It is thus used for the separation of closely related compounds in mixture and also to separate widely different compounds[57].

Separation techniques combined with mass spectrometry are important enhancement to the mass resolving and mass determining capabilities of mass spectrometry where it is used in tandem with chromatographic, and other separation techniques [58].

1.9.1.1- Liquid chromatography

Liquid chromatography- mass spectrometry (LC/MS) separates compounds chromatically before they are introduced to the ion source and mass spectrometer. It differs from (GC/MS) in that the mobile phase is liquid usually which is a mixture of water and an organic solvent instead of gas. Most commonly electrospray ionization source is used in (LC/MS). Other popular and commercially available (LC/MS) ion sources are atmospheric pressure chemical ionization and atmospheric pressure photoionization. There are also some newly developed ionization techniques like laser spray[60].

1.9.1.2. Gas chromatography-mass spectrometry

In the technique of gas chromatography-mass spectrometry the feature of gas chromatography and mass spectrometry are combined to identify different substances within a sample. Applications of (GC/MS) include:

- Drug detection
- Fire investigation
- Environment analysis
- Explosive investigation
-Identification of unknown samples
- Airport security

This powerful analytical tool can identify trace elements in materials that were previously thought to have disintegrated beyond identification. This technique allows analysis and detection even of tiny amount of substance. Since (GC/MS) is used to perform 100% specific test which positively identifies the presence of particular substance, then it is used as a gold standard for forensic substance identification[63].

The need to un equivocally identify the constituents of complex matrix was the motivation for the development of different instrumental coupling techniques (tandem), including the widely and successfully used gas chromatography (GC) coupled with mass- spectrometery (MS) This technique is an extremely favorable, synergistic union, as the compounds susceptible to be analyzed by GC (low –molecular weight, medium or low polarity in ppm concentration) are also compatible with the MS requirements. Besides both analyses proceed in the same aggregation state (vapor phase).

However, the only conflict (short term and already resolved) between GC and MS were the different working pressure, for example atmospheric at the GC column exit and low in the ionization chamber, respectively. This drawback

was overcome by technically introducing an efficient vacuum pump (turbo molecular and gas-jet pumps) and above all due to the introduction of gas chromatography capillary columns (internal diameter 0.18 to 0.32 mm)[63,62].

1.9.2. Capillary electrophoresis – mass spectrometry

The technique of capillary electrophoresis-mass spectrometry (CE\MS) combines the liquid separation process of capillary electrophoresis with mass spectrometry[59]. (CE\MS) is typically copied to electrospray ionization [60].

1.9.3. Mass spectrometry

Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions on the of basis of their mass to charge ratio in simpler terms, a mass spectrum is a measure masses within sample. The technique is used in many different fields and is applied to pure samples as well as complex mixtures. Mass spectrum can be defined as a plot of the ion signals as a function of the mass –to-charge-ratio. These spectra are used to determine the elemental or isotopic signature of a sample as well as the masses of particles and of molecules, and to elucidate the chemical structure of molecules, such as peptides and other chemical compounds.

In a typical MS procedure, a sample, which may be solid, liquid or gas, is ionized, for example by bombarding it with electrons. This may cause some samples molecules to break into charged fragments. The fragmented ions are then separated according to their mass-to-change ratio, typically by accelerating them and subjecting them to an electric or magnetic field[63].

1.10. The target plant species

1.10.1. *Pimpinella anisum*

Anise (*Pimpinella anisum*) is a plant in the family Umbelliferae. It is indigenous to the Mediterranean region[64.] The major production area in Sudan is north Sudan, while there is a very limit production in Khartoum state [59]. Anise seeds contain 1.5-5 % essential oils and is used as flavor, digestive, carminative and for the relief of gastrointestinal spasms. Consumption of anise by lactating women increase milk and also reliefs their infants from gastrointestinal problems[66]. In food industry, anise is used as flavoring and aromatic agent for fish products, ice cream, sweats, and gums[67,68]. Essential oils of the genus *Pimpinella* are a complex mixture of various components that contain sesqueterpenes, phenolic compound (C_6-C_3) and alkanes. The essential oil is located in the oil ducts of fruits, shoots and roots[69]. Anise seeds as well as anise essential oil has medicinal values. The anise tea is used for children' s flatulence upper respiratory tract problems and bronchial asthmatic attacks[70]. It can also be used for scabies, where it may be applied externally in an ointment base [71].

Figure (1.1) *Pimpinella ansium*

The composition of anise varies considerably with origin and cultivation method. Here are typical values for the main constituents: moisture (9-13%) protein (18 %) fatty oil (8-23%) essential oil (2-7%), starch 5% N-free extract (22-28%) crude fiber (12-25%)[70]. Anise oil obtained by distillation is generally around (2-3%) and anethole- a phytoestrogen- makes up (80-90%) of the oil[73]. The essential oil has reportedly been used as insecticide against head lice and mites. The oil is very famous oil throughout the world. It was used in confectioneries, pharmaceutical, tooth paste, and other industrial uses[74].

Anatomical features of anise are a vital process for clear identification of the plant, and furthermore investigations in the plant anatomy. Phytochemical studies of anise oil are important for identification and quality control and for other uses of essential oil for medicinal and food purposes. Due to variation in

climate, topography, soil and cultural practices, anise essential oil can be different, there for more investigation are important to clarify this changes.

Some spices like anise are reported to have bactericidal or bacteriostatic activities. The inhibitory effects of spices are mostly due to the volatile oils present in their composition. The main factors that determine the antimicrobial activity are the type and composition of the spice as well as the type of microorganism.[75.]

1.10.2. *Prosopis juliflora*

Prosopis juliflora is a perennial deciduous thorny shrub or small tree up to12 m tall, trunk up to 1.5m diameter, bark thick, brown or blackish, shallowly fissured, leaves compound, commonly many more than 9 pairs, the leaf lets mostly 5-10 mm long- linear-oblong, glabrous, often hairy, commonly rounded at the apex, spike like, corolla deeply l0 bate. Pods several seeded, strongly compressed when young, think at maturity, more or less constricted between the seeds 10-25 cm long brown or yellowish, 10-30 seeds. Seed compressed and oval or elliptic, 2.5-7 long brown[77]. Per 100g the flower is reported to contain 21g protein, 3.2g fat, 65.8g total carbohydrates, 15.5g fiber, 10g ash, 1.31mg Ca and 400mg P. Leaves contain 19g protein, 2.9g fat, 69.6g total carbohydrate, 21.6g fiber.8.5g ash, 2.08mg Ca and 220g P. Fruits 13.9g protein, 3g fat, 78.3g total carbohydrates, 27.7g fiber and 4.8g ash. Seeds contain 65.2g proteins, 7.8g fat, 21.8g carbohydrates, 2.8g fiber, and 5.2g ash.

Another analysis of the fruit shows 14.35% water (hygroscopic), 1.64% oil, 16.36% starch, 30.25% glucose, 0.85% nitrogenous material, 5.81%tannin-like material, 3.5% mineral salts, and 27.24% cellulose. Mesquite gum readily hydrolyses with dilute sulfuric acid to yield L-arabinose: D-galactose: 4-O-methyl -D-glucuronic acid at 4:2:1. Owing to the high content arabinose, the gum is an excellent source of sugar. Roots contain 6.7% tannin, bark 3-8.4% and dry wood o.9%. The alkaloids hydroxytryptamine and tyranine are reported from this species[78].

Figure (1.2) *Prosopi juliflora*

Mesquite pods are among the earliest known foods of prehistoric man in the new world. Today flour products made from the pods are still popular, although only sporadically prepared, mostly by Amerindians. Pods are made into gruels, sometimes fermented to make a mesquite wine. The leave can be used for forage, providing good bee pasturage. Also nectar from mesquite yield a superior honey. The wood is used for parquet floor, furniture, and

turnery items, fencepost, pilings, as a substrate for producing single-cell-protein, but most of all for fuel. Toasted seeds added to coffee. Bark which is rich in tannin, is used for roofing in some countries. The gums forms are used as adhesive mucilage and as an emulsifying agent. Gum is also used in confectionary and mending pottery. Roots contain 6-7% tannins[79].

Mesquite is also used as folk medicine. The juice is used in folk remedies for cancerous condition. Reported to be cathartic, emetic, stomachic, mesquite is a folk remedy for catarrh, colds, diarrhea, dysentery, inflammation, itch, stomachache, sore throat, and wounds. Pima Indians drink the hot tea for sore throat. Aqueous and alcoholic extracts are markedly antibacterial[80].

1.10.3. *Medicago sativa*

Alfalfa (*Medicago sativa*) also called Lucerne, is a perennial flowering plant in the pea family (Fabaceae). The plant is cultivated as an important forage crop worldwide. It is used for grazing, hay, as well as a green manure. It has clusters of small purple flowers followed by fruits spiraled in 2 to3 turns containing 10-20 seeds. Alfalfa is native to warmer temperature climates [79].

Alfalfa is rich in chlorophyll, carotene, protein, calcium and other minerals, vitamins, in the B group, vitamin C and D, E, and vitamin K [82]. The sun dried hay of alfalfa has been found to be source of vitamin D containing 48mg/g vitamin D_2 and 0.63mg/g vitamin D_3[86].

Alfalfa like other leguminous crops, is a known source of phytoestrogens, including spinasterol, because of this grazing on alfalfa has caused reduced fertility in sheep and in dairy cattle[86].

Figure (1.3) *Medicago sativa*

Raw alfalfa seeds and sprouts are a source of the amino acid canavanine. Much of the canavanine is converted into other amino acids during germination so sprouts contain much less caravanine than unsprouts seeds. The United State National institutes of health (US-NiH) reports that there is insufficient evidence to rate effectiveness of alfalfa for high cholesterol[87]. Taking alfalfa seeds seems to lower total low density lipoproteins (LDL) in people with high cholesterol level. Also alfalfa is traditionally used in kidney problems, bladder problems, prostate problems, asthma, arthritis, diabetes, upset stomach as well as other condition[88].

1.10.4. *Corchorus olitorius*

Corchorus is a genus of about 40-100 species of flowering plants in the family Malvaceae, native to tropical and subtropical regions throughout the world[90]. The plants are tall, usually annual herbs, reaching a height to 2-4m, unbranched or with only a few side branches. The leaves are alternate, simple, lancelets, 5-15cm long, with an acuminate tip and are finely serrated or lobed

margin. The flowers are small (2-3 diameter) and yellow, with five petals, the fruit is a many seeded capsule. The genus *Corchorus* is classified under the sub family Grewioideae of the family Malvaceae[91].

Figure (1.4) *Corchorus olitorius*

Per 100g the leaves are reported to contain :43-58 calories, 80.4-84.1g H_2O, 4.5-5.6g protein, 0.3g fat, 7.6-12.4g total carbohydrates, 1.7-2g fiber, 2.4g ash, 226-366mg Ca, 97-122mg P, 7.2-7.7mg Fe, 12mg Na, 444µg K, 6,410-7.850µg beta carotene equivalent 0.13-0.15mg thiamine, 0.26-0.53mg riboflavin, 1.1-1.2 mg niacin and 53-80mg ascorbic acid. Leaves contain oxydase and chlorogenic acid. The folic acid content is substantially higher than that of other folacin-rich vegetables, acid 800 micro grains per 100g (Ca 75%moisture) or Ca 3200 micro grams on a zero moisture basis [83]. The seeds contain 11.3-14.8% oil[85] reportedly estrogenic [86] which contain 16.9% palmitic, 3.7% stearic, 1.8% behenic, 1.1`% lignoceic, 9.1% oleic, 62,5% linoleic, and 0.9%linoleic acids as well as large portions of B, Mn, Mo, and

Zn. Reported to be demulcent, lactagogue, purgative, and tonic. This plant is a folk remedy for aches and pain, dysentery, fever, pectoral pains, and tumors [89,93,94].

1.10.5. *Solenostemma argel*

Solenostemma argle (Apocynaceae) is a desert plant widely distributed in Egypt with the common name ‘‘hargel’’[95], and in Sudan which is its richest source[96]. It is the most important one from the many Egyption plants which are known to be of potential medicinal value in herbal medicine[97]. An extract from the leaves of this plant showed fungitoxic activity[98]. The leaves are used in herbal medicine for the treatment of some diseases such as of liver and kidney and allergies. It is an effective remedy for bronchitis and is used to treat neuraigia and sciatica. It is used in the treatment of measles, and sometimes crushed and used as remedy for suppurating wounds. The leaves are infused to treat gastro-intestinal cramps, stomach-ache, colic, cold, and urinary tract infections and is effective as an anti-syphilitic where it is used for prolonged periods of 40-80 days [99,100]. Leaves possess purgative properties which may be due to the latex present in the stems. Several active compounds have been extracted from *S. argle*. The native Sudanese have commonly used *solenostemma argle* to suppress stomach pain, pains due to child birth, and loss of appetite. It has been proved that its crude aqueous extracts possessed larvicidal activity against *mosquito larvae*. From the previous phytochemical studies, it was found that the leaves are characterized by high carbohydrates, low crude fiber, proteins, crude oil, ash, and high potassium, calcium,

magnesium, sodium, and low copper, ferrous, manganese, lead, and contained phytic acid and tannins[101].

Figure (1.5) *Solenostemma argle*

Also *S. argel* contains acylated phenolic, glycosides, pregnene glycosides (solenoside A) kaempferol-3-o-glucoside and 3-o-rutinoside(R). Also it was found that its aerial parts contained two monoterpene glucosides, a pregnane glucoside, benzyl alcohol β-apiofuranosyl (1-6), β-glucopyranoside (1-6), β-glucopyranoside, astragalin and kaempferol-3-O-neohesperidose[101].

1.10.6. *Acacia seyal*

Acacia seyal –Mimosaceae- is native to the Sahelian zone from Senegal to Sudan, it also occurs in Egypt and eastern and southern Africa, from Somalia to Mozambique and Namibia[103.] The Seyal tree is 3-12m tall, crown flat-topped, bark powdery, white to greenish-yellow or orange-red, sparsely branched, horizontal or ascending. Pod, bark, or wood are harvested in season from tree or shrub in native habitats. Gum also obtained from native plantings,

in manner similar to that for other gum Arabic plants. This species has been reported to contain 18-20% tannin.

Wood is white to yellow-brown, finely striated with dark lines coarse-grained, soft, easy to work, polishes well, but discolors easily, with mold and is susceptible to insect attack. Trees also yield a gum of good quality. Bark contains tannins and yield red liquid extract. The leaves are important for forage and the wood is a fuel where the trees are abundant. Both, leaves and young pod are eaten.

The plant is reportedly resistant to insect attacks felled logs may be severely damaged by wood borers. The gum is believed to be aphrodisiac. The bark decoction is used for dysentery and leprosy. Tang any Ikans African tribes use the bark as a stimulant in tropical Africa. The gum is used as emollient and astringent for colds, diarrhea, hemorrhage and ophthalima. Mixed with *Acacia sieberana* DC it is used for intestinal ailments. Wood is used as fumigant for rheumatic pains, and to protect puerperal mothers from colds and fever. Eating the gum is supposed to afford some protection against bronchitis and rheumatism[103-104].

Figure (1.6) *Acacia seyal*

1.10.7. *Coriandrum sativum*

Coriandrum sativum, also known as citantro[104] or Chinese parsley, is an annual herb in the family Apiaceae. All parts of the plant are edible, but the fresh leaves and the dried seeds are the part most traditionally used in cooking. Coriander grows wild over a wide area of western Asia and southern Europe. It is hard to define exactly where this plant is wild and where it only recently established itself[106].

The coriander leaves contain vitamins: vitamin A 42%, beta carotene 36%, thiamine B_1 6% riboflavin B_2 14%, niacin B_3 7%, pantothenic acid B_5 11, % vitamin B_6 16%, folate B_9 16%, vitamin C 33%, vitamin E 17%, vitamin K 295%, and mineral like calcium 7%, iron 14%, manganese 20%, magnesium 7%, phosphorus 7%, potassium 11%, sodium 3%, zinc 5%, and other

constituents. The leaves are variously referred to as coriander leaves, dhania, Chinese parsley. The leaves have a different taste from the seeds, with citrus overtones.

Figure (1.7) *Coriandrumm sativum*

Some people may be genetically predisposed to find the leaves to have unpleasant soapy taste or a rank smell. The leaves spoil quickly when removed from the plant, and lose their aroma when dried or frozen. The dry fruits are known as coriander seeds. The seeds have a lemony citrus flavor when crushed, due to terpenes, linalool and pinene. It is described as warm, nutty, spicy, and orange flavoured[107].The roots having a deeper, more intense flavor than the leaves, coriander roots are used in a variety of Asian cuisines. One preliminary study showed that coriander essential oil is sensitive to Gram-positive and Gram–negative bacteria, including *Staphylococcus aureus*, *Enterococcus faecailis*, *Pseudomonas aeriginosa*, and *Escherichia*[108].

Aim of this study

This study was aimed to:

- Extract the oils from some key species in Sudanese ethanomedicine.
- Investigate the oil constituents via GC-MS.
- Detect the antimicrobial activity of target oils.

Materials and Methods

2-1-Materials

2-1-1-Plant materials

Prosopis juliflora seeds were collected from Shendi-Sudan. Seeds of *Pimpinella anisum, Corchorus olitorius, Medicago sativa, Coriandrum sativum, Solenostemma argel* and *Acacia seyal* were purchased from the local market-Shendi.

Prosopis juliflora seeds were collected and authenticated by the Department of Phytochemistry and Taxonomy, Institute of Medicinal and Aromatic Plants, Khartoum-Sudan.

2-1-2-Instruments

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

2-1-3-Test organisms

Prosopis juliflora, *Pimpinella anisum, Corchorus olitorius, Medicago sativa, Coriandrum sativum*, *Solenostemma argel* and *Acacia seyal* seeds oil were screened for antibacterial and antifungal activities using standard microorganisms shown in table (2-1)

Table 2-1: Test microorganisms

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

2-2-Methods

2-2-1-Extraction of oil

Dry powdered plant material (300g) was exhaustively extracted with n-hexane at room temperature for 72h. 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 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) n-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 target oils of 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 given in Table (2.2), while other chromatographic condition was depicted in Table (2.3)

Table 2.2: Oven temperature program

Rate	Temperature ©	Hold time
---	150.0	1.00
4.00	300.0	0.00

Tale 2.3: Chromatographic condition

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

2-2-3-Antimicrobial test

2-2-3-1-Preparation of bacterial suspensions

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

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9 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 the surface viable counting technique.

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

2-2-3-2-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.

2-2-3-3-Testing for antibacterial activity

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

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

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

Results and Discussion

Seven plants which are key species in Sudanese ethnomedicine have been investigated. The fixed oils of these species were extracted and studied by GC-MS. Furthermore, the oils have been assessed for antimicrobial activity via the cup plate agar diffusion bioassay against five standard human pathogens.

3.1-GC-MS analysis

3.1.1-*Prosopis juliflora*

The oil from *Prosopis juliflora* was analyzed by GC-MS. Identification of the constituents was accomplished by consulting the MS library (NIST). The observed fragmentation pattern was also interpreted.

3.1.1.1-Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 24 components (table3.1). The typical total ion chromatograms (TIC) is depicted in Fig.3.1.

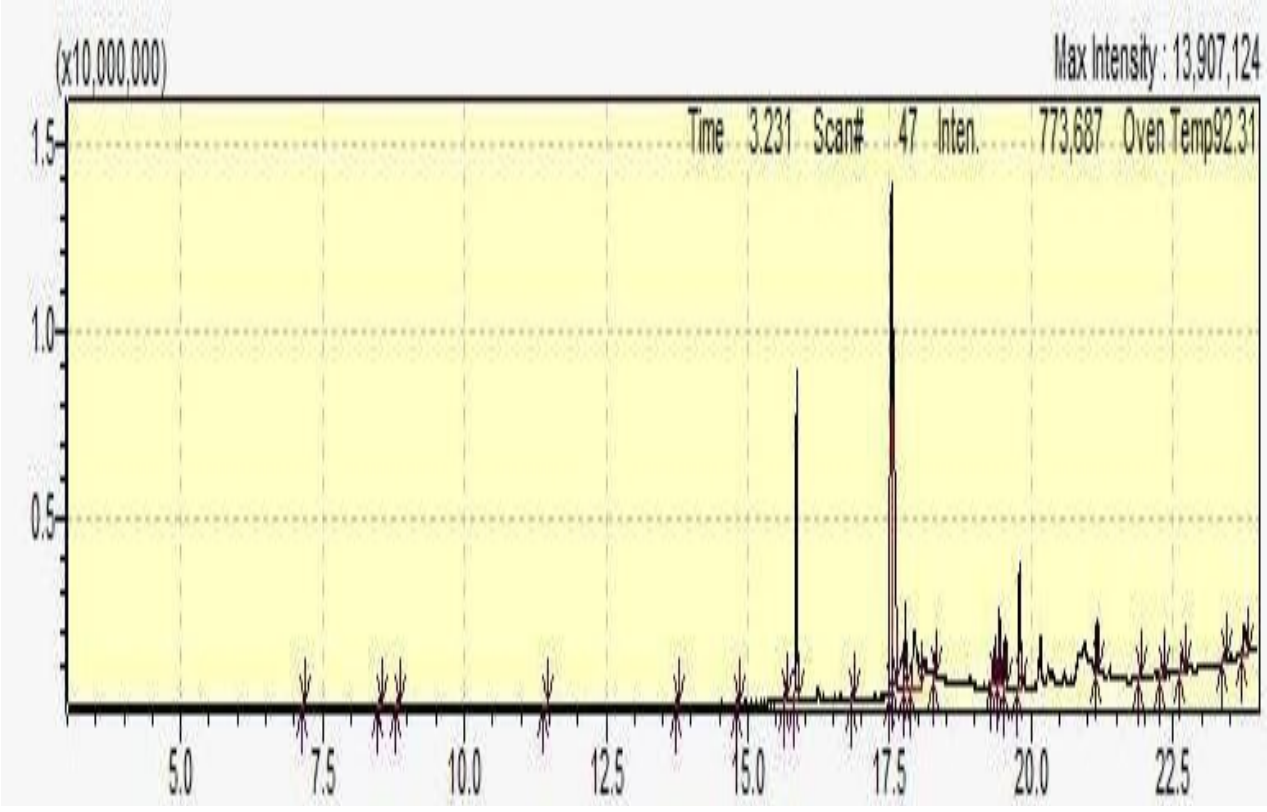

Fig.3.1: Typical total ion chromatograms

Table 3.1- Constituents of *Prosopis juliflora* oil:

ID#	Name	Ret. Time	Area	Area%
1.	.alpha.-Terpineol	7.154	86888	0.07
2.	2,4-Decadienal, (E,E)-	8.515	336658	0.29
3.	2,4-Decadienal	8.829	357584	0.30
4.	Dodecanoic acid, methyl ester	11.428	74520	0.06
5.	Methyl tetradecanoate	13.749	210956	0.18
6.	Pentadecanoic acid, methyl ester	14.825	186323	0.16
7.	9-Hexadecenoic acid, methyl ester, (Z)-	15.664	624711	0.53
8.	Hexadecanoic acid, meth yl ester	15.860	16488903	14.04
9.	Heptadecanoic acid, methyl ester	16.836	172667	0.15
10.	Methyl 10-trans,12-cis-octadecadienoate	17.537	38568516	32.83
11.	9-Octadecenoic acid, methyl ester, (E)-	17.572	22976375	19.56
12.	Methyl stearate	17.775	3615214	3.08
13.	Z,Z-8,10-Hexadecadien-1-ol	17.932	10479114	8.92
14.	Hexadecanoic acid, butyl ester	18.305	1017911	0.87
15.	11-Eicosenoic acid, methyl ester	19.333	1987895	1.69
16.	10-Octadecynoic acid, methyl ester	19.439	3097091	2.64
17.	Eicosanoic acid, methyl ester	19.534	2345210	2.00
18.	Methyl 5,13-docosadienoate	19.792	6873275	5.85
19.	Docosanoic acid, methyl ester	21.154	2571039	2.19
20.	Tricosanoic acid, methyl ester	21.921	333501	0.28
21.	9,12-Octadecadienoic acid (Z,Z)-, octyl ester	22.291	625259	0.53
22.	Tetracosanoic acid, methyl ester	22.659	1096336	0.93
23.	Squalene	23.402	520105	0.44
24.	gama,-Ergosterol	23.762	2830762	2.41

Some important constituents are discussed below:

Methyl -10-trans-12-cis-Octadecadienoate (32.83%)

The EI mass spectrum of methyl-10-trans-12-cis-octadecadienoate is shown in Fig.3.2. The peak at m\z 294 which appeared at R. T 17.537 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m\z 263 corresponds to loss methoxyl function.

9-Octadecadienoic acid methyl ester (E) (19.56%)

The mass spectrum of 9. octadecadienoic acid methyl esters is shown in Fig.3.3. The peak at m\z 296 which appeared at R. T 17.572 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]$ The peak at m\z 264 corresponds to loss of methoxyl function.

Hexadecanoic acid methyl ester (14.04%)

The mass spectrum of hexadecanoic acid methyl esters is depicted in Fig.3.4. The peak at m\z 270, which appeared at R.T.15.860 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

Hexadecanoic acid (Palmitic acid) is saturated fatty acid. It is wide spread in plants and human. The acid is produced first during the synthesis of fatty acid and is considered as precursor of long chain fatty acid. Palmitic acid is a major lipid components of human breast milk. The acid finds applications in soap and Cosmetics industries, it is also used in food industry.

Z, Z-8,10-Hexadecadien-1-ol (8.92%)

Fig.3.5 shows the mass spectrum of z, z-8,10-hexadecadien-1-ol. The signal at m\z 238, which appeared at R.T.17.932 corresponds to $M^+[C_{16}H_{30}O]^+$ while the peak at m\z 185 is attributed to loss of methoxyl function.

Methyl 5,13- Docosadienoate (5.85 %)

The mass spectrum of methyl 5,13- docosadienoate is shown in Fig.3.6. The peak at m\z 350, which appeared at RT 19.792 correspond $M^+[C_{23}H_{42}O_2]^+$.The peak at m\z 318 corresponds to loss of methoxyl function.

Methyl stearate (3.08%)

The EI mass spectrum of methyl stearate is shown in Fig.3.7. The peak at m\z 298which appeared at R.T. 17.775 corresponds to $M^+[C_{19}H_{38}O_2]$. The peak at m\z 267 corresponds to loss of methoxyl function.

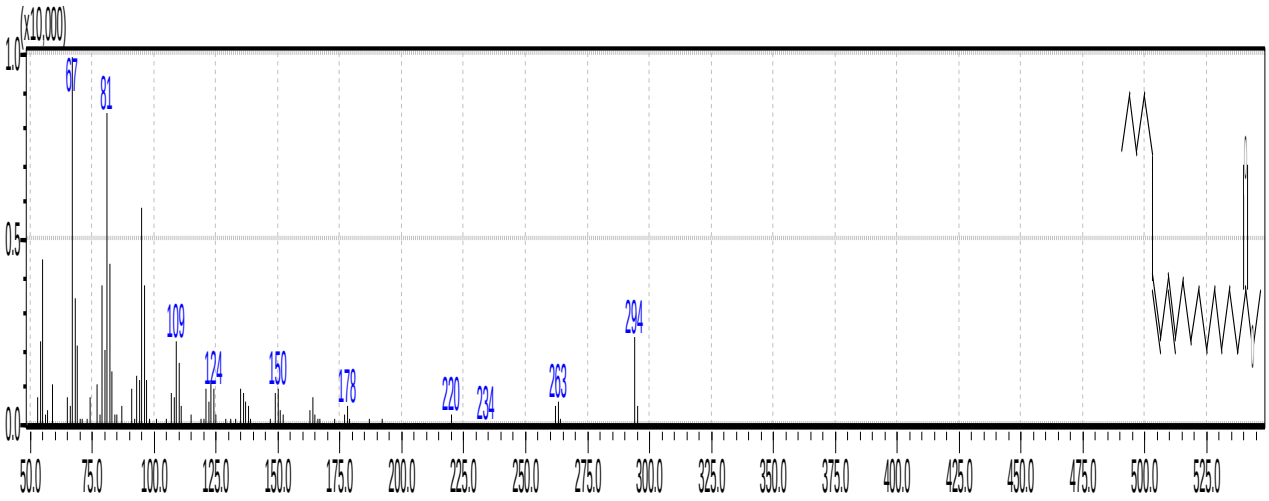

Fig.3.2. Mass spectrum of Methyl 10-trans,12-cis- octadecadienoate

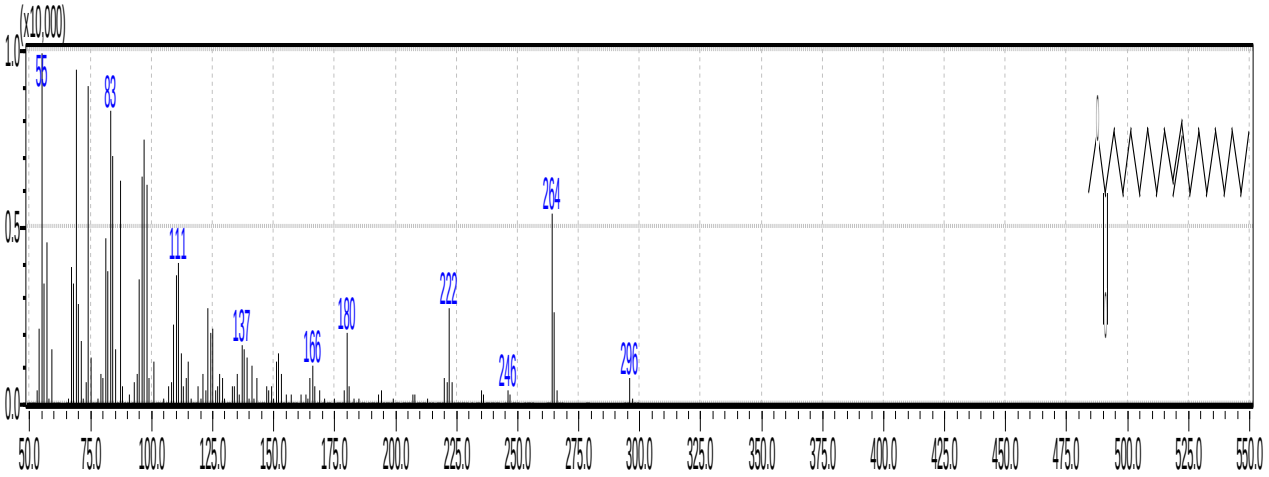

Fig.3.3. Mass spectrum of 9- octadecenoic acid, methyl ester.

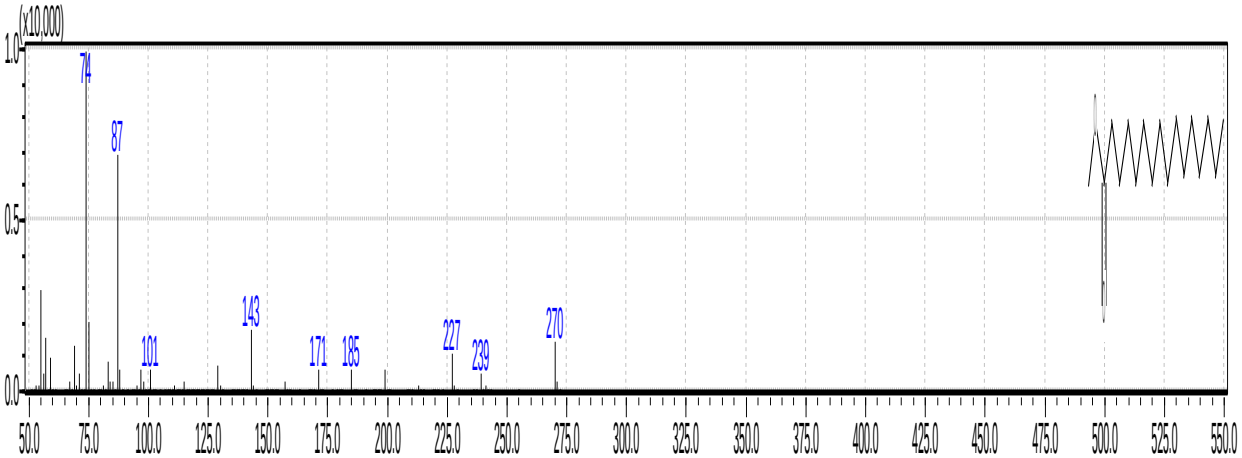

Fig.3.4. Mass spectrum of hexadecanoic acid methyl ester.

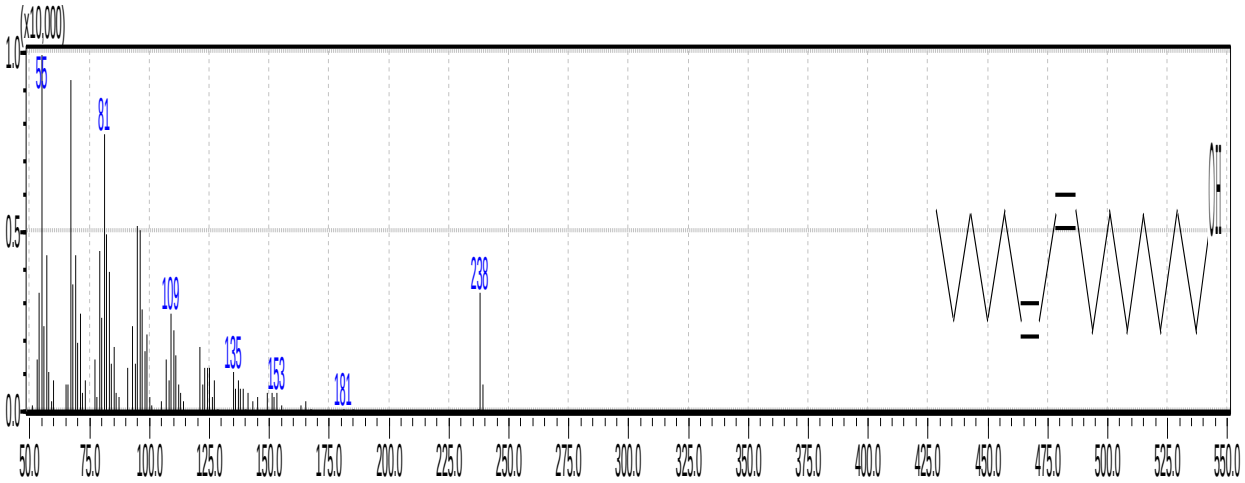

Fig.3.5. Mass spectrum of z,z-8,10-hexadecadien-1-ol

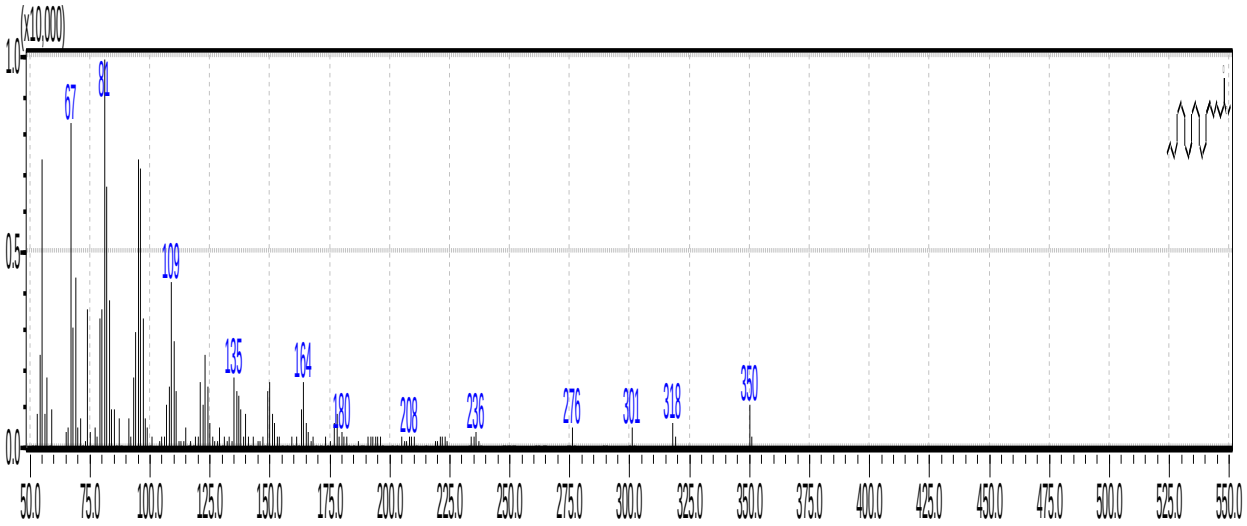

Fig.3.6. Mass spectrum of methyl 5,13-docosadienoate

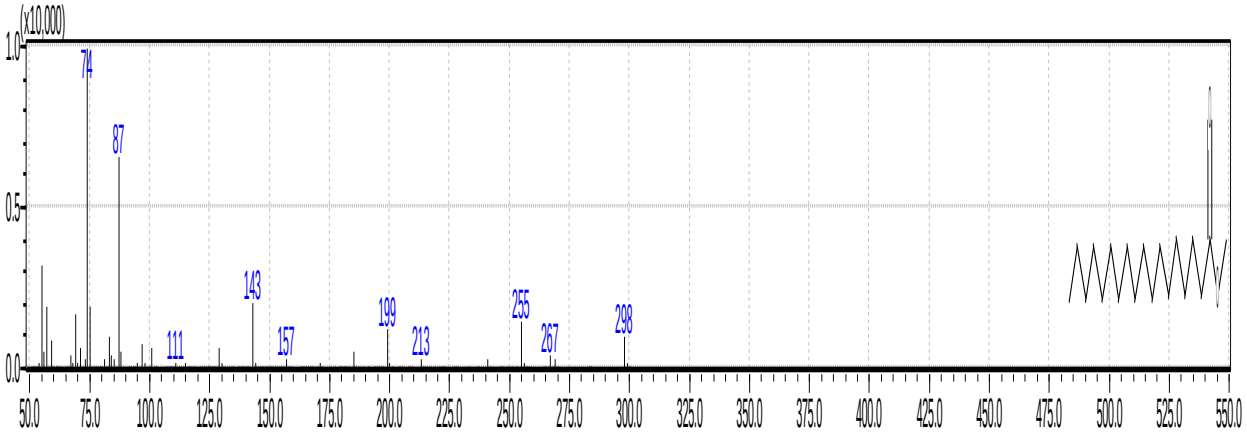

Fig.3.7. Mass spectrum of methyl stearate

3.1.2-*Acacia seyal*

GC-MS analysis of *Acacia seyal* oil has revealed the presence of 41 components Table.3.2. The typical total ion chromatograms(TIC) is depicted in Fig.3.8.

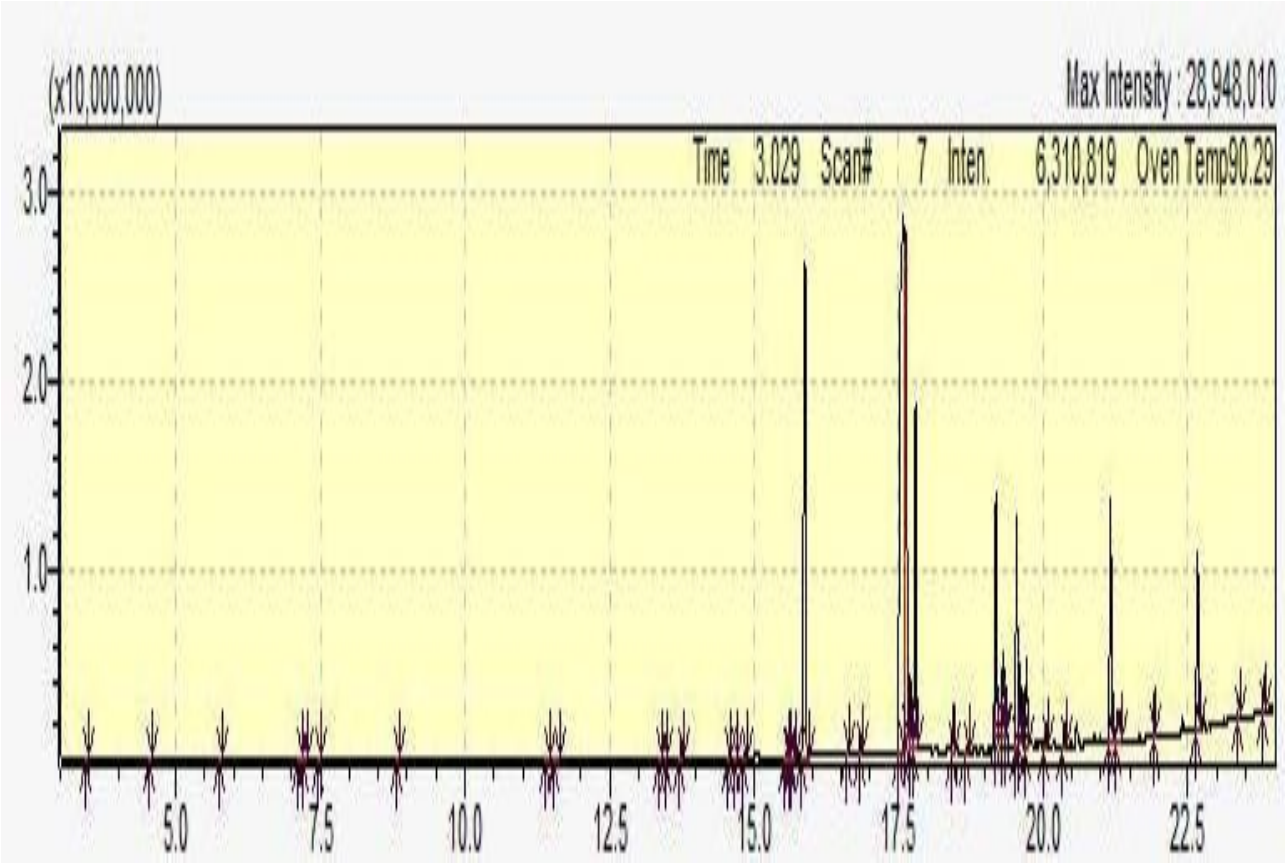

Fig.3.8. Typical total ion chromatograms

Table.3.2: Constituents of *Acacia seyal* oil

ID#	Name	Ret. Time	Area	Area%
1.	Hexanoic acid, methyl ester	3.460	63984	0.01
2.	6-Heptenoic acid, methyl ester	4.581	25168	0.01
3.	Benzoic acid, methyl ester	5.775	38588	0.01
4.	.alpha.-Terpineol	7.152	185218	0.04
5.	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-	7.240	32807	0.01
6.	Nonanoic acid, methyl ester	7.479	37804	0.01
7.	Decanoic acid, methyl ester	8.856	29426	0.01
8.	Dodecanoic acid, methyl ester	11.425	130397	0.03

9.	1-Naphthalenol	11.578	134504	0.03
10.	Methyl 5,11,14-eicosatrienoate	13.396	61876	0.01
11.	cis-5-Dodecenoic acid, methyl ester	13.475	43982	0.01
12.	Methyl tetradecanoate	13.750	1429960	0.30
13.	5-Octadecenoic acid, methyl ester	14.563	259361	0.05
14.	6-Octadecenoic acid, methyl ester, (Z)-	14.667	107683	0.02
15.	Pentadecanoic acid, methyl ester	14.829	601481	0.13
16.	7,10-Hexadecadienoic acid, methyl ester	15.561	342474	0.07
17.	9-Hexadecenoic acid, methyl ester, (Z)-	15.623	925120	0.20
18.	cis-10-Nonadecenoic acid, methyl ester	15.666	2147248	0.45
19.	Hexadecanoic acid, methyl ester	15.882	6798847	14.38
20.	cis-10-Heptadecenoic acid, methyl ester	16.629	1209289	0.26
21.	Heptadecanoic acid, methyl ester	16.840	1510407	0.32
22.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.583	1474723	31.18
23.	9-Octadecenoic acid (Z)-, methyl ester	17.619	6309372	13.34
24.	Phytol	17.706	5494922	1.16
25.	Methyl stearate	17.793	3642122	7.70
26.	Hexadeca-2,6,10,14-tetraen-1-ol,3,7,11, 16-tetramethyl-	18.447	4281397	0.91
27.	Nonadecanoic acid, methyl ester	18.677	440187	0.09
28.	Tridecanedial	19.186	2375343	5.02
29.	Oxiraneoctanoic acid, 3-octyl-, methyl ester	19.306	8406739	1.78
30.	11-Eicosenoic acid, methyl ester	19.343	4005619	0.85
31.	Eicosanoic acid, methyl ester	19.543	2310450	4.89
32.	PGH1, methyl ester	19.603	7111848	1.50
33.	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	19.713	8292833	1.75
34.	Methyl 15-hydroxy-9,12-octadecadienoate	20.051	2982991	0.63
35.	Heneicosanoic acid, methyl ester	20.366	2118192	0.45
36.	Docosanoic acid, methyl ester	21.168	2577015	5.45
37.	Tetrapentacontane	21.316	6276421	1.33
38.	Tricosanoic acid, methyl ester	21.929	4078163	0.86
39.	Tetracosanoic acid, methyl ester	22.671	1803974	3.81
40.	Methyl 22-methyl-tetracosanoate	23.385	1530849	0.32
41.	Dotriacontane	23.818	2937863	0.62

Some major constituents are discussed below:

9,12-Octadecadienoic acid (z,z)methyl esters (31.18%)

Mass spectrum of 9.12 octadecadienoic acid (z,z) methyl esters is depicted in Fig.3.9.The peak at m\z 294 which appeared at R.T 17.583 corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The at m\z 263 corresponds of loss methoxyl function.

Hexadecanoic acid methyl ester (14.38%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.10. The peak at m\z 270, which appeared at R.T.15.882 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

9-Octadecenoic acid(Z)methyl ester (13.34%)

The mass spectrum of 9-octadecenoic acid(z)methyl ester is shown in Fig.3.11. The peak at m\z 296 which appeared at R.T.17.619 corresponds to $M^+[C_{19}H_{36}O_2]$. The peak at m\z 264 is corresponds to loss of methoxyl function.

Methyl stearate (7.70%)

Mass spectrum of methyl stearate is shown in Fig.3.12. The peak at m\z 298 which appeared at R.T. 17.793 corresponds to $M^+[C_{19}H_{38}O_2]$. The peak at m\z 267 corresponds to loss of a methoxyl function.

Docosanoic acid, methyl ester (5.45%)

Fig.3.13 displays the mass spectrum of docosanoic acid, methyl. The peak at m\z 354 which appeared at R.T.21.168 corresponds to $M^+[C_{23}H_{46}O_2]^+$. The peak at m\z 323 corresponds loss of methoxyl function.

Tridecanedial (5.02%)

Mass spectrum of tridecanedial shown in Fig.3.14. The peak at m\z 213 which appeared at R.T.19.186 corresponds to $M^+[C_{13}H_{24}O_2]$. The peak at m\z 194.

Eicosanoic acid, methyl ester (4.89%)

Fig.3.15 shows the mass spectrum of eicosanoic acid, methyl ester. The peak at m\z 326 which appeared at R.T.19.543 corresponds to $M^+[C_{21}H_{42}O_2]^+$. The peak at m\z 263.

Tetracosanoic acid, methyl ester (3.81%)

EI mass spectrum of tetracosanoic acid, methyl ester depicted in Fig.3.16. The peak at m\z 382 which appeared at R.T.22.671 corresponds to $C_{25}H_{50}O_2$. The peak at m\z 351 corresponds to loss of methoxyl function.

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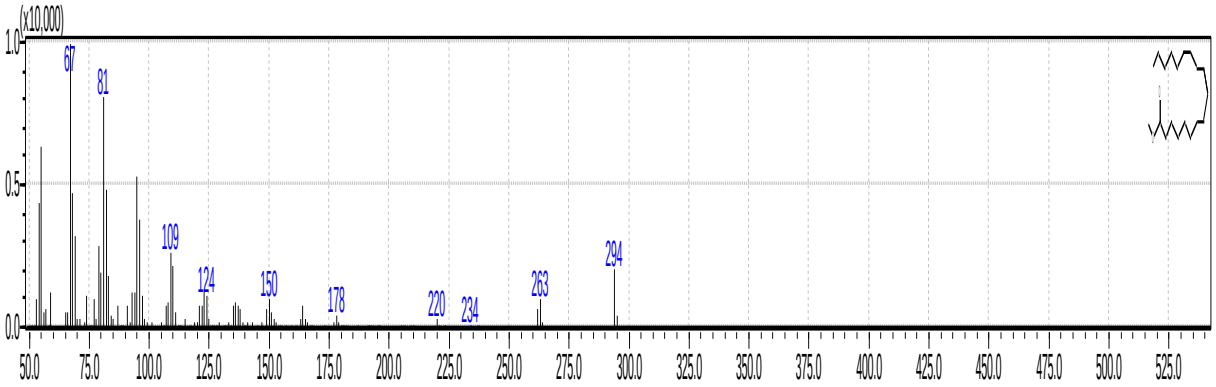

Fig.3.9. Mass spectrum of 9-12octadienoic acid(z,z)methyl ester.

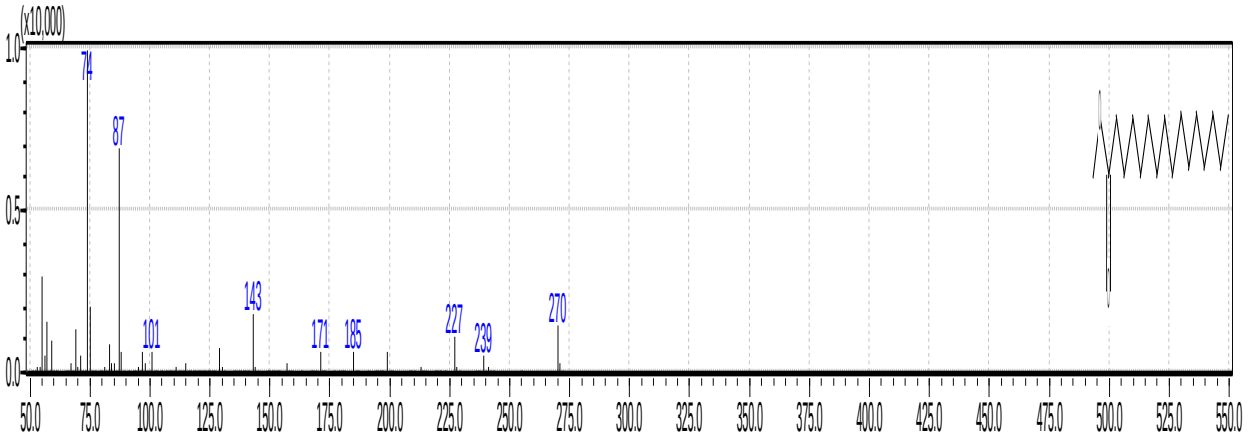

Fig.3.10. Hexadecanoic acid methyl ester.

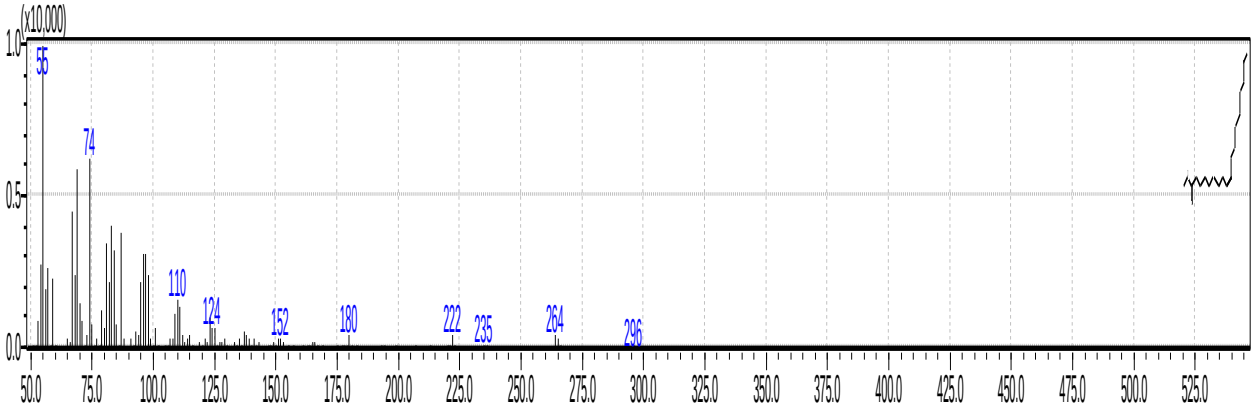

Fig.3.11. 9-Octadecenoic acid (Z), methyl

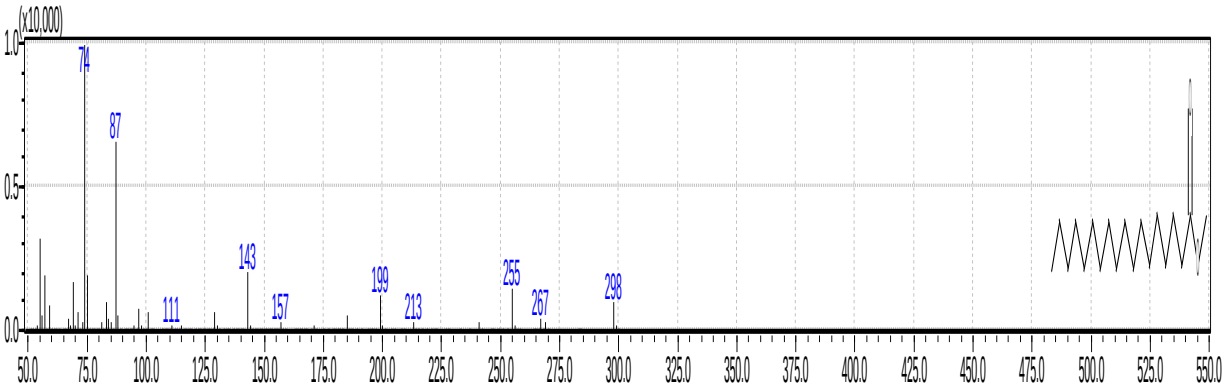

Fig.3.12. Mass spectrum of methyl stearate

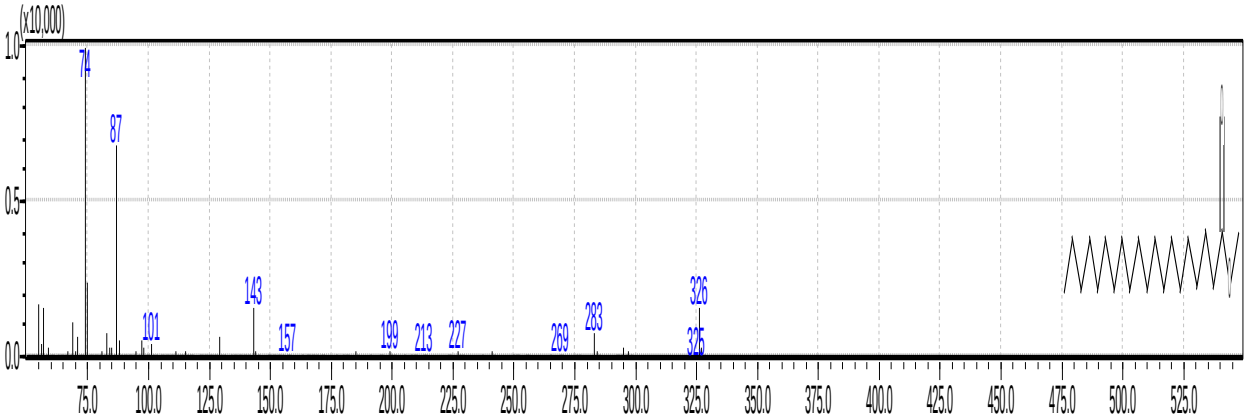

Fig.3.13. Mass spectrum of docosanoic acid, methyl ester

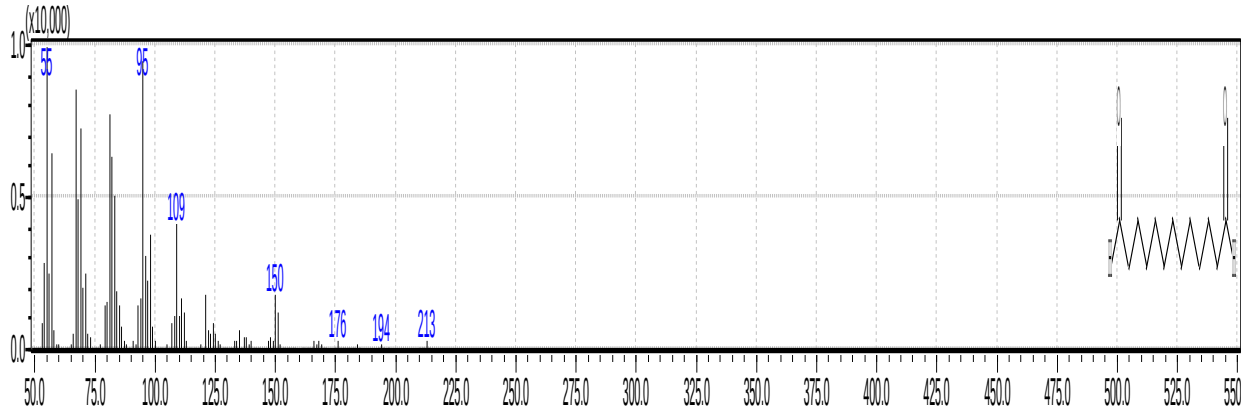

Fig.3.14. Mass spectrum of tridecanedial

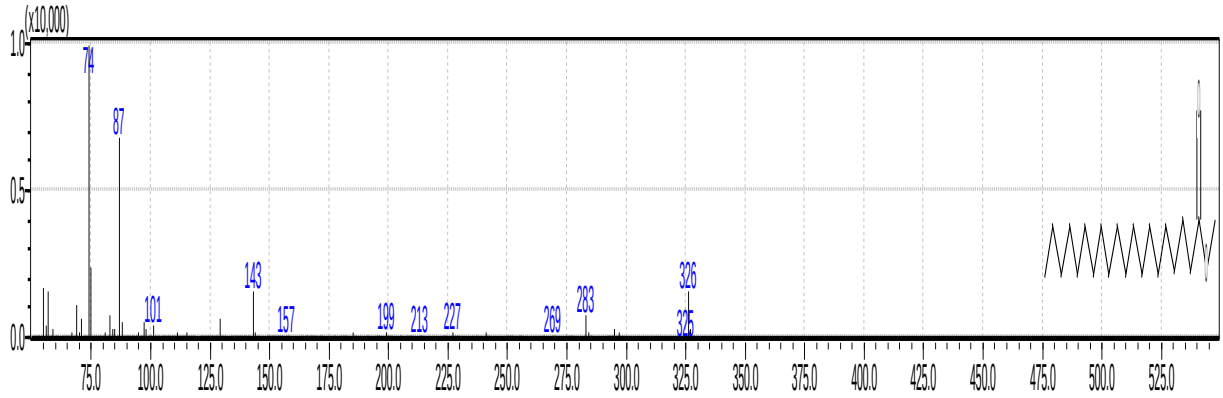

Fig.3.15. Mass spectrum of eicosanoic acid methyl ester

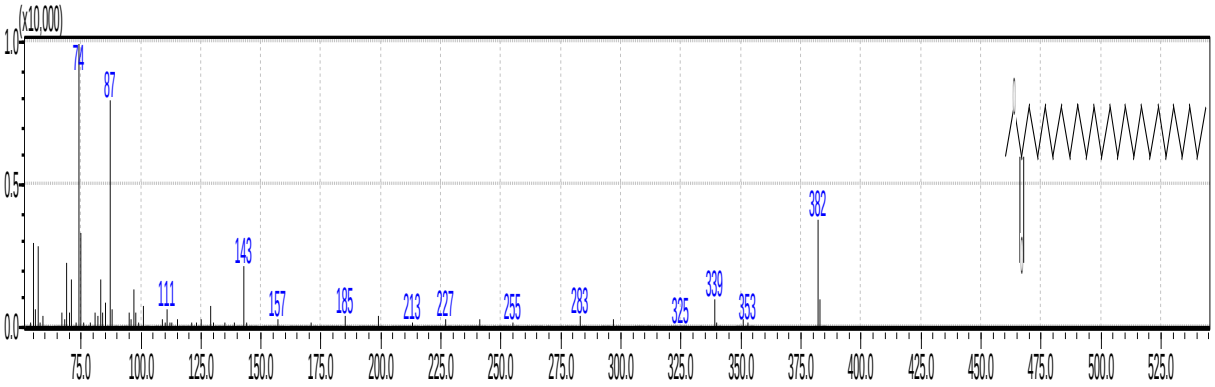

Fig.3.16. Mass spectrum of tetracosanoic acid methyl ester

3.1.3-*Solenostemma argel*

GC-MS analysis of *Solenostemma argel* oils was conducted. It revealed the presence of 31 components table.3.3. The typical total ion chromatograms(TIC) is depicted in Fig.3.17 .

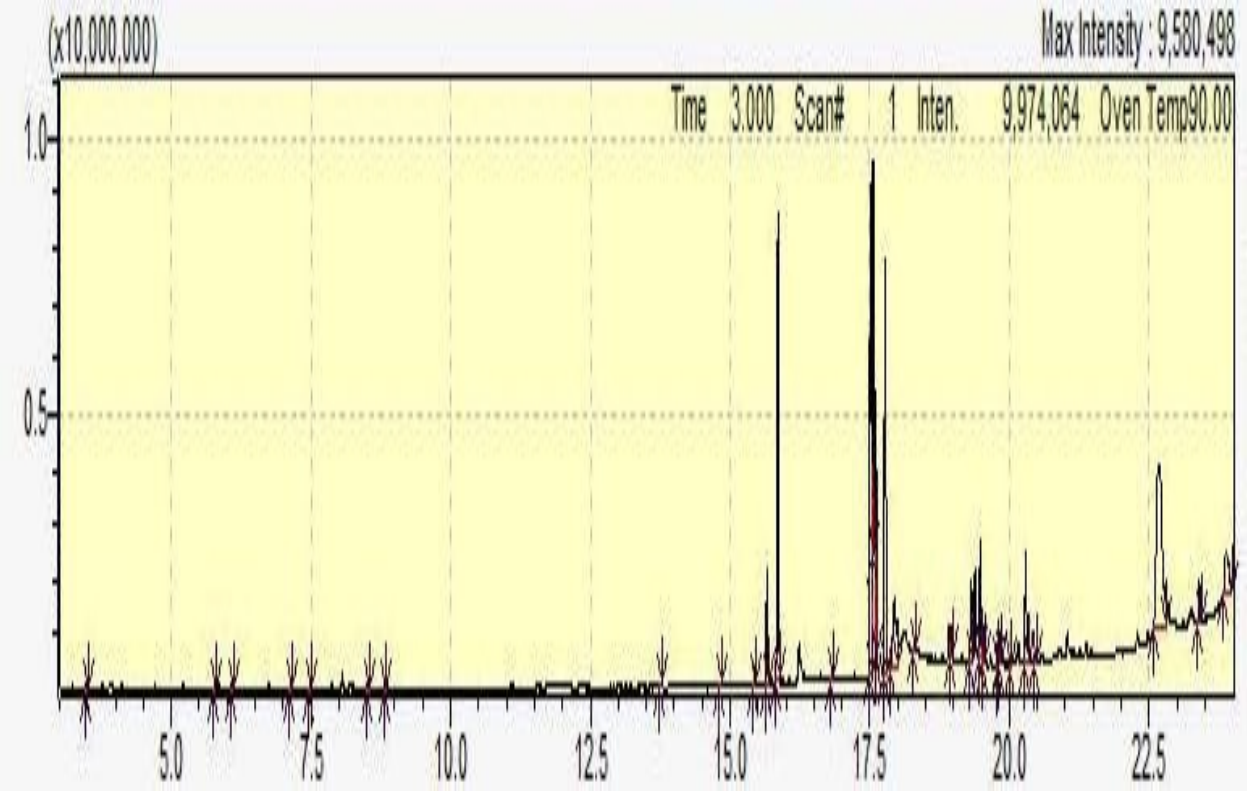

Fig.3.17. Typical total ion chromatogram

Table.3.3 Constituents of *Solenostemma argel* oil

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.480	144785	0.12	Hexanoic acid, methyl ester
2	5.783	69690	0.06	1,6-Octadien-3-ol, 3,7-dimethyl-
3	6.085	103725	0.09	Octanoic acid, methyl ester
4	7.151	155514	0.13	L-.alpha.-Terpineol
5	7.486	270614	0.22	Nonanoic acid, methyl ester
6	8.512	210736	0.17	2,4-Decadienal
7	8.827	351695	0.29	2,4-Decadienal, (E,E)-
8	13.746	110588	0.09	Methyl tetradecanoate
9	14.823	122182	0.10	Pentadecanoic acid, methyl ester
10	15.440	598062	0.49	Tonalid
11	15.661	3181745	2.62	9-Hexadecenoic acid, methyl ester, (Z)-
12	15.857	15443492	12.72	Hexadecanoic acid, methyl ester
13	16.832	286107	0.24	Heptadecanoic acid, methyl ester
14	17.516	19072090	15.71	9,12-Octadecadienoic acid (Z,Z)-, methyl e
15	17.562	11943428	9.84	9-Octadecenoic acid (Z)-, methyl ester
16	17.608	7395521	6.09	9-Octadecenoic acid, methyl ester, (E)-
17	17.776	14348504	11.82	Methyl stearate
18	17.941	5359711	4.42	Oleic Acid
19	18.302	461279	0.38	Hexadecanoic acid, butyl ester
20	18.951	840076	0.69	4,8-Ethano-4H-1,3-benzodioxin, hexahydr
21	19.318	2492312	2.05	4-Oxo-.beta.-isodamascol
22	19.476	3368614	2.78	Octanoic acid, 2-propenyl ester
23	19.531	954705	0.79	Eicosanoic acid, methyl ester
24	19.791	620150	0.51	n-Propyl 9,12-octadecadienoate
25	19.818	846611	0.70	2,3-Dihydroxypropyl elaidate
26	20.015	324356	0.27	Octadecanoic acid, butyl ester
27	20.289	4440203	3.66	Octadecanoic acid, 9,10-dihydroxy-, methy
28	20.464	393729	0.32	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
29	22.686	22120646	18.23	7-Hexadecenal, (Z)-
30	23.400	1194043	0.98	Squalene
31	23.875	4143332	3.41	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
		121368245	100.00	

Some important constituents are discussed below:

7-Hexadecenal, (z) (18.23%)

The EI mass spectrum of 7-Hexadecenal, (z) is shown in Fig.3.18. The peak at m\z 238 which appeared at R.T. 22.686 corresponds to $M^+[C_{16}H_{30}O]^+$.The peak at m\z 220.

9,12 Octadecadienoic acid (z,z)methyl ester (15.71%)

Mass spectrum of 9.12 octadecadienoic acid (z,z) methyl ester is depicted in Fig.3.19.The signal peak at m\z 294 which appeared at R.T 17.516 corresponds to $M^+[C_{19}H_{34}O_2]^+$. The at m\z 263 corresponds of loss methoxyl function.

Hexadecanoic acid methyl ester (12.72 %)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.20. The peak at m\z 270, which appeared at R.T.15.857 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

Methyl stearate (11.82%)

Mass spectrum of methyl stearate is shown in Fig.3.21. The peak at m\z 298which appeared at R.T. 17.775 corresponds to $M^+[C_{19}H_{38}O_2]$. The peak at m\z 267 corresponds to loss of methoxyl function.

9-Octadecenoic acid (Z), methyl ester (9.84%)

Fig.3.22 displays the mass spectrum of 9-octadecenoic acid (z)-, methyl ester. The peak at m\z 296which appeared at R.T.

17. 562 corresponds to $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z 264 is attributed to loss of methoxyl function**.**

9-Octadecenoic acid, methyl ester, (E) (6.09%)

Fig.3.23 shows the mass spectrum of 9-octadecenoic acid, methyl ester. The peak at m\z 296which appeared at R.T.17. 608 corresponds to$M^+[C_{19}H_{36}O_2]^+$. The peak at m\z 264 accounts to loss of methoxyl function**.**

Oleic Acid (4.42%)

Mass spectrum of Oleic acid) is shown in Fig.3.24. The peak at m\z 282 which appeared at R.T 17.941 corresponds to$C_{18}H_{34}O_{2.}$ The peak at m\z 264.

Octadecanoic acid,9,10-dihydroxy-, methyl ester (3.66%)

Mass spectrum of octadecanoic acid,9,1.0-dihydroxy-, methyl is shown in Fig.3.25. The peak at m\z 330 which appeared at R.T 20.289 corresponds to $C_{19}H_{38}O_{4.}$ The peak at m\z 281.

E,E,Z-1,3,12-Nonadecatriene-5,14-diol (3.41%)

Mass spectrum E, E, Z-1,3,12-Nonadecatriene-5,14-diol is shown in Fig.3.26. The peak at m\z 294 which appeared at R.T 23.875 corresponds to $C_{19}H_{38}O_{4.}$ The peak at m\z 262 corresponds to loss of methoxyl function**.**

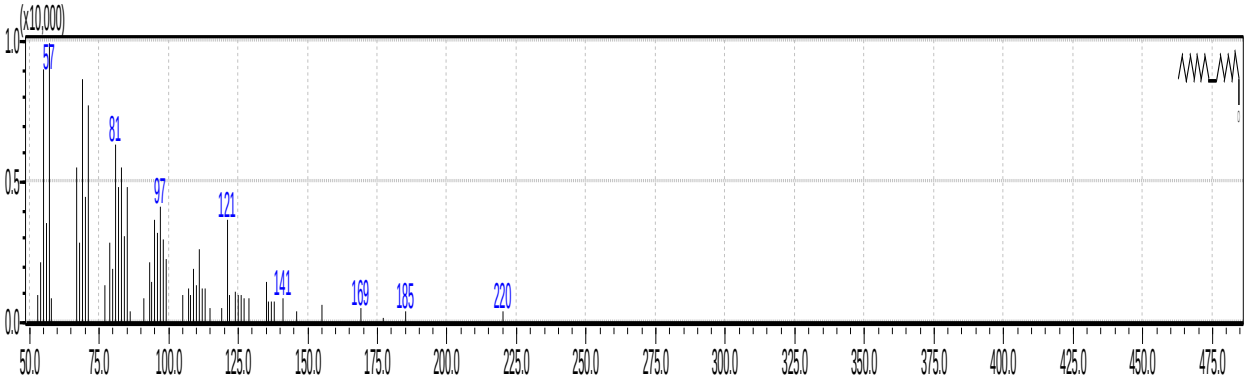

Fig.3.18: Mass spectrum of 7-Hexadecenal, (z)

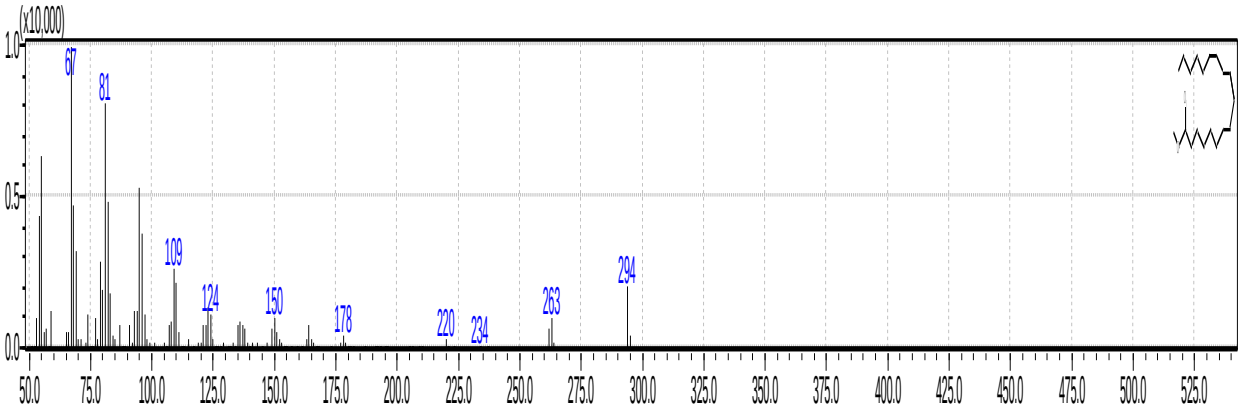

Fig.3.19: Mass spectrum of 9-12 octadienoic acid(z,z) methy ester.

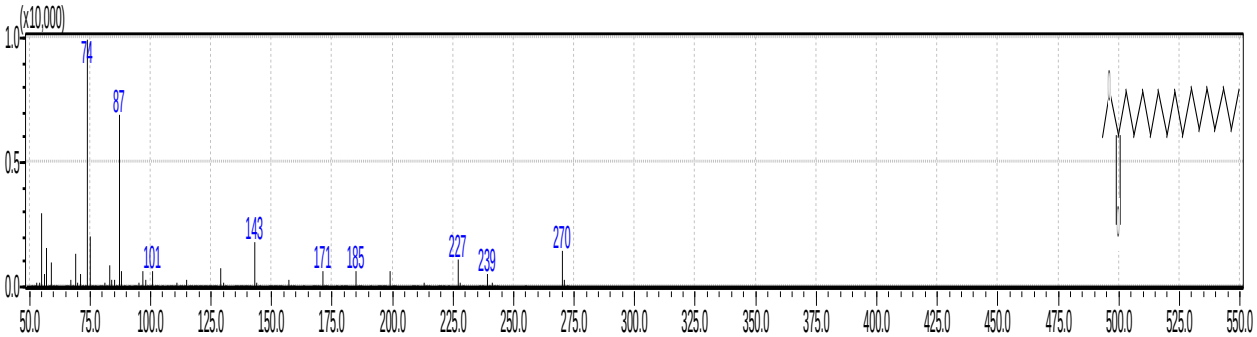

Fig.3.20: Mass spectrum of hexadecanoic acid methyl ester.

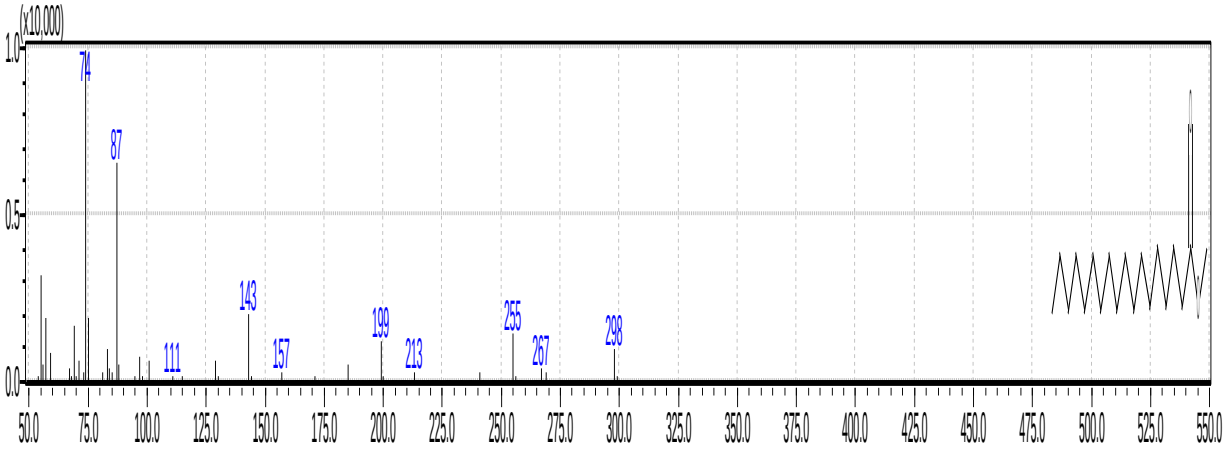

Fig.3.21: Mass spectrum of methyl stearate

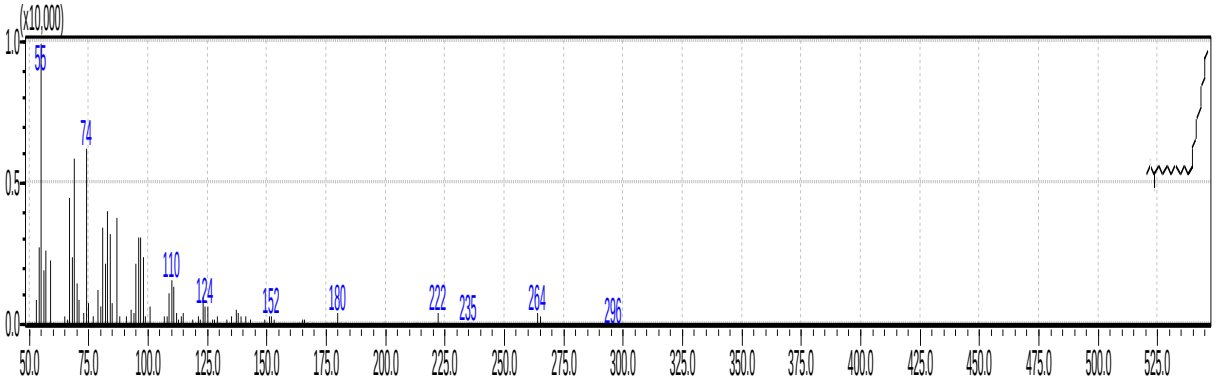

Fig.3.22: Mass spectrum of 9-octadecenoic acid (z)-, methyl ester

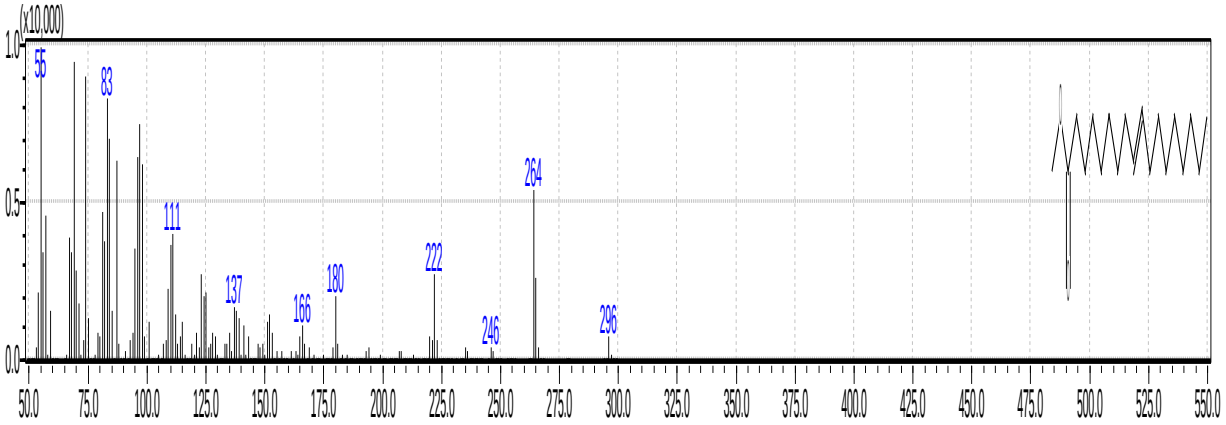

Fig.3.23: Mass spectrum of 9-octadecenoic acid, methyl ester(E)

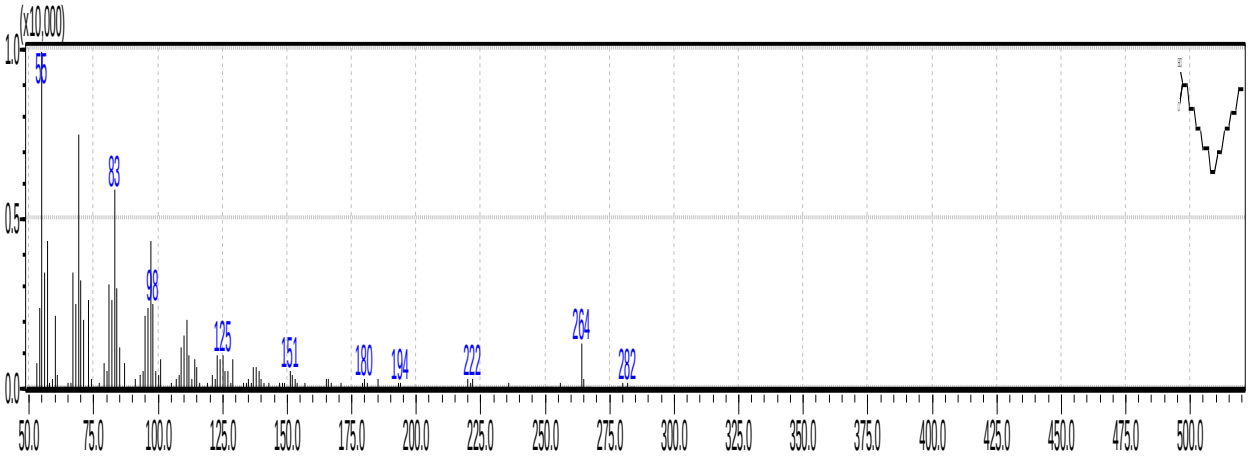

Fig.3.24: Mass spectrum of oleic acid

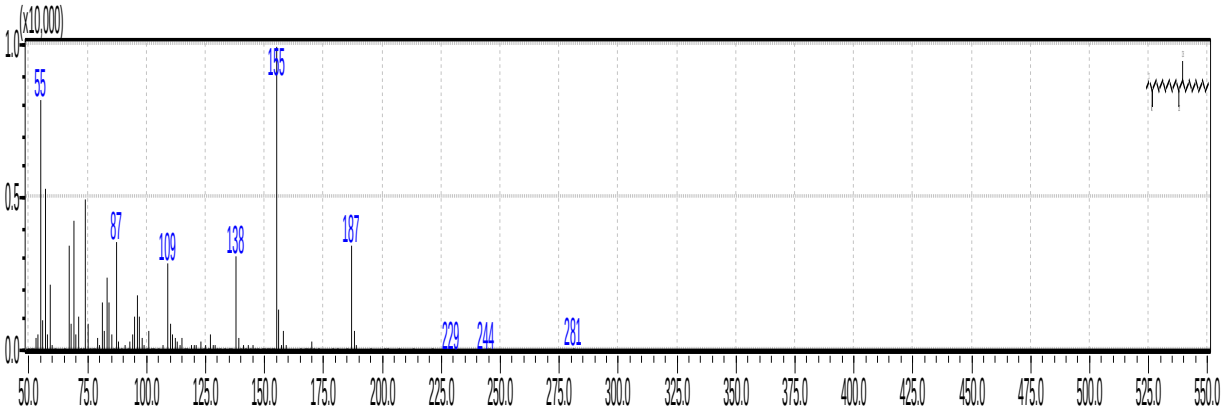

Fig.3.25: Mass spectrum of octadecanoic acid,9,10-dihydroxy-, methyl ester

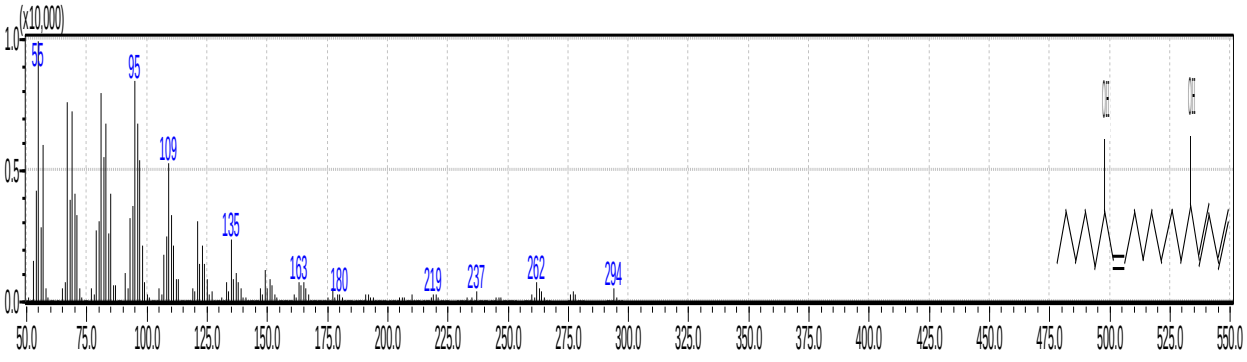

Fig.3.26. Mass spectrum E,E,Z-1,3,12-Nonadecatriene-5,14-diol

3.1.4-*Coriandrumm sativum*

GC-MS analysis of *Coriandrumm sativum* oil revealed the presence of 52 components table 3.4. The typical total ion chromatograms(TIC) is depicted in Fig.3.27

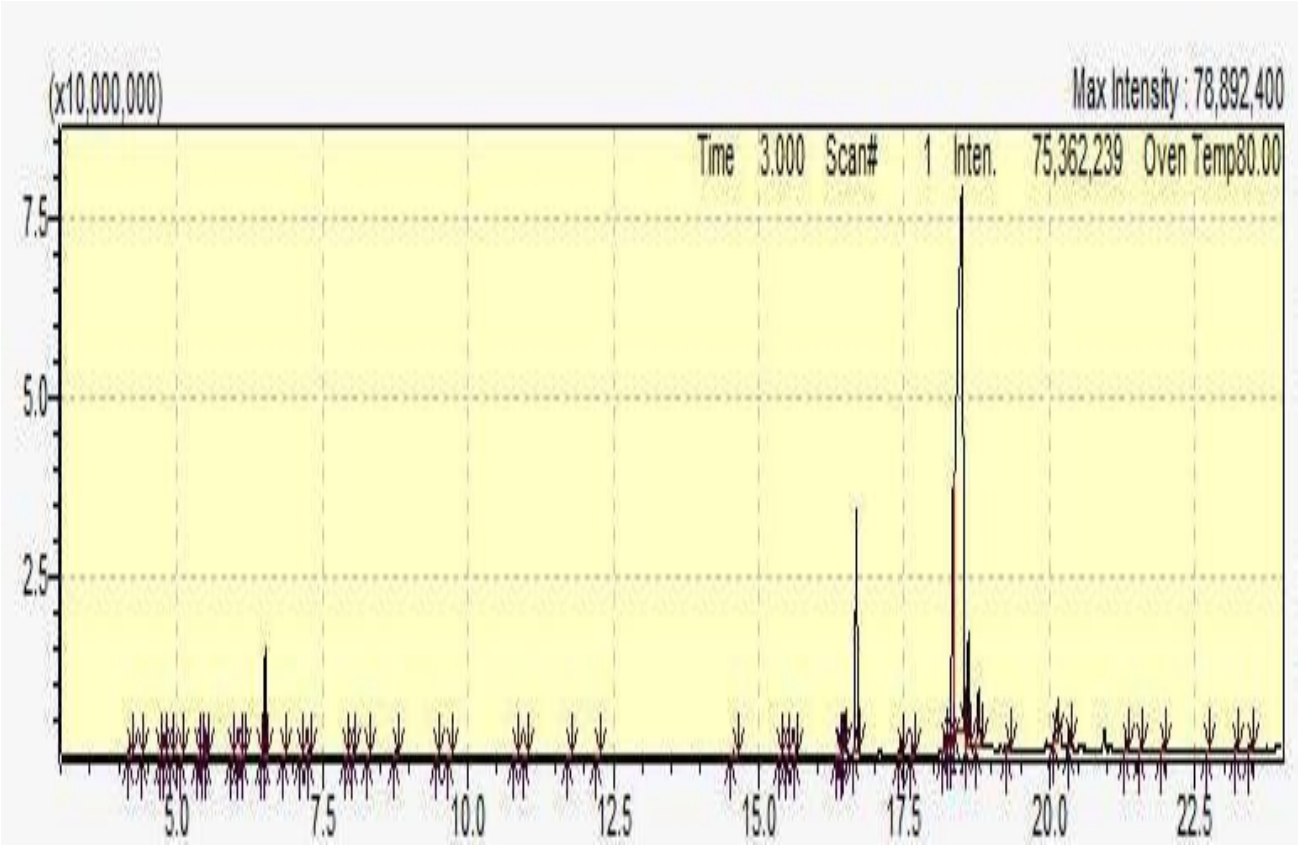

Fig.3.27. Typical total ion chromatograms

Table 3.4: Constituents of *Coriandrumm sativum* oil

ID#	Name	Ret.Time	Area	Area%
1.	.alpha.-Pinene	4.186	3414165	0.41
2.	Camphene	4.402	415376	0.05
3.	Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl)-	4.720	95322	0.01
4.	.beta.-Pinene	4.788	327293	0.04
5.	.beta.-Myrcene	4.910	624749	0.07
6.	Octanal	5.092	103650	0.01
7.	Heptanoic acid, methyl ester	5.377	66410	0.01
8.	p-Cymene	5.442	123215	0.01
9.	D-Limonene	5.503	1401622	0.17
10.	.gamma.-Terpinene	5.936	470879	0.06

11.	1-Octanol	6.076	62489	0.01
12.	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	6.146	33725	0.00
13.	Undecane	6.460	268865	0.03
14.	1,6-Octadien-3-ol, 3,7-dimethyl-	6.516	22273211	2.67
15.	Octanoic acid, methyl ester	6.839	120323	0.01
16.	Methyl 1-cyclohexene-1-carboxylate	7.129	104561	0.01
17.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	7.277	2442106	0.29
18.	L-.alpha.-Terpineol	7.919	454710	0.05
19.	Decanal	8.042	520174	0.06
20.	Nonanoic acid, methyl ester	8.283	125492	0.02
21.	Geraniol	8.756	1326269	0.16
22.	Undecanal	9.466	187747	0.02
23.	Decanoic acid, methyl ester	9.674	423212	0.05
24.	Dodecanal	10.821	415488	0.05
25.	Undecanoic acid, methyl ester	10.994	102231	0.01
26.	1,E-11,Z-13-Octadecatriene	11.747	88424	0.01
27.	Dodecanoic acid, methyl ester	12.250	484844	0.06
28.	Methyl tetradecanoate	14.567	1670774	0.20
29.	5-Octadecenoic acid, methyl ester	15.376	65185	0.01
30.	4-Octadecenoic acid, methyl ester	15.483	110150	0.01
31.	Pentadecanoic acid, methyl ester	15.642	1088860	0.13
32.	7,10-Hexadecadienoic acid, methyl Ester	16.371	226122	0.03
33.	7-Hexadecenoic acid, methyl ester, (Z)-	16.450	6455686	0.77
34.	9-Hexadecenoic acid, methyl ester, (Z)-	16.474	7444450	0.89
35.	Hexadecanoic acid, methyl ester	16.679	66947485	8.04
36.	14,17-Octadecadienoic acid, methyl Ester	17.437	3778590	0.45
37.	Heptadecanoic acid, methyl ester	17.649	1553399	0.19
38.	6,9-Octadecadienoic acid, methyl Ester	18.203	6179904	0.74
39.	5,8-Octadecadienoic acid, methyl Ester	18.252	1760109	0.21

40.	9,12-Octadecadienoic acid (Z,Z)-, ester	18.370	11787077	14.15
41.	9-Octadecenoic acid (Z)-, methyl Ester	18.498	51314698	61.62
42.	Methyl stearate	18.608	25988268	3.12
43.	6-Octadecenoic acid, (Z)-	18.783	16807311	2.02
44.	cis-10-Nonadecenoic acid, methyl ester	19.280	2514817	0.30
45.	cis-11-Eicosenoic acid, methyl ester	20.117	11137876	1.34
46.	Eicosanoic acid, methyl ester	20.340	3446015	0.41
47.	Ethyl stearate, 9,12-diepoxy	21.323	2176449	0.26
48.	9,12-Octadecadienoyl chloride, (Z,Z)-	21.572	2054112	0.25
49.	Docosanoic acid, methyl ester	21.957	1496863	0.18
50.	Tricosanoic acid, methyl ester	22.724	298620	0.04
51.	Heneicosane	23.201	640730	0.08
52.	Tetracosanoic acid, methyl ester	23.459	1720021	0.21

Some important Constituents are discussed below:

9-Octadecenoic acid(z)-, methyl ester (61.62%)

The EI mass spectrum of 9-octadecenoic acid(z)-, methyl ester is shown in Fig.3.28. The peak at m\z 296 which appeared at R.T. 18.498 corresponds to $M^+[C_{19}H_{36}O_2]^+$.The peak at m\z 264 corresponds to loss methoxyl function.

9,12-Octadecadienoic acid (z,z)methyl ester (14.15%)

Mass spectrum of 9.12 octadecadienoic acid (z,z) methyl ester is depicted in Fig.3.29.The peak at m\z 294 which appeared at R.T 18.370 corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The at m\z 263 corresponds of loss methoxyal function.

Hexadecanoic acid methyl ester (8.04%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.30. The peak at m\z 270, which appeared at R.T.16.679 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

Methyl stearate (3.12%)

Mass spectrum of methyl stearate is shown in Fig.3.31. The peak at m\z 298which appeared at R.T. 18.608 corresponds to $M^{+}[C_{19}H_{38}O_{2}]$. The peak at m\z 267 corresponds to loss of methoxyl function.

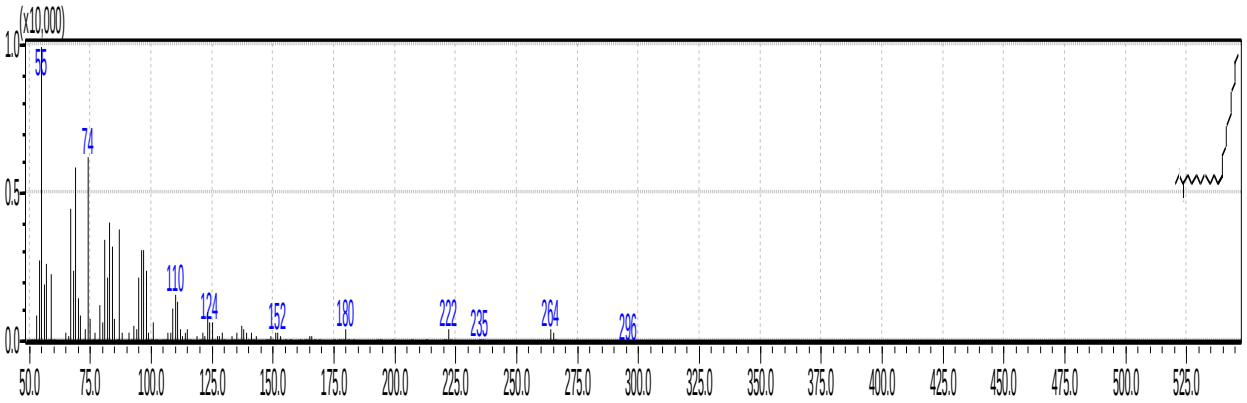

Fig.3.28. Mass spectrum of 9-octadecenoic acid(z)-, methyl ester

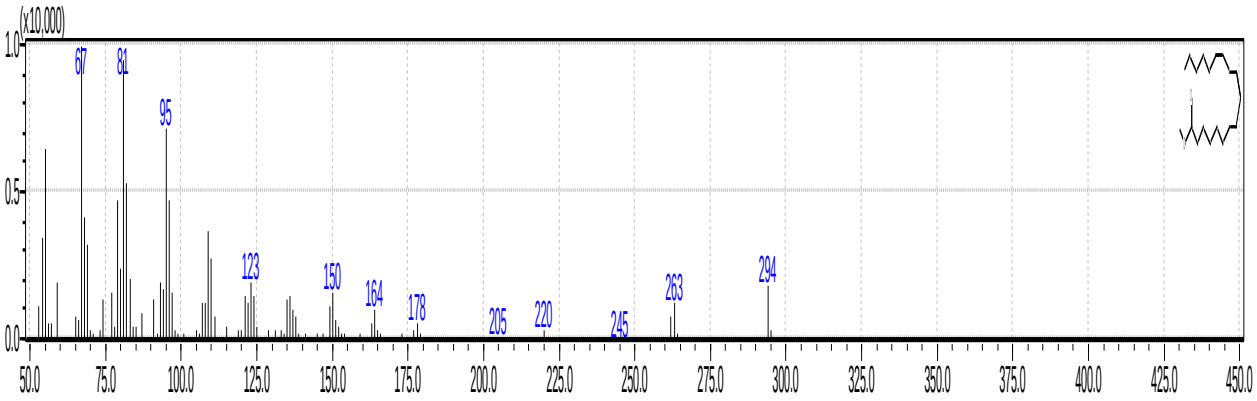

Fig.3.29. Mass spectrum of 9-12 octadienoic acid(z,z)methy ester

.

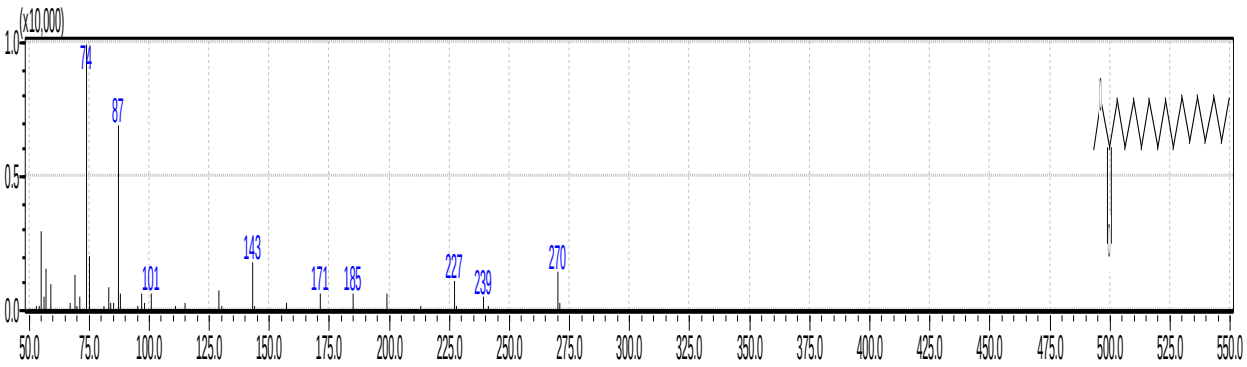

Fig.3.30. Mass spectrum of hexadecanoic acid methyl ester.

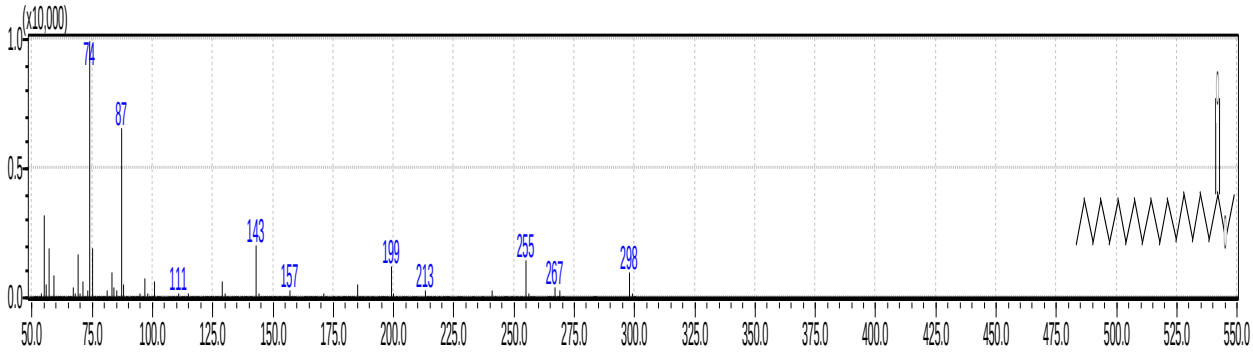

Fig.3.31. Mass spectrum of methyl stearate

3.1.5-*Medicago-sativa*

GC-MS analysis of *Medicago-sativa* oils was conducted.GC-MS analysis revealed the presence of 26 components, see Table 3.5. The typical total ion chromatograms(TIC) is depicted in Fig.3.32.

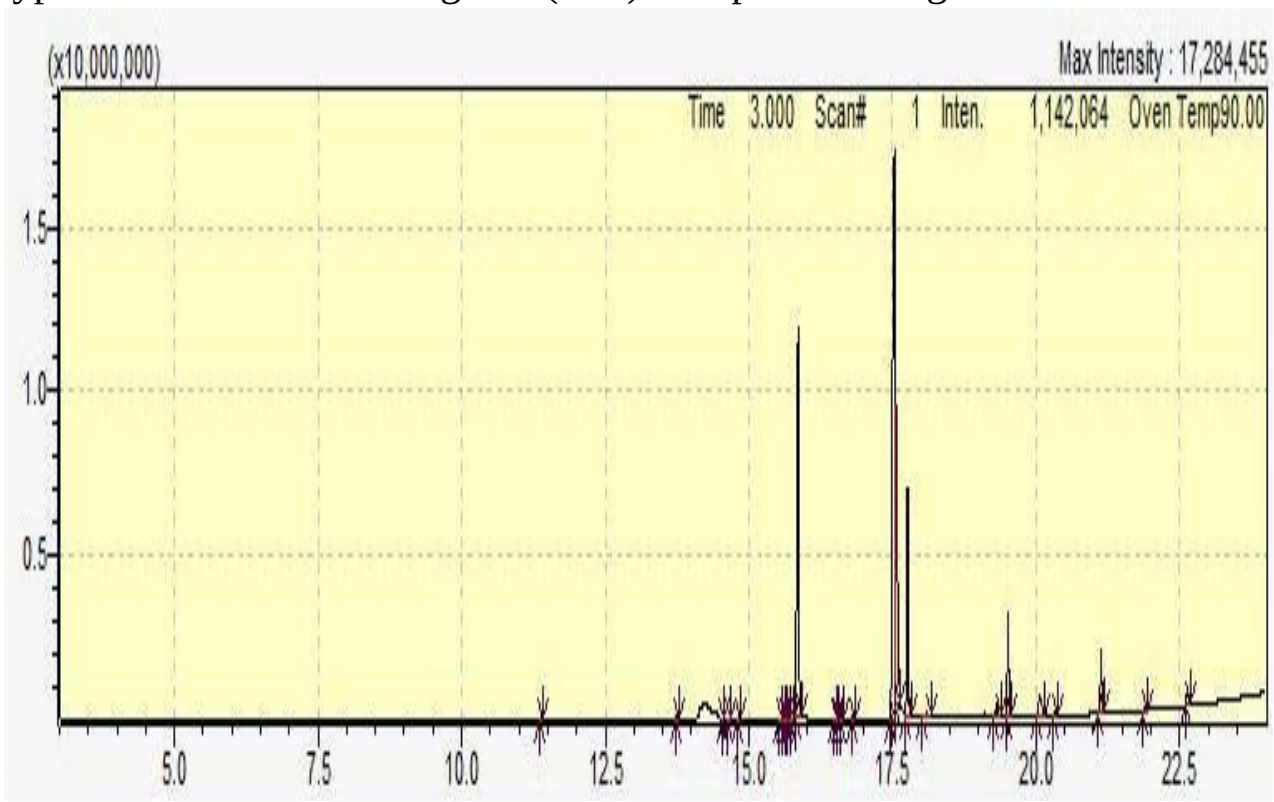

Fig.3.32. Typical total ion chromatograms

Table3.5 constituents of *Medicago-sativa* oil

ID#	Name	Ret.Time	Area	Area%
1.	Butylated Hydroxytoluene	11.392	174713	0.13

2.	Methyl tetradecanoate	13.743	178597	0.14
3.	4-Octadecenoic acid, methyl ester	14.554	46970	0.04
4.	5-Octadecenoic acid, methyl ester	14.659	33851	0.03
5.	Pentadecanoic acid, methyl ester	14.818	69594	0.05
6.	7,10-Hexadecadienoic acid, methyl Ester	15.550	45973	0.03
7.	7-Hexadecenoic acid, methyl ester, (Z)-	15.608	89410	0.07
8.	cis-10-Nonadecenoic acid, methyl ester	15.653	408125	0.31
9.	9,12-Hexadecadienoic acid, methyl Ester	15.693	67763	0.05
10.	cis-10-Nonadecenoic acid, methyl ester	15.746	34462	0.03
11.	Hexadecanoic acid, methyl ester	15.858	27119676	20.57
12.	Hexadecanoic acid, ethyl ester	16.505	27110	0.02
13.	Hexadecanoic acid, 14-methyl-, methyl ester	16.552	30317	0.02
14.	cis-11,14-Eicosadienoic acid, methyl	16.613	117914	0.09
15.	Heptadecanoic acid, methyl ester	16.822	320091	0.24
16.	Methyl 10-trans,12-cis-octadecadienoate	17.535	57285855	43.45
17.	9-Octadecenoic acid (Z)-, methyl Ester	17.560	16671468	12.65
18.	Methyl stearate	17.763	13840256	10.50
19.	17-Octadecynoic acid, methyl ester	18.101	479190	0.36
20.	11-Eicosenoic acid, methyl ester	19.316	1054631	0.80
21.	Methyl 18-methylnonadecanoate	19.513	5756283	4.37
22.	Tetracosanoic acid, methyl ester	20.061	2524738	1.92
23.	Heneicosanoic acid, methyl ester	20.340	133886	0.10
24.	Methyl 20-methyl-heneicosanoate	21.133	3871363	2.94
25.	Tricosanoic acid, methyl ester	21.898	299551	0.23
26.	Tetracosanoic acid, methyl ester	22.635	1135464	0.86

Major constituents of the oil are discussed below:

Methyl -10-trans-12-cis-Octadecadienoate (43.45%)

The EI mass spectrum of methyl-10-trans-12-cis- octadecadienoate is shown in Fig.3.33. The peak at m\z 294 which appeared at R. T 17.535 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$.The peak at m\z 263 corresponds to loss methoxyl function.

Hexadecanoic acid, methyl ester (20.57%)

Fig.3.34. shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m\z 270, which appeared at R.T.15.858 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

9-Octadecenoic acid (Z), methyl ester (12.65%)

Mass spectrum of 9-Octadecenoic acid (Z) methyl ester is shown in fig.3.35. The peak at m\z 296 which appeared at R.T. 17.560 corresponds to $M^+[C_{19}H_{36}\ O_2]$. The peak at m\z 264 corresponds to loss of methoxyl function.

Methyl stearate (10.50%)

Mass spectrum of methyl stearate is displayed in Fig.3.36. The peak at m\z 298which appeared at R.T. 17.763 corresponds to $M^+\{C_{19}H_{38}O_2\}$. The peak at m\z 267 corresponds to loss of methoxyl function.

Methyl 18-methylnonadecanoate (4.37%)

Mass spectrum of methyl 18-methylnonadecanoate is shown in Fig.3.37. The peak at m\z 326 which appeared at R.T.19.513 corresponds to M^+ $[C_{21}H_{42}O_2]^+$. The peak at m\z 263.

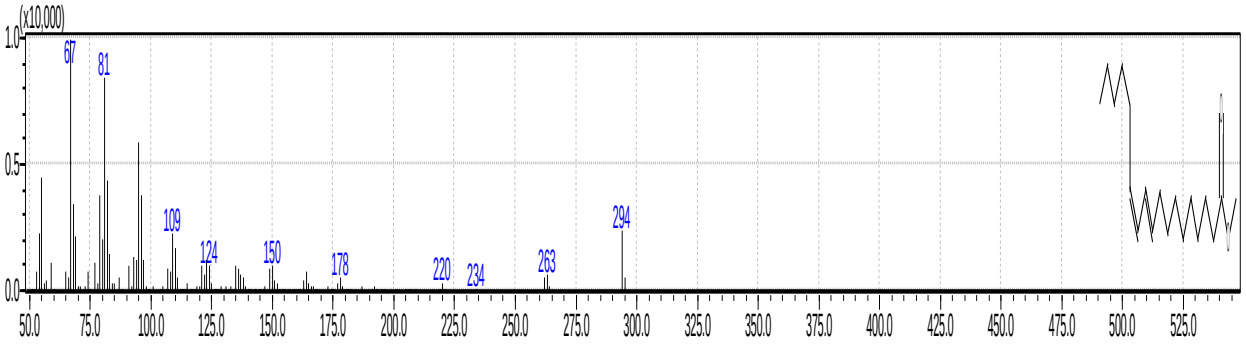

Fig.3.33. Mass spectrum of methyl 10-trans,12-cis-octadecadienoate

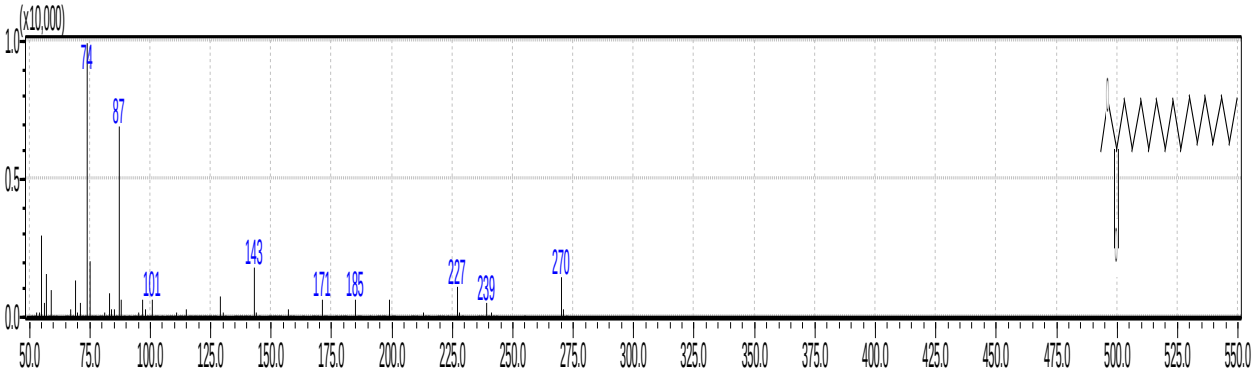

Fig.3.34. Mass spectrum of hexadecanoic acid methyl ester

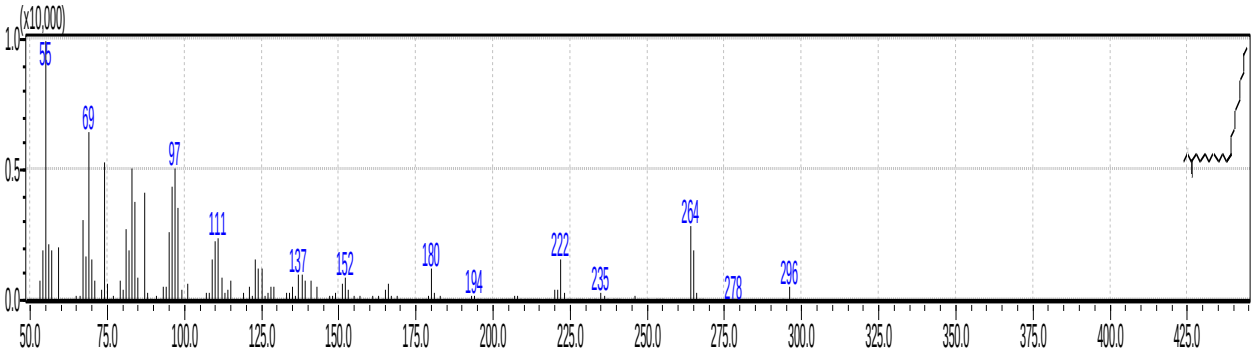

Fig.3.35. Mass spectrum of 9- octadecenoic acid, (z)-, methyl ester

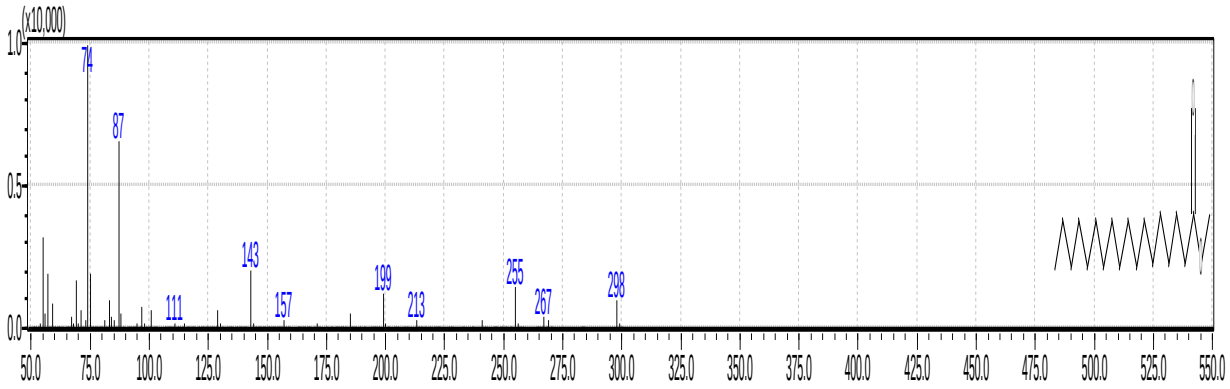

Fig.3.36. Mass spectrum of methyl stearate

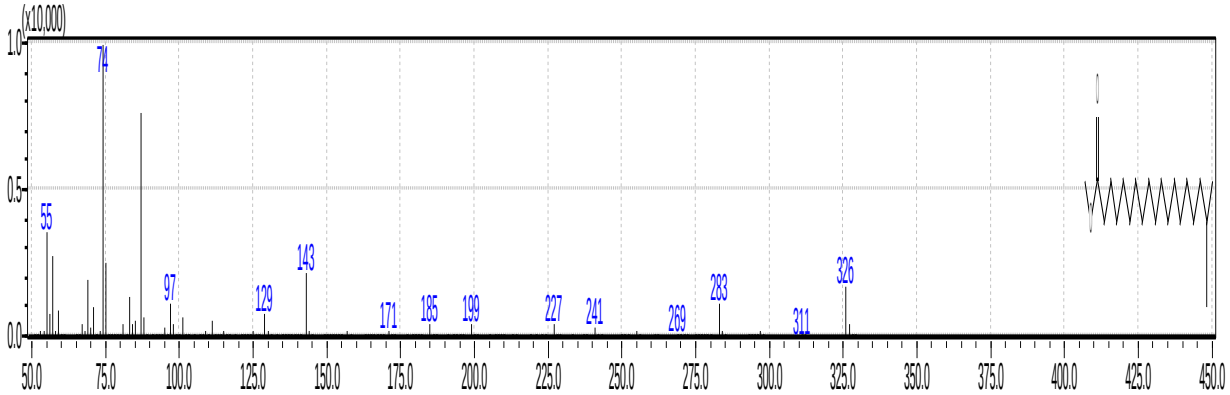

Fig.3.37. Mass spectrum of methyl- 18- methyl lnonadecanoate

3.1.6-*Corchorus olitorius*

GC-MS analysis of *Corchorus olitorius* was conducted showing the presence of 28 components- Table.3.6. The typical total ion hromatograms(TIC) is depicted in Fg.3.38

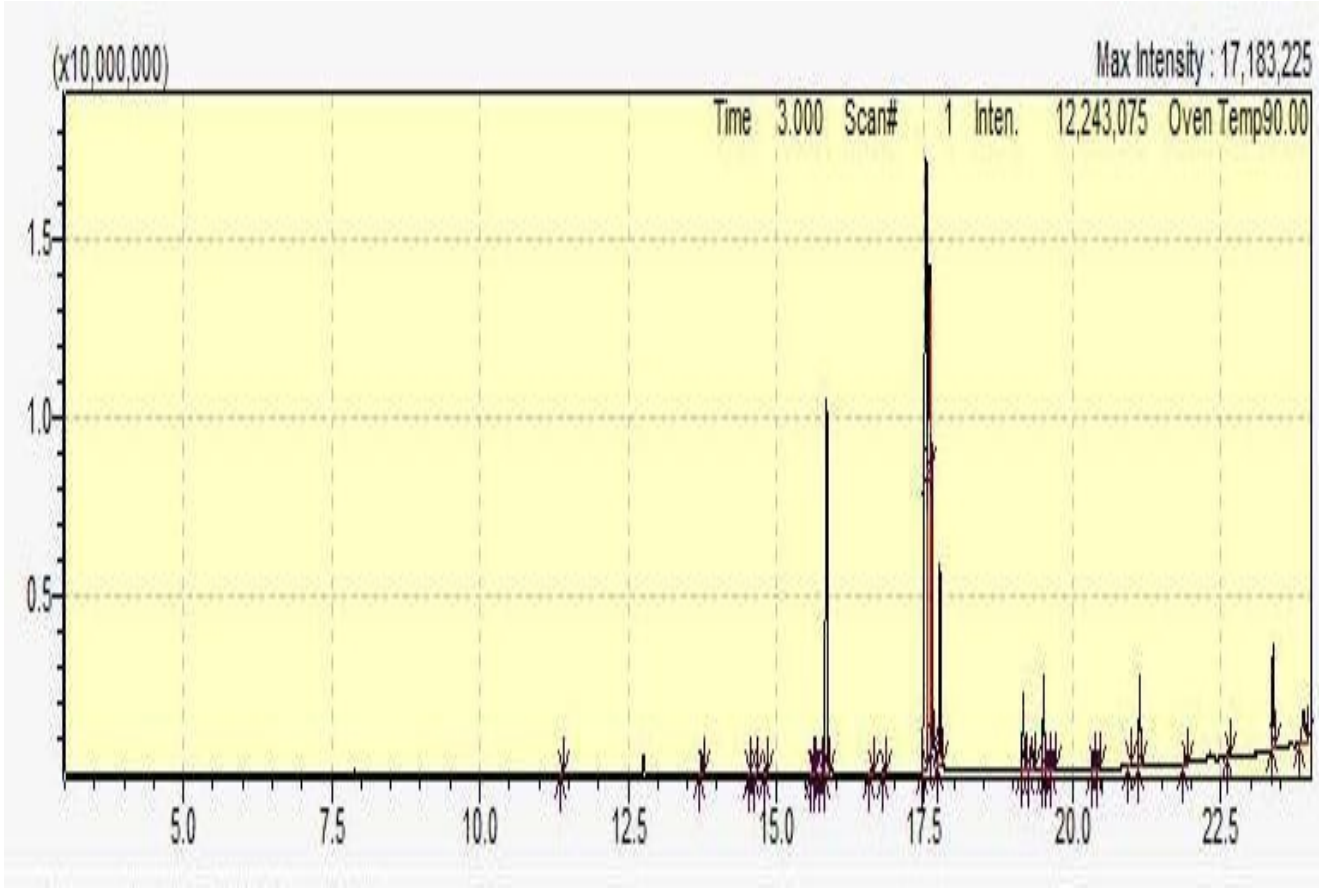

Fig.3.38. Typical total ion chromatogram

Table3.6: Constituents of *Corchorus olitorius*:

ID#	Name	Ret.Time	Area	Area%
1.	Butylated Hydroxytoluene	11.389	224460	0.15
2.	Methyl tetradecanoate	13.740	909662	0.62
3.	6-Octadecenoic acid, methyl ester, (Z)-	14.551	20414	0.01
4.	4-Octadecenoic acid, methyl ester	14.655	19622	0.01
5.	Pentadecanoic acid, methyl ester	14.815	273761	0.19
6.	Methyl hexadec-9-enoate	15.606	114460	0.08
7.	9-Hexadecenoic acid, methyl ester, (Z)-	15.650	391584	0.27
8.	cis-10-Nonadecenoic acid, methyl ester	15.744	55299	0.04

9.	Hexadecanoic acid, methyl ester	15.849	22559459	15.31
10.	cis-10-Heptadecenoic acid, methyl ester	16.612	230144	0.16
11.	Heptadecanoic acid, methyl ester	16.819	304709	0.21
12.	9,12-Octadecadienoic acid (Z,Z)-, ester	17.532	57670035	39.11
13.	9-Octadecenoic acid, methyl ester, (E	17.585	9474172	6.43
14.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.605	18700396	12.69
15.	Methyl stearate	17.760	10268895	6.97
16.	Methyl 8,11,14-heptadecatrienoate	19.167	3818288	2.59
17.	11-Eicosenoic acid, methyl ester	19.313	1541179	1.05
18.	Methyl 18-methylnonadecanoate	19.511	4449383	3.02
19.	Methyl9.cis.,11.trans.t,13.trans.-octadecatrienoate	19.559	446765	0.30
20.	6,9-Octadecadienoic acid, methyl este	19.669	344266	0.23
21.	Heneicosanoic acid, methyl ester	20.336	733328	0.50
22.	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.425	67599	0.05
23.	13-Docosenoic acid, methyl ester	20.957	165893	0.11
24.	Methyl 20-methyl-heneicosanoate	21.130	4426479	3.00
25.	Tricosanoic acid, methyl ester	21.895	365628	0.25
26.	Tetracosanoic acid, methyl ester	22.632	592307	0.40
27.	Squalene	23.379	5546942	3.76
28.	Stigmast-7-en-3-ol, (3.beta.,5. alpha.,24S)-	23.896	3669260	2.49

Some important constituents are discussed below:

9,12 Octadecadienoic acid (z,z)methyl esters (39.11%)

Mass spectrum of 9.12 octadecadienoic acid (z,z) methyl ester is depicted in Fig.3.39.The peak at m\z 294 which appeared at R.T 17.532 corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ ·The at m\z 263 corresponds of loss methoxyl function.

Hexadecanoic acid methyl ester (15.31%)

Fig.3.40 displays the mass spectrum of hexadecanoic acid methyl esters. The signal at m\z 270, which appeared at R.T.15.849 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

9,12,15-Octadecatrienoic acid, methyl ester (12.69%)

Mass spectrum of9,12,15-Octadecatrienoic acid, methyl ester is depicted in Fig.3.41. The peak at m\z,292 which appeared at R.T.17.605 corresponds to $M^+[C_{19}H_{32}O_2]^+$ while the peak at m\z 261 is attributed to loss of methoxyl function

Methyl stearate (6.97%)

Mass spectrum of methyl stearate is shown in Fig.3.42. The peak at m\z 298which appeared at R.T. 17.760 corresponds to $M^+[C_{19}H_{38}O_2]$. The peak at m\z 267 corresponds to loss of methoxyl function.

9-Octadecenoic acid, methyl ester, (E) (6.43 %)

Fig.3.43 shows the mass spectrum of 9-octadecenoic acid, methyl ester, (E). The peak at m\z 296 which appeared at R.T. 17. 585 corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m\z 264 corresponds to loss of methoxyl function.

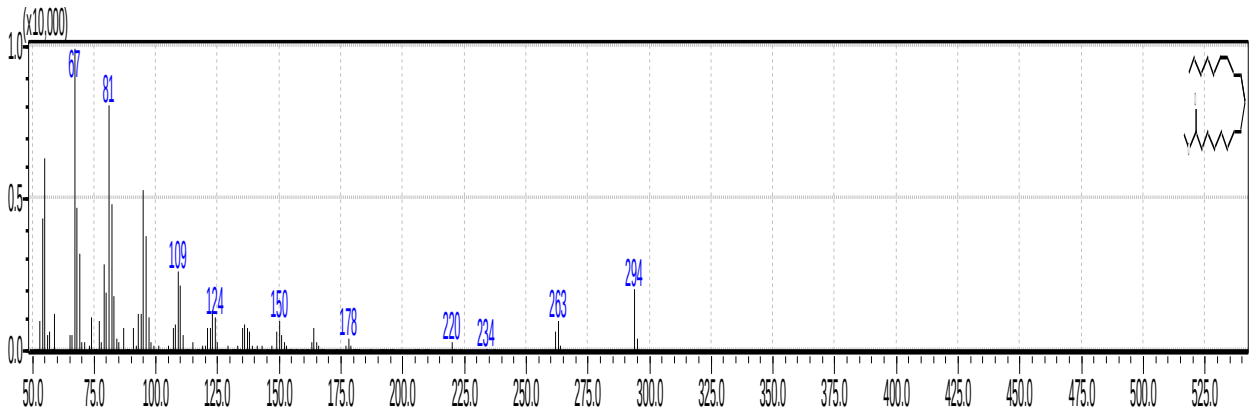

Fig.3.39. Mass spectrum 9.12 octadecadienoic acid (z,z)methyl ester

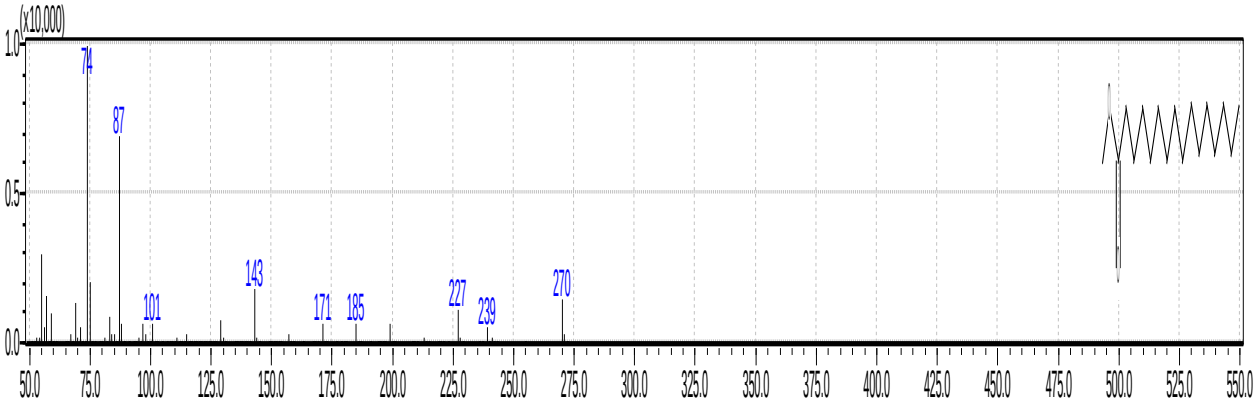

Fig.3.40. Mass spectrum hexadecanoic acid methyl ester.

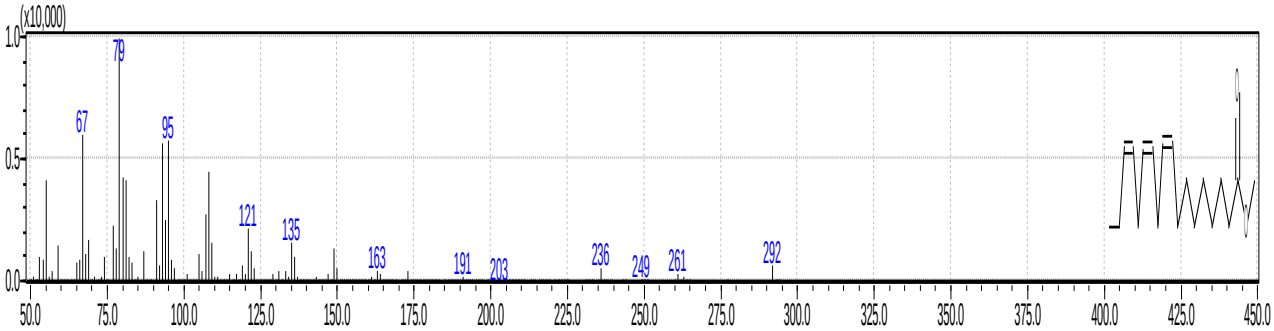

Fig.3.41. Mass spectrum 9,12,15-octadecatrienoic acid, methyl ester

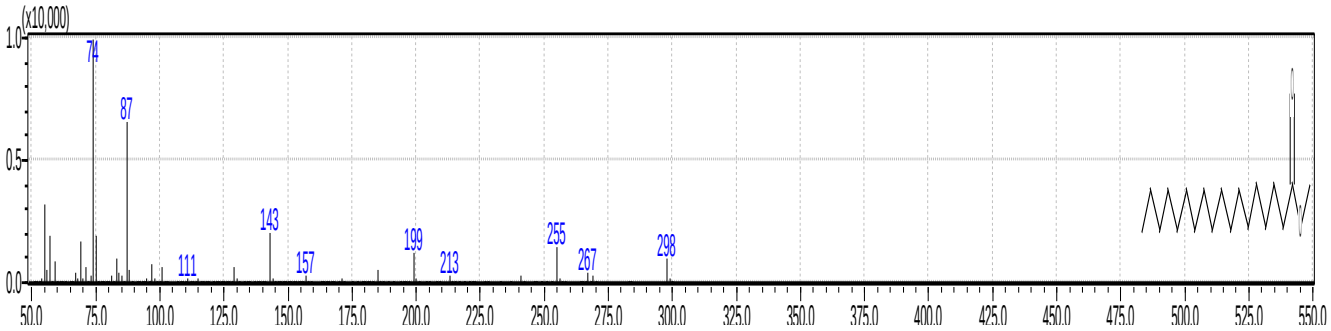

Fig.3.42. Mass spectrum of methyl stearate

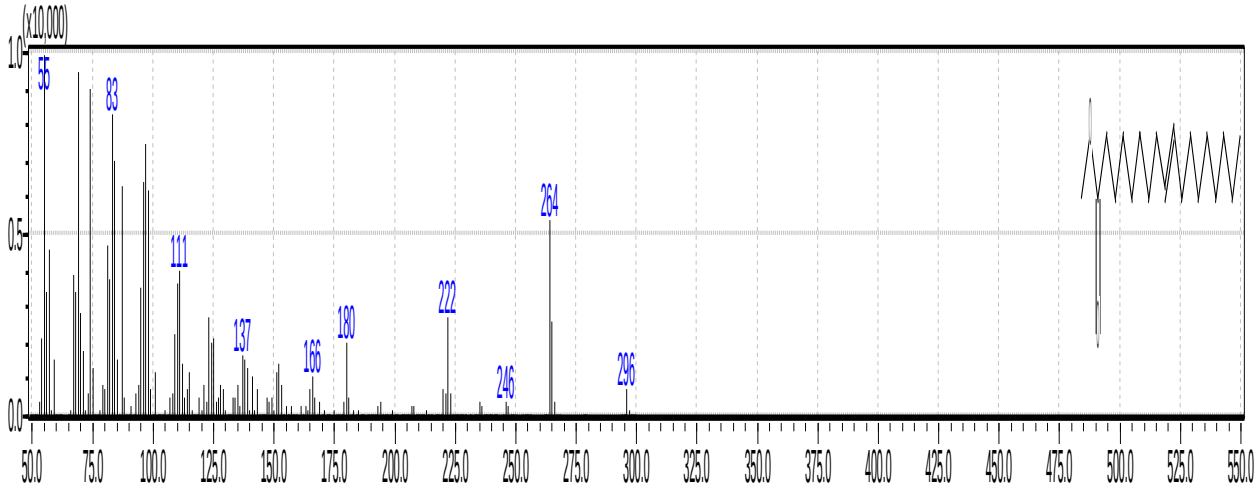

Fig.3.43. Mass spectrum of 9-octadecenoic acid, methyl ester, (E)

3.1.7- *Pimpinella anisum*

The oil of *Pimpinella anisum* was analyzed by GC-MS. The analysis revealed the presence of 49 components table 3.7. The typical total ion chromatograms(TIC) is depicted in Fig.3.44.

Fig.3.44. Typical total ion chromatogram

Table.3.7: Constituents of *Pimpinella anisum*

ID#	Name	Ret.Time	Area	Area%
1.	Pentanoic acid, 4-methyl-, methyl ester	3.137	8877	0.00
2.	Hexanoic acid, methyl ester	3.488	24592	0.01
3.	Octanoic acid, 4,6-dimethyl-, methyl ester, (4S,6S)-(+)-	4.378	17752	0.01
4.	.alpha.-Phellandrene	4.540	17255	0.01
5.	D-Limonene	4.858	108091	0.05
6.	Cyclohexanol,2-methyl-5-(1-methylethenyl)-	7.186	21496	0.01
7.	Cyclohexanone,2-methyl-5-(1-methylethenyl)-, trans-	7.238	1664375	0.85
8.	Cyclodecene, 1-methyl-	7.358	299693	0.15
9.	Cyclohexanol,2-methyl-5-(1-methylet , (1.alpha.,2.beta.,5.alpha.)-	7.474	71976	0.04
10.	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1.alpha.,3.alpha.,4.beta.,6.alpha.)-	7.665	111909	0.06
11.	D-Carvone	7.900	11762147	5.98
12.	1,1-Dimethyl-4-methylene Cyclohexane	8.175	174486	0.09
13.	Cyclopentanol, 1-(1-methylene-2-propenyl)-	8.362	2086823	1.06
14.	4-(1-Ethyl-piperidin-3-ylamino)-1-ox spiro[4.5]dec-3-en-2-one	9.235	77580	0.04
15.	11-Oxa-tricyclo[4.4.1.0(1,6)] undecan-2-ol	9.696	45189	0.02
16.	1,6-Cyclodecadiene, 1-methyl-5-me 8-(1-methylethyl)-, [S-(E,E)]-	11.100	52605	0.03
17.	Butylated Hydroxytoluene	11.390	102533	0.05
18.	Dodecanoic acid, methyl ester	11.423	143621	0.07
19.	1,3-Benzodioxole,4-methoxy-6-(2-propenyl)-	11.535	405837	0.21
20.	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	11.869	233069	0.12

21.	Apiol	12.800	32512778	16.52
22.	Methyl tetradecanoate	13.741	960825	0.49
23.	5-Octadecenoic acid, methyl ester	14.657	82831	0.04
24.	Pentadecanoic acid, methyl ester	14.816	452546	0.23
25.	2-Pentadecanone, 6,10,14-trimethyl-	15.036	162471	0.08
26.	11,14-Eicosadienoic acid, methyl ester	15.549	91106	0.05
27.	7,10,13-Hexadecatrienoic acid, methy	15.623	1615293	0.82
28.	Methyl hexadec-9-enoate	15.648	1820114	0.93
29.	Hexadecanoic acid, methyl ester	15.848	16895277	8.59
30.	6-Octadecenoic acid, methyl ester, (Z)-	16.611	350924	0.18
31.	Heptadecanoic acid, methyl ester	16.820	315259	0.16
32.	Methyl 6,11-octadecadienoate	17.372	745142	0.38
33.	6,9-Octadecadienoic acid, methyl ester	17.422	397508	0.20
34.	9,12-Octadecadienoic acid (Z,Z)-, ester	17.512	26591350	13.51
35.	9-Octadecenoic acid (Z)-, methyl ester	17.617	81624785	41.47
36.	Methyl stearate	17.762	6981593	3.55
37.	Cyclopropaneoctanoic acid, 2-octyl-, ester, cis-	18.454	183155	0.09
38.	Methyl 5,13-docosadienoate	19.118	287910	0.15
39.	11-Eicosenoic acid, methyl ester	19.293	1825419	0.93
40.	Methyl 18-methylnonadecanoate	19.511	1316733	0.67
41.	Stigmast-8(14)-en-3.beta.-ol	20.035	580707	0.30
42.	Heneicosanoic acid, methyl ester	20.338	115692	0.06
43.	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	20.426	81436	0.04
44.	Hexacontane	20.863	203106	0.10
45.	13-Docosenoic acid, methyl ester	20.957	176183	0.09
46.	Methyl 20-methyl-heneicosanoate	21.131	799184	0.41
47.	Dotriacontane	22.375	490948	0.25
48.	Tetracosanoic acid, methyl ester	22.632	547563	0.28
49.	Tetracosane	23.779	1121107	0.57

Some important constituents are discussed below:

9-Octadecenoic acid(Z)methyl ester (41.47%)

Mass EI spectrum of 9-Octadecenoic acid(Z)methyl ester is shown in Fig.3.45. The peak at m\z 296 which appeared at R.T.17.617 corresponds to $M^+[C_{19}H_{36}O_2]$. The peak at m\z 264 is corresponds to loss of methoxyl function.

Apiol (16.52%)

Fig.3.46 displays the mass spectrum of apiol. The signal at m\z 222 which appeared at R.T.12.800 corresponds to $C_{12}H_{14}O_4$, whilst the peak at m\z 207 is due to loss of methoxyl function.

9,12 Octadecadienoic acid (z,z)methyl esters (13.51 %)

Mass EI spectrum of 9.12 octadecadienoic acid (z,z) methyl esters is depicted in Fig.3.47.The peak at m\z 294 which appeared at R.T 17.512 corresponds to $M^+[C_{19}H_{34}O_2]^+$ The at m\z 262 corresponds of loss methoxyl function.

Hexadecanoic acid methyl ester (8.59%)

Mass EI spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.48. The peak at m\z 270, which appeared at R.T.15.848 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is attributed to loss of methoxyl function.

D-Carvone (5.98%)

Mass EI spectrum of D-Carvone shown in Fig.3.49. The peak at m\z 150 which appeared at R.T.7.900 corresponds to $C_{10}H_{14}O$. The peak at m\z122 corresponds to loss of a methoxyl function

Methyl stearate (3.55%)

Mass EI spectrum of methyl stearate is shown in Fig.3.50. The peak at m\z 298 which appeared at R.T. 17.762 corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m\z 267 corresponds to loss of a methoxyl function.

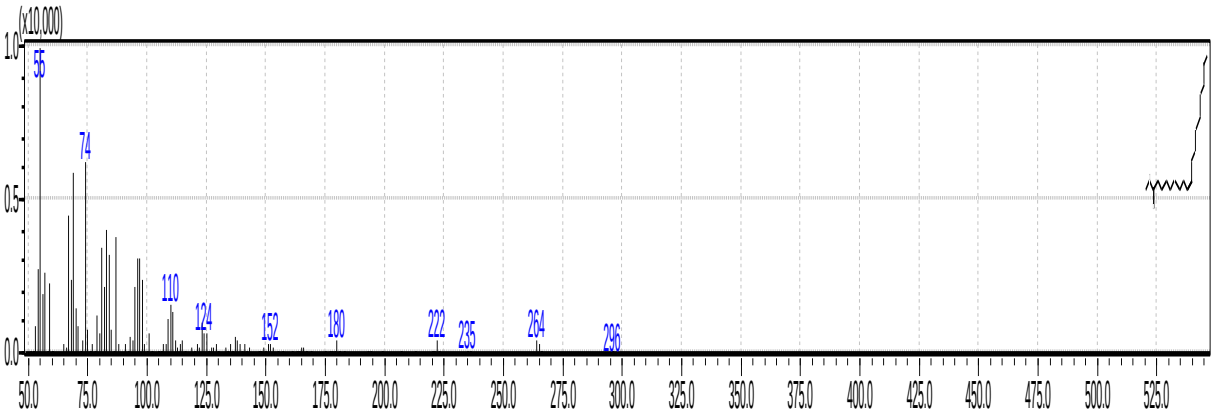

Fig.3.45. Mass spectrum of 9-Octadecenoic acid (Z), methyl ester

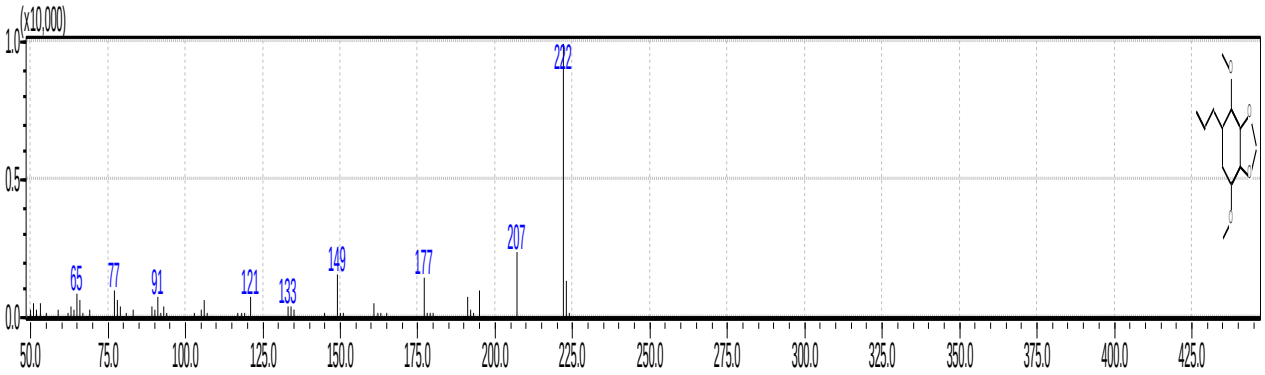

Fig.3.46. Mass spectrum of apiol

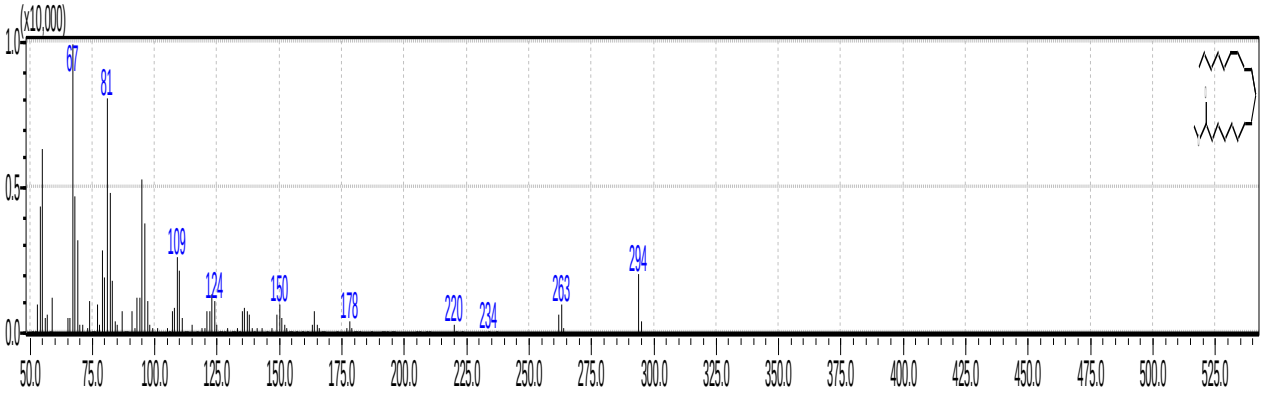

Fig.3.47. Mass spectrum of 9-12 octadienoic acid(z,z)methyl ester.

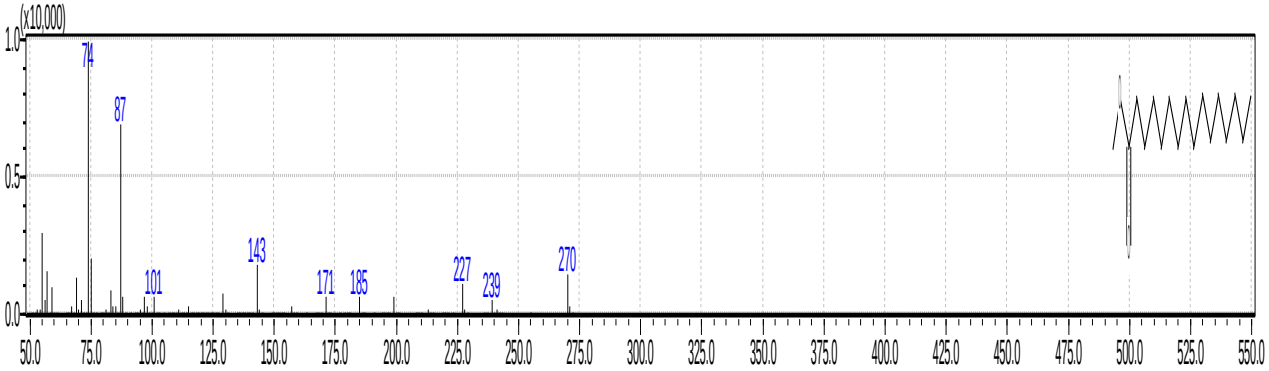

Fig.3.48. Mass spectrum of hexadecanoic acid methyl ester.

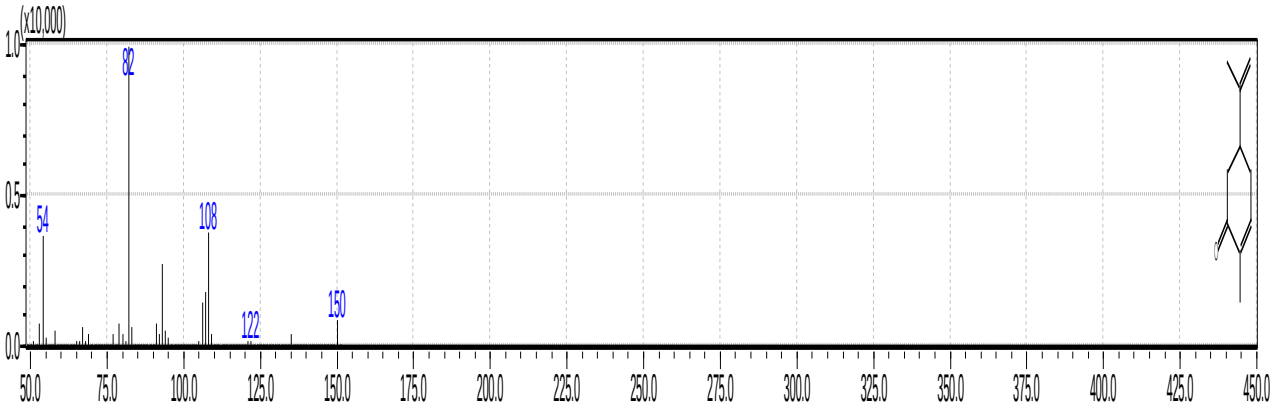

Fig.3.49. D-Carvone

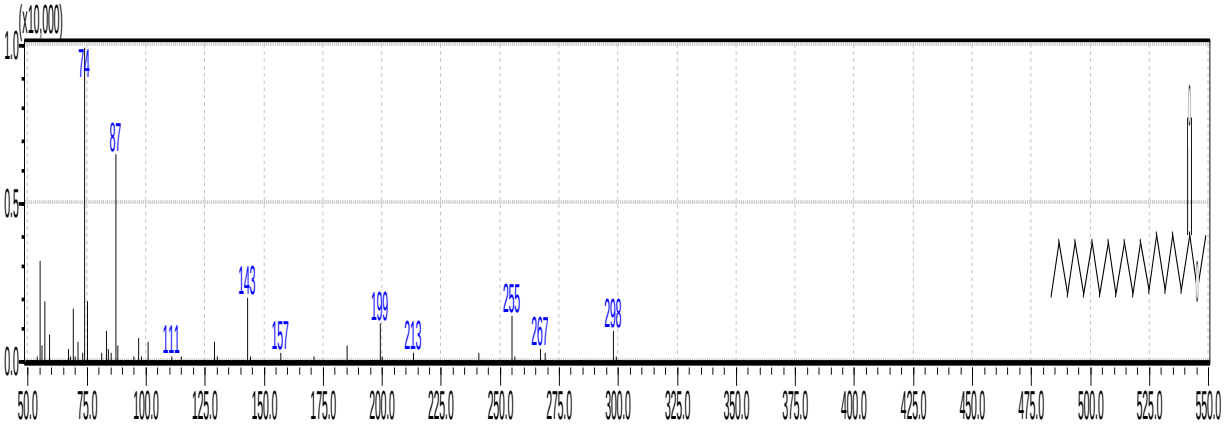

Fig.3.50. Mass spectrum of methyl stearate

3.2.-Antimicrobial activity

In cup plates agar diffusion bioassay, the target oil was assessed for antimicrobial activity against five standard human pathogen. The averages of the diameters of the growth of inhibition zones are depicted. The result was interpreted in term of commonly used terms <9mm in active; 9-12mm partially active; 13-18 active; >18mm very active. Table 3.8 and 3.9 represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi

Table 3.8: Antibacterial activity of standard chemotherapeutic agents

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

Table3.9: Antifungal activity of standard chemotherapeutic agent.

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

.

Ec: Escherichia coli. *Ps: Pseudomonas aeruginosa.*

Bs: Bacillus subtilis. *Ca: Candida albicans*.

Sa: Staphylococcus aureus.

3.2.1-*Prosopi juliflora*

Table 3.10: Antimicrobial activity of *Prospis juliflora* oils

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	18	14	16	17	16

The *Prosopis juliflora* oil showed significant activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. It also showed very good anti-candid potential

3.2.2-*Acacia seyal*

Table 3.11 Antimicrobial activity of *Acacia seyal* oils

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	18	17	18	18	15

Acacia seyal oil showed significant activity against all test bacteria. It also gave very good anti-candid potency

3.2.3 *Solenostemma argel*

Table 3.12 Antimicrobial activity of *Solenostemma argel* oil

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	22	18	16	-	15

Solenostemma argel oil showed excellent activity against Escherichia coli and Pseudomonas aeruginosa. The oil exhibited very good activity against *Staphylococcus aureus*.

3.2.4 –*Mediacago sativa*

The average of the diameters of the growth of inhibition zones displayed by target oil are depicted in Table 3.13

Table3.13 Antimicrobial activity of *Medicago-sativa* oil at conc.100mg/ml

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	-	11	10	14	9

Medicago-sativa oil showed moderate activity against *Bacillus subtilis*. However, it exhibited partial activity against other test organisms

3.2.5- Corchorus olitorius

Table 3.14: Antimicrobial activity of *Corchorus olitorius* oil

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	7	7	16	--	7

Corchorus olitorius oil only showed activity against *Staphylococcus aureus*

3.2.6- *Pimpinella anisum*

The oil has been assessed for antimicrobial activity and the results are depicted in Table 3.15:

Table 3.15: Antimicrobial activity of *Pimpinella anisum* oils

Type of microbial	*Ec*	*Ps*	*Sa*	*Bs*	*Ca*
Diameter average	-	20	18	17	8

Pimpinella anisum oil showed excellent activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. It was inactive against *Escherichia coli* and the fungi *Candida albicans*.

Conclusion

Seven plants which are key species in Sudanese ethnomedicine have been investigated. The fixed oils of these species were extracted and studied by GC-MS and the major constituents of these oils have been discussed. Furthermore, the oils have been assessed for antimicrobial activity via the cup plate agar diffusion bioassay against five standard human pathogens and significant antimicrobial activity has been reported for some of the target oils.

Recommendations

- Other biological activity (anti-inflammatory, antileishmenial …ect) of the target oils may be investigated.
- Other phytochemicals of the target plants may be extracted and isolated in a chromatographically pure form and then their structure could be elucidated and their biological activities may be screened.

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