Sudan University of Science and Technology

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GC/MS Analysis and Antioxidant Activity of Some Medicinal Plants

تحليل الكروموتوغرافيا الغازيه – طيف الكتله وفعالية مضاد الاكسده لبعض النباتات الطبيه

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

By

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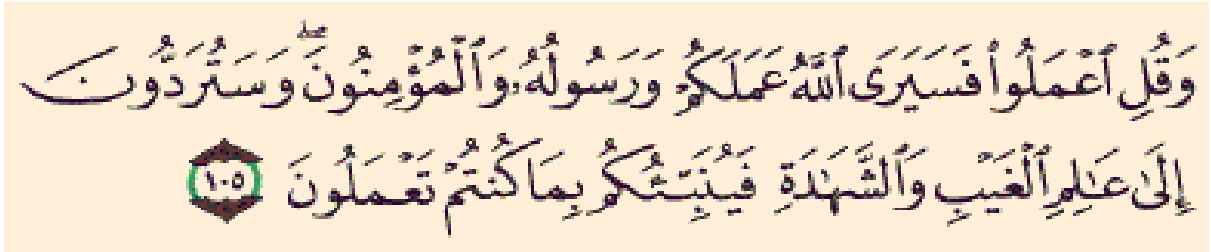
استهلال



(التوبة-105)

Dedication

To….

my parents

husband

son

brothers and sisters

Acknowledgement

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

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

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

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Deep thanks to my family for their infinite support.

Abstract

In this study the oils from six plants of medicinal attributes **(***Acacia seiberiana, Acacia alata, Leucaena leucocephala, Pongamia pinnata* , *Delonix regia* and *Cassia auriculata*) have been investigated by GC.MS and the antimicrobial activity has been screened. Gas chromatography - mass spectrometry has been used for the identification and quantification of *Acacia seiberiana* oil. The analysis revealed the presence of 22 components dominated by 9, 12-octadecadienoic acid methyl ester (31.21 %). *Acacia seiberiana oil* has been screened for antioxidant activity against stable DPPH radicals .The oil exhibited weak antioxidant activity. Gas chromatography - mass spectrometry analysis of *Leucaena leucocephala* oil showed the presence of 18 components dominated by 12-octadecadienoic acid methyl ester (38.70 %). The oil did not exhibit any antioxidant activity. GC/MS was conducted for *Acacia alata* oil. The analysis revealed the presence of 23 components. Major constituent being 9, 12-octadecadienoic acid methyl ester (22.73 %). *Acacia alata* oil has been screened for antioxidant activity against stable DPPH radicals.The oil exhibited very weak antioxidant activity. GC/MS was conducted for *Pongamia pinnata* oil. The analysis revealed the presence of 15 components dominated by 9-octadecenoic acid methyl ester(53.58%). The oil exhibited moderate antioxidant activity. Analysis of *Cassia auriculata* oil revealed the

presence of 16 components . Major component being 9, 12-octadecadienoic acid methyl ester (33.02 %). The oil exhibited moderate antioxidant activity. GC/MS for *Delonix regia* oil showed the presence of 16 components dominated by 9, 12-octadecadienoic acid methyl ester (34.07 %). The oil exhibited moderate antioxidant activity.

المستخلص

فى هذا البحث تمت دراسة ستة زيوت لنباتات طبيه هامه هى : الكوك , اللوسينا , الشباهى , البنقامى , الاركولاتا والقولدمور. تم تحديد مكونات هذه الزيوت بتقنيه الكروموتوغرافيا الغازيه- طيف الكتله, كما واجرى اختبار مضاد الميكروبات لهذه الزيوت. احتوى زيت نبات الكوك على 22 مكونا اهمها : حمض 9,12- اوكتاديكادابنويك مثيل استر (31.21%). وقد اعطى الزيت نشاطا ضعيفا فى اختبار مضاد الاكسده. اما زيت نبات اللوسينا فقد احتوى على 18 مكونا اهمها : حمض 9,12- اوكتاديكادابنويك مثيل استر(38.70%) . وفى اختبار مضاد الاكسده لم يعطى هذا الزيت الى نشاط . نبات الشباهى احتوى على23 مكونا اهمها : حمض 9,12- اوكتاديكادابنويك مثيل استر (22.73%) . اعطى هذا الزيت نشاطا ضعيفا فى اختبار مضاد الاكسده. احتوى نبات البنقامى على 15مكونا اهمها: حمض 9-اوكتاديكينويك(53.58%) . وفى اختبار مضاد الاكسده اعطى الزيت نشاطا متوسطا. نبات الاركولاتا احتوى على 16 مركبا تصدرها : : حمض 9,12- اوكتاديكادابنويك مثيل استر (33.02%). وفى اختبار مضاد الاكسده اعطى الزيت نشاطا متوسطا. اما نبات قولدمور فقد احتوى على 16 مكونا اهمها : حمض 9,12- اوكتاديكادابنويك مثيل استر (22.73%). وفى اختبار مضاد الاكسده اعطى الزيت نشاطا متوسطا .

1-Introduction

1.1- General approach

Vegetable oils have been used for centuries, oil bearing nuts and animal fats were consumed as sources of energy long before nutrition concepts were envisioned. Vegetable oils are mainly fluid hydrophobic compounds at ambient temperature obtained from crushed seed from different plants such as sunflower, canola, soybean, Jatropha, rapeseed, peanut, cottonseed [1,2]..

Sources of vegetable oils include seeds of annual plants grown in relatively temperate climates, most of these annual plants not only are cultivated as a source of oil, but are also utilized as protein-rich foods; a second source of vegetable oil is oil-bearing trees. However, some oils like olive, coconut, and palm oils are extracted from the fruit pulp rather than the seed of the fruit. Recently, the production of vegetable oils like sunflower and linseed oils has significantly increased; oils are being considered as a major economic resource[3].

In the future the economic value of these oils and fats will increase considerably in the future because they represent a vast

potential of naturally renewable raw materials in which the chemical and pharmaceutical industries have a special interest[4].

Oils extracted from natural sources are used in various industrial applications such as emulsifiers, lubricants, plasticizers, surfactants, plastics, solvents and resins .Most plants contain fats or oils, chiefly in their seeds; the amount varies from very little to as much as 40 - 70%[5] .

The nature of molecules of the fats and oils determines the physical and chemical characteristics of these fats and oils[6]. The fatty acid composition of vegetable oils is the main factor influencing their nutritional value and properties[7].

In fat and oil processing plants, there are analytical requirements for process quality control. In refining, for example, evaluating the free fatty acids (FFA) content of the oil it is necessary to determine the caustic treat, and to serve as a quality indicator in other areas. Evaluation of melting points, fat solids content, and other physical parameters indicate that the product will function as developed. For final edible-oil products, organoleptic evaluations, peroxide value, free fatty acids, and other analyses are utilized for assurance that the product has the required bland flavor, with predictive analysis, such as active oxygen method (AOM) stability being utilized to ensure proper shelf life[6].

Nowadays, there are only around twelve out of about 500000 known plant species exploited for the commercial production of vegetable oils[8]. Oils obtained from plants are an important part of the human diets world-wide. The supply of vegetable oil is in excess of 100 million metric tons in the world.

With the increasing demands of fats and oils, several plant species have become the target of researchers in exploring their uses and functional properties . However, there is an urgent need for exploring the production of alternative sources of vegetable oils[8].

1.2-Target Plants

1.2.1-*Delonix regia*

Delonix regia (royal Poinciana) is a plant in the legume family (Leguminacea). This plant is well known for its seasonal red-orange bloom[9].

Delonix regia

It has been reported that *Delonix regia* contains many biologically interesting phytochemicals including flavonoids, alkaloids, steroids, saponins, tannins and carotenoids[10-14]. Seeds contain galacomannon[15], while leaves are rich source of β-sitosterol and lupeol[15]. *Delonix regia* is used traditionally against malaria and bacterial infections[16-18]. It has been shown that the leave extract possesses a hypoglycemic effect[19].

1.2.2-*Pongamia pinnata*

Pongamia pinnata is a medium-sized, evergreen tree in the family Luguminaceae[20]. The plant contains some bioactive molecules including flavonoids which are known antioxidants.

Pengomata pinnata

Some flavones and chalcones have been isolated from the leaves and stem[21]. The fruits and sprouts have been used traditionally against cancer[21]. Seeds are claimed to treat fever, bronchitis, whooping cough, rheumatism and skin diseases[22]. Seed oil is

used by local healers against piles, leprosy, ulcers and scabies[23] . Plant juice and oil possess antiplasmodial, anti-inflammatory[24], antinociceptive, antihyperglycemic, antiulcer, antidiarrhoeal and antihyperammonemic activity[25]. The antiulcer activity of root extract has been reported[26-28]. It has been shown that a decoction of *Pongamia pinnata* possesses selective antidiarrhoeal effect with efficacy against cholera[29]. Leave extract detoxified ammonia, urea and creatinine in ammonium chloride – induced hyperammonium models[30-32]. It has been shown that the flower extracts exhibited significant antihyperglycemic and antilipidperoxidative activity[32]. The extract also enhanced the antioxidant defense system in alloxan-induced diabetic models[32;33]. The ethanol extract of the leaves showed significant antiinflammatory effect[34;35]. The antiviral[36;37] and antibacterial activities of the leaves have been documented[38-40] .

1.2.3-*Cassia auriculata*

Cassia auriculata L. is a plant of many medicinal attributes in the family Caesalpiniaceae. Phytochemical investigation of pod husk revealed the presence of emodin, rubiadin, β-sitosterol, anthracene derivatives and flavonoids[41]. Leaves contain , among others, α-tocopherol derivative, octadecenal and hexadecanoic acid.

Cassia auriculata

The flowers contain sitosterol, emodin and some anthraquinones[42] . The ethanolic extract of leaves contain many secondary metabolites including alkaloids, tannins, flavonoids and saponins[43]. *Cassia auriculata* is used traditionally as antidiabetic[44], antipyretic[45], hepatoprotective, antiviral and antispasmodic. The plant is also used against diarrhea, leprosy, intestinal worms, female infertility[46], rheumatism[47] and conjunctivitis[48]. Flowers are used as a natural remedy for nocturnal emissions, throat irritation, diabetes, and urinary discharge. The bark is used in the treatment of skin diseases[49] , while seeds are used against eye inflammation[50]. The antioxidant[43], anthelmintic[51], antihyperglycemic[52], antilipidemic[53], antipyretic[45], hepatoprotective and antiinflammatory properties of *Cassia auriculata* have been documented[54;55] .

1.2.4-*Cassia sieberiana*

Cassia sieberiana (also known as West African laburnum) is a tropical deciduous small tree. It is characterized by bright yellow flowers that form into groups (upright or hanging).

Cassia seiberiana

Cassia sieberiana belongs to the kingdom Plantae, subkingdom Tracheobionta, phylum Angiospermophyta, superdivision Spermatophyta, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Fabales, family Fabaceae, genus Cassia and species sieberiana[56]. *C. sieberiana* has several common and local names.

C. sieberiana is a shrub native to Africa. Its distribution spans across Africa including the southern part of the Sahel[57] . It grows best in well drained, humid soils with an annual rainfall of approximately 20 inches. These shrubs grow in groups of other plants, they usually never grow alone[56] . It also grows in

wooded grassland and savannah, secondary bush, on lateritic soils, roadsides, gravel and thickets, secondary (closed) forest, coastal scrub and sandstone plateau[58] .

Individuals range from 10-20m in height. The color of the bark ranges from dark grey to black. The lenticels are horizontal and reddish in colour. The leaves are arranged in leaflets that contain 7-10 pair of opposite leaves. The upper side of the leaf is moderately shiny while the bottom has very fine nerves with stipules that are deciduous[57]. This plant has both flowers and fruit. The flowers are arranged either uprightly or in pendulous racemes ranging from 30–50 cm[57].

The leaves, roots and pods are widely used in traditional medicine[58] . Sleeping sickness is treated using the twigs. The liquid obtained after soaking the roots in water is used for a bath to remedy tiredness and also for body massage . A decoction of the bark, leaves or root is used for the treatment of dysentery, diarrhoea and vomiting the twigs are also used for the treatment of trypanosomiasis[58]. The encapsulated root bark is used in the treatment of dysmenorrhea and pain associated with gastric ulcer.

1.2.5-*Leucaena leucocephala*

Leucaena leucocephala is a medium sized fast growing tree belonging to the family Fabaceae. It is native to Southern Mexico and Northern Central America[59] and now it has

naturalized in many tropical and sub-tropical locations[60]. The specific name ‘leucocephala’ comes from ‘leu’ meaning white and ‘cephala’, meaning head, referring to the flowers. It is commonly known as White Lead tree, White Popinac, Jumbay and Wild Tamarind[61].

Leucaena leucocephala

This plant has also been described as a "conflict tree" because it has been promoted for its forage production and naturally spreads like a weed[62]. It grows up to 20m height. Leaves are looking like that of tamarind having white flowers tinged with yellow, and having long flattened pods. Seeds are dark brown with hard shining seed coat. The tree has multivarious uses like firewood, timber, greens, fodder, green manure, provide shade, controls soil erosion[63-66]. The kernel of seeds contains more than 20% oil and it can be used as a bio energy crop. The seeds may also be used as concentrates for dairy animals, as manure[67;68] ,

as a protein source[69] , as an oil seed[70] and as a potential source of commercial gum[71;72].

L. leucocephala is a legume and in the symbiosis with Rhizobia bacteria the tree is able to fix about 500 kg nitrogen per ha annually. The nitrogen fixing nodules are found on the small lateral roots near the soil surface[71].

Leucaena leucocephala leaves and seeds contain lipids, crude protein and carbohydrates. The seeds contain tannin and oxalic acid[73;74]. The kernel contains oil content of about 17 -20 % . The leaves and seeds also contain a toxic and a non-protein substance known as mimosine.

The seeds of *leucocephala* have great medicinal properties and are used to control stomachache, as contraception and abortifacient. The seed gum is used as a binder in tablet formulation[76;77]. Sulfated glycosylated form of polysaccharides from the seeds was reported to possess significant cancer chemo-preventive and anti-proliferative activities[78]. The extracts of the seeds has been reported as anthelmintic, antidiabetic and has a broad spectrum antibacterial activity[79] .Recently, the seed oil was used in engineering as a novel biodevice useful in biomembrane modeling in lipophilicity determination of drugs and xenobiotics. The plant is reported to be a worm repellent.

1.2.6-*Cassia alata*

Cassia alata , commonly named ringworm cassia since the plant leaves are used for treatment of ringworm. It is also, commonly named candle due to the erect flower spikes when in bud appear like yellow candles.

Cassia alata

Cassia alata is a tropical tree that typically grows to 4 m in height with horizontal branches. The fruits are straight, up to 25 cm long, black and winged pods. *Cassia alata* is often used specifically as treatment against ringworm and as fungicidal. It also has antibacterial, laxative, anti-inflammatory, anti-tumor, and diuretic properties[80].

1.3- Essential oils

Essential oils are liquids containing volatile aroma compounds from the plant. Essential oils are also known as aromatic oils,

fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of" the plant material from which they were extractedEssential oils are those products obtained from a vegetable raw material. The essential oil is then separated from the aqueous phase by physical means[81]., such as oil of clove. The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majorities of essential oils are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits[82] .

Essential oil may occur in:

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

ii)Leaves, most often, including: eucalyptus, mint, thyme, bay leaf,savory, sage, pine needles,and tree underground organs,

e.g., roots (vetiver),

iii)Rhizomes (ginger, sweet flag),

iv)Seeds (carvi, coriander),

v)Fruits, including: fennel, anise, Citrus epicarps,

vi)Wood and bark, including: cinnamon, sandalwood, rosewood.

1.3.1-Extraction of essential oils

Essential oils are extracted from their natural sources by many techniques as described below[83 ,84.]

i)Hydrodistillation

The technique of hydrodistillation is used for the extraction of essential oils, in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser.The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively[85,86].

ii)Solvent extraction

The process of solvent exraction, is employed mainly to separate a compound based on the solubility of its parts. This is done by using two liquids that don't mix, for example, water and an organic solvent . In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent[87].

iii)Soxhlet extraction

Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid [87].

iv)Pressing

During cold pressing the oil is extracted from plant material at low temperatures and pressure. This process in considered as one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils [88]. In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

v)Steam distillation

Steam distillation is specially designed for the extraction of heat-sensitive plant constituents such as natural aromatic compounds.

In this technique the plant materials which are charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material . This steam functions as agents that break up the pores of the raw material and release the

essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further and the essential oil is collected [89]. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.

Some efficient modern extraction techniques have some advantages over the conventional methods mentioned above including: reduction of extraction times, reduction of energy consumption, reduction of volumes of solvents used . Some of these innovative techniques are discussed briefly below:

a)Supercritical fluid extraction

During this innovative technique of extraction a supercritical fluid is employed as the extracting solvent. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction. In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32^{o}C), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract [90]. The main drawback of CO_2 is its

lack of polarity for the extraction of polar analytes [91]. These essential oils can include limonene and other straight solvents.Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods[92]. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts proved to be of superior quality, with better functional and biological activities [93]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

b)Extraction by microwave hydrodiffusion

The technique known as microwave hydrodiffusion and gravity (MHG) is a green technique used for the extraction of essential oils. It is originally a microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants [93]. Microwave hydrodiffusion and gravity (MHG) become clear not

only as economic and efficient but also as environment-friendly, not require solvent or water and as it does require less energy[94]. The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental impact and power saving [95 ,96].

c)Solvent-free microwave extraction

Another innovative extraction technique used for the extraction of essential oils is the so called solvent-free microwave extraction (SFME) . The extraction is performed by the water which exists within the matrix without using any solvent [97]. Based on the integration of dry distillation and microwave heating energy, . It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent [98]. In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature, and extraction time were controlled by the panel in the instrument.

d)Ultrasonic-assisted extraction

A technique that can achieve high valuable compounds is the so called ultrasonic-assisted extraction. This process is reputed as an excellent technique and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material[99]. The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation . In these applications the power ultrasonic increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer[100].

In ultrasonic-assisted extraction the plant material is immersed in water or another solvent (methanol or ethanol or any other solvent) and at the same time, it is subjected to the work of ultrasound[101]. This technique has been used for the extraction of many essential oils especially from the flower, leaves or seeds [102].

e)Microwave-Assisted Hydrodistillation

A microwave oven is used in the extraction process known as microwave-assisted hydrodistillation. The efficiacy of this extraction process is strongly dependent on the dielectric constant of water and the sample [103]. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique(Scheme 8). Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere [104,105]and consuming only a fraction of the energy used in conventional extraction methods[106].The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions. Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process.The principle of heating using Microwave-assisted hydrodistillation is based

upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously[107].

1.3.2-Composition of essential oils

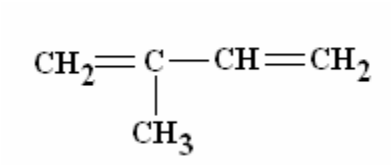
Usually Eessential oils are composed of mixtures of a large number of components. They contain terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups[108 ,109]:

a-Volatile fraction: essential oil which are constituting of 90–95% of the oil in weight and containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

b-Nonvolatile residue: that comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids.

i)Hydrocarbons

Those hydrocarbon found in plants mainly consist of isoprene units.The isoprene is illustrated below[109].



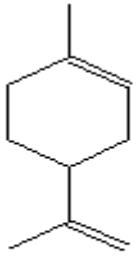
(Isoprene)

ii)Terpenes

In essential oils terpenes possess diverse biological activites including: anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes, diterpenes , triterpenes and polyterpenes. Referring back to isoprene units under the hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes[109].

i)Monoterpenes

A Mono-terpene has the molecular formula : **[$C_{10}H_{16}$].**The biological activity of monoterpense include: analgesic, bactericidal, expectorant, and stimulant effects. Some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids.An Example of monoterpenes is limonene [109]. Monoterpenes are branched-chain C_{10} hydrocarbons with two isoprene units and they are of wide distribution in nature with more than 400 naturally occurring monoterpenes identified.Some of these being linear derivatives (geraniol, citronellol).



Limonene

Monoterpenes ccould be monocyclic like camphor – bicyclic like pinenes (**α** and **β**) or tricyclic . Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium*_(wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses[109].

ii)Sesquiterpenes

Sesquiterpenes are widely distributed in essential oils and constitute a large group of secondary metabolites .These secondary metabolites are endowed with diverse biological activies including: anti-inflammatory, anti-septic, analgesic, anti-allergic.The secondary metabolites – sesquiterpenes- are biogenetically derived from farensyl pyrophosphate and their structure may be linear, monocyclic or bicyclic., some have been shown to be stress compounds formed as a result of disease or injury.

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

allergies in humans and they can also act as insect feeding deterrents[109].

Such lactones are classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc[109].

Eudesmanolides Xantnanolides

iii)Diterpene

Diterpenes are characterized by four isoprene units. Diterpenes exhibit diverse pharmacological effects including: hormonal balancers, hypotensive, anti-fungal, expectorant,.

Diterpenes are rarely found in distilled essential oils since they are too heavy to allow for evaporation with steam in the distillation process.

Diterpenes usually occur different plant families and consist of compounds having a C_{20} skeleton. Up to date there are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis

occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate [109].

iv)Alcohols

Some essential oils may contain alcohols. Alcohols exert many biological activities including: antiseptic, antiviral, bactericidal and germicidal.

Alcohols may combine with a terpenes or may exist naturally in a free form. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use[109].

v)Aldehydes

Essenial oils may contain aldehydes. Natural aldehydes show diverse pharmacological properties like anantifungal, anti-inflammatory, anti-eptic, antiviral, bactericidal, disinfectant, sedative. Medicinally, essential oils containing aldehydes are effective in treating *Candida* and other fungal infections[109].

vi)Esters

Essential oils esters are antimicrobial agents and are mainly used traditionally for their soothing, and balancing effects. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally

are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system[109].

vii)Ketones

Some essential oils contain ketones which are anti-catarrhal, cell proliferant and expectorant.Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic. The most toxic ketone is thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil[109].

viii)Lactones

Lactone-bearing essential oils can reduce prostaglandin synthesis and may act as expectorant. They possess antiinflammatory, antiphlogistic, expectorant and febrifuge activity. Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the actions. Lactones have an even stronger expectorant action than ketones[109].

1.4-Gas Chromatography coupled with mass spectrometry

Essential oil analysis is usually based on separation procedures giving the best performance and achieved by the most effective tool. The most popular tool used by scientists for separation techniques is the chromatography and coupled to that is often the mass spectrometry for the identification of components. Analysis of EOs have recently known major developments with varying methods adapted from the conventional gas chromatography coupled to mass spectrometry technique. The driving force of this surge has been the characterization and identification of the structure of known and novel molecules. The advantage of using a gas chromatograph is that it provides the conditions required for achieving the separation of analyte components without lowering the performance of the column when it comes to more complex analysis. However gas Chromatography can be insufficient or difficult to interpret. Presently, we have seen in the literature the use of the gas chromatography coupled to Tandem mass spectrometry. It is a powerful analytical technique which offers the possibility of detecting specific, targeted compounds whether present in large amount or in trace[110].

Following the separation by gas chromatography, the Tandem mass spectrometry operates by selecting the target ions having specific and known mass. These ions are then dislocated by collision with helium molecules. The product ion resulting from

this collision gives a spectrum which confirms the target analyte as even if there is another ion with the same mass, the spectrum will be different. This factor increases the selectivity of the tandem mass spectrometry. The target gas, which can be argon, xenon, helium or other (according to choice of energy desired for the collision ion dissociation process), can play an important role in the results as the pressure and temperature of the target gas affect the internal energy distribution and thus affecting also the mass spectrum. Hence, low energy target gas is less reproducible. Whereas the high energy target gas for the collision ion dissociation process was found to be more reproducible and to give less rearrangement in the mass spectrum making it less complex to analyze[110]. According to the literature, gas chromatography coupled to Tandem mass spectrometry is not only commonly used for the regular analysis of EOs but it remains however an accurate tool for the separation and detection of trace elements found in a complex mixture.

1.5-Examples of oils extracted from some Sudanese plants

I.5.1-*Haplophyllum tuberculatum* oil

Haplophyllum tuberculatum seed oil was studied by GC-MS. The analysis showed 22 constituents. Major constituents of the oil are[111]:

9,12-octadecadienoic acid methyl ester (49.60%); 9,12,15-octadecatrienoic acid methyl ester (17.91%); hexadecanoic

acid methyl ester (11.70%) and methyl stereate (8.07%).

The antimicrobial activity of the oil was examined against Gram positive bacteria *Bacillus subtilis,* and *staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseud*om*onas aeruginose* and fungus *candida albicans*. The oil exhibited good activity against *Bacillus subtilis.*

1.5.2-*Brassica juncea* oil

Brassica juncea oil was analyzed[111] by GC-MS. The analysis showed 15 constituents. Major constituents are:

-13-Docosenic acid methyl ester(43.61%)

-.9 12-Octadecadienoic acid methyl ester(17.50%).

-9 12 15-Octadectrienoic acid methyl ester(12.49%)

- cis-13-Eicosenoic acid methyl ester(7.83%).

The oil exhibited partial activity against *Bacillus subtilis,Escherichia coli* and *Pseudomonas aeruginosa*.

1.5.3-*Momordica chantia* oil

The GC-MS analysis of *Momordica chantia* oil showed 21 constituents.Major components are: i-9,12-octadecadienoic acid methyl ester(47.64%) ; ii)hexadecanoic acid , methyl ester (17.06 %); iii) 9,12,15-Octadecatrienoic acid methyl ester(13.29%).The oil exhibited[111] good activity against *Escherichia coli.*

1.5.4-*Cordia myxa* oil

The GC-MS analysis of *Cordia myxa* oil exhibited[111] the presence of 17 components dominated by : i-9,12-octadecadienoic acid methyl ester(28.81%) ii)hexadecanoic acid , methyl ester (23.24 %) iii)11-octadecenoic acidmethyl ester(16.89%) and iv)9-Octadecenoic acid methyl ester(11.03%).

The oil exhibited good activity against *Bacillus subtilis* , *staphylococcus aureus*, *Pseud*om*onas aeruginose* beside significant anticandidal activity .

1.5.5-*Helianthus annuus* oil

The GC-MS analysis of *Helianthus annuus* oil exhibited the presence of 31 constituents[111]. Major components of the oil are :i-9,12-octadecadienoic acid methyl ester(44.67%) ii)9-octadecenoic acid methyl ester(19.71%) and iii)hexadecanoic acid , methyl ester (17.16 %).

The antimicrobial activity of the oil was examined against Gram positive bacteria :*staphylococcus aureus*, Gram negative bacteria *Escherichia coli*, *Pseud*om*onas aeruginose* and the fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole).The oil exhibited good activity against all test organisms.

1.5.6-*Securidaca longipedum* oil

Securidaca longipedum oil was analyzed[111] by GC-MS. The analysis showed 22 constituents. The GC-MS analysis showed the following major components:

i)9 12-Octadecadienoic acid methyl ester(29.04%)

ii)Hexadecanoic acid methyl ester(22.90%).

iii)Methyl stearate(15.30%).

iv)9 12 15-Octadecatrienoic acid methyl ester(7.10%).

The oil exhibited good activity against *Staphylococcus aureus* It was also active against *Pseudomonas aeruginosa* and *Candida albicans.*However the oil exhibited weak activity against *Bacillus subtilis*.

1.5.7-*Lycopersicum esculentum* seed oil

Lycopersicum esculentum seed oil was analyzed by GC-MS. The following major constituents have been detected[112] by GC-MS analysis:

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

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

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

The oil showed significant activity against *Bacillus subtilis*. It also exhibited moderate activity against *Pseudomonas*

aeroginosa, Escherichia coli, Staphylococcus aureus and *Candida albicans.*

1.5.8-*Sterculia setigera* oil

The GC-MS analysis of *Sterculia setigera* oil revealed[112] the presence of 20 components. Major components of the oil are:

i)-cis-9-Hexadecenal

(32.66%)

ii)-Oleic acid (20.29%)

Sterculia speciesare oil showed partial activity against *Staphylococcus aureus* and *Escherichia coli.*

1.5.9-*Carthamus tinclorius* oil

GC-MS analysis of *Carthamus tinclorius* oil was conducted[112].The GC-MS analysis of the oil revealed the presence of 21components. The analysis revealed the following major constituents **:**

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

ii) ii) Hexadecanoic acid methyl ester (14.04%)

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

The oil showed good activity against *Pseudomonas aeruginosa.*

1.5.10-*Ocimum basilicum* oil

The oil extracted from *Ocimum basilicum* was investigated by GC-MS analysis[112]. Fifty six components were detected in total

ion chromatogram. The GC-MS analysis revealed the following major constituents:

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

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

iii-Hexdecanoic acid methyl ester(14.74%)

iv-Methyl stearate (9.83%).

the oil failed to give antimicrobial potential against the following microbial isolates : *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*

1.5.11-*Prunus mahaleb* oil

Prunus mahaleb oil has been investigated[112] by GC-MS. The analysis showed 40 constituents. Major constituents are:

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

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

ii) Hexdecanoic acid (7.91 %)

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

1.5.12-*Beta vulgaris* oil

The GC-MS analysis of *Beta vulgaris* oil showed the presence of 35 components.. Fatty acids constituted major bulk of the oil(99.67%). terpenes(0.03%) and hydrocarbons(0.03%) appeared as minor constituents. The studied oil showed

significant activity against *Bacillus subtilis* and moderate anticandidal activity[113].

1.5.13-*Tephrosia apollina* oil

Tephrosia apollina oil was analyzed by GC-MS . The analysis showed 22 components. Fatty acids constituted 97.96% , the rest is ϐ-sitosterol (2.04%)[113].The studied oil showed significant activity against *Bacillus subtilis* , *Staphylococcus aureus* and *Escherichia coli.*

1.5.14-*Eucalyptus camaldulensis* oil

GC-MS analysis of *Eucalyptus camaldulensis* volatile oil was conducted[113]. Thirty seven constituents were identified by GC-MS.Major constituent was : 9,12-Octadecadienoic acid methyl ester (54.74%).The oil showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.*

1.5.15-*Annona squamosa* oil

GC-MS analysis of *Annona squamosa* oil was conducted[113]. Twenty two constituents were identified. The oil showed moderate anticandidal activity.

1.5.16-*Dichrostachys cinera* oil

Dichrostachys cinera oil was analyzed[114] by GC-MS. Twenty three constituents were identified.. Major components are: 9,12-octadecadienoic acid methyl ester(30.87%) and 9-octadecenoic

acid methyl ester(27.02%).The oil showed significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

1.5.17-*Kegalia Africana* oil

The oil from *Kegalia Africana* was analyzed[114] by GC-MS. Twenty three constituents were identified.Major components are: 9,12,15-octadecatrienoic acid methyl ester(31.32%) and 9,12-octadecadienoic acid methyl ester(29.44%).The oil showed moderate anticandidal activity.

Aim of this study

This study was designed to:

-Investigate the oils from six Sudanese plants of potential medicinal attributes.

-Analyze the targeted oils by GC/MS to identify and quantify the constituents of the oils.

-Evaluate the targeted oils for antioxidant activity.

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Acacia seiberiana, Acacia alata, Leucaena leucocephala, Pongamia pinnata , Delonix regia* and *Cassia auriculata* were collected from Damazin- Sudan. The plants were identified and authenticated by The Medicinal and Aromatic Plants Research Institute-Khartoum-Sudan.

2.1.2-Instruments

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

2.1.3-Test organisms

The following test organisms used in this study: *Bacillus subtilis (*G+ve) ,*Staphylococcus aureus(*G+ve), *Pseudomonas aeroginosa* (G-ve) ,*Escherichia coli* (G-ve) and *Candida albicans* (fungus).

2.2-Methods

2.2.1-Extraction of oil

Powdered plant material (400g) were macerated with n-hexane for 48h.The solvent was removed under reduced pressure giving the oil.

2.2.2-GC-MS analysis

(2ml) of the oil was mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving 2 g in 100 ml methanol. (7 ml) alcoholic sulfuric acid (1ml H_2SO_4 in 100 ml methanol) was added. The mixture was then shacked for 5 minutes .The content of the test tube was left to stand overnight. Then (1ml) of supersaturated sodium chloride was added and the tube was shaken for 5 min. (2ml) of normal hexane were added and the contents were shaken thoroughly for 5 minutes. (5 µl) of the n-hexane were diluted with (5ml) of diethyl ether and dried over anhydrous sodium sulphite . (1µl) of the diluted sample was injected in the GC.MS vial.

The qualitative and quantitative analysis of the sample was carried out by using a Shimadzu machine- model (GC/MS-QP2010-Ultra) The sample was injected under the following chromatographic conditions:column oven temperature :150.0^oC ; injection temperature:300.0^oC ;injection mode : split; flow mode: linear velocity; pressure:139KPa; total flow: 50.0ml/min ; column flow:1.54ml/sec. ;

linear velocity: 47.2cm/sec. ;purge flow:3.0 ml/min. ; split ratio: -1.0. Oven temperature program is presented Table 1

Table 1: Oven temperature program

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

2.2.3-Antioxidant assay

The targeted oils have been screened for antioxidant activity against stable DPPH radicals and the decrease in absorbance at λ_{Max} 217 nm has been measured via UV spectroscopy.

3-Results and Discussion

In this study the oils from six plants of medicinal attributes (*Acacia seiberiana, Acacia alata, Leucaena leucocephala, Pongamia pinnata , Delonix regia* and *Cassia auriculata*) have been investigated by GC.MS and the antimicrobial activity has been screened.

3.1-*Acacia seiberiana*

3.1.1-GC/MS analysis of *Acacia seiberiana* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of the constituents of *Acacia seiberiana* oil. The analysis revealed the presence of 22 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

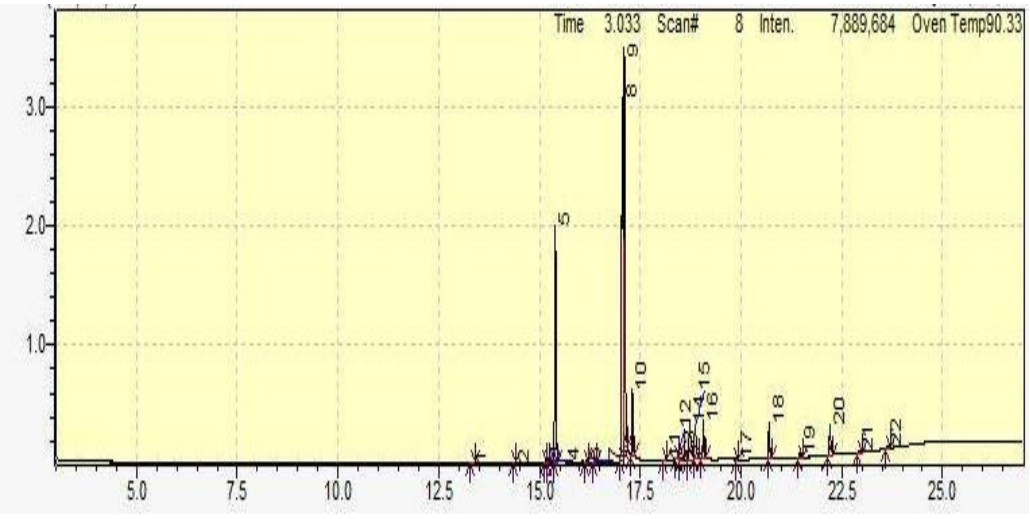


Fig. 3.1: Total ion chromatograms

Table 3.1: Constituent of the oil

ID#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.313	0.06
2.	Pentadecanoic acid, methyl ester	14.371	0.04
3.	7-Hexadecenoic acid, methyl ester, (Z)-	15.153	0.06
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.195	0.17
5.	Hexadecanoic acid, methyl ester	15.392	15.95
6.	cis-10-Heptadecenoic acid, methyl ester	16.156	0.12
7.	Heptadecanoic acid, methyl ester	16.366	0.14
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.057	31.21
9.	9-Octadecenoic acid (Z)-, methyl ester	17.105	29.55
10.	Methyl stearate	17.304	4.35
11.	10-Nonadecenoic acid, methyl ester	18.117	0.11
12.	.gamma.-Linolenic acid, methyl ester	18.388	0.17
13.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.503	0.33
14.	11,14-Eicosadienoic acid, methyl ester	18.728	4.81
15.	cis-11-Eicosenoic acid, methyl ester	18.866	3.33
16.	Eicosanoic acid, methyl ester	19.069	2.63
17.	Heneicosanoic acid, methyl ester	19.898	0.09
18.	Docosanoic acid, methyl ester	20.696	2.80
19.	Tricosanoic acid, methyl ester	21.464	0.35
20.	Tetracosanoic acid, methyl ester	22.205	2.93
21.	Pentacosanoic acid, methyl ester	22.926	0.34
22.	Hexacosanoic acid, methyl ester	23.618	0.46

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (31.21 %)
ii) 9-Octadecenoic acid methyl ester(29.55%)
iii) Hexdecanoic acid methyl ester(15.95 %)

The GC-MS analysis showed a mass spectrum(Fig.3.2) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT.17.057)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The analysis

showed a mass spectrum(Fig.3.3) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT.17.105) corresponds : $M^{+}[C_{19}H_{36}O_2]^{+}$.

The GC-MS analysis also gave a spectrum(Fig.3.4) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT.15.392) accounts for: M^{+} $[C_{17}H_{34}O_2]^{+}$.

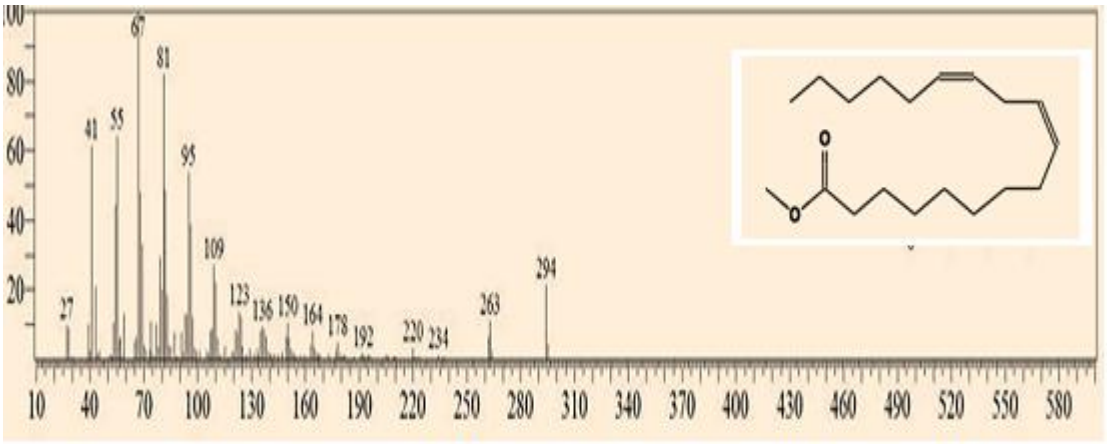


Fig. 3.2 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

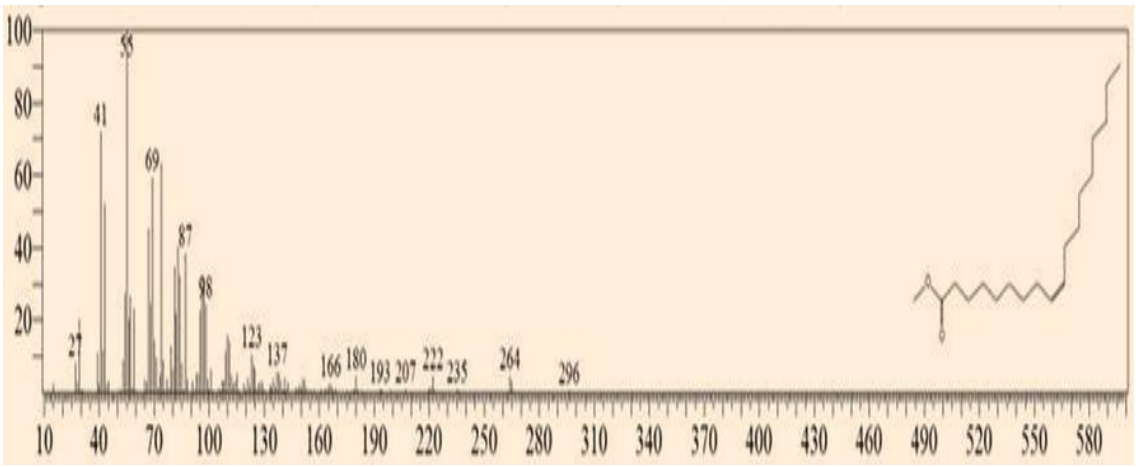


Fig.3.3: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

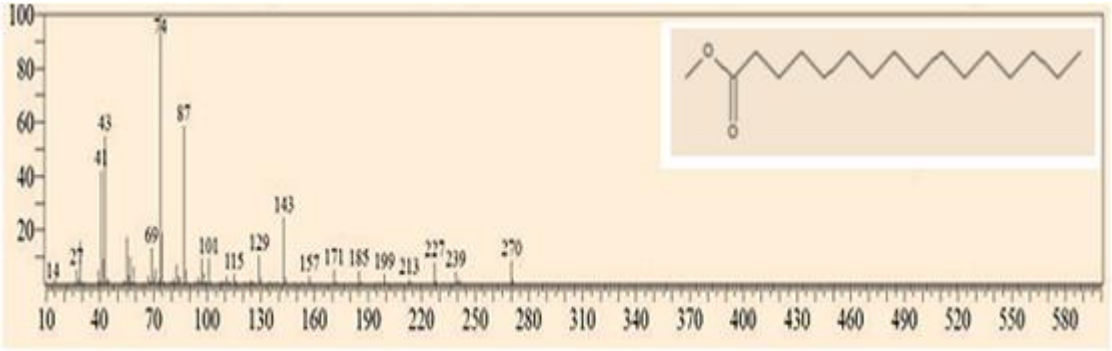


Fig 3.4 : Mass spectrum of hexadecanoic acid, methyl ester

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3.1.2-Antioxidant activity of *Acacia seiberiana* oil

Acacia seiberiana oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.2.The oil exhibited weak antioxidant activity.

Table 3.2: Antioxidant activity of *Acacia seiberiana* oil

Sample	**%RSA± SD (DPPH)**
Oil 100mg/ml	19 ± 0.01
Propyl gallate	89± 0.01

3.2-*Leucaena leucocephala*

3.2.1-GC-MS analysis of *Leucaena leucocephala* oil

Gas chromatography - mass spectrometry analysis of *Leucaena leucocephala* oil showed the presence of 18 components - Table (3.3).The total ion chromatogram is presented in Fig.3.5.

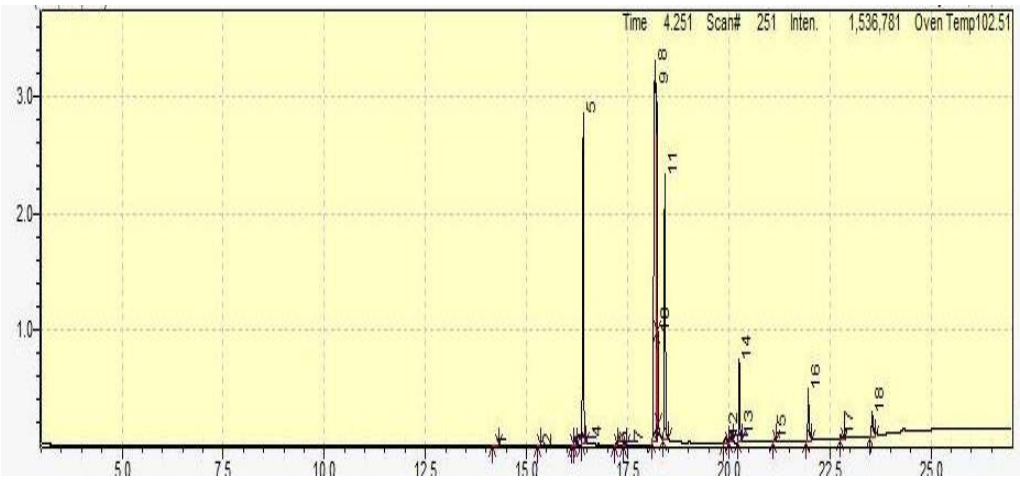

Fig. 3.5 :Total ions chromatograms

Table 3.3 : Constituents of the oil

No.	Name	Ret.Time	Area%
1	Methyl tetradecanoate	14.196	0.07
2	Pentadecanoic acid, methyl ester	15.316	0.02
3	7-Hexadecenoic acid, methyl ester, (Z)-	16.155	0.02
4	9-Hexadecenoic acid, methyl ester, (Z)-	16.193	0.45
5	Hexadecanoic acid, methyl ester	16.404	19.90
6	cis-10-Heptadecenoic acid, methyl ester	17.210	0.08
7	Heptadecanoic acid, methyl ester	17.423	0.15
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.182	38.70
9	9-Octadecenoic acid (Z)-, methyl ester	18.217	11.29
10	6-Octadecenoic acid, methyl ester, (Z)-	18.248	3.00
11	Methyl stearate	18.420	15.00
12	9,12-Octadecadienoyl chloride, (Z,Z)-	19.918	0.53
13	cis-11-Eicosenoic acid, methyl ester	20.055	0.42
14	Eicosanoic acid, methyl ester	20.261	4.49
15	Heneicosanoic acid, methyl ester	21.129	0.19
16	Docosanoic acid, methyl ester	21.967	3.48
17	Tricosanoic acid, methyl ester	22.769	0.29
18	Tetracosanoic acid, methyl ester	23.546	1.92

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (38.70 %)

ii) Hexdecanoic acid methyl ester(19.90 %)

iii) Methyl stearate(15.00%)

iv) 9-Octadecenoic acid methyl ester(11.29%)

The GC-MS analysis showed a mass spectrum(Fig.3.6) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.182)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The GC-MS analysis also gave a spectrum(Fig.3.7) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT. 16.404) accounts for: M^+ $[C_{17}H_{34}O_2]^+$. The analysis also exhibited a mass spectrum (Fig. 3.8) identical with that of methyl stearate. The peak at m/z 298 (RT. 18.420) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$. The analysis showed a mass spectrum(Fig.3.9) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 18.217) corresponds : $M^+[C_{19}H_{36}O_2]^+$.

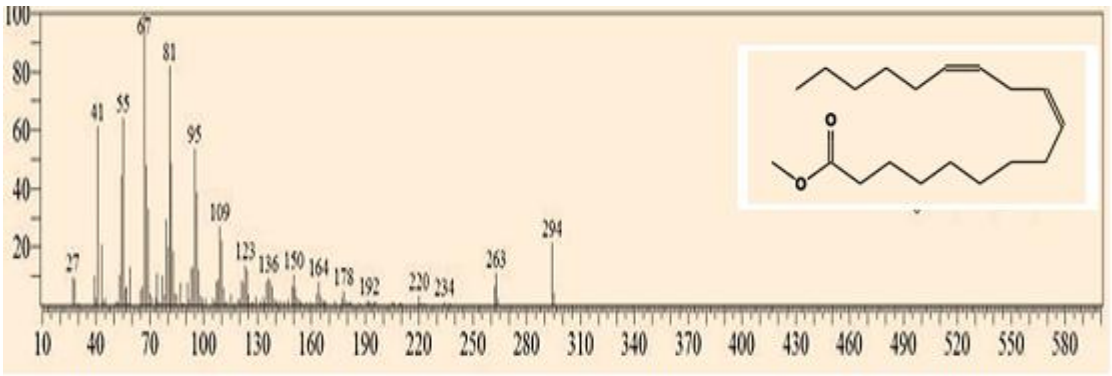

Fig. 3.6 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

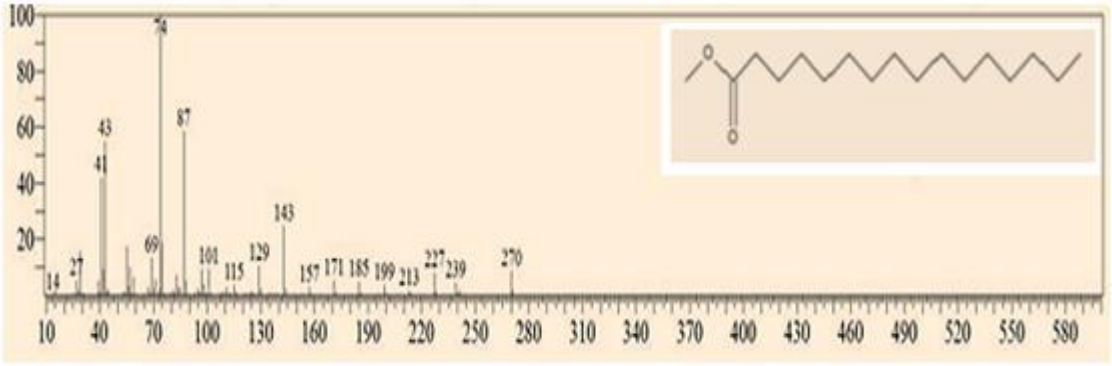

Fig .3.7 : Mass spectrum of hexadecanoic acid, methyl ester

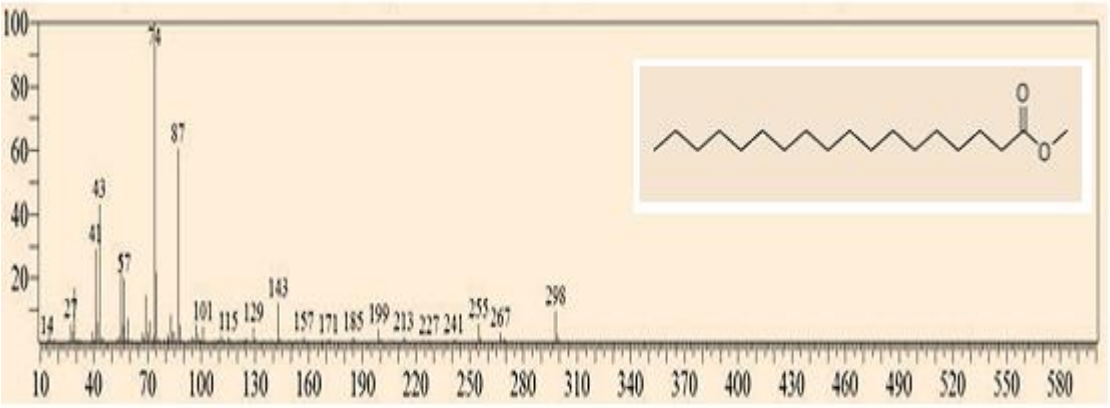

Fig.3.8 : Mass spectrum of methyl stearate

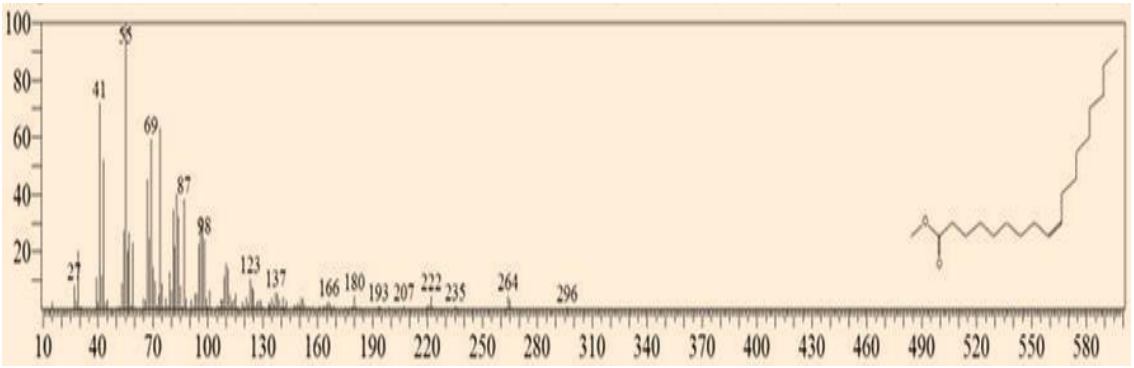

Fig.3.9: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

3.2.2-Antioxidant activity of *Leucaena leucocephala* oil

Leucaena leucocephala oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.4.The oil did not exhibit any antioxidant activity.

Table 3.4: Antioxidant activity of *Leucaena leucocephala* oil

Sample	**%RSA± SD (DPPH)**
Oil (100mg/ml)	Inactive
Propyl gallate	89± 0.01

3.3-*Acacia alata*

3.3.1-GC-MS analysis

GC/MS was conducted for *Acacia alata* oil. The analysis revealed the presence of 23 components - Table (3.5).The total ion chromatogram is presented in Fig.3.10.

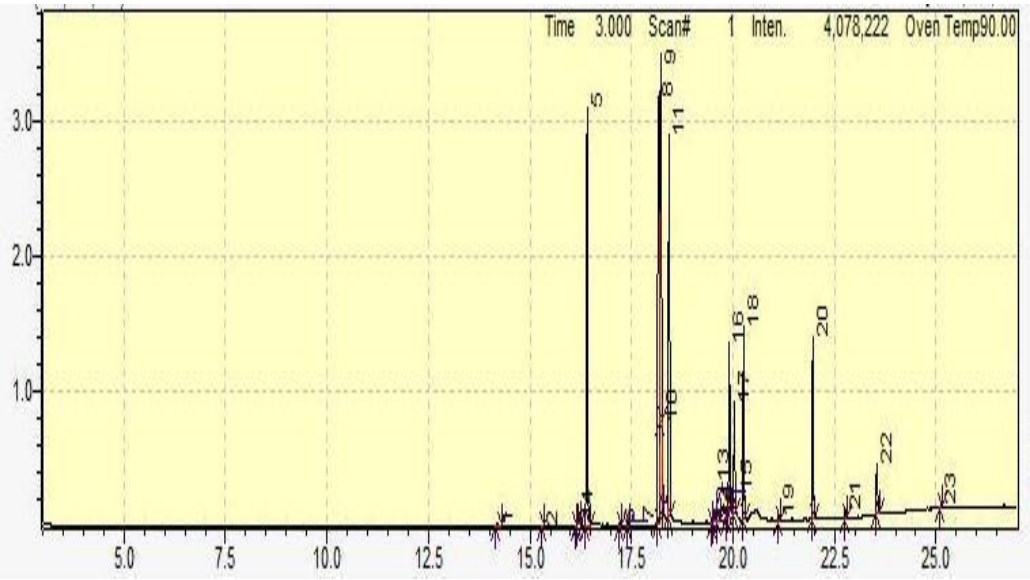

Fig. 3.10 :Total ions chromatograms

Table 3.5 : Constituents of the oil

	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.196	0.10
2.	Pentadecanoic acid, methyl ester	15.318	0.01
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.151	0.04
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.194	0.60
5.	Hexadecanoic acid, methyl ester	16.409	17.53
6.	cis-10-Heptadecenoic acid, methyl ester	17.211	0.09
7.	Heptadecanoic acid, methyl ester	17.423	0.17
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.171	22.73
9.	9-Octadecenoic acid (Z)-, methyl ester	18.226	16.51
10.	9-Octadecenoic acid, methyl ester, (E)-	18.255	1.16
11.	Methyl stearate	18.428	14.83
12.	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	19.500	0.21
13.	.gamma.-Linolenic acid, methyl ester	19.525	0.28
14.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.644	0.32
15.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	19.875	0.56
16.	11-Octadecynoic acid, methyl ester	19.908	5.93
17.	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	20.031	4.01
18.	Eicosanoic acid, methyl ester	20.262	6.26
19.	Heneicosanoic acid, methyl ester	21.124	0.20
20.	Docosanoic acid, methyl ester	21.964	6.11
21.	Tricosanoic acid, methyl ester	22.765	0.26
22.	Tetracosanoic acid, methyl ester	23.541	1.97
23.	Hexacosanoic acid, methyl ester	25.118	0.12

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (22.73 %)
ii) Hexdecanoic acid methyl ester(17.53 %)
iii) 9-Octadecenoic acid methyl ester(16.51%)
iv) Methyl stearate(14.83%)

The GC-MS analysis showed a mass spectrum(Fig.3.11) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.171)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The GC-MS analysis also gave a spectrum(Fig.3.12) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT. 16.409) accounts for: M^+ $[C_{17}H_{34}O_2]^+$. The analysis showed a mass spectrum(Fig.3.13) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 18.226) corresponds : $M^+[C_{19}H_{36}O_2]^+$.The GC-MS analysis also exhibited a mass spectrum (Fig. 3.14) identical with that of methyl stearate. The peak at m/z 298 (RT. 18.429) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$

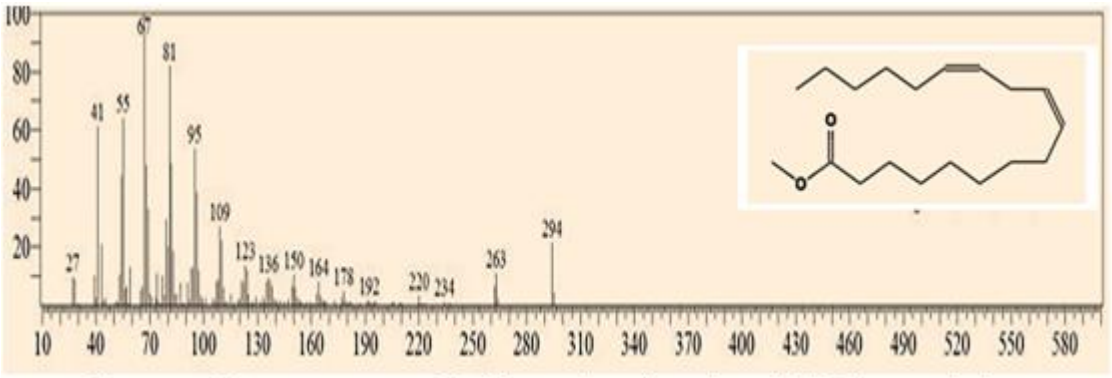

Fig.3.11: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

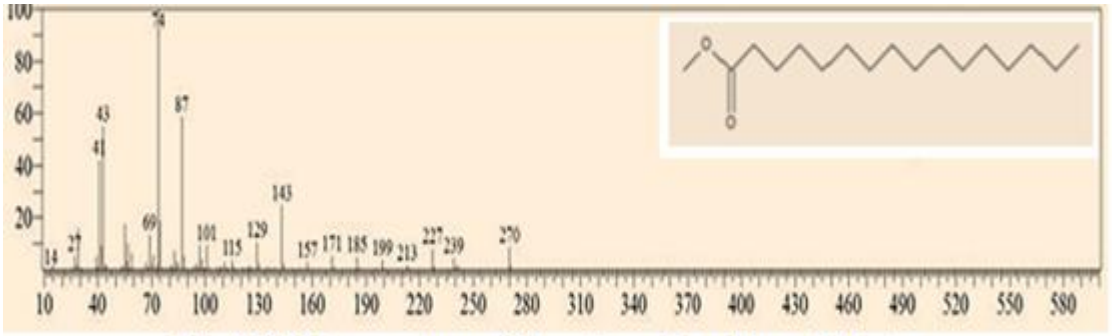

Fig 3.12: Mass spectrum of hexadecanoic acid, methyl ester

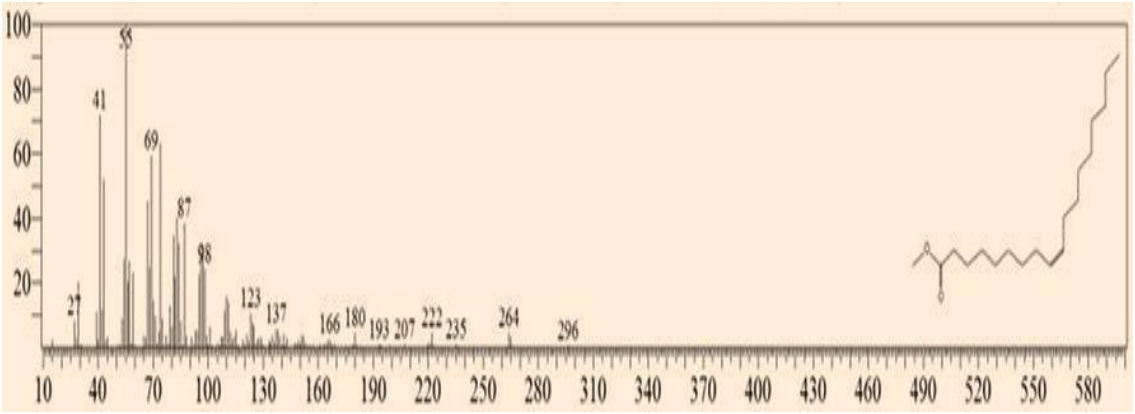

Fig.3.13: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

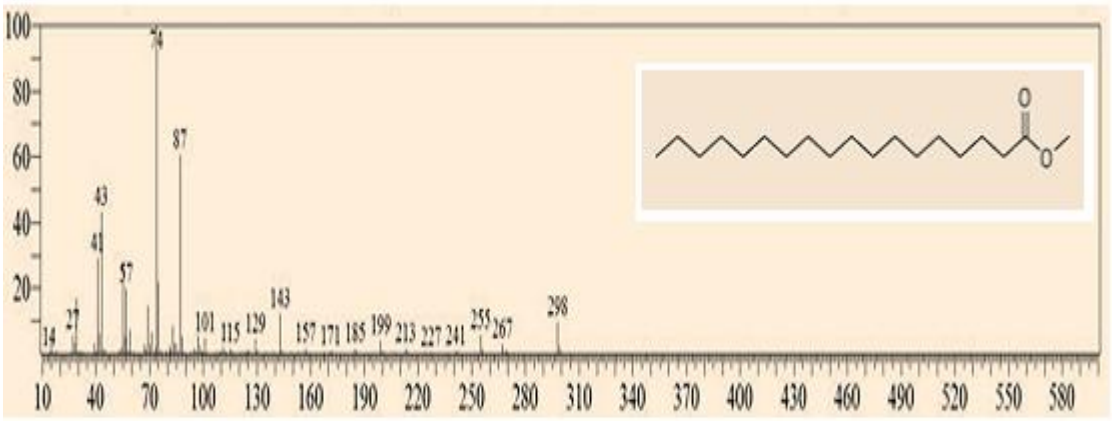

Fig.3.14 : Mass spectrum of methyl stearate

3.2.2-Antioxidant activity of *Acacia alata* oil

Acacia alata oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.6.The oil did not exhibited very weak antioxidant activity.

Table 3.6: Antioxidant activity of *Acacia alata* oil

Sample	**%RSA± SD (DPPH)**
Oil (100mg/ml)	2.3 ± 0.01
Propyl gallate	89± 0.01

3.4-*Pongamia pinnata*

3.4.1-GC-MS analysis

GC/MS was conducted for *Pongamia pinnata* oil. The analysis revealed the presence of 15 components - Table (3.7).The total ion chromatogram is presented in Fig.3.15.

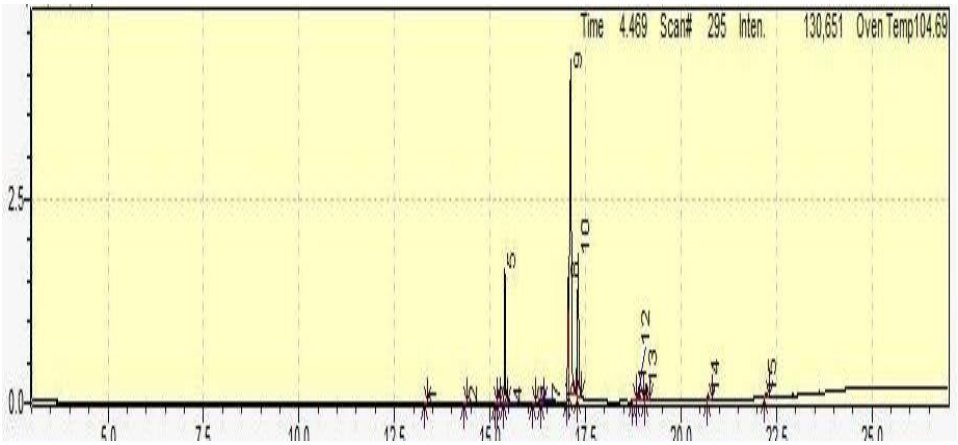

Fig.3.15: Total ions chromatograms

Table 3.7 : Constituents of the oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.314	0.03
2.	Pentadecanoic acid, methyl ester	14.370	0.02
3.	7-Hexadecenoic acid, methyl ester, (Z)-	15.152	0.05
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.196	0.06
5.	Hexadecanoic acid, methyl ester	15.390	12.39
6.	cis-10-Heptadecenoic acid, methyl ester	16.157	0.06
7.	Heptadecanoic acid, methyl ester	16.365	0.14
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.045	13.31
9.	9-Octadecenoic acid (Z)-, methyl ester	17.118	53.58
10.	Methyl stearate	17.308	15.00
11.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.798	0.41
12.	cis-11-Eicosenoic acid, methyl ester	18.884	1.01
13.	Eicosanoic acid, methyl ester	19.083	2.09
14.	Docosanoic acid, methyl ester	20.712	0.91
15.	Tetracosanoic acid, methyl ester	22.219	0.94

The following compounds were detected in the chromatogram as major constituents:

i) 9-Octadecenoic acid methyl ester(53.58%)
ii) Methyl stearate(15.00%)
iii) 9, 12-octadecadienoic acid methyl ester (13.31 %)
iv) Hexdecanoic acid methyl ester(12.39 %)

The GC-MS analysis showed a mass spectrum(Fig.3.16) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 17.118) corresponds : $M^+[C_{19}H_{36}O_2]^+$. The GC-MS analysis also exhibited a mass spectrum (Fig. 3.17) identical with that of methyl stearate. The peak at m/z 298 (RT. 17.308) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$. It also revealed a mass spectrum(Fig.3.18) characteristic of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.045)corresponds M^+ $[C_{19}H_{34}O_2]^+$.The GC-MS analysis also gave a spectrum(Fig.3.19) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT. 15.390) accounts for: M^+ $[C_{17}H_{34}O_2]^+$.

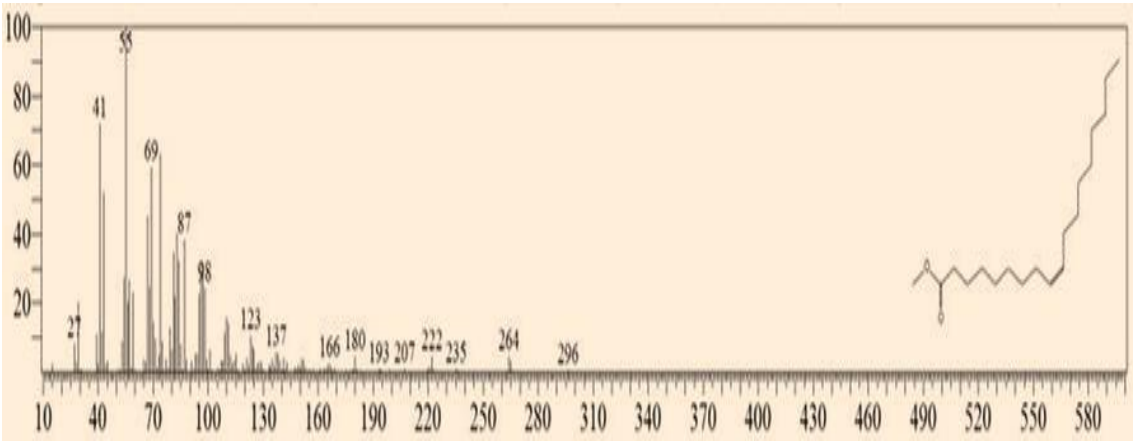

Fig.3.16: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

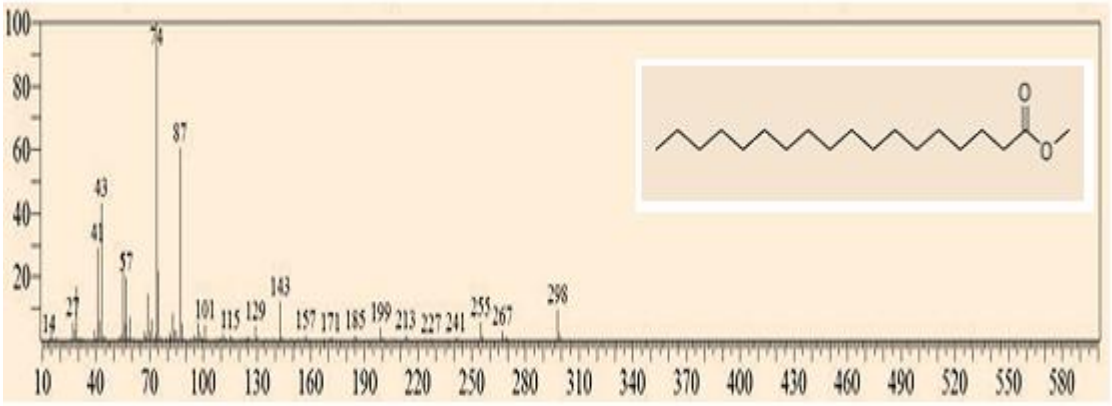

Fig.3.17 : Mass spectrum of methyl stearate

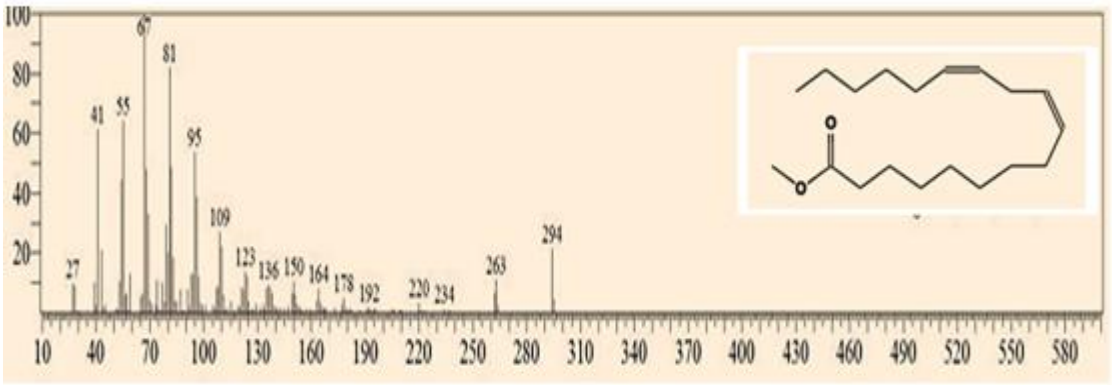

Fig.3.18: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

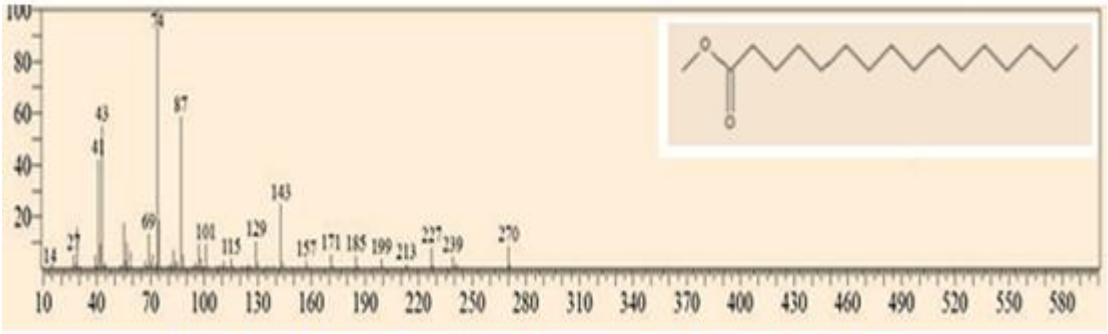

Fig 3.19 : Mass spectrum of hexadecanoic acid, methyl ester

3.4.2-Antioxidant activity of *Pongamia pinnata oil*

Pongmia pinnata oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.8.The oil exhibited moderate antioxidant activity.

Table 3.8:Antioxidant activity of *Pongmia pinnata* oil

Sample	**%RSA± SD (DPPH)**
Oil (100mg/ml)	46 ± 0.08
Propyl gallate	89± 0.01

3.5-*Cassia auriculata*

3.5.1-*GC-MS analysis*

GC/MS was conducted for *Cassia auriculata* oil. The analysis revealed the presence of 16 components - Table (3.9).The total ion chromatogram is presented in Fig.3.20.

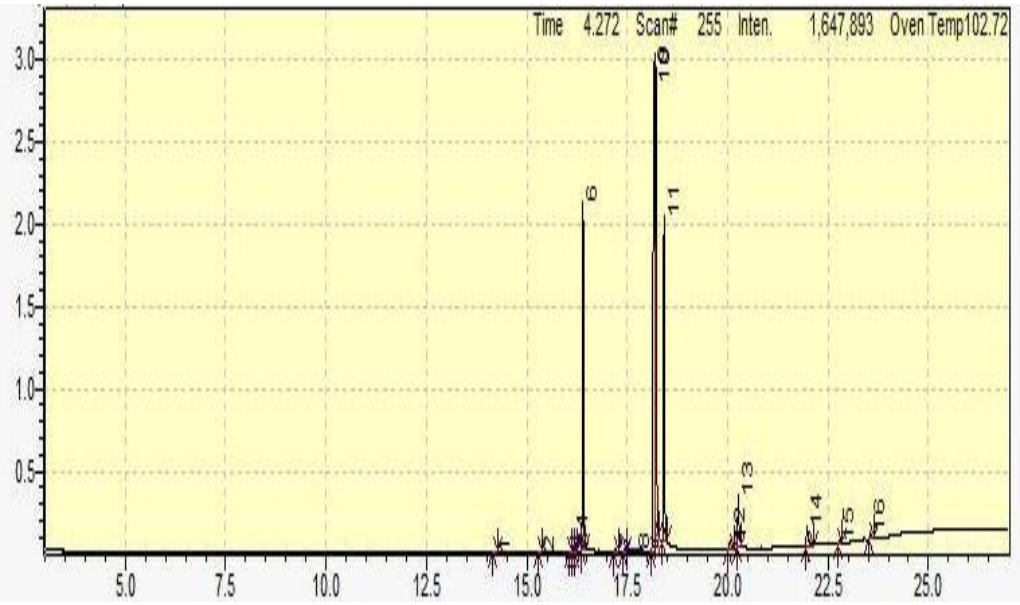

Fig.3.20: Total ions chromatograms

Table 3.9 : Constituents of the oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.200	0.06
2.	Pentadecanoic acid, methyl ester	15.320	0.04
3.	7,10-Hexadecadienoic acid, methyl ester	16.092	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	16.150	0.08
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.196	0.13
6.	Hexadecanoic acid, methyl ester	16.399	17.59
7.	cis-10-Heptadecenoic acid, methyl ester	17.212	0.05
8.	Heptadecanoic acid, methyl ester	17.424	0.12
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.164	33.02
10.	9-Octadecenoic acid (Z)-, methyl ester	18.209	26.77
11.	Methyl stearate	18.417	15.86
12.	cis-11-Eicosenoic acid, methyl ester	20.063	0.50
13.	Eicosanoic acid, methyl ester	20.266	3.21
14.	Docosanoic acid, methyl ester	21.976	1.84
15.	Tricosanoic acid, methyl ester	22.781	0.20
16.	Tetracosanoic acid, methyl ester	23.555	0.50

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (33.02 %)
ii) 9-Octadecenoic acid methyl ester(26.77%)
iii) Hexdecanoic acid methyl ester(17.59 %)
iv) Methyl stearate(15.86%)

The GC-MS analysis revealed a mass spectrum(Fig.3.21) characteristic of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.164)corresponds M^+ $[C_{19}H_{34}O_2]^+$. It also showed a mass spectrum(Fig.3.22) identical with that of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 18.209) corresponds : $M^+[C_{19}H_{36}O_2]^+$. The GC-MS analysis exhibited a mass spectrum (Fig. 3.23) identical with that of

hexadecanoic acid methyl ester .The peak at m/z 270(RT. 16.399) accounts for: M^+ $[C_{17}H_{34}O_2]^+$. It also revealed a mass spectrum(Fig.3.24) characteristic of methyl stearate. The peak at m/z 298 (RT. 18.417) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$.

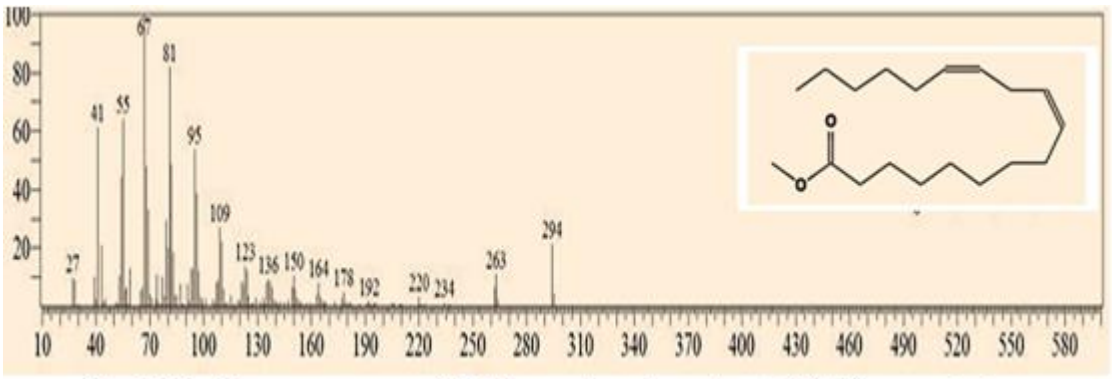

Fig.3.21: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

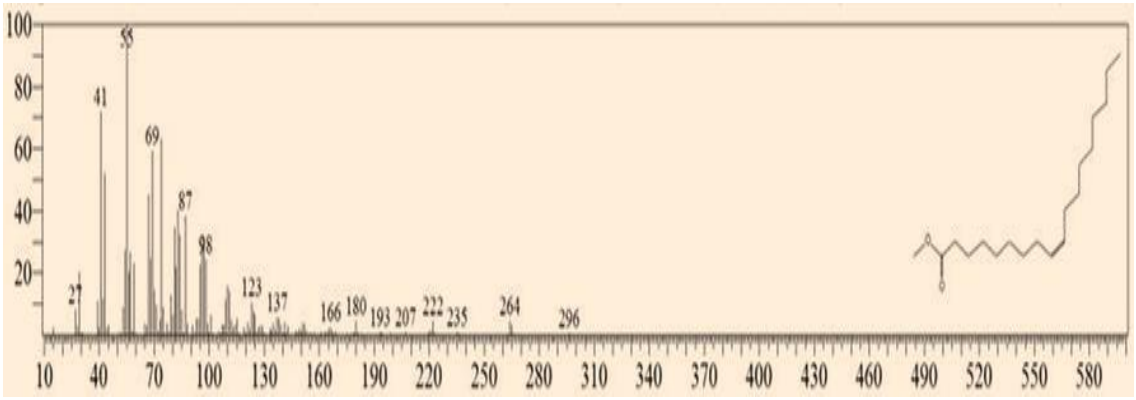

Fig.3.22: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

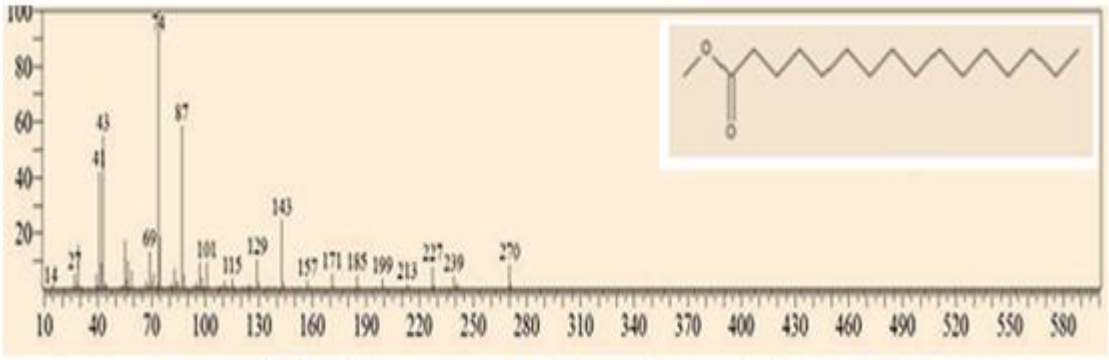

Fig 3.23: Mass spectrum of hexadecanoic acid, methyl ester

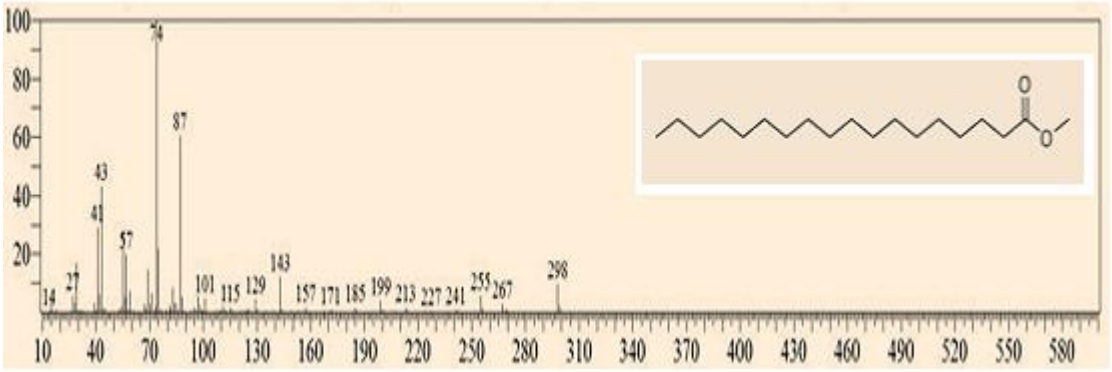

Fig.3.24 : Mass spectrum of methyl stearate

3.5.2-Antioxidant activity of *Cassia auriculata* oil

Cassia auriculata oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.10.The oil exhibited moderate antioxidant activity.

Table 3.10: Antioxidant activity of *Cassia auriculata* oil

Sample	**%RSA± SD (DPPH)**
Oil(100mg/ml)	23.3 ± 0.18
Propyl gallate	89± 0.01

3.6-*Delonix regia*

3.6.1-GC-MS analysis of *Delonix regia* oil

GC/MS was conducted for *Delonix regia* oil. The analysis revealed the presence of 16 components - Table (3.11).The total ion chromatogram is presented in Fig.3.25.

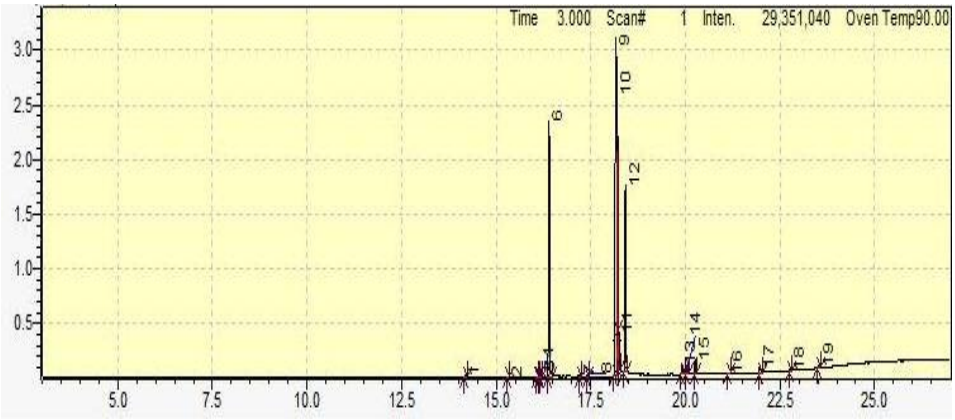

Fig.3.25: Total ions chromatograms

Table 3.11 : Constituents of the oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.181	0.13
2.	Pentadecanoic acid, methyl ester	15.311	0.04
3.	7,10-Hexadecadienoic acid, methyl ester	16.083	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	16.148	0.03
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.191	0.38
6.	Hexadecanoic acid, methyl ester	16.399	21.33
7.	cis-10-Heptadecenoic acid, methyl ester	17.208	0.08
8.	Heptadecanoic acid, methyl ester	17.420	0.16
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.170	43.07
10.	9-Octadecenoic acid (Z)-, methyl ester	18.205	14.51
11.	9-Octadecenoic acid, methyl ester, (E)-	18.241	2.04
12.	Methyl stearate	18.414	14.12
13.	11-Octadecynoic acid, methyl ester	19.911	1.32
14.	cis-11-Eicosenoic acid, methyl ester	20.042	0.41
15.	Eicosanoic acid, methyl ester	20.258	1.18
16.	Heneicosanoic acid, methyl ester	21.127	0.04
17.	Docosanoic acid, methyl ester	21.964	0.63
18.	Tricosanoic acid, methyl ester	22.770	0.16
19.	Tetracosanoic acid, methyl ester	23.543	0.34

The following compounds were detected in the chromatogram as major constituents:

i) 9, 12-octadecadienoic acid methyl ester (34.07 %)

ii) Hexdecanoic acid methyl ester(21.33 %)

iii) 9-Octadecenoic acid methyl ester(14.51%)

iv) Methyl stearate(14.12%)

The GC-MS analysis revealed a mass spectrum(Fig.3.26) characteristic of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.170)corresponds M^+ $[C_{19}H_{34}O_2]^+$. It also showed a mass spectrum(Fig.3.27)identical with that of hexadecanoic acid methyl ester .The peak at m/z 270(RT. 16.399) accounts for: M^+ $[C_{17}H_{34}O_2]^+$.The analysis gave a mass spectrum(Fig.3.28) characteristic of 9-octadecenoic acid methyl ester. The signal at m/z 296 (RT. 18.205) corresponds : $M^+[C_{19}H_{36}O_2]^+$. It also revealed a mass spectrum(Fig.3.29) characteristic of methyl stearate. The peak at m/z 298 (RT. 18.414) is due to the molecular ion : $M^+[C_{19}H_{38}O_2]^+$.

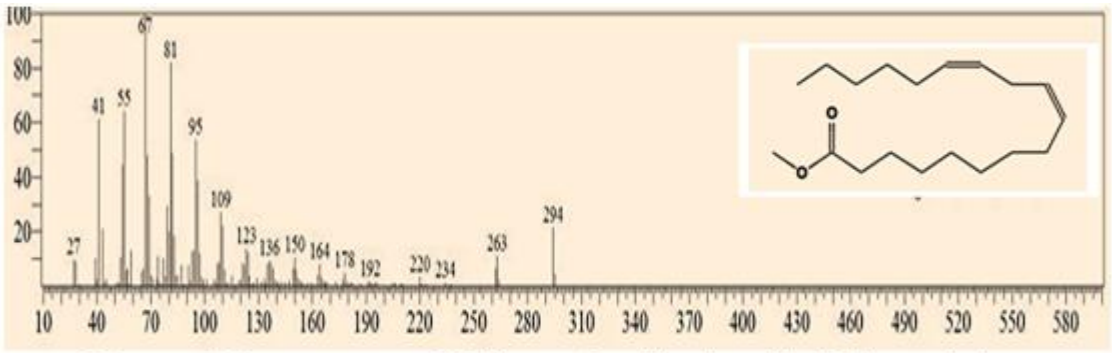

Fig.3.26: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

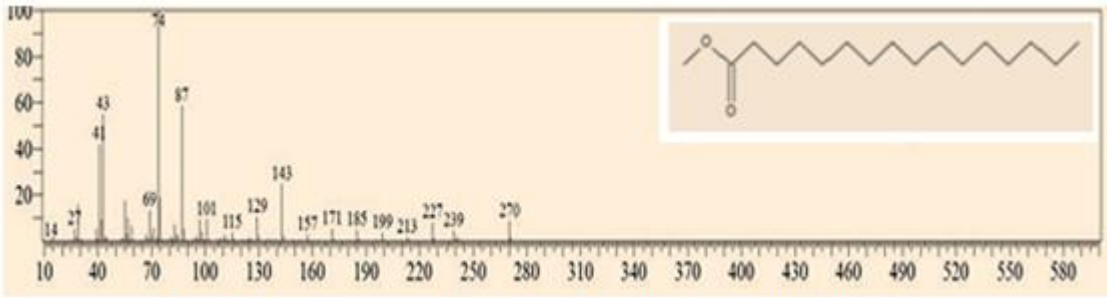

Fig 3.27: Mass spectrum of hexadecanoic acid, methyl ester

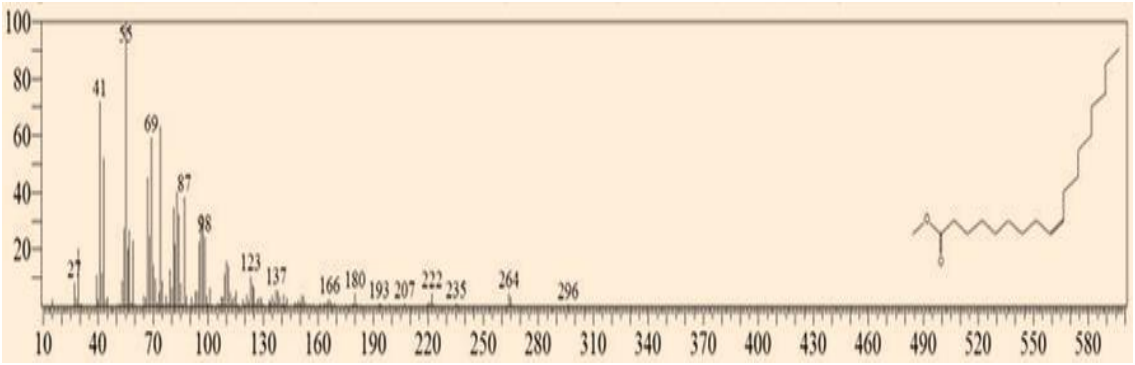

Fig.3.28: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

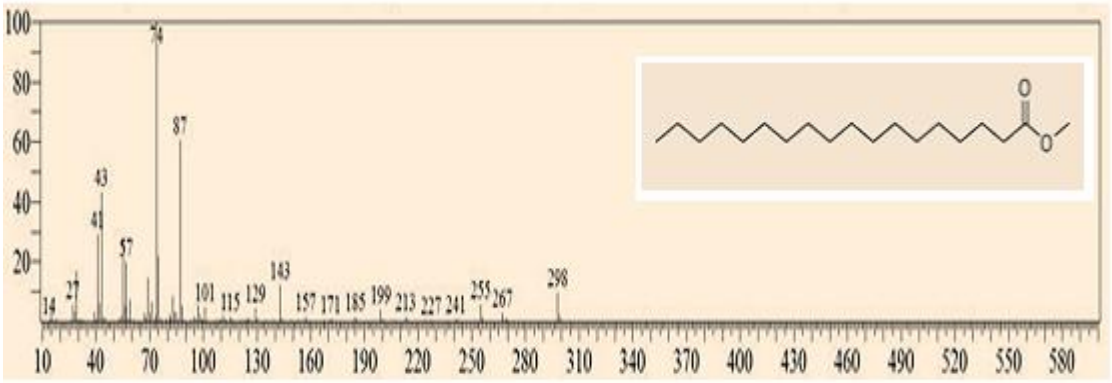

Fig.3.29 : Mass spectrum of methyl stearate

3.6.2-Antioxidant activity of *Delonix regia* oil

Delonix regia oil has been screened for antioxidant activity against stable DPPH radicals and the results are depicted in Table 3.12.The oil exhibited moderate antioxidant activity.

Table 3.12:Antioxidant activity of *Delonix regia* oil

Sample	**%RSA± SD (DPPH)**
Oil(100mg/ml)	50.1 ± 0.25
Propyl gallate	89± 0.01

Conclusion

In this study , the oils from five plants **(***Acacia seiberiana, Acacia alata, Leucaena leucocephala, Pongamia pinnata , Delonix regia* and *Cassia auriculata*) of medicinal attributes have been extracted and the constituents of the oils have been characterized by the technique of GC/MS. The target oils have been assessed for their antioxidant activity using the DPPH bioassay.

Recommendations

1- The extracted oils may be assessed for other biological activities including : antiviral, antimalarial , antiinflammatory …etc.

2- Other biologically interesting molecules of the studied plant species(like alkaloids , flavonoids….etc) may be isolated and identified by spectroscopic tools. Furthermore they may be evaluated for their biological activity.

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