Sudan University of Science and Technology

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Chemical Constituents and Antimicrobial Activity of Oils Extracted From Five Sudanese Medicinal plants

المكونات الكيميائيه و نشاط مضادات المكروبات لمستخلص زيوت خمسة نباتات سودانية طبية

A thesis Submitted in Fulfillment of the Requirements of the

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استهلال

قال تعالى:

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ ﴿105﴾

صدق الله العظيم

سورة التوبة الآية 105

Dedication

To …..

My parents ……

Wife…..

Sons……

Brothers and sisters……

Acknowledgement

First of all , Iwoud 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 interst close supervision and continuous advice.

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

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Deep thanks to my family for their infinite support

Abstract

In this study the oils from five plants ofmedicinal atteributtes (*Sesabania sesaban , Dichrostachys cinarea , Cordia sinensis , Acacia polyacantha ,and Peganum harmala)* have been studied by GC-MS and antimicrobial activity has been screened . Gas chromatography – mass spectrometry has been used for the identification and quantification Sesabania sesban oil. The analysis revealed the presence of 20 components dominated by cis-9-Hexadecenal (14.75%).The oil however failed to show any activity in the cup plate agar diffusion bioassay . Gas chromatography – mass spectrometry analysis of *Dichrostachys cinarea* oil showed the presence of 11 components dominated by 9-Octadecenoic acid (Z)-, methyl ester (41.66%) . The oil did not exhibit any antimicrobial activity. The GC-MS was conducted for *Cordia sinensis* oil . The analysis revealed the presence of 32 components . Major constituent being 9-Octadecenoic acid (Z)-, methyl ester (27.09%) . The studied oil failed to show any activity against

all test organisms. The GC-MS was conducted for *Acacia polyacantha oil.* The analysis revealed the presence of 37 components dominated by 9,12-Octadecadienoic acid (Z,Z), mthyl ester (31.24 %). The studied oil exhibited significant activity against Echarichia coly(G-ve) and Psendomonasaeroginasa (G-ve) Candida albicans (fungus).

Analysis of *Peganum harmala* oil revealed the presence of 23 components components dominated by 9,12-Octadecadienoic acid (Z,Z), mthyl ester (54.14 %). The studied oil exhibited significant activity against Echarichia coly(G-ve) and Psendomonasaeroginasa (G-ve) Candida albicans (fungus). Staphylococcus aureus(G+ve). The oil exhibit significant antioxidant activity compared to the positive control

المستخلص

في هذا البحث تمت دراسة خمسة نباتات طبية هامه هي : السيسبان الكاكموت الكداد الاندراب والحرمل .تم تحديدمكونات هذه النباتات بتقنية الكروماتوغرافيا الغازيه – طيف الكتله كما اجرى اختبار مضاد الميكروبات .

اعطى زيت نبات السيسبان 20 مركبا اهمها cis-9-Hexadecenal (14.75%).

في اختبارمضاد الميكروبات لم يبدي هذا الزيت فعاليه . احتوى نبات الكداد على 11 مكونا

اهمها : 9-Octadecenoic acid (Z)-, methyl ester (41.66%) . وفي اختبار مضاد الميكروبات لم يبدي هذا الزيت اي فعاليه . احتوى نبات الاندراب على 32مكونا اهمها : 9-Octadecenoic acid (Z)-, methyl ester (27.09%)

ولم يبدي هذا الزيت فعاليه في اختبار مضادات الميكروبات.

اما نبات الكاكموت فقد ابدى 37 مكونا اهمها : 9,12-Octadecadienoic acid (Z,Z), mthyl ester (31.24 %) وفي اختبار مضاد الميكروبات ابدى هذا الزيت فعاليه عاليه ضد Echarichia coly(G-ve) and Psendomonasaeroginasa (G-ve) Candida albicans (fungus).

احتوى نبات الحرمل 23 مركبا اهمها :

9,12-Octadecadienoic acid (Z,Z), mthyl ester (54.14 %).

وفي اختبار مضاد الميكروبات ابدى هذا الزيت فعاليه عاليه ضد Echarichia coly(G-ve) and Psendomonasaeroginasa (G-ve) Candida albicans (fungus) اعطى هذا الزيت فعاليه عاليه في اختبار مضاد الاكسدة.

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1-Introduction

1.1. History of essential oils

Essential oils are oldest form of medicine, made up of different chemical compounds extracted from seeds, .bark, leaves, roots, flowers and all parts of trees, shrubs and herbs. The major compounds of essential oils were alcohol, hydrocarbons, phenols, a1dyhides, esters, ketones (Younis et. al., 2008) . The word “pharmacopoeias” itself which is derived from Greak word “pharmakon” means drug which is obtained from dry herbs (Pawanider et al 2007). A great deal of volatile oils has been published, in books and papers on pharmacology, pathology and physiology, especially on the antiseptic and bactericidal activities (Malcolm et al 1833).

About 1866 the name “terpens” was mentioned in a textbook written by (Kekule 1896) who apparently coined this term.Name of essential oil is a very old one and refers to the alchemists' idea of the most sublime extractive, the Quinia Essentia. Later the name was applied to volatile materials responsible for the fragrance of plants.(Engineering and science 1961)

Essential oils were, in general, the active ingredient of pharmacologically important drugs. Later the name was applied to volatile materials responsible for the fragrance of plants. The manifold aspects of these materials have been studied by chemists, plant physiologists, and pharmacologists as well as historians. These highly valued products have played a very important role in the economy of many countries - especially in classical times. In the ancient countries of the Orient, in Greece, and in Rome the essence of flowers and roots was extracted by placing them in fatty oils. (Engineering and Science ,May 1961).

In the East also began the history of essential oils; the technical basis of the essential oil distillation was conceived and first employed in the Orient, especially in Egypt, Persia and India.

As in many other fields of human endeavor, it was in the Occident, however, that these first attempts reached their occidental full development. However, data on the methods, objectives and results of distillation of essential oils in ancient times are scarce and extremely vague. Indeed, it appears that the only essential oil of which the preparation (by a somewhat crude distillation) has been definitely established is oil of turpentine and, if we care to mention it in connection with essential oils, camphor. Dioscorides the author of the treatise "De Materia Medica" which dominated therapy for more than 1,500 years mention oil of turpentine and give partial information about methods of producing it.No other oil was described by this author.

Until the early Middle Ages (and even later) the art of distillation was used primarily for the preparation of distilled waters. Where this process resulted in a precipitation of essential oils, as in the crystallization

of rose oil on the surface of distilled rose water, it is likely that the oil was regarded as an undesired by-product rather than as a new and welcome one.

An extensive trade in odoriferous oils and ointments was carried on in the ancient countries of the Orient and in ancient Greece and Rome.'- The oils used, however, were not essential oils, nor were they produced by mixing the latter with fatty oils; they were obtained by placing flowers, roots, etc., into a fatty oil of best quality, submitting the glass bottles containing these mixtures to the warming influence of the sun and, finally, separating the oil

Of considerable importance in the further development of the chemistry of volatile oils were the investigations of the French chemist, (Berthelot 1907), devoted primarily to the hydrocarbons contained in these oils. About 1866 the name Terpene was mentioned in a textbook written by (Kekule 1896) who apparently coined this term. In 1875 one of the greatest English chemists emerging from pharmacy, (Tilden 1926), introduced nitrosyl chloride as a reagent for terpenes, a reaction perfected and used to such an extent.

1.2.Extraction of essential oils in past

In the past essential oils were extracted from flowers as follows: flowers were macerated with wine before the fatty oil was added, and the product obtained by digestion was filtered and then boiled down to honey consistency.The same way of preparing odoriferous oils is described in the "Grabaddin"written by the somewhat mysterious JoannesMesue, and published probably in the middle of the thirteenth century. This very widely used book did not list a single essential oil. However, two oils prepared by destructive distillation (oil of juniper wood or cade, and oil of asphaltum) were mentioned.

1.3. Traditional extraction methods

Water or hydrodistillation is one of the oldest and easiest method to extract oils (Meyer et. al. 1984) ,also the steam which considered as roughly traditional method used for extraction is still important in certain industrial sectors(Fahlbusch et. al.2003).The old way of producing the oil was to press by hand and collect the oil in a sponge. When the sponge was saturated, the oil was squeezed out. This process is still used in Italy and Spain ,the oils so obtained are practically insoluble in water, just like fatty oils; however, unlike these, they are volatile. This process doesn't fit too well into our present day economy and is gradually being replaced by solvent extraction with low-boiling petroleum fractions

1.4. Production of essential oils

Countries produce different kinds of essential oils India ranks second in the world trade (Rao. et. al. 2005).An extensive trade in odoriferous oils and ointments was carried on in the ancient countries of the

Orient and in ancient Greece and Rome. The oils used, however, were not essential oils, nor were they produced by mixing the latter with fatty oils they were obtained by placing flowers, roots, etc.,into a fatty oil of best quality.(Urdang et al 1943) .

1.5. Use of essential oils

The use of essential oils during the latter half of the nineteenth century in medicinal drugs became quite subordinate to their employment in the production of perfumes, beverage, and foodstuff, some of them luxuries. Many of these products contribute directly to the health and general wellbeing. Some volatile oils are more or less powerful external or internal antiseptics, etc (U R L-1 -1).

The use of essential oils did not become general until the second half of the sixteenth century .Official pharmacopoeias have always been more or less conservative. The non-polar extracts of aromatic medicinal plant species are mainly composed of essential oils.

The ancient Egyptian ,Chinese ,Arab ,Greek and Roman used essential oils to heal the sick.Also Chinese culture used essential oils as drug .

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The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits (Somesh *et. al*., 2015).

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are increasing very fast .

1.6. Herbal medicine

The use of plants as medicines has a long history in the treatment of various diseases. The earliest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies (Koehn et.al. 2005). Many plants possess antimicrobial activities are used for treatment of different diseases (Najafi et. al.,2010). Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (Recio et. al. 2005).

According to the World Health Organization (WHO), a medicinal plants is defined as any plant, which one or more

of its content contain substance that can be used for treatment of numerous diseases

The use of traditional medicine is widespread throughout the world (WHO , 1978). The term traditional medicine is interchangeably used with herbal medicine and natural medicine (Hazan et. al. 2005).

evolution of these plant-based medicine systems, primarily based on plants within a local area, produced the well-known traditional medicine systems in several local systems within Africa . Asia and other worlds.

The use of traditional medicine is wide spread throughout the world (WHO , 1978). The term traditional medicine is interchangeably used with herbal medicine and natural medicine (Hazan et. al., 2005). evolution of these plant-based medicine systems, primarily based on plants within a local area, produced the well-known traditional medicine systems in several local systems within Africa and other worlds. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties According to World Health Organization (WHO) report, nearly 4 billion peoples (80%) utilize herbal medicine as part of treatment. This can reduce utilization of chemical drugs and their side effects .

Many researchers studied and experimented diverse groups of plants for research based on the antibacterial herbs. The extracts of medicinal plants showed an excellent effect on bacterial growth and their effects were observed within the limits of antibiotic effects. Most concentrations of the extracts of the studied plants showed a high antibacterial activity against *P. aeruginosa. .Pseudomonas aeruginosa* resistant to a variety of new antibiotics.

Researchers now are looking for new methods, such as herbal medicines for the treatment and prevention of infections caused by antibiotic -resistine bacteria. Due to prevalence of β-lactamase in *Pseudomonas aeruginosa* and resistance to antibiotics, researchers proposed herbal medicine. (Rabie et. al . 2016) .

1.7. Importance of essencial oils

extracts containing volatile oil however additionally contain non-volatile flavour elements and these have wide application within the food and pharmaceutical industries.

1.8.Essential used as antioxidant agents

The human body has defense mechanisms against free radicals present in most of the cells. Balance between free radicals and antioxidants can be recovered from an external supply of antioxidants such as essential oils(Garner et al 1997) .

Essential oils are rich in phenolic compounds, and for this reason, attract investigators to gauge their activity as antioxidants or free radical scavengers.

1.9. Essential oils as antimicrobials

Essential oils will act as antibacterial agents against a wide spectrum of bacterial strains, however essential oils might favor the emergence of resistant colonies and conjointly gift a possible for the

disruption of intestinal microbial flora, that is liable for facet effects (WHO 2002) . Such chance introduced the use of essential oils as food additives that may act as antibacterial and antifungal additives. Essential oils are made up of different chemical compounds including alcohols, hydrocarbons, phenols, aldehydes, esters and ketones (Younis et al., 2008).

At present time oils from hundreds of plant species are available commercially (Kubeczka,et .al. 1982). Numerous studies have demonstrated the efficiency of essential oils in low doses in the fight against bacterial pathogens (Oussalah et al., 2006,) and even the fight against multi-resistant bacteria (Burt et al., 2004). The effectiveness of these procedures has been attributed mainly to the presence of active phytochemicals or bioactive compounds in plants giving the scope of searching new antimicrobial agents (Seoussen et al., 2016).

Antimicrobials derived from plant materials are often regarded as natural and safe compared to industrial chemicals. Of late, plant-based medicine has become more popular due to the increasing concern of consumers with regard to the use of synthetic chemical preparations and use of artificial antimicrobial preservatives, especially in modern food protection practices. The worldwide market for essential oil rapidly developed and nowadays a lot of scientific research focused on the industrial development together with environmental preservation of essential oils by using different techniques as hydrodistillation (HD), supercritical fluid extraction (SFE), microwave-assisted hydrodistillation (MAHD) and ultrasound-assisted extraction (UAE

1.10.Essential oils extraction methods

There are several methods of extraction behavior of essential oils. Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on a commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production.

i- **Hydrodistillation (HD)**

Hydrodistillation is normally used for the isolation essential oils from the aromatic and medicinal plant.. The principle of extraction is based on the isotropic distillation. Hydro-distillation (HD) is a variant of steam distillation, which is bespoke by the French.(Rassem et al 2016),

ii- Solvent extraction

Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts.. In the solvent-extraction method of essential oils , an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. (Rassem et al, 2016)

iii- Steam distillation

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic compounds but has become obsolete by vacuity distillation. Steam distillation still important in certain industrial sectors(Fahlbusch et al., 2003).

iv-Cold pressing method

Cold pressing technique 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.. The cold pressed method is also known as scarification method. Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils (Arnould et al., 1981).

v- Soxhlet extraction

Although the method of Soxhlet extraction is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility (Dawidowicz et al., 2008). A Soxhlet extractor is a piece of laboratory apparatus (Harwood 1989) invented in 1879 by Franz von Soxhlet. (Soxhlet et al., 1879)

vi- Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. 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 (Rassem et al, 2016)

1.11.Non-traditional extraction techniques

Novel techniques, for example, abide by green extraction concept and principles have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of official methods. New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO_2 emissions. .

i- Microwave-assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizing a microwave oven in the extraction process. (Golmakani et al., 2008). Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required and thus minimizing environmental impact (Lucchesi et al., 2004)

ii- Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could be involved with the increase in the estimate of some food by-products when used as sources of natural compounds or plant material (Bhaskaracharya et al., 2009).

iii- Maceration method

Maceration method is Simple and not expensive expensive technique that produces higher yield of essential oils , becuse the principle of extraction is not based on heat and distillation. The method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time

1.12. Targeted plants taxonomy

The plant materials were taxonomically authenticated at herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute,National center for research ,Khartoum ,Sudan.

This study focused on the extraction of oils from 5 potential medicinal plants grouped in table (1-1). The constituents of the oils were studied by GC.-MS. Furthermore the antimicrobial potential of the oils has been investigated.

Table (1.1) : The targeted Plants species

Family	Botanical name	Local name
Fabaceae	*Sesbania sesban* (L) Merr	Sesaban
Mimosaceae	*Dichrostachys cinerea*	Kadad
Cordiaceae	*Cordia sinensis*	Anderab
Mimosaceae	*Acacia polyacantha*	Kacamout
Zygophyllaceae	*Peganum harmala (L)*	Harmal

1.12.1.*Sesbania sesban*

The tree have different local names . Rivierboontjie [Afrikans] ,Girangire [Amharic] ,Sesaban[Arabic] ,Rattlepod [Egyptian] (Allen et al 1981) .

1.12.1.1. Taxanomy

Kingdom: Plantae

Superdivision :Spermatophyta

Class: Magnoliposides

Order :Fabals

Family :Leguminosae

Genus: Sesbania

S**pecies**: *Sesbania sesban*

1.12. 1.2. Botanical description

Sesbania sesban is narrow-crowned deep rooting single or multi- stemmed shrub or small tree 1-7m tall ,the trees usually have a main stem but may develop many side branches if widely spaced .Leaves paripinate ,long narrow leaflets in many pairs, rounded or oblong ,usually asymmetric at the base , stipules minute or absent (Orwa et al 2009). Flowers attractive , yellow, red, purplish white large or small. Sometimes pale yellow to orange yellow which may sometimes having dark purple or purplish brown (Char 1983) .Pods pale yellow linear usually 10-20 cm long .Most species' seeds have impermeable seed coats and require scarification. Seed coat impermeability allows for survival over time, for transmittal along waterways(Trivedi 1955),Seeds have variable hardseededness (Sharma et al 1978) Sesbanias propagate themselves readily, some

Sesbania species are relatively short-lived perennial, the majority are annuals. In Egypt, Sesbania and alfalfa are major pollen sources in spring and summer (Ibrahim 1976)

There were different spp. of Sesabani : *S.javanica* from Australia and *S.grandiflora S.speciosa S.marginat S.tetraptera* growing in different regions of Africa exhibit varying growth durations .where they exhibit a determinate growth habit characterized by early flowering and senescence after a reproductive phase (Evans 1983).

Sesabania sesaban seeds

1. 12.1.3. Uses of *Sesbania sesbanis* (L.) Merrill

S. sesbanis is a multipurpose tree with different parts of the plant (bark, root, seed, leaf and stem) used for various purposes. Leaves are used for reproduction and milk production (Sabara et al 2010) ,whole tree is used for green manure and nitrogine fixation (Patra et al 2006) , .Leaves having anti-inflammatry

effect .Roots and leaves are used as traditional medicine(Pravin et al 2012,Orwa et al 2009).Leaves and seeds are used as antidiabetic (Pandhare et al 2011) .Flowers possess antioxidant and antimicrobial effect.Seeds are used as anti fertility agent ,barks possess stimulating effect In southeast Asia(URL 2-1) the leaves are used in Ayurveda for treating itchness, fever, toxicity, sinus, respiratory disorders and as anthelmintic, purgative, diuretic and laxative . The flowers are used traditionally by tribals to treat night blindness, cataract and headache . The leaves of Sesabania have high nutritive value. Agathikeerai, is a south Indian dish prepared from the young edible leaves . Leaves are also used for treating anemia, they are also used as antidote for tobacco and cigarette smoking related respiratory problems. Steamed flowers are used as a traditional Indonesian dish, Peal (Duke 1983 , Ramesh 2007).The antipyretic, anti-inflammatory, antioxidant, antimicrobial, thrombolytic and membrane stabilizing properties of Sesbania is attributed b to the phytochemicals of this plant. Recent scientific studies has revealed potent hepatoprotective, cardio protective,

*S. sesban*when incorporated into soil serving as a green manure (Patra et al., 2006) in alley cropping (Heering, 1995) which could bring about substantial increment in crop available nitrogen and soil organic carbon .*S. sesban* tree has a high level of foliage nitrogen and is an excellent supplement to protein-poor roughage (Sabra et. al., 2010;). The leaves and tender branches of this tree have high levels of protein, and easily digestible when consumed by ruminants (Pravin et al., 2012). It has a long history of use as a source of cut-and-carry forage (Naik et al., 2011). In Ethiopia, feeding *Sesbania* leaves and young twigs have become increasingly important as a protein rich supplement to a basal diet of either grass or poor quality forage for ruminants (Tessema et. al. 2004).

1.12. 1.4. Pharmacological profile

Seed, bark and leaves of *S. sesban* are used in traditional medicine. Seeds are used in diarrhea, excessive menstrual flow, to reduce enlargement of spleen and in skin disease. Leaves are used in inflammatory rheumatic swelling and as anthelmintic (Nadkarni et al 1982) .

Anthocyanins were extracted with acidified methanol from the *Sesbania sesban* flower petals and their antioxidant properties were investigated. Anthocyanins from Sesbania sesban flower petals exhibited a dose dependent free-radical scavenging activity against DPPH radical, superoxide anions and hydroxyl radical (Kathresh et al 2011) . The leaves of Sesbania sesban evaluated the topical anti-inflammatory activity of the crude saponins extract by carrageenan induced rat paw edema method by preparing the gel formulation. The activity was carried on Wistar albino rats, receiving two strengths of crude saponin gel at a concentration of 1% w/w and 2%w/w respectively and Diclofenac sodium gel (1%w/w) was used as reference drug. A 2% w/w gel formulation of saponins extracted from leaves showed significant anti-inflammatory effect(Dandi et al 2010) It has been shown that the exogenous administration of petroleum

ether, chloroform and methanol extracts of bark of *Sesbania sesban* and *Sesbania grandiflora* showed significant anti-inflammatory activity .

1.12.2. Dichrostachys cinerea

1.12.1. Taxanomy

Kingdom: Plantea
Class: Dicotyledons
Order: Fabales
Family: Fabaceae
Genus: Dichrostachys
Species: *Dichrostachys cinerea.L*

1.12.2.2. Botanica description

Semi-deciduous tree to 7m tall. Bark brown except on new branches where it is green and hairy .Alternate spines up to 8cm ,leaves stalks hairy ,flowers in aspink hanging spike of dense flowers [Bottle brush like fruits a narrow pod yellow to brown twisted10 cm long .In southern Africa flowering is from October to February and fruiting from May to September. The structure of the inflorescence suggests pollination by bats. The inflorescence has a strong aroma, which probably attracts animals to feed on the pods.

1.12.2.3. Ecology and distribution of D. cinerea

D. cinerea penetrates clear-cut areas far into the rainforest zone. In Malaysia, it occurs in areas with strong seasonal climate, usually on poor, occasionally clay soils, in brushwood, thickets, hedges, teak forest and grassland. Forms dense hammocks on lateritic soils in Senegal and Sudan, while in India it occurs in dry deciduous forests. It can be an indicator of overgrazing in low rainfall areas. Usually resistance and tolerance is less on poor soils, but definitely drought- resistant. It is fire resistant and does not tolerate water logging. It is a weedy species. For instance in Cuba, the tree is unchecked and forms veritable forests on hill land or in areas on which cane growing has been discontinued. In some parts of central Cuba, there are reports that whole farms have been rendered useless by this foreign weed. Altitude: Up to 2 000 m, Mean annual temperature: -2 to 50 deg. C, Mean annual rainfall: 200-900 mm . Soil type: best growth occurs on deep, sandy loamy soils; it can tolerate a wide pH range. ***(***Orwa et. al.2009) .

Dichrosachys cinerea pods

Many studies have demonstrated antimicrobial ,anti-periopathic and anti-fungal properties of both aqueous and ethanol extract of various chewing stick(Rotimi et, al,1988). Many spp of this biodiversity have been found to be useful in traditional medicine for cure of diseases (Iwu et. al. 1993).Alkaloid extract incorporated into various dentifrices and oral rinses have been shown to posses broad-spectrum in vitro activity against a wide variety of microorganism (Socrasky et. al. 1985). A Nigerian chewing stick has been shown to provide beneficial effects for the oral hygiene of some rural natives (Solowora et. al. 1979) . the plant is used in Sahel regions for the treatment of furunculosis, eczema and wounds (Maydell et al 1990) .

Malinkés peoples (North of Côte d'Ivoire) also use the decoction of Dichrostachys cinerea roots to make mouthwashes in case of tooth decays, being able to be of infectious origin. Besides, (Kamizi et al 2001) , this herbal is widely prescribed in traditional medicine of Zimbabwe for the treatment of sexually transmitted infections, the roots being the most used part of the plant. It has been shown that in Sudan that leaves of the plant possessed anti-infective activities(Eisa et al 2000) . According to the findings of Sinon (Sinon et al 2001) , the leaves extracts of this plant would be active on bacterial strains including *Staphylococus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*. These antibacterial properties would be due to to some constituents of the whole plant, such as tannins (Banson et al 2007)

1.12.3. *Cordia sinensis*

2.1.3.1.Botanical description of *C. sinensis*

Cordia sinensis is low leafy shrub or bush multi-stemmed tree 3 to 12m high and often with slender branches tending to droop. The bark is brown to pale creamy, leaves opposite or alternate ovate about 10mm long with long pair hairs .Flowers white or cream in terminal cymes .Fruits conical bright red orange when ripe 7-20 mm long ,with conspicuous long tip and hang in conspicuous clusters. Seeds 1-4 hard rough yellowish cream .Flowering occurs in December to February .

1.12.3.2. Taxanomy

Kingdom: Plantae

Subkingdom: Tracheobionta;

Super division: Spermatophyta;

Division: Magnoliophyta;

Class: Magnoliopsida;

Subclass: Asteridae;

Order: Lamiales,

Family: Boraginaceae,

Genus: *Cordia*L.,

Species: *Cordia sinensis*

1.12.3.3. Distribution of *C.sinensis*

Cordia sinensis is found in the drier areas of India,Africa ,and Sudiaarabia(Araque et. al. 2009). Some species of Cordia are utilized in production of furniture in Ethiopia Sudan ,Saudia arabia and tropical Africa (Derero et. al. 2011).The plant is native in Asia temperate and Asia tropics. *Cordia.*originated from the area stretching from the eastern Mediterranean region to eastern India, and was introduced long ago in tropical Africa, tropical Asia and Australia, and more recently also in the Americs.

The genus *Cordia* belongs to the family Boraginaceae, with some 300 species distributed worldwide, mostly in the warmer regions of the World (Thirupathi et al 2008) . *C.sinensis* grows in the Middle East, Pakistan, India, Srilanka and in Africa from West Africa to Ethiopia, Somalia, Sudan, Egypt, south to Namibia and north-east South Africa. The species is found in dry riverine vegetation, usually with *Salvadora persica*, or in open bush land, usually from sea level to 1,400 m in alluvial, sandy, red loam and rocky soils (Maundu et al., 1999).

C. sinensis fruits

1.12.3.4. Traditional and native uses of *C. sinensis*

Fruits are eaten to suppress cough and for the treatment of respiratory infections and a sore throat, as it has demulcent properties. The pulp was also applied as an emollient to mature abscesses, to calm rheumatic pain and as an anthelminthic. In Tanzania the fruit pulp is applied on ringworm. In Mali and Côte d'Ivoire the leaves are applied to wounds and ulcers. A macerate of the leaves is taken to treat trypanosomiasis, and is externally applied as a lotion to tse-tse fly bites. In the Comoros the powdered bark is applied to the skin in cases of broken bones before a plaster was applied, to improve healing. Bark powder has been used externally in the treatment of skin diseases. Bark juice together with coconut oil is taken to treat colic (Oudhia et.al.2007)

The analgesic, anti-inflammatory and anti-arthritic activities of different extracts of several species of Cordia was evaluated in rat. The results obtained showed that the petroleum ether and alcoholic extracts of *Cordia* leaves exerted a significant analgesic, anti-inflammatory and anti-arthritic activity in rat(Almeida et. al. 2001) Accordingly, aqueous extract was found to stimulate cell mediated and immune responses in mice(Ali et al 2015).

According to a literature survey, several uses in traditional medicine have been reported for different Cordia species (Ioset et al 2000 ,Ioset et al 1998).

Specifically the medicinal value of Co rdia species lies in some chemical substances that produce a definite physiological action on the human or animal body (Edeoga et al., 2005). The most important of these bioactive constituents are mainly secondary metabolites like flavonoids, triterpenes, tannins and alkaloids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species (Thirupathi et al., 2007).

The roots and bark of Co*rdia sinensis* are used for stomach disorders in both children and adults. A decoction of boiled roots is used to treat malaria but can cause an abortion. Bark and roots are mixed to treat conjunctivitis in cattle (Orwa et al., 2009). The bark of *C. sinensis* is used for stomach disorders and for chest pains (Richard et al 2010) . A literature survey revealed that very little phytochemical work has so far been carried out on *C. sinensis.* A methanolic extract of this plant showed strong toxicity in the brine shrimp lethality test and on subsequent fractionation mucilage specifically antagonized nicotine-induced hypotensive effect on rabbit and nicotine ganglionic stimulant effect on the isolated guinea pig ileum(Abuo 1989).

1.12.4. *Acacia polyacantha*

1.12.4.1.Taxanomy

Kingdom: Plantae
Order: Fabales
Family: Fabaceae
Genus: *Acacia*
Species: *A. polyacantha*

1.12.4.2.Botanic description *A. polyacantha*

Acacia polyacantha known as white thorn, is a flowering tree which can grow up 25m tall.A.polyacantha has ameaning (many thorns in latin .A. polyacantha is alargedeciduostree,barck yellow-brown petiole 5-4 cm long usually with conspicous flattened oblong gland rhachis pubescent ,rarely subglabrousagland at the junction of the top 3-17 pinnae pairs 14-60 pairs leaflets .Iflorescencespicate,solitary 3-12 cm long flowers yellowish-white.Fruitsastraight flat pods brown ,seeds 9-7 compressed .The seeding period can be observed approximately 6 months.The species occurs in wooded grasslands ,decidous wood land reverine and ground water forests in altitudes ,it prefers sites with ahigh ground water table .A. polyacantha distribute in Sudan ,Zambia ,Etheopea,Gambia,India,Kinia,Senigal,Sieraleon ,Southafrica ,Srilanka, Swaziland,Tanzania.

Acacia polyacantha pods

1.12.4.3. **Traditional uses of *A.polyacantha***

Acacia polyacantha is native to Africa, India and Asia but it introduced to the Caribbean .The root of *A. polyacantha* have chemical compounds that repel animals including rats ,snakes and croccodiels . Synergistic activity of antibiotics and plant extracts was investigated with *E. coli* and *Proteus* isolates possessing antibiotic resistant ,. Prominently methanolic extracts of both hot and cold preparations of *A. polyacantha* leaves and bark produced maximum zone of inhibition .Developing resistance against antibiotics in bacterial species was attempted to control by synergistic action of the plant *A. polyacantha.*This surely remarked the success of synergistic action against the pathogens. Earlier also, many studies highlighted similar success of plant extracts where plant based product's antimicrobial properties was recorded for great implication in therapeutic treatments. In the last thirty years, a number of studies with several plants have been conducted to prove such efficiency.(Almagboul et. al. 1985) . Other work done in the bark by (Okpanachi et al 2012) noted the presence of flavonoids and anthraquinones in the stem bark of *A. polyacantha*. The presence of tannins and flavonoids in this plants would justify their use in the treatment of diarrheal diseases and gastrointestinal diseases of animals (Min et al 2001). As per earlier study, *A. polyacantha* contains many secondary metabolites such as saponins, catechic tannins, alkaloids, leuco anthocyanin, coumarins, sterols flavonoids, anthraquinones and terpenes (Alain et al 2015 ,Okpanachi et al 2012) .

1.12.5. ***Peganum harmala***

1.12. 5.1. Taxanomy

Kingdom: Plantae

Order: Sapindales

Family: Nitrariaceae

Genus: *Peganum*

Species: *P. harmala*

1.12.5.2. Botanic description of *P. harmala*

P. harmala is native for the eastern Mediterranian region and India ..It is known as wild Rue or Syrian Rue because of its resemblance to plants of the Rue family . It is a perenial plant which can grow to about 0.8 m tall (URL-2-3) . The roots of the plant can reach a depth of up ,6m if the soil is very dry . The plant blossoms between June and August in the northern hemisphere. .The flowers are white The round seed capsules measure about 1-1`.5 cm have three chambers and carry more than 50 seeds(URL-2-4).

Peganum harmala seeds

1.12.5.3. Medicinal uses of *P. harmala*

Peganum harmala is used as analgesic and anti-inflammatory (Edward et. al. 2002) .In Yaman it is used to treat depression(URL-2-7). Smoke from the seeds kills algae ,bacteria ,intestinal parasites (URL-2-6).The roots is applied to kill lice and other insects. Also seeds are used as anthelmintic(URL-2-8]) .Greeks use powder of seeds to get rid of tapeworms and to treat recurring fevers(Randa 2000).Large quantity can reduce spermatogenesis and mail fertility in rats(Dwairi 2007).Harmal is effective against protozoa ,leishmania .Peganine hydrochloride dehydrate was found to induce apoptosis in both the stages of *L.donovani* via loss of mitocondarial transmembrane potential(Misra et al 2008) .The beta carbonile alkaloids present in Peganum harmala which inhibit DNA poisomerases to interfere with DNA synthessis (Liang et al 2007) .Harmal has antioxidant and antimutagenic properties(Moura et al 2007) .Harmal exhibit cytotoxicity with regards to HL60 and K562 leukemia cell lines ,seed are used to treat skin cancer and subcutaneous cancer the couting of the seeds are known to contain large amounts of harmin(Jahaniani et. al 2005)

Smoke from the seeds kills algae, bacteria, intestinal parasites and molds. *Peganum harmala* has antibacterial activity (Prashanth et al 1999) including antibacterial activity against drug-resistant bacteria (Arshad et al 2008). It is also used as an anthelmintic (to expel parasitic worms) (URL -2-9).
Fresh plant was used against rheumatism by rubbing. Smelling vapors of burnt plant was used to cure headache and also neurotic pains while dried powdered plant was used for purulent conjunctivitis (Boulos, 1983). Also, alkaloids of *P. harmala* have significant antitumour activities, which would be useful as novel anticancer therapy . However, its seeds were known to have hypothermic and hallucinogenic characteristics. The active ingredient of the seeds and its derivatives causes visual troubles and at high doses can cause paralysis

Aim of this study

This study was designed to :

-Extract oils from five potential medicinal plants (*Sesabaniasesaban ,Dichrostachyscinarea , Cordiasinensis , Acacia polyacantha ,and Peganumharmala)*

- Identifying the constituents of the oils by **GC-MS**

- Evaluating the antimicrobial activity of the extracted oils .

Chapter Two

Material and Methods

2. **Materials and Methods**

2.1 Materials

2.1.1 . Plant materials

Seeds of *Sesabaniasesaban , Dichrostachyscinarea , Cordiasinensis , Acacia polyacantha ,and Peganumharmala* were collected from Gedarif western 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 (30 m , length ; 0.25 mm **d**iameter ; 0.25 mm thickness) was analysis

2. 1. 3 Test organisms

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

2.2. **Methods**

2.2.1. Extraction of oil

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

2.2.2. GC-MS analysis

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

The qualitative and quantitative analysis of the sample was carried out by using a Shimadzo machine model (GC-MS-QP2010-Ultra). The sample was injected under the following chromatographic conditions : column , oven temperature 150 C ,

injection temperature 300 C, injection mode : split , flow mode : linear velocity , pressure : 139 KPa ; total flow : 50 mL/min.; column flow : 1.54 mL/sec.;

linear velocity :47.2 cm/sec. ; purge flow : 3mL/min.; split ratio: -1.0.

Oven temperature program is presented . Table 2.1

Rate	Temperature (^{0}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 antioxidantactivity again**ststable** DPPH radicalsand the decrease in absorbance at λ_{max} 217 nm has been measured via UV spectroscopy **.**

2.2.4. Antimicrobial assay

(i) Bacterial suspensions

One mLaliquots of 24 hours , culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37 C for 24 hours .

The bacterial growth was harvested and washed off with sterile normal saline , and finally suspended in 100 mLof normal saline to produce a suspension containing about 10^8 - 10^9 colony forming units per mL. The suspension was stored in the refrigerator at 4 C until used . The average

number of viable organism per mLof the stock suspension was determined by means of the surface viable counting technique , Serial dilutions of stock suspension were made sterile normal saline in tubes and one drop volumes (0,02 mL) of the appropriate dilutions were transferred by adjustable volume micro pipette on to the surface of dried nutrient agar plates . The plates were allowed to stand for two hours at room temperature for the drop to dry , and then incubated at 37 C for 24 hours .

(ii) Fungal suspensions

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

(iii) Antibacterial suspensions

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

Were designed for standard antibacterial chemotherapeutic (ampicillin and gentamicin).

The agar discs were removed , alternate cup were filled with 0.1 mLof test sample 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 37 C for 24 hours .

Alter incubation , the diameter of the resultant growth inhibition zones were measured as average of two replicates .

Chapter Three

Result and Discussion

3-Results and Discussion

In this study five plants of medicinal attributes have been studied. The oils from the targeted species were extracted by hexane and the constituents of the oils have been investigated by GC-MS. Furthermore the antimicrobial potential of the oils has been evaluated.

3.1. *Sesabania sesaban*

3.1.1.GC-MS analysis

The oil from *Sesabania sesaban* was analyzed by GC-MS . The analysis revealed the presence of 20 compenents(Table 3.1), The total ion chromatogram is displayed below in Fig. 3.1.

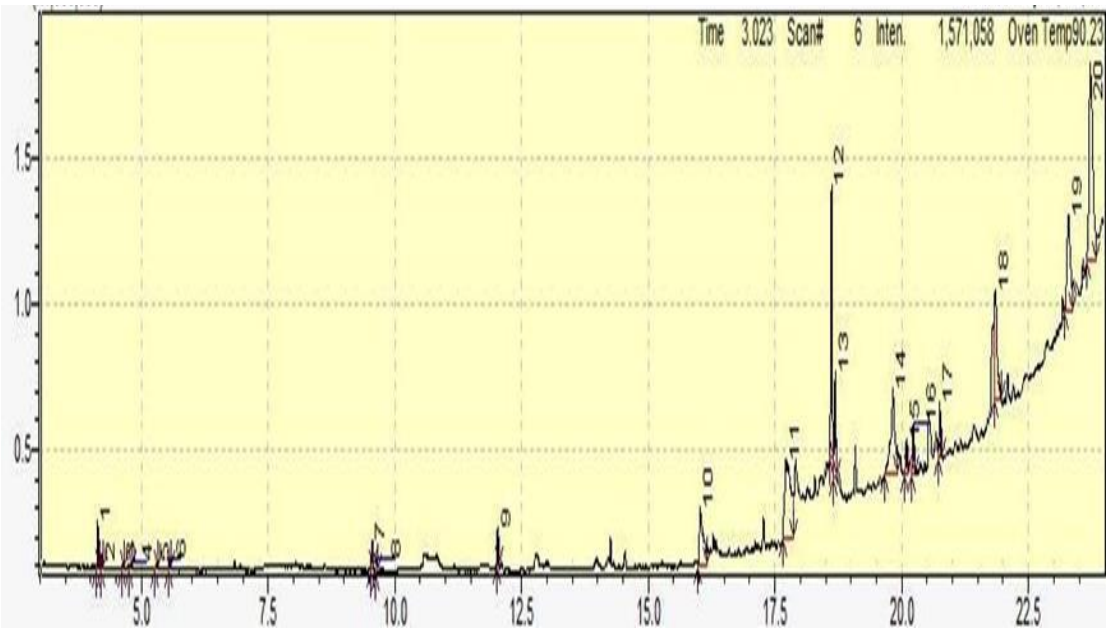

Fig (3-1) typical total ion chromatograms of *S. sesaban* oil

Table (3-1) Constituents of *Sesabania sesaban* seed oil

No	Area%	RT	Name
1.	1.34	4.140	**Heptane, 2,2,4,6,6-pentamethyl-**
2.	0.22	4.219	**Undecane**
3.	0.17	4.633	**2,2,4,4-Tetramethyloctane**
4.	0.15	4.792	**Ether, 2-ethylhexyl tert-butyl**
5.	0.18	5.296	**Butanoic acid, 3,3-dimethyl-, methyl ester**
6.	0.29	5.553	**Undecane**
7.	0.84	9.549	**1-Pentadecene**
8.	0.36	9.644	**Tetradecane**
9.	1.13	12.023	**1-Heptadecene**
10.	6.71	16.024	**n-Hexadecanoic acid**

11.	14.75	17.716	**cis-9-Hexadecenal**
12.	10.36	18.614	**13-Hexyloxacyclotridec-10-en-2-one**
13.	4.84	18.688	**9-Tetradecenal, (Z)-**
14.	10.79	19.828	**.gamma.-Tocopherol**
15.	1.22	20.090	**2-Ethyl-1-cyclohexyldimethylsilyloxyhexane**
16.	1.93	20.231	**Phenol, 2,2'-methylenebis[6-(1,1 dimethyl ethyl)-4-methyl-**
17.	2.28	20.750	**Octadecanoic acid, 2-hydroxy-1,3-propanediylester**
18.	9.39	21.848	**9,19-Cyclolanost-24-en-3-ol, (3.beta.)-**
19.	10.63	23.293	**9,19-Cyclocholestan-3-ol, 14-methyl-, (3.beta., 5. alpha .)-**
20.	22.42	23.721	**Stigmasterol**

S. sesaban seed oil was dominated by the following constituents:

i) cis-9-Hexadecenal(14.75%).

ii) gamma.-Tocopherol(10.79%).

iii) 9,19-Cyclocholestan-3-ol, 14-methyl-, (3.beta., 5. alpha .)- (10.63%).

iv) 13-Hexyloxacyclotridec-10-en-2-one(10.36%).

The fragmentation pattern of these major components is discussed below:

Fig (3.2) shows the mass spectrum of cis-9- hexadecenal . The signal at m/z238(RT.17.716) is due to the molecular ion $[C_{16}H_{30}O]^+$.

Fig.3.3 displays the mass spectrum of gamma.-tocopherol. The signal at m/z416 (R.T. 19.828 in total ion chromatogram) is due to the molecular ion : $[C_{28}H_{48}O_2]^+$.

The mass spectrum of 9,19-cyclocholestan-3-ol, 14-methyl-, (3.beta., 5. alpha .)- is shown in Fog. 3.4. The molecular ion $[C_{30}H_{50}O]^+$appeared at m/z 426(R.T. 23.721).

Fig.3.5 illustrates the mass spectrum of 13-hexyloxacyclotridec-10-en-2-one. The peak at m/z280(R.T. 18.614) is due to the molecular ion $[C_{18}H_{32}O_2]^+$.

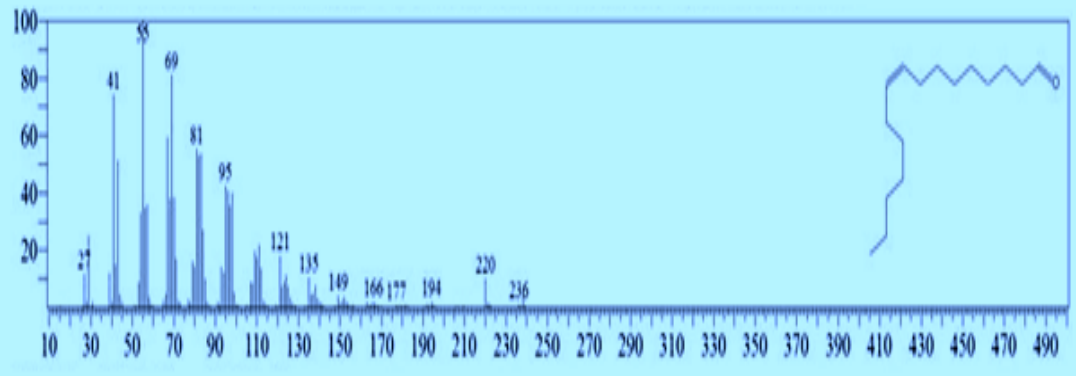

Fig. 3.2: Mass spectrum of cis-9-hexadecenal

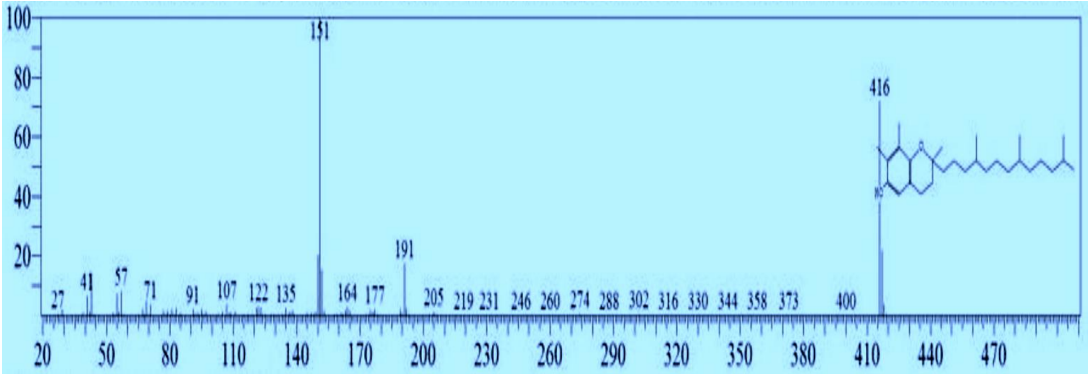

Fig.3.3: Mass spectrum of gamma.-tocopherol

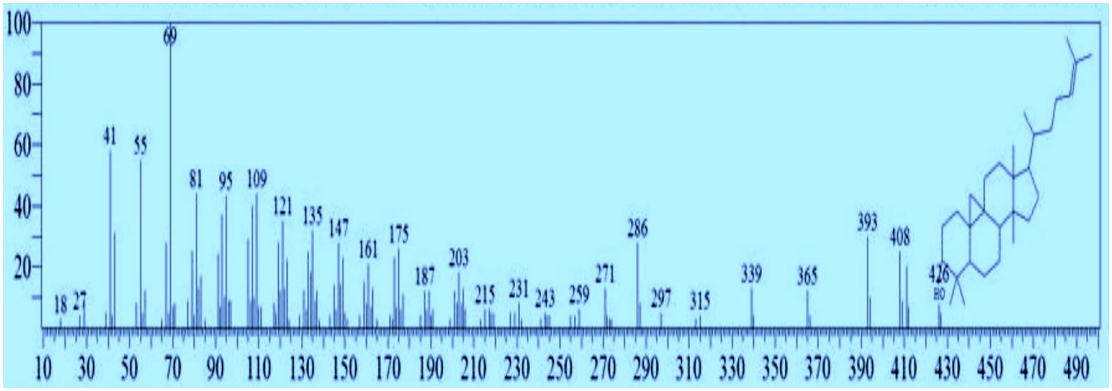

Fig.3.4: Mass spectrum for 9,19-cyclolanost-24-en-3-ol, (3.beta.)-

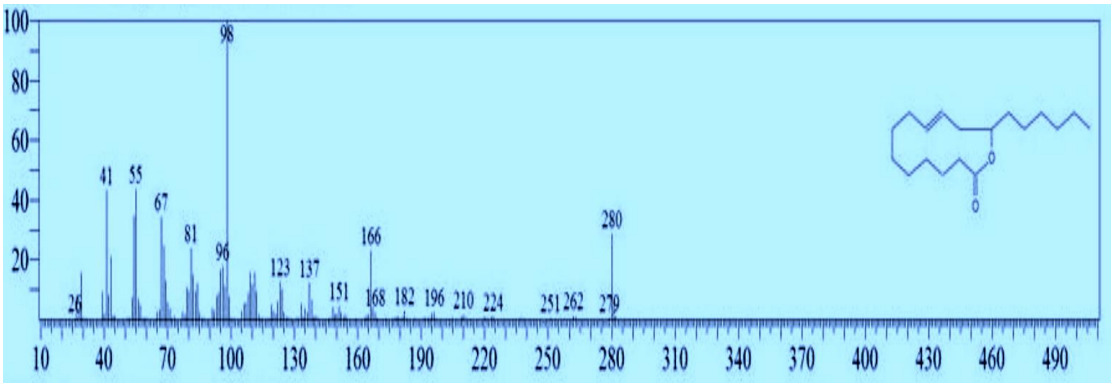

Fig. 3.5: Mass spectrum of 13-hexyloxacyclotridec-10-en-2-one

3.1.2. Antimicobial activity

The antimicrobial activity of the oil was examined against Gram positive bacteria *Bacillus subtilis,* and *staphylococcus aureus,* Gram negative bacteria *Escherichia coli, Pseud*o*monas aeruginose* and fungus *candida albicans*. The obtained results are compared with reference drugs (ampicilin, gentamicin and clotrimazole). As shown in Table 3.2, the oil failed to give any activity against all test organisms.

Table 3.2: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	C
Oil (100mg/ml)	--	--	--	--	--
Ampicilin	30	15	--	--	--
Gentamicin	19	25	22	21	--
Clotrimazole	--	--	--	--	38

<9mm : Inactive; 9-12mm : partially active; 13-18mm: active ; >18mm very active
Sa.: *Staphylococcus aureus*; Bs.: *Bacillus subtilis*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Ca.: *Candida albicans*

3.2. *Dichrostachys cinarea*

3.2.1. GC/MS analysis

The GC-MS analysis of the n-hexane extract of oil seed of *Dichrostachys cinarea* showed eleven compounds **(**Table.3.3). The total ion chromatogram is displayed in Fig.3.6.

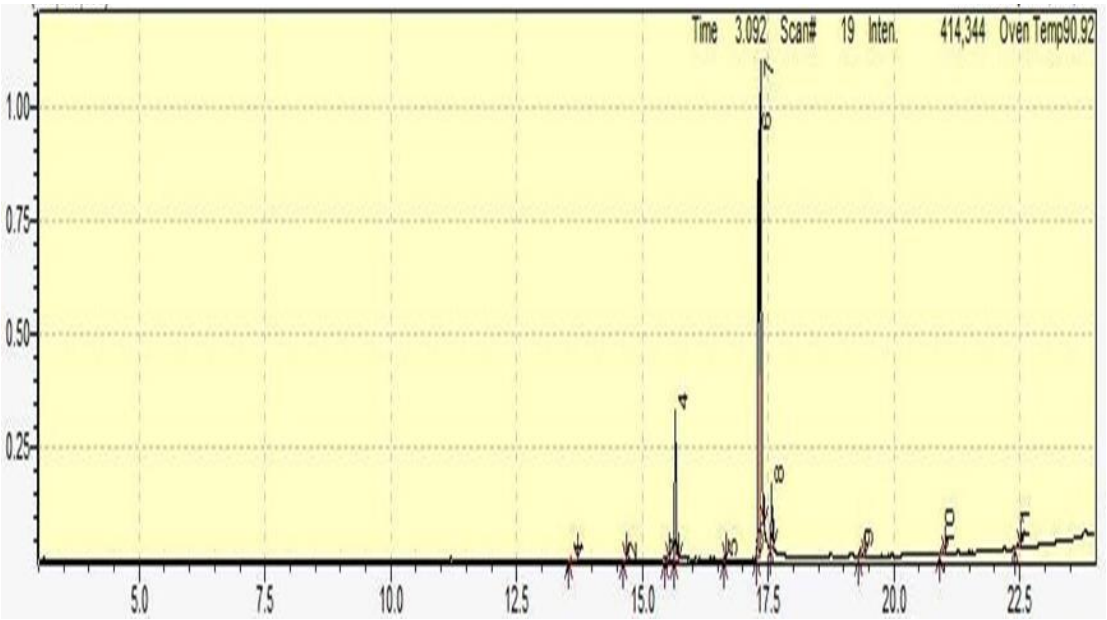

Fig.3.6 :Typical total ion chromatograms of D .cinerea oil

Table 3.3 : Constituents of *D. cenarea* oil

No	Area%	Ret.Time	Name
1.	0.27	13.579	Methyl tetradecanoate
2.	0.07	14.640	Pentadecanoic acid, methyl ester
3.	0.24	15.466	9-Hexadecenoic acid, methyl ester, (Z)-
4.	12.62	15.655	Hexadecanoic acid, methyl ester
5.	0.08	16.633	Heptadecanoic acid, methyl ester
6.	37.22	17.308	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
7.	41.66	17.352	9-Octadecenoic acid (Z)-, methyl ester
8.	5.34	17.567	Methyl stearate

9.	0.92	19.331	Eicosanoic acid, methyl ester
10.	0.74	20.952	Docosanoic acid, methyl ester
11.	0.84	22.457	Tetracosanoic acid, methyl ester

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

i)9-Octadecenoic acid methyl ester(41.66%)

ii) 9 12-Octadecenoic acid methyl ester (37.22%)
ii) Hexdecanoic acid (12.62% %)
iii) Methyl stearate(5.34%).

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3.7. The signal at m/z296 (RT.17.352) corresponds M^+ $[C_{19}H_{36}O_2]^+$.

Fig. 3.8 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.430)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.9.The peak at m/z 270 which appeared at (RT.15.702) is due to M^+ $[C_{17}H_{32}O_2]^+$.

Fig. 3.10 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.625) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 accounts for loss of a methoxyl.

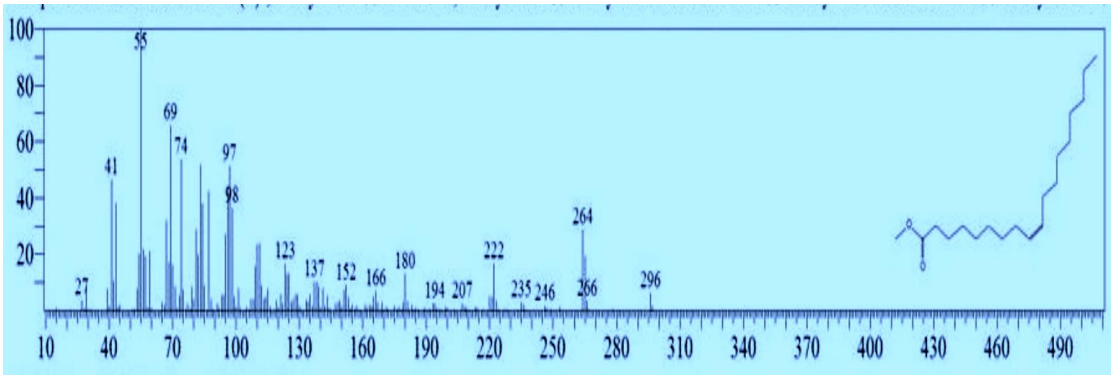

Fig.3.7 : Mass spectrum for 9-octadecenoic acid[z]-,methyl ester

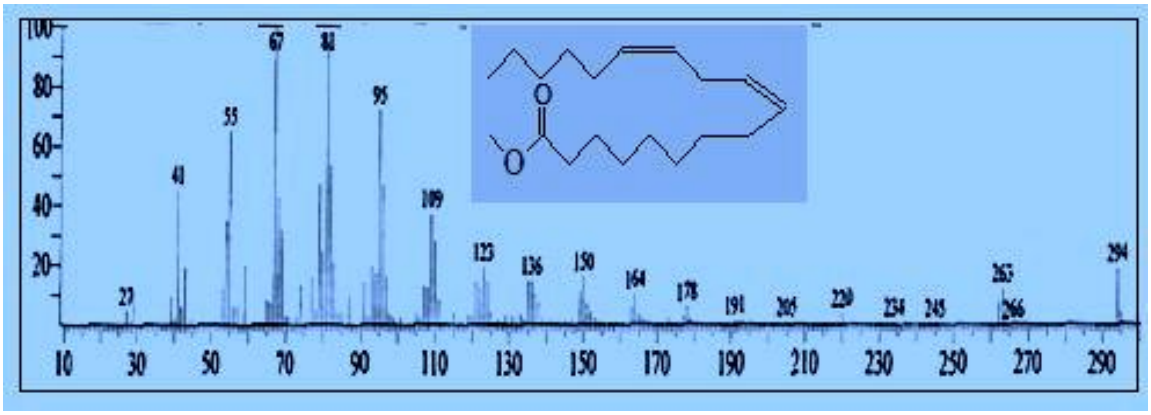

Fig.3.8: Mass spectrum of 9,12-octadecadienoic acid

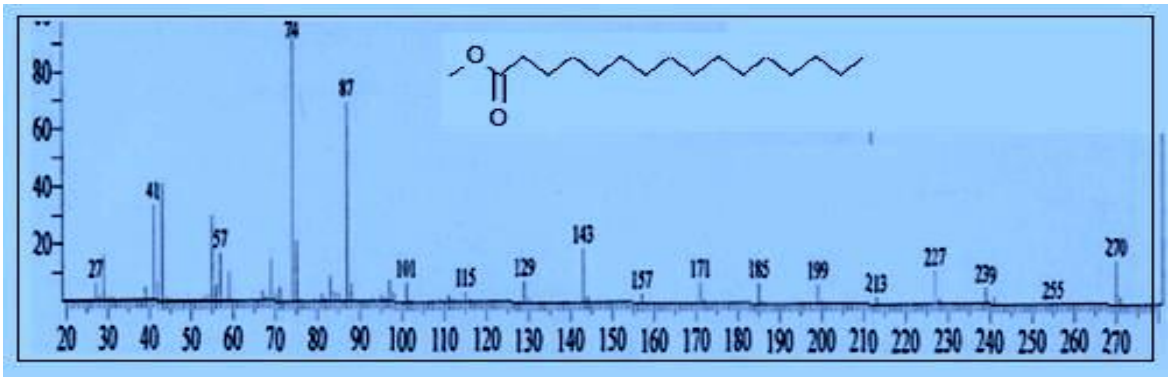

Fig. 3.9: Mass spectrum of hexadecanoic acid methyl ester

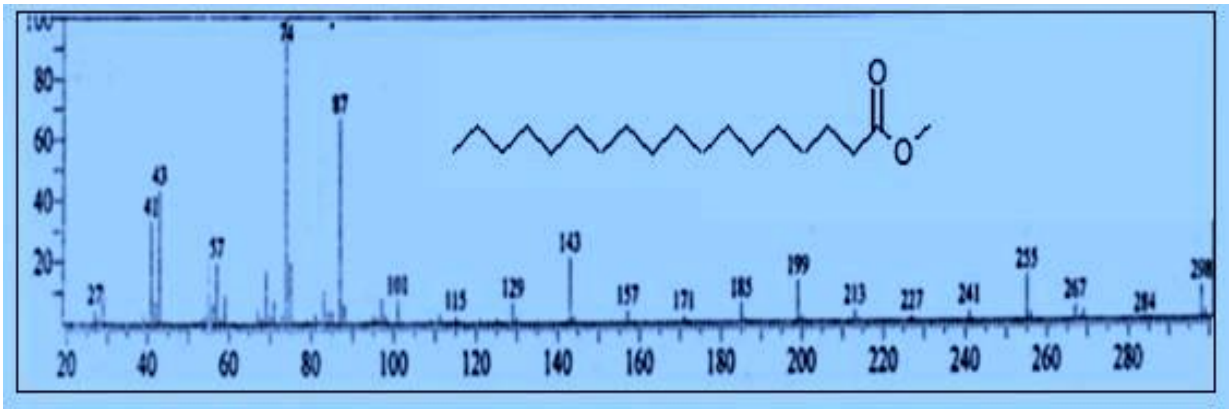

Fig.3. 10: Mass spectrum of methyl stearate

3.2.2. Antimicrobial activity

Dichrostachys cinarea oil was assessed for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.4).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active; >18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil failed to show activity against tall test organisms.

Table : 3.4 : Inhibition zones(mm/mg sample)

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

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

3.3. *Cordia cinensis*

3.3.1. GC-MS anlysis

The GC-MS analysis of the oil extracted from *Cordia sinensis* revealed the presence of 32 components as presented in Fig.3.11 and Table 3.5.

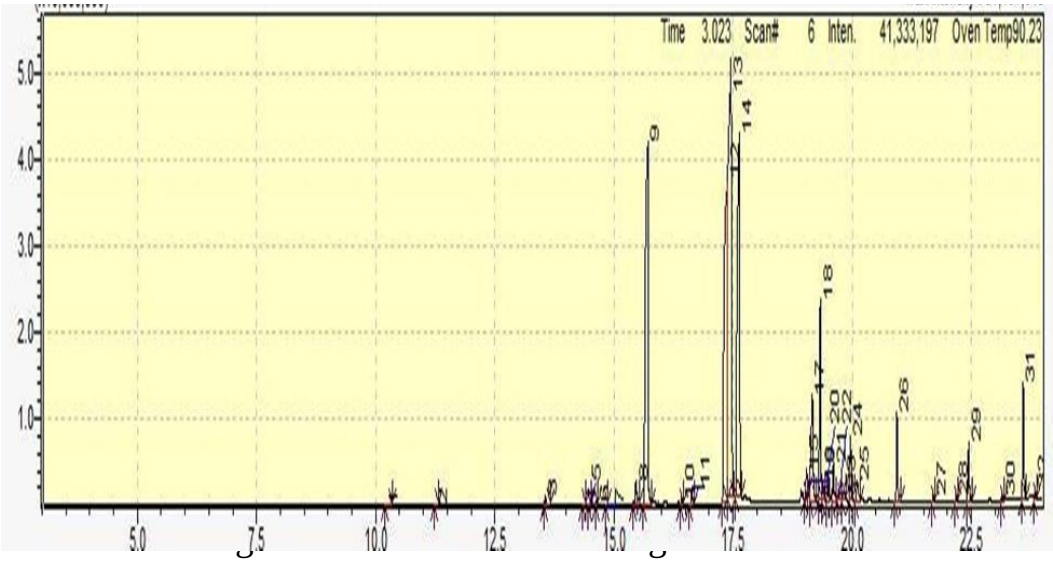

Table 3.5 : Constituents of *C. sinensis* oil

No	**Area%**	**Ret.Time**	**Name**
1.	0.05	10.219	Nonanoic acid, 9-oxo-, methyl ester
2.	0.01	11.253	Dodecanoic acid, methyl ester
3.	0.19	13.558	Methyl tetradecanoate
4.	0.01	14.367	5-Octadecenoic acid, methyl ester
5.	0.01	14.472	6-Octadecenoic acid, methyl ester, (Z)-
6.	0.04	14.632	Pentadecanoic acid, methyl ester
7.	0.03	14.848	2-Pentadecanone, 6,10,14-trimethyl-
8.	0.56	15.463	9-Hexadecenoic acid, methyl ester, (Z)-
9.	18.22	15.709	Hexadecanoic acid, methyl ester
10.	0.09	16.427	cis-10-Heptadecenoic acid, methyl ester
11.	0.34	16.634	Heptadecanoic acid, methyl ester
12.	16.63	17.375	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
13.	27.06	17.454	9-Octadecenoic acid (Z)-, methyl ester
14.	16.38	17.629	Methyl stearate
15.	0.76	19.043	2-Hydroxy-(Z)9-pentadecenyl propanoate
16.	0.15	19.089	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-
17.	4.04	19.163	2-Furanhexanoic acid, 5-(24,26-dimethyloctacosyl)tetrahydro-, methyl ester
18.	4.54	19.330	Eicosanoic acid, methyl ester
19.	0.41	19.381	PGH1, methyl ester
20.	0.44	19.488	9,12-Octadecadienoyl chloride, (Z,Z)-
21.	0.93	19.605	2-Butyl-3-methyl-5-(2-methylprop-2-

			enyl)cyclohexanone
22.	0.25	19.720	Methyl (11R,12R,13S)-(Z)-12,13-epoxy-11-methoxy- 9-octadecenoate
23.	0.33	19.826	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22), 7 (16) - diepoxy-
24.	1.51	19.965	2-Furanpentanoic acid, tetrahydro-5-nonyl-, methyl ester
25.	0.65	20.103	Hexadecane-1,2-diol
26.	1.91	20.941	Docosanoic acid, methyl ester
27.	0.15	21.704	Tricosanoic acid, methyl ester
28.	0.15	22.183	Hexatriacontane
29.	1.34	22.444	Tetracosanoic acid, methyl ester
30.	0.08	23.155	Pentacosanoic acid, methyl ester
31.	2.58	23.590	Tetratriacontane
32.	0.16	23.843	Hexacosanoic acid, methyl ester

Four components were predominant in the oil:

ii)9-Octadecenoic acid (Z)- methyl ester(27.09%).

iii)Hexadecanoic acid. Methyl ester (18.22%)

iv)9,12-Octadecadienoic acid (Z,Z)- methyl ester (16.63%)

v)Methyl stearate (16.38)

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3.12. The signal at m/z296 (RT.17.352) corresponds M^+ $[C_{19}H_{36}O_2]^+$.

Fig. 3.13 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.430)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.14.The peak at m/z 270 which appeared at (RT.15.702) is due to M^+ $[C_{17}H_{32}O_2]^+$.

Fig. 3.15 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.625) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak

at m/z 267 accounts for loss of a methoxyl.

methoxyl.

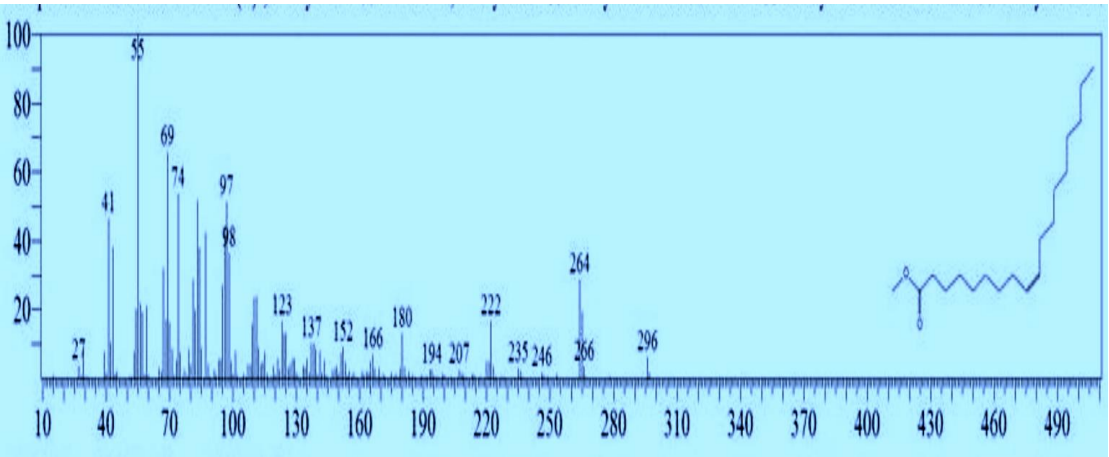

Fig.3.12 : Mass spectrum for 9-octadecenoic acid[z]-,methyl ester

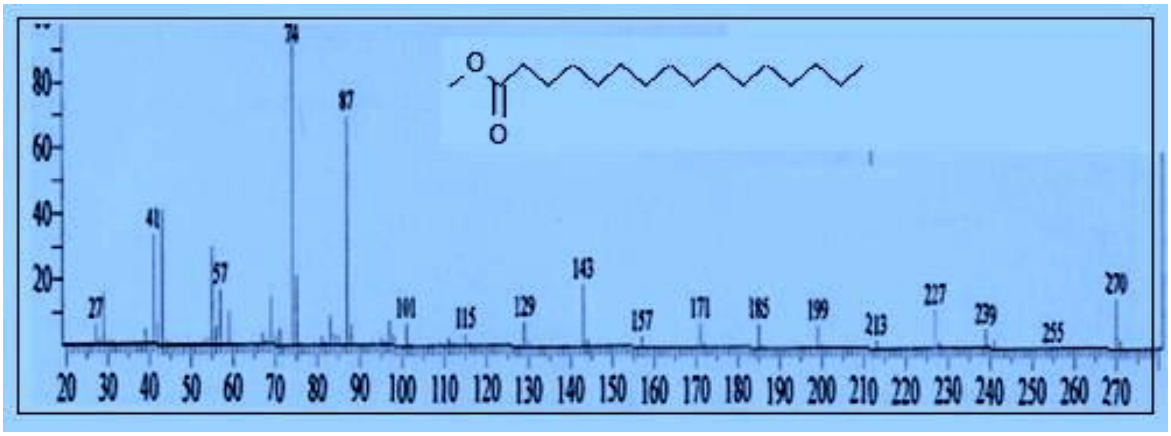

Fig. 3.13: Mass spectrum of
hexadecanoic acid methyl ester

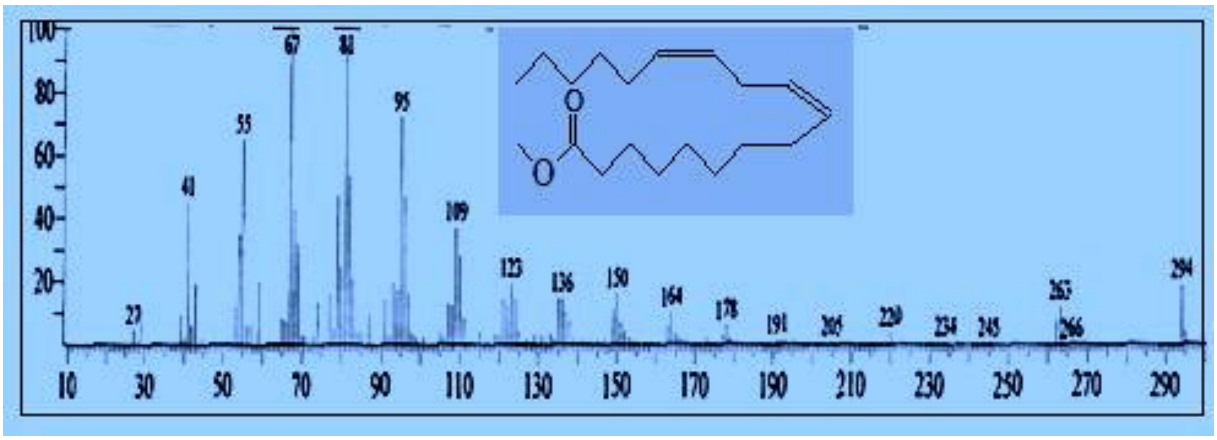

Fig.3.14: Mass spectrum of 9,12-octadecadienoic acid

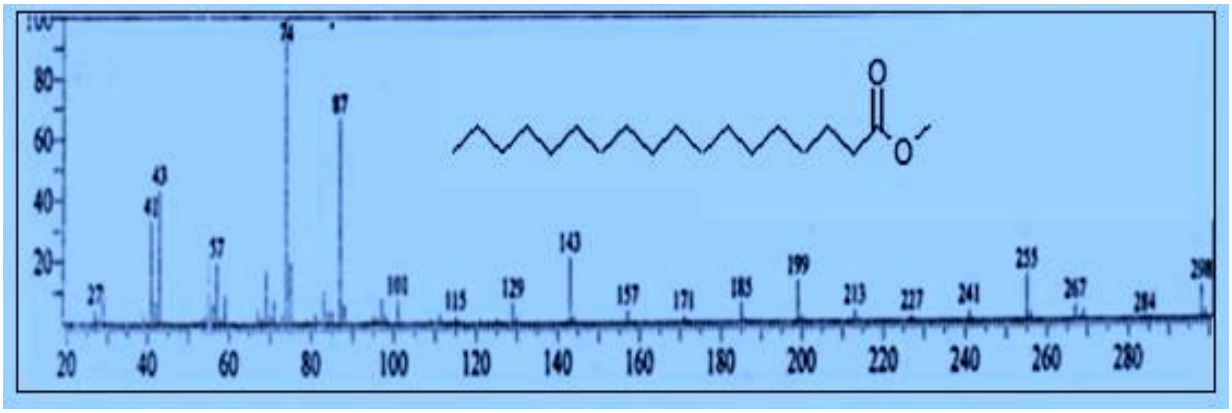

Fig.3. 15: Mass spectrum of methyl stearate

3.3.2. Antimicrobial activity

Cordia sinensis oil was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.6).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active; >18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil failed to show any activity against all test organisms.

Table : 3.6 : Inhibition zones(mm/mg sample)

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

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

3.4. *Acacia polyacantha* seed oil

3.4.1. GC-MS analysis

The typical GC chromatogram of oil extracted from *A. polyacantha* revealed the presence of thirty seven components presented in table 3.7.

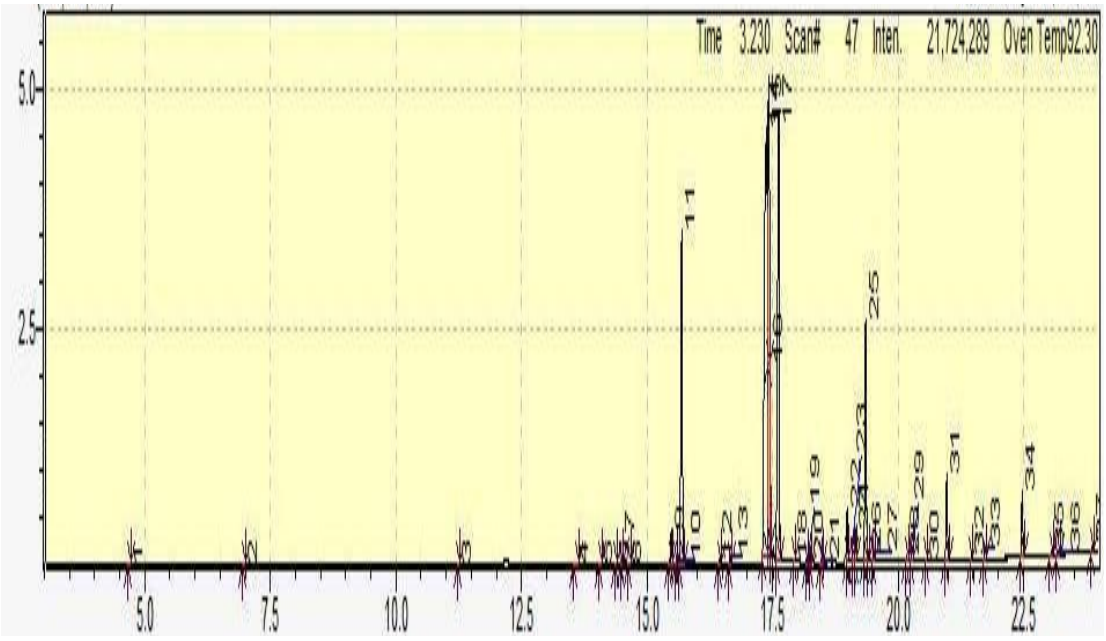

Fig.3.16: Total ion chromatograms of A. polyacantha oil

Table 3.7: Constituents of *A. polyacantha* oil

No	**Area%**	**Ret.Time**	**Name**
1.	0.03	4.702	D-Limonene
2.	0.02	6.986	L-.alpha.-Terpineol
3.	010.	11.258	Dodecanoic acid, methyl ester
4.	0.06	13.574	Methyl tetradecanoate
5.	0.02	14.072	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans-
6.	0.01	14.382	5-Octadecenoic acid, methyl ester
7.	0.00	14.489	4-Octadecenoic acid, methyl ester
8.	0.02	14.649	Pentadecanoic acid, methyl ester
9.	1.10	15.482	7-Hexadecenoic acid, methyl ester, (Z)-
10.	0.05	15.576	9-Hexadecenoic acid, methyl ester, (Z)-
11.	12.81	15.691	Hexadecanoic acid, methyl ester
12.	0.17	16.444	cis-10-Heptadecenoic acid, methyl ester
13.	0.24	16.653	Heptadecanoic acid, methyl ester
14.	31.24	17.378	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
15.	11.45	17.428	11-Octadecenoic acid, methyl ester
16.	2.40	17.450	11-Octadecenoic acid, methyl ester
17.	21.54	17.623	Methyl stearate
18.	0.06	17.935	Linoleic acid ethyl ester
19.	0.04	18.188	Octadecanoic acid, ethyl ester
20.	0.06	18.257	trans-Geranylgeraniol
21.	0.12	18.485	Nonadecanoic acid, methyl ester
22.	1.51	18.986	9,12-Octadecadienoyl chloride, (Z,Z)-
23.	0.81	19.111	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-

24.	0.75	19.147	cis-11-Eicosenoic acid, methyl ester
25.	8.28	19.353	Eicosanoic acid, methyl ester
26.	0.19	19.404	Cyclohexylidenecyclohexane
27.	0.30	19.514	8,11,14-Docosatrienoic acid, methyl ester
28.	0.17	20.171	Heneicosanoic acid, methyl ester
29.	0.03	20.265	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
30.	0.04	20.562	Isoxazole, 5-chloro-4-(2-phenylethyl)-
31.	2.95	20.968	Docosanoic acid, methyl ester
32.	0.10	21.465	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
33.	0.39	21.731	Tricosanoic acid, methyl ester
34.	2.42	22.469	Tetracosanoic acid, methyl ester
35.	0.17	23.058	Vitamin E
36.	0.22	23.181	Pentacosanoic acid, methyl ester
37.	0.22	23.871	Hexacosanoic acid, methyl ester

The predominant components of the oil are: 9,12-octadecadienoic acid (Z,Z),methyl ether(31.24%), methyl stearate(21.54%), hexadecanoic acid, methyl ester(12.81%) , 11-octadecenoic acid, methyl ester(11.45%).

Fig. 3.17 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.378)corresponds $M^+ [C_{19}H_{34}O_2]^+$. Fig. 3.18 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.623) corresponds M+$[C_{19}H_{38}O_2]$+ , while the peak at m/z 267 accounts for loss of a methoxyl.

The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.19.The peak at m/z 270 which appeared at (RT.15.691) is due to $M^+ [C_{17}H_{32}O_2]^+$. Fig.3.20 present the mass spectrum of 11-octadecenoic acid methyl ester.The peak at m/z 296 which appeared at (RT.17.428) is due to $M^+[C_{21}H_{42}O_2]$.

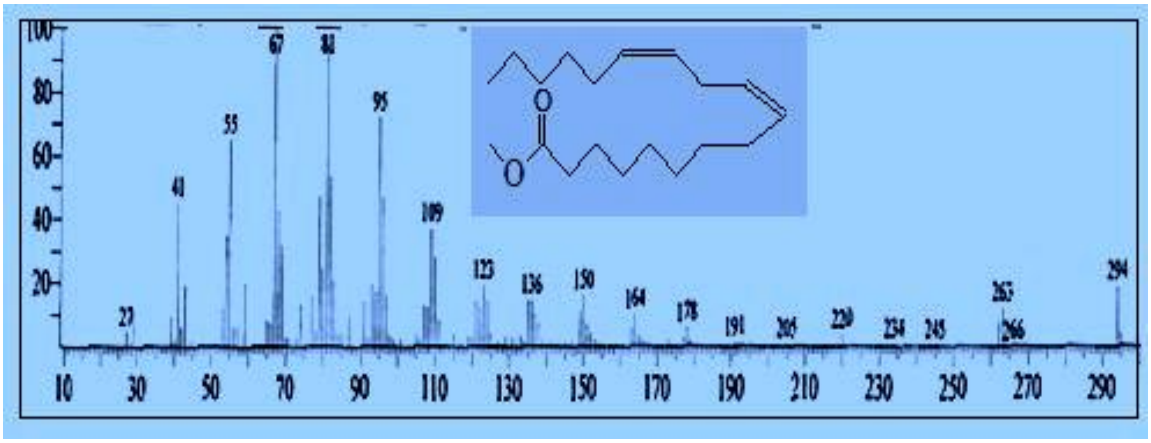

Fig.3.17 Mass spectrum of 9,12-octadecadienoic acid

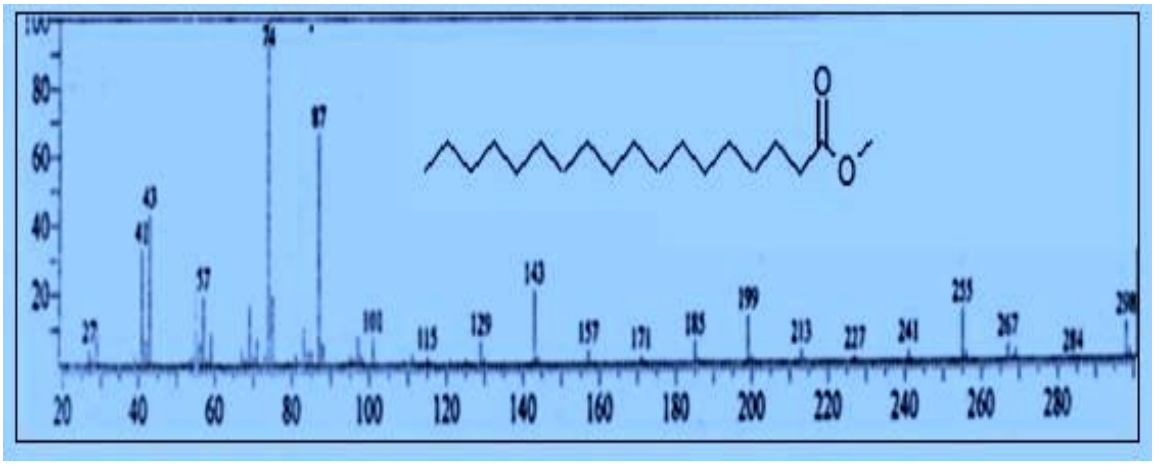

Fig.3. 18: Mass spectrum of methyl stearate

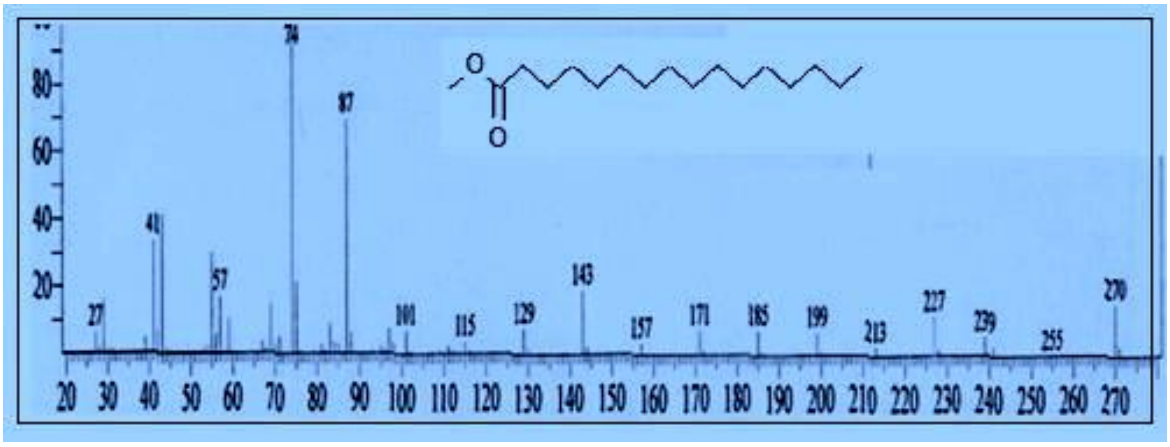

Fig. 3.19: Mass spectrum of
hexadecanoic acid methyl ester

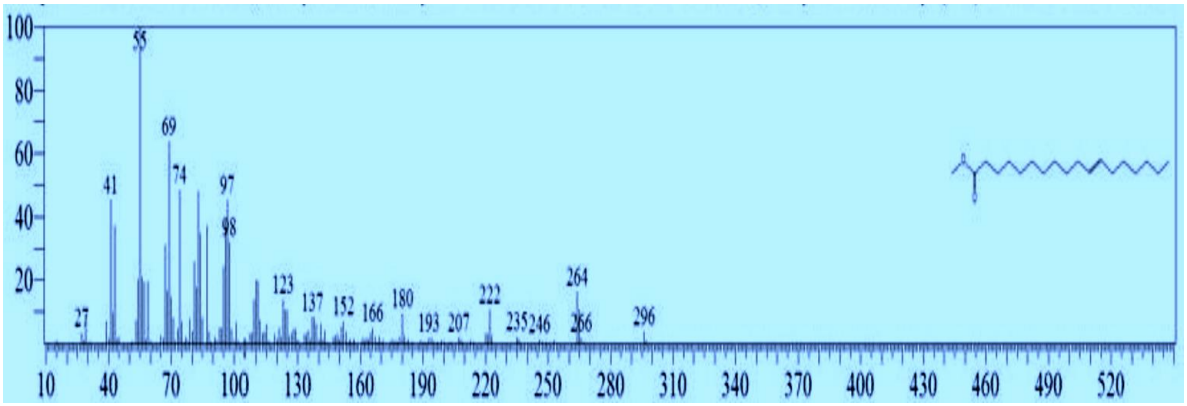

Fig.3.20 : Mass spectrum for11-octadecenoic acid, methyl ester

3.4.2. Antimicrobial activity

Acacia polycantha oil was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.8).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active; >18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil exhibited significant activity against *Escherichia coli, Pseudomonas aeruginosa* and and the fungal species *Candida albicans*. It also showed moderate activity against *Staphylococcus aureus.*However, it was inactive against *Bacillus subtilis.*

Table 3.8 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	15	--	18	17	17
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

3.5. *Peganum harmala* seed oil

3.5.1. GC-MS analysis

GC-MS analysis of seed oil of *Peganum harmala* revealed the presence of 23 components displayed in Table 3.9. The total ions chromatograms is shown in Fig. 3.21.

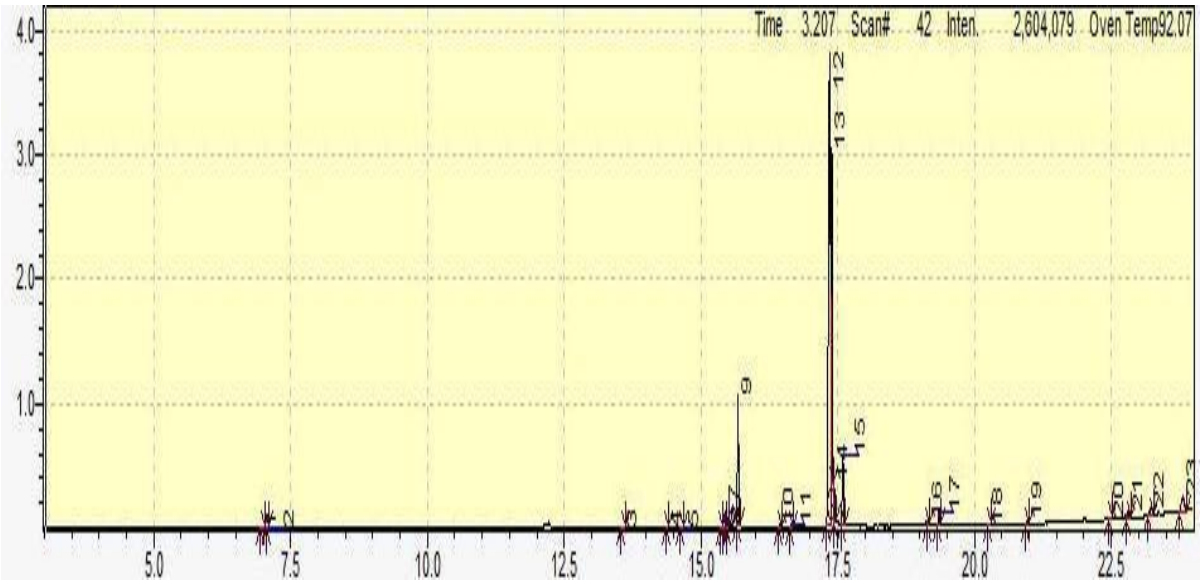

Fig.3.21 :Total ion chromatograms of *P. harmala* oil

Table 3.9: Constituents of *P. harmala* oil

No	**Area%**	**Ret.Time**	**Name**
1.	0.09	6.985	L-.alpha.-Terpineol
2.	0.01	7.076	Bicyclo[2.2.1]heptane, 2-chloro-2,3,3-trimethyl-
3.	0.11	13.577	Methyl tetradecanoate
4.	0.01	14.387	5-Octadecenoic acid, methyl ester
5.	0.03	14.651	Pentadecanoic acid, methyl ester
6.	0.03	15.380	7,10-Hexadecadienoic acid, methyl ester
7.	0.11	15.442	7-Hexadecenoic acid, methyl ester, (Z)-
8.	0.18	15.484	9-Hexadecenoic acid, methyl ester, (Z)-
9.	9.33	15.677	Hexadecanoic acid, methyl ester
10.	0.16	16.447	cis-10-Heptadecenoic acid, methyl ester
11.	0.13	16.655	Heptadecanoic acid, methyl ester
12.	54.14	17.353	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
13.	21.75	17.392	9-Octadecenoic acid (Z)-, methyl ester
14.	2.24	17.428	11-Octadecenoic acid, methyl ester

15.	4.86	17.593	Methyl stearate
16.	0.37	19.151	cis-11-Eicosenoic acid, methyl ester
17.	1.00	19.351	Eicosanoic acid, methyl ester
18.	0.12	20.269	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
19.	0.53	20.973	Docosanoic acid, methyl ester
20.	0.18	22.475	Tetracosanoic acid, methyl ester
21.	0.59	22.811	(-)-Isolongifolol, methyl ether
22.	1.27	23.222	D-Norandrostane-16-methanol, (5.alpha.,16.beta.)-
23.	2.76	23.807	Stigmasterol

Major components of the oil are : 9,12-octadecadienoic acid (Z,Z),methyl ether (54.14%), 9-octadecenoic acid (z)-, methyl ester (21.75%) , hexadecanoic acid, methyl ester (9.33%) .

Fig. 3.22 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.353)corresponds M^+ $[C_{19}H_{34}O_2]^+$.

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3.23. The signal at m/z296 (RT.17.392) corresponds M^+ $[C_{19}H_{36}O_2]^+$.

The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.24.The peak at m/z 270 which appeared at (RT.16.677) is due to M^+ $[C_{17}H_{32}O_2]^+$.

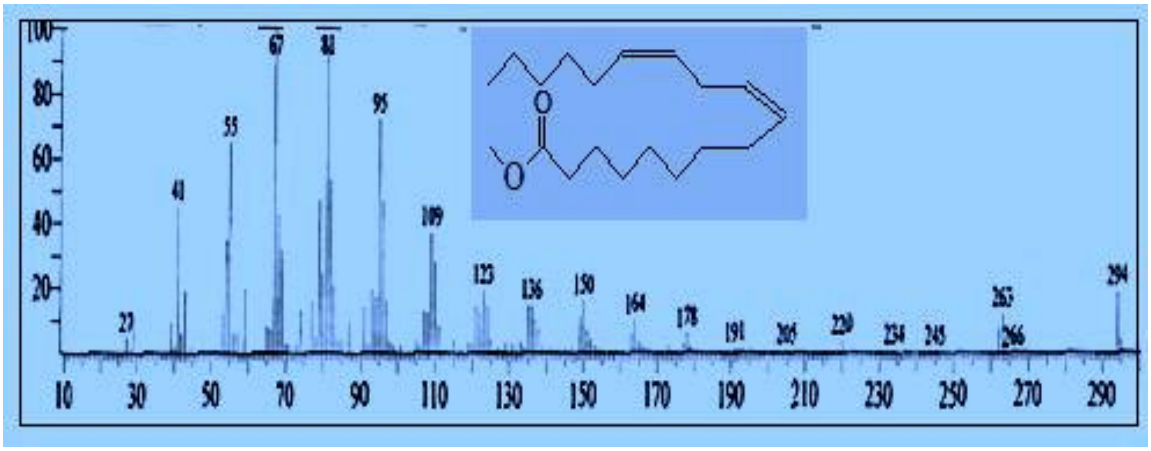

Fig.3.22: Mass spectrum of 9,12-octadecadienoic acid

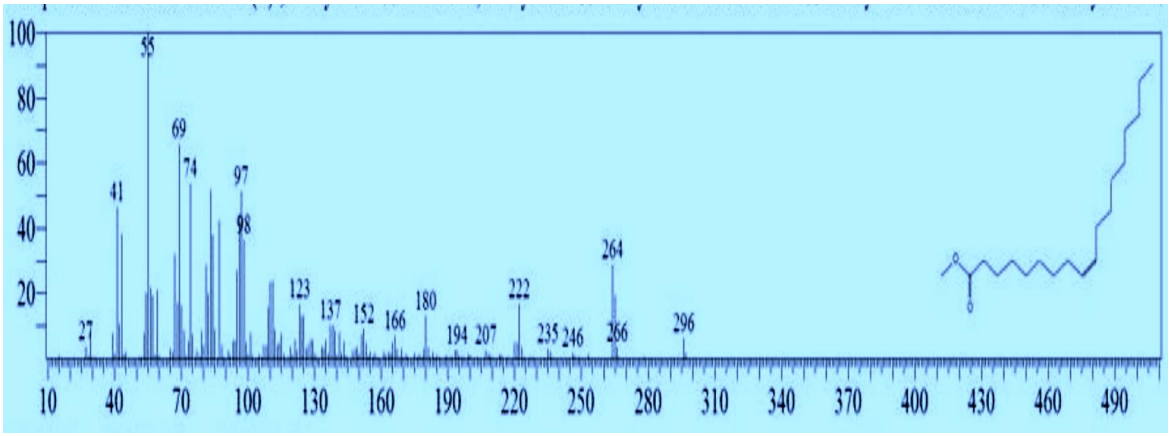

Fig.3.23 : Mass spectrum for 9-octadecenoic acid[z]-,methyl ester

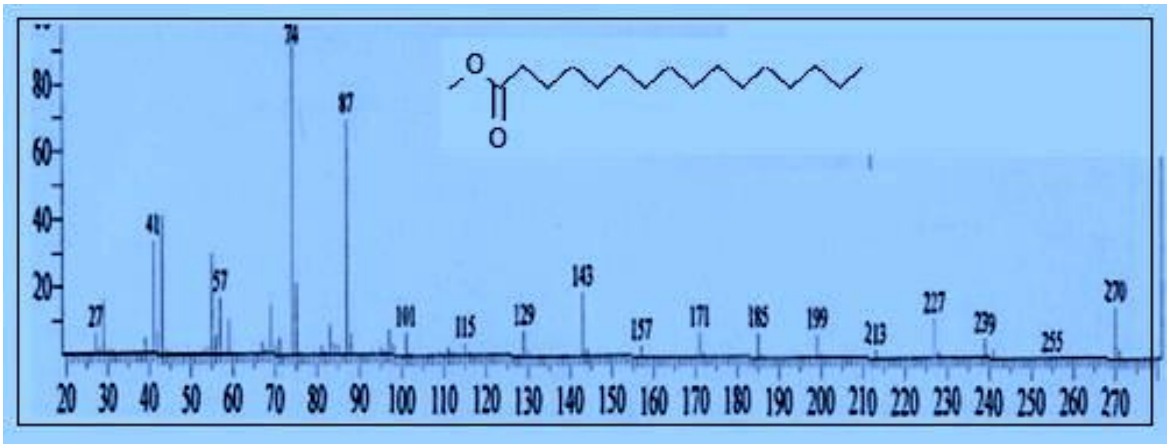

Fig. 3.24: Mass spectrum of
hexadecanoic acid methyl ester

3.5.2. Antimicrobial activity

***Peganum harmala* seed oil** was evaluated for antimicrobial activity against five standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.10).Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active; >18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The studied oil showed moderate activity against *Pseudomonas aeruginosa Escherichia coli* and *Candida albicans*.

Table 3.10 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	14	--	18	16	20
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*
Bs.: *Bacillus subtilis*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Ca.: *Candida albicans*
Cucurbita maxima oil

3.6. Conclusion

1. Essential oils are natural products which consist of many volatile molecules. They have been used for several applications in pharmaceutical, cosmetic, agricultural and bioactivity example
2. Fatty acid methyl oil (obtained from seed oils of tested plants) might be good candidates these esters presented better radical-scavenging activity
3. The results of experiment which was carried out on Sesbaniasesabanaseed oil definitely prove that the plant has a good analgesic and CNS depressant activity, these findings could open a new window on the use of this plant in traditional.
4. The seed oil extract of the plant Cissusquadrangularishave therapeutic efficacy and are known to possess antioxidant, antimicrobial activity, the plant is considered as a versatile medicinal plant in both Ayurvedic and modern drug development areas for its valuable medicinal uses, it is a very rich source of some minerals which are necessary for proper functioning of human body.
5. The study of components present in the oil seeds of Daturastramoniumshowed promising medicinal properties compared to other oil extracts, so could be used as a natural antibiotics for dif-ferent diseases, also this plant could serve as use fullsource of new antimicrobial agents
6. GC-MS analysis of P. harmala seed oil illustrate its potential as a therapeutic agent, including anti-tumor effect, anti-oxidant activity, leukemic healing, hypoglycemic effect, analgesic and anti-inflammatory properties and antinociceptive effects, also it has been reported that this plant has antibacterial, antifungal and antiviral effects. As the current information shows, it is also possible that the seed oil might be useful in the development of new drugs to treat various diseases.
7. This research discuss the chemical constituent, pharmacological and therapeutic effects of Cordiasinensisas promising herbal drug because of its safety and effectiveness
8. Eleven compounds were identified from D. cinerea, seed oil predominant in the extract is
9. octadecenoic acid[z]-,methyl ester(41.66%) which is a common monounsaturated fat in human diet, It was used as anti-oxidant , anti-carcinogenic Human Blood ,dermatitigenic flavor ,and other compounds that used as fuel and fuel additives, potential cancer preventive, anti-inflammatory and anti-arthritic activities.
10. A. polyacantha seeds contain soluble glactomannas which might be expected to have wide applications in food and pharmaceutical industries.
11. The chiefly constituents of C.cordofanus seed oil is the richest dietary source and good physical properties like pleasant odur and taste, indicates that it can be utilized as a raw material for the production of steroid hormones and manufacturing cosmetic products.
12. The oil seed of A. polyacantha, and C. quadrangularis showed anti-bacterial activity against Pseudomonas aeruginosa Staphylococcus aureus Escherichia coli and Candida ablicans.

13. 0il seed of Datura showed antibacterial activity against Staphylococcus aureus and Psedomonasaeruginosa .The oil seed of S. sesaban ,C. senensis, D. cinerea, did not exert any effect against all tested spp of bacteria

3.7. Recommendations

1. Field study is required to obtain good yield of the seeds qualitatively and quantitatively
2. All medicinal plants should be protected from beingcut down through the expansion of mechanized farms, over grazing, traditional cultivation and repeating burning.
3. More studyandfurther researches is required to know more about the oil seeds compositions.
4. Future investigations must carry out on the extracts of seeds oil of the plants so that they could get some medicinally important drugs .

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