Sudan University of Science and Technology

College of Graduate Studies

Constituents and Antimicrobial Activity of Oils From Five Plants of Medicinal Attributes

المكونات والنشاط المضاد للميكروبات لزيوت خمسه نباتات طبيه

A Thesis Submitted in Fulfillment for the Requirements of the M.Sc. Degree in Chemistry

By

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الاستـهلال

بسم الله الرحمن الرحيم

قال تعالى :

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَاعَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة – الآية (32).

Dedication

To

The Soul of my dear father…..

My bothers and sisters

Acknowledgment

Thanks at first and last for **Almighty Allah** who gave me the strength while doing this research .

I would like to express my special and deep gratitude to Prof . Mohamed Abedel Karim for his excellent guidance and efforts in supervising and leading this research.

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Abstract

Five medicinal plants widely used in Sudanese system of traditional medicine have been investigated for constituents by GC-MS analysis. The oils from these plant species have been extracted by maceration and their antimicrobial potential has been evaluated. Gas chromatography - mass spectrometry has been used for the identification and quantification of *Xemania aegyptiaca* oil. The analysis revealed the presence of 34 components dominated by: cis-13-eicosanoic acid methyl ester(13.78%) .*Commiera africana* oil was studied by GC-MS. The GC-MS analysis showed 61 constituents. Major component is :(1S,2E,4S,5R,7E,11E) - cembra-2,7,11- trien-4,5-diol (20.62%) . Gas chromatography - mass spectrometry has been used for the identification and quantification of *Trachyspermum ammi* oil. The analysis revealed the presence of 11 components dominated by:9-octadecenoic acid (Z)-, methyl ester(61.11%) .Gas chromatography - mass spectrometry analysis of *Vangueria madagascarinsis* oil showed 19 components dominated by: 9 , 12-octadecenoic acid methyl ester (53.68%).GC-MS analysis of *Vigna subterrenea* oil revealed the presence of 30 components dominated by:13-docosenoic acid methyl ester(31.47%).The target oils showed different inhibition zones in the antimicrobial assay.

المستخلص

فى هذا البحث تمت دراسة خمسة نباتات طبيه تنمو فى السودان. تم استخلاص الزيوت من هذه النباتات عن طريق النقع. تم تحديد مكونات هذه الزيوت بتقنيه الكروموتوغرافيا الغازيه- طيف الكتله. ايضا اخضعت هذه الزيوت لاختبار مضاد الميكروبات. اوضح تحليل زيت اللالوب الاصفر وجود 34 مكونا اهمها : -cis-13- eicosanoic acid methyl ester(13.78%) . احتوى زيت نبات الكندر على 61 مكونا اهمها: (1S,2E,4S,5R,7E,11E)-cembra-2,7,11-trien-.4,5-diol (20.62%) . كما واحتوى زيت نبات النخوه على 11 مكونا اهمها حمض 9- octadecenoic acid .(Z)-, methyl ester(61.11%).اوضح تحليل زيت نبات الكركر وجود 19 مكونا اهمها: 12-octadecenoic acid methyl ester , 9 . (53.68%).احتوى زيت نبات فول ابو القوى على 30 مكونا اهمها: -13 docosenoic .acid methyl ester(31.47%) . وفى اختبار مضاد الميكوبات ابدت الزيوت قيد الدراسه اقطار منع متفاوته.

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1. Introduction

1.1-General overview

Since the dawn of civilization Humans have used plants for healing diseases. From this tradition the use of aromatic plant compounds inmedicine began.

Since time immemorial ,essential oilshave beenused in the treatment of human disorders .There are also over 200 references to aromatics and ointments in the old and new testaments; noted for being used for healing of the sick.

Now it is known that the 'fragrant pharmacy' contains moleculeswith an extremely broad range of biochemical effects. Today there are over 300 essential oils used by herbalists[1].

With the increasing alarm of viral, bacterial, parasitic and fungal contamination in our planet, oils and especially essential oils are a great benefit to help protect human beings from these pathogens[1].

Our immune systems need an external support and oils can efficiently do that. Oils have long been revered for their healing and aromatherapy properties. Several oils can fight bacteria, fungi, and viruses. These precious oils not only can fight and prevent infection in your body, but they also prevent illness, treat skin conditions, disinfect the surfaces of your home, and eliminate microbes floating in the air of your environment[1].

However, lots of products of oils now circulated on the market have been polluted with lower quality,commercial – grade oils or contain other chemical substances to reduce the cost or increase the profit margin–a fact not usually revealed on the label.This is why it is important to study the chemical composition of the natural oil extracted[1].

This is characterized by the complexity in the separation of its components, which belong to various classes of compounds and which are present in a wide range of concentrations. Therefore it is complicated to establish a composition profile of the oils.

The technique of gas chromatography- mass spectroscopy is usually used for the qualitative analysis of oils. Also study of various physicochemical characteristics explores the practical importance of herbal oils in daily life[1].

The physicochemical properties of oils like odor, density, color, peroxide value, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly influence the quality of both essential and fixed oils. The commercial importance of oils mostly depends on these physicochemical properties, which provide baseline data to determine its suitability for consumption[1].

1.2. **Gas chromatography (GC)**

Gas chromatography is an analytical tool used in various fields of science including : environmental, food, forensic, agriculture and petrochemical industries[1].

In the technique of gas chromatography, the separation is mainly achieved as a result of partitioning of analytes between the gaseous mobile phase and a static phase (stationary phase) while transporting the capillary column. A capillary GC column is coated with a thin film of liquid-like stationary phase which serves to retain the gaseous analytes transported by the mobile phase. The most commonly used mobile phases, referred to as carrier gases, include helium or hydrogen. Stationary phases are differentiated based on their polarity

Differential partitioning of sample constituents , in GC , occurs as a function of properties such as polarity and boiling points[6]. Those components which have greater affinity for the stationary phase spend more time in the column, whereas those with lower affinity spend less time in the stationary phase and thus elute earlier[1].

Mainly the GC instrument consists of a carrier gas supply-like helium or nitrogen- , sample introduction unit (injector), capillary column, oven and a detector; the operation of the most important instrumental parts will be discussed briefly below.

The gas chromatography equipment has an injection port that allows the volatiles to be introduced in vapour form via the carrier gas stream(like Helium) into the capillary column. The

most common injector used in contemporary GC is the vaporising split/splitless injector. This injector was invented to prevent overloading of the capillary column due to its low volume and capacity, which may affect resolution. The sample is introduced into a heated chamber, where vaporisation occurs. Two modes of injection, split and splitless, can be used depending on the concentration of the target analyte. Split mode is mostly used when the analyte is present at high concentrations, while splitless is used when the concentration of the analyte is low[7]. Splitless injection requires effective utilisation of focusing mechanisms such as the solvent effect, cold trapping and stationary phase focusing to avoid injection band broadening[1].

The capillary column, in GC instrument, is coated with a stationary phase that permits separation of different constituents of an analyte. Stationary phases in a capillary GC are differentiated according to their polarity. Non-polar stationary phases such as PDMS, sometimes with 5 to 50% phenyl PDMS groups added are commonly used for the separation of compounds ranging from non-polar to medium polar. In these phases, separation is governed primarily by differences in vapour pressure, since non-specific dispersion interactions occur between the analytes and the stationary phase. A polar phases have been used for the analysis of petrochemical samples and also for TDOs, since they primarily contain hydrocarbons. On

semi-polar (14% cyanopropylphenyl 86% PDMS) or polar phases, mostly PEG, selective interactions such as hydrogen bonding and dipole interactions occur, and compounds are separated according to their polarity. The column is housed in an oven for accurate temperature control. Since the separation of compounds in GC is primarily based on differences in the vapour pressures of compounds, temperature plays a crucial role[1].

There is a temperature programming in GC , where an initial low oven temperature is increased as a function of time, is used to provide optimal resolution for a range of weakly and strongly retained analytes within an acceptable analysis time. Column dimensions such as length, internal diameter, film thickness and stationary phase are selected based on the analysis goals. Short (10-20 m) columns are used for fast separation of relatively simple mixtures. For complex samples, longer columns (50-60 m) provide improved separation efficiencies at the cost of longer analyses. Furthermore, reduction in the internal diameter (from e.g. from standard 0.25 mm i.d. to 0.1-0.18 mm) increases the efficiency per unit length and also provides higher optimal mobile phase flow rates, thereby allowing speeding up of the analysis. This is evident from the relationship between the column length, efficiency and optimal flow rate and the internal diameter: N = L H = L dc (1.1) $uopt = 2DMrc$ (1.2) Where N is the plate number, L is the length of the column, H is the height

equivalent of a theoretical plate, uopt is the optimal mobile phase linear velocity, DM is the diffusion coefficient of the analyte in the mobile phase and dc and rc are the column internal diameter and radius, respectively[2,3].

The efficiency of separation in GC may be checked via the peak capacity. Peak capacity is defined as number of peaks that can theoretically be separated within the retention window[4]. The peak capacity of a chromatographic separation depends[5] on the plate number (N), the mobile phase linear velocity and the temperature.

Peak capacity can be calculated[4] using the following equation below:

$$nc = 1 + tg\ wav$$

Where:

- nc : is the peak capacity
-tg : denotes the gradient run time and
-wav : avarage peak width at baseline.

The oven ramping rate (˚C/min) affects the achievable peak capacity[6]. Slow ramping rates result in longer gradient times and generally higher peak capacities, although for very slow temperature programming rates peak widths increase and peak capacity decreases again. Evaluation of peak capacity in GC provides a measure of the separation performance as well as the optimum conditions for better separation. Detectors in GC are very important and they should ideally obey certain characteristics such as adequate sensitivity, stability and

reproducibility, linear response range to solute concentration over a wide dynamic range, as well as being reliable and easy to use[6].

Now there is a wide array of detectors which are compatible with GC, such as the nitrogen phosphorus detector (NPD), atomic emission detector (AED), thermal conductivity detector (TCD), sulphur chemiluminescence detector (SCD), electron capture detector (ECD), FID and MS, amongst others. Detectors are normally selected depending on the analyte of interest and the analysis goals (i.e. selective detection of the target analytes or screening of unknowns, trace level analysis, etc). Some detectors are universal, meaning that they respond to any or most sample constituents, for example FID, MS and AED. In contrast, selective detectors respond to certain group of compounds, for example the NPD (for nitrogen and phosphorus containing compounds) and the ECD (for halogenated compounds). Among all these detectors, MS and FID are the most commonly used detectors for analysis of a wide range of samples containing organic compounds. Detectors are also most commonly used in petrochemical analysis. MS is the most powerful and universal detector that provides detailed information about the identity of the chemical constituents, while FID only gives information about the quantitative chemical composition of the sample[6].

A common detector in gas chromatography is the flame ion detector(FID) is. Here, the effluent from the column is directed into a small air and hydrogen flame; ions formed in the combustion of organic compounds in the flame are detected. Detection involves monitoring the current produced by collection of these ions by the collector electrode. The response of the FID is related to the number of carbon atoms entering the detector, thus it is a mass sensitive detector. This detector is not sensitive towards non-combustible permanent gases such as CO2, SO2, NO2, etc.[1]. For qualitative and quantitative analyses[7-9], the FID is extensively used in a variety of fields. Being a mass sensitive universal detector for hydrocarbons, it can be used to estimate the mass % composition of hydrocarbon mixtures[10], and indeed has been used for this purpose in TDO analysis.

1.3 Mass spectrometry

The main function of a mass spectrometer is to measure the mass /charge ratio (m/z) of ions produced by the analyte. The technique of MS detection essentially involves:(i) ionization (ii) separation and (iii)detection[10].

In the technique of GC, the analyte enters the mass spectrometer via the ionization source. Two types of ionization sources are used in GC ; (i) electron impact (EI) and (ii)chemical ionization (CI), with the former being more common.

During electron ionization, the molecules of the analyte are bombarded with a high energy beam of electrons (around 7eV) that ionize the molecules entering the ion source in the gas phase by removing an electron[10].

Since the formed molecular ions are unstable under such low pressure conditions, they fragment easily, and may be identified according to the characteristic fragmentation patterns formed .

Subsequently,the formed ions are separated according to their mass / charge ratio (m/z) in vacuum in the mass analyser. Two of the most common mass analyzers are : quadrupole (q) and time-of-flight (TOF) analyzers[10].

In quadrupole MS (qMS), separation according to mass to charge ratio is performed by changing the rf and dc voltages applied across the four rods comprising the quadrupole[11] . This changes the field in the quadrupole and allows only ions of a particular m/z ratio through to the detector for a given rf/dc ratio. By varying this ratio, ions of different m/z ratios can be detected. The quadrupole mass analyser consists of four parallel rods around the flight path of the ions. On two opposite rods a radio frequency (rf) is applied, whilst on the remaining two a direct current (dc) voltage is applied. This results in a magnetic field through which the ions travel which is changed continuously so that at any given setting of the rf and DC voltages only one ion will be resonant and arrive at the detector[12], while other ions are non-resonant and collide with the

rods . MS instruments can be operated in one of two modes: full scan mode, which is used for identification of unknown compounds, and selected ion monitoring (SIM), which is used for analysis of target compounds and is more sensitive than scan mode. In time of-flight mass analysers , ions formed in the source are accelerated into a flight tube by application of an extraction field on a back-plate or repeller. Ions attain the same kinetic energy in this process, and are forced through the acceleration region into the field-free drift region[12]. Because all ions have the same kinetic energy, but different masses, the time taken by the ions to travel through the flight tube depends on their mass to charge ratios[13]. Lighter ions reach the detector earlier, while the heavier ones reach the detector last. TOFMS detectors are capable of high resolution acquisition and/or very fast acquisition speeds, which makes them the MS detector of choice for GC×GC. The final step of MS involves detection of ions. This is typically done in qMS detectors using an electron multiplier, whereas in TOFMS detectors multi-channel plates are more common.

1.4 Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry(abbreviated as GC-MS) is usually employed to identify unknown compounds based on their retention parameters, and interpretation of mass spectral fragmentation patterns[14].

A Combination of two powerful analytical tools (GC and MS) is capable of detecting a wide range of constituents, providing high selectivity and sensitivity, as well as providing structural information about these compounds.[14,15]

A wide array of detectors have been used in detection of the different constituents of samples but it is generally agreed that[18] confirmation of the presence of such constituents requires a further identification by MS[14,15].

Portable GC-MS system is adequate for organic compound detection and identification. Currently, such instruments have suffered from low sensitivity and low resolution compared to their traditional counterparts. Nevertheless a need exists for a portable GC-MS system. This is because of the need for immediate answers in certain applications and also because in-laboratory confirmation runs the risk of losing volatile compounds during transportation. Field portable GC-MS systems have been successfully used in four major application categories:[16] environmental, diagnostics, forensics, and emergency response. When the technology was first used, field-portable referred to transportable, man-portable, and even better, hand-portable.

Field portable GC-MS systems can obtain information about transient events on a near real-time basis, it can map out complex gradients beside accurately locate point sources.

Different types of detection methods are used in such portable machines which have been used as rapid screening techniques in the field. These include colorimetric, infrared, surface acoustic wave (SAW) sensors, and ion mobility spectrometry (IMS)[16].

It must be stressed that these methods are effective only when used for analyzing specific classes of chemicals, particularly those having low background interferents[16].

Portable GC-MS instruments may provide detailed information about specific compounds with high sensitivity; these advantages make field-portable GC-MS more widely used than other methods[15].

During a GC-MS experiment, a mixture usually is first sampled and injected into the GC (sample introduction zone) then it is separated into its components (separation zone), and detected (detection zone) via the MS. Data are finally processed and analyzed (data analyzing zone). Each of these zones is separate, yet interrelated[16].

GC-MS involves primarily the GC oven and MS analyzer. Different researchers ,in the field of chromatography, have approached a variety of methods to design and construct field-portable GC-MS systems.

A prototype, portable GC-MS was developed at Lawrence Livermore National Laboratory[17]. This system was used to analyze hazardous substances.

Lawrence Livermore laboratory has developed a newer version of this portable GC-MS system[18] based on a commercially available portable GC (Model 8610, SRI instruments, Torrance, CA, USA) and compact double-focusing MS[19,20], allowing separation of three samples within 30 s using a 30 m long column, 2 s scan rate, resolving power of 125, and 10-150 Da mass range[21].

A portable GC system with a Paul quadrupole ITMS. has been designed by Shortt et al.[22] .This equipment required only 42 W of power. The mass range was,however, narrow, but all other aspects, such as mass resolution, sensitivity, etc., were all comparable to its commercial counterpart. Other systems include Hewlett Packard MSD based GC-MS systems[23,24]and a hand-portable prototype[25].

Low thermal mass GC-MS is a novel approach that is well suited for field GC-MS[26]. This technique was first described by Hail et al.[27]

Another portable GC-MS equipment is called transfer line GC (TLGC). In this machine a short, narrow-bore column is used as an interface between the MS and a sampling system, typically for a process that must be analyzed on line[28]. The column acts both as the flow restrictor for the MS pumping system and as the separation column for the sample.

The process of separation by GC-MS involves two steps for analyzing and identifying a sample. GC is a technique based on

the partition (or adsorption) of analytes between a gaseous mobile phase and a liquid (or solid) phase immobilized on the surface of an inert solid in a small diameter tube (the column). A GC is an apparatus that is used to separate a mixture into its different components according to the interaction of the components with the liquid (or solid) stationary phase[28].

Once the constituents of the analyte are separated, they arrive at the output of the GC where they are identified. A conventional GC requires a bulky oven, which is power inefficient, heavy to carry, and has relatively slow temperature programming rates, usually allowing at most 75˚C/min. Miniaturizing GC usually involves miniaturizing the column oven, which has the largest thermal mass in a GC system. There are four possible ways of reducing the 5 mass of the traditional oven and thus increasing its maximum heating rate[29]: (a) build a smaller traditional oven or reduce the volume of a traditional oven by adding insulating material; (b) wrap a capillary column around a heated metal cylinder, thus allowing the components to take up a smaller volume; (c) micro-machine a capillary column into a small silicon wafer; and (d) sheath the capillary column with an electrically conducting material[30,31].

A Compact GC probe was designed[26] for use in GC-MS system. Aluminum-clad columns coiled around a 2.5 in. diameter Teflon spool were covered with Nextel glass braid insulation. Copper connector slides were used to electrically connect the ends of the

columns, allowing them to be heated resistively. Because of the very low thermal mass of a column, it could be heated at a rate of 524 ˚C/min (with an initial rate of 2400 ˚C/min) from 50 ˚C to 150 ˚C and cooled down at a rate of 165 ˚C/min (also with a initial rate of 2400˚C/min) from 150˚C to 50 ˚C. The maximum heating rate for a traditional GC oven from 50 ˚C to 150 ˚C is approximately 50 ˚C/min38, with an approximate cool-down time of 4 min for the same temperature range, or about 7 times longer. The total power required to resistively heat the 2.3 m column to 150 ˚C was approximately 35 W whereas 2400 W is needed for a conventional oven under exactly the same conditions[31].

1.5 Essential oils

An acceptable definition of essential oils is: highly concentrated hydrophobic liquid of complex mixtures containing volatile aroma compounds and can be extracted from several parts of plant , for example ,leaves, peels, barks, flowers , buds, seeds, and so on which serve as the major source of essential oil[32].

Through decades many cultures used essential oils for their medicinal and therapeutic benefits. In medicinal practice, the Egyptians used essential oils extensively for beauty treatment, food preparation, and in religious ceremony. Frankincense, sandalwood, myrrh and cinnamon were considered very valuable cargo along the ancient caravan trade routes and were

sometimes exchanged for gold. Greeks also used essential oils in their practices of therapeutic massage and aromatherapy. The Romans utilized essential oils to promote health and personal hygiene. Also the Romans, the Persians began to refine distillation methods for extracting essential oils from aromatic plants Essential oil extracts were used throughout the dark ages in Europe for their antibacterial and fragrant properties[32].

The potential healing effect of essential oils were perceived in 1937 by a French chemist, Rene Maurice Gattefosse, who plunged his badly burnt hand into a vat of lavender oil (mistaking it for water) and was surprised to see no injury or scarring. Therapeutic-grade essential oils have been used successfully to treat injured soldiers during World War II.

However, after the invention of modern drugs, the use of plants and herbs for their therapeutic properties temporarily declined. That trend is reversing as health scientists and medical practitioners continue to research and validate the benefits of therapeutic-grade essential oils to protect the body, boost the immune system, and revitalize the mind and influence mood, without the adverse effects commonly associated with synthetic drugs [32].

Essential oils can be extracted from various parts of the plants : leaves (cedar); bark(cinnamon), root (ginger),seed (caraway),grass (citronella); gum (myrrh and balsam oils); florwers (rose) ; buds (cloves), wood(amyris) ;

heartwood(cedar).and saw dust(cedar oil). These plants are processed to yield their quintessence or essential oils by separation from cellulose, glycerides, starches.sugars, tannins, salts and minerais in the botanicals[32].

Co-distillation with steam is a widespread technique for isolating essential oils from plants is A small group of products are exception to the simple definition of an essential oil.To extract some oils like garlic oil , mustard oil, enzymatic release of the volatile components is required before they can be freed from their matrix via steam distillation. There are other flower oils or resin oils obtained by extraction which contain only a small portion of volatile oil, but nonetheless are called essential oils. Some products obtained through dry-distillation contain only a limited amount of volatiles. Such products do not fall within the designation of essential oil.Getting the odorous principal from the botanical is called ‘expressing' the essential oil[32].

In the past , oils from citrus plants were obtained by tedious hand-pressing or sponge-pressing, but now they are produced by modern high speed multi-functional units.

Enfleurage is an old extraction technique in which delicate flower petals were physically stuck onto a purified fat, is no longer in common use.One popular extraction procedure is known as maceration[32].

It must be stressed that yield of essential oils from their natural matrix varies largely. Nutmegs yield (10-12wt %) of oil, whereas onions yield less than 0.01 % after enzymatic treatment. Thuga wood oil yield is within the range : 0.60-1.00% wet material .Essential oils are typically liquid at room temperature[32].

The exact biological function of essential oil in the plants is not well understood. Microscopicexamination of plant parts that contain the oil sacs readily shows their presence when the sac on the foliage is pricked and the aroma appears.

Flowers odor are beleived to act as attractants for insects involved in pollination, hence they help in preservation of species and natural selection. Essential oils are mostly bacteriostatic(slow the growth of pathogenic bacteria) and often bactericidal(killing bacteria)[32].

Essential oils usually contain many components with biological activity. They are sources of plant metaboiic energy if present in large quantities . Thoughsome chemists have considered essential oils as waste products of plant metabolism, exudates like balsam and resinscontain essential oils that act as protective seals againstdisease or parasite. They prevent loss of sap and are formed readily when the tree trunks are damaged[33].

1.5.1 Diverse uses of essential oils

For centuries , essential oils have been utilized by various communities for health purposes.Essential oil benefits come

from their antioxidant, antimicrobial and anti-inflammatory properties. Essential oil uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments[33].

However, the yield of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods[33].

Very useful bioactive molecules are derived from essential oils. These oils are good source of several bioactive compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved[33].

Essential oils find a wide spectrum of practical applications including: cosmetics, soaps, perfumes, pharmaceutical formulations. They are used for flavoring food and drink, and for adding scents to incense and household cleaning products and have been used medicinally throughout history[33].

Herbalist recipes range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of

medical treatments, and treatment of cancers in particular, are now subject to regulation in most communities[34].

1.6 Antimicrobials

An antimicrobial is an agent that kills microorganisms or stops theirgrowth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They canalso be classified according to their function. Agents that kill microbes are called microbicide, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis[35].

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness. Antiseptics are applied to living tissue and help reduce infection during surgery while antibiotics destroy microorganisms within the body. The term"antibiotic" originally described only those formulations derived fromliving microorganisms but is now also applied to synthetic antimicrobials, such as the sulfonamides, or fluoroquinolones. The term was initially restricted to antibacterials but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, who kill

bacteria, and bacteriostatic agents, which slow down or stall bacterial growth[35].

Antimicrobial use is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection.

In the 19th century, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine. In1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as penicillin rubens and named the extracted substance penicillin which in 1942 was successfully used to treat a *Streptococcus* infection[35].

Antibacterials are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterials is generally considered low. Prolonged use of certain antibacterials can decrease the number of gut flora, which may have a negative impact on health. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty in recovering after prolonged antibiotic treatment[35].

The discovery, development and use of antibacterial during the 20thcentury has reduced mortality from bacterial infections. The antibiotic began with the pneumatic application of nitroglycerine drugs, followed by a “golden” period of discovery from about 1945 to 1970, when a number of structurally diverse and highly effective agents were discovered

and developed. Since 1980 the introduction of new antimicrobial agents for clinical use has declined, in part because of the enormous expense of developing and testing new drugs. In parallel there has been an alarming increase in antimicrobial resistance of bacteria,fungi, parasites and some viruses to multiple existing agents[35].

Antibacterials are among the most commonly used drugs and among the drugs commonly misused by physicians, for example, in viral respiratory tract infections. As a consequence of widespread and injudicious use of antibacterial, there has been an accelerated emergence of antibiotic resistant pathogens, resulting in a serious threat to global public health.The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibacterial. Possible strategies towards this objective include increased sampling from diverse environments and application of metagenomics to identify bioactive compounds produced by currently unknown and uncultured microorganisms as well as the development of small-molecule libraries customized for bacterial targets. Antifungals are used to kill or prevent further growth of fungi. In medicine, they are used as a treatment for infections such as athlete's foot, ringworm and thrush and work by exploiting differences between mammalian and fungal cells. They kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi- and humans are eukaryotes.Thus, fungal and human cells are similar at the molecular level, making it more difficult

to find a target for anantifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can belife-threatening if the drug is not used properly[35].

As well as their use in medicine, antifungals are frequently sought afterto control mold growth in damp or wet home materials. Sodium bicarbonate (baking soda) blasted on to surfaces acts as an antifungal.

Another antifungal serum applied after or without blasting by soda is a mix of hydrogen peroxide and a thin surface coating that neutralizes mold and encapsulates the surface to prevent spore release. Some paints are also manufactured with an added antifungal agent for use in high humidity areas such as bathrooms or kitchens. Other antifungal surface treatments typically contain variants of metals known to suppress mold growth e.g. pigments or solutions containing copper, silver or zinc. These solutions are not usually available to the general public because of their toxicity[35].

Antiviral drugs are a class of medication used specifically for treating

viral infections. Like antibiotics, specific antivirals are used for specific viruses. They are relatively harmless to the host and therefore can be used to treat infections. They should be distinguished from viricides,which actively deactivate virus particles outside the body.

Traditional herbalists used plants to treat infectious disease. Many of these plants have been investigated scientifically for

antimicrobial activity, and some plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal[35,]

1.7 The targeted plant species

1.7.1 *Commifera Africana*

-Taxanomic profile

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Sapindales
Family:	Burseraceae
Genus:	*Commiphora*
Species:	*C. africana*

Commifera is a large genus. Different species of this genus yield gums, oils and resins. *Commifera Africana* (A.Rich.) Engl. , commonly known as African myrrh is a small tree or shrub in the family Burseraceae(Dalziel). *Commifera Africana*is widely distributed throughout the African continent,Asia and the Middle East. Different parts of this plant-leaves, roots and barks- are used traditionally against a wide range of ailments[36].

Commifera Africana

Commifera Africana is used in herbal medicine against heart burn,dysentery,stomachache and snake bite. In some African communities, the bark is cooked with sorghum and taken for heart burn. Gum resins finds many industrial applications including manufacture of films,fibre,plasters and varnishes[37,38].

1.7.2 *Vigna subterrenea*

-Taxonomic profile

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Genus:	*Vigna*
Species:	***V. subterranea***

Vigna subterrenea is a legume indigenous to the African contintenant.This plant is widely distributed in Africa extending

from Sahara to south Africa[39-41]. Seeds of *Vigna subterranea*may vary in color being brown, black or red[43,44].This plant contains ,among others,some bioactive molecules including flavonoids and alkaloids.

Vigna subterrenea

The traditional uses of *Vigna subterranea*is based on inherited information.The plant is used as a natural remedy against a range of human disorders . It is used traditionally against diarrhea and dysentery[45].*Vigna subterrenea* is antimicrobial ,antioxidant , antiallergic, anti-inflammatory and antitumor[46].

1.7.3 *Xiemenia aegyptiaca*

-Taxonomical profile

Kingdom:	Plantae
Order:	Zygophyllales
Family:	Zygophyllaceae
Genus:	*Balanites*
Species:	***B. aegyptiaca***

Xelenia aegyptiaca

Xelenia aegyptiaca Del. is a potential , but underestimated ,tree in the family Zygophyllaceae.This tree is one of the most common trees in Sudan and Senegal[47-50].This neglected wild plant is also distributed in dry land areas of Africa[51,52]. *Xelenia aegyptiaca* is native to most of the African countries and parts of the Middle East[53].

Xelenia aegyptiaca is multibranched ,spiny shruber tree up to 10m in height. The trunk is short and often branching; bark is dark brown-grey;branches are armed with stout yellow-green thorns;leaves have two separate leaflet; flaowers are in fascicles in the leaf axils and are fragrant;fruit rather long ,narrow drupe; pulp is bitter-sweet and is edible[54-56].

The fruit aqueous extract exhibited spermicidal activity in human subjects.Fruit pulp showed hyperglycemic – induced testicular anthelmintic, purgative[57],expectorant and antifungal[58]. Seeds are also used as febrifuge. Seed oil is said to treat tumors and wounds[59].The oil is also used as laxative and to treat

hemorrhoid, stomacache, yellow fever, syphilis, jaundice and epilepsy[60]. Fruits are natural remedy for leucoderma, skin infections and whooping cough[58]. Fruits are used traditionally as hypoglycemic[61].Fruit mesocarp is a remedy for jaundice[62].Fruits are purgative, they are also used for liver disorders[63,64]. is a The bark is used traditionally as spasmolytic[58]. It is also used in the treatment of syphilis and round worm infections. Latex of *Xelenia aegyptiaca* is used by herbalist in the treatment of epilepsy[59].

Leaves of *Xelenia aegyptiaca* contain, among others, saponins, flavonoids and furanocoumarins[65,66].Fruit mesocarp contains sugars, protein and organic acids[67].Root contains steroidal saponons and some glycosides[68,69].

Many pharmacological effects have been associated with *Xelenia aegyptiaca* including : antioxidant[70],anthelmintic[71],antibacterial[72-74],antivenin[75],anticancer[76,77],antiinflammatory[78],analgesic[78], antinociceptive[79],mosquito larvicidal[80], hepatoprotective[81],wound –healing[82],diuretic[82] and hypocholesterolemic activity[83].

1.7.4 *Vangueria madagascarinsis*

-Taxonomy

Kingdom:	Plantae
Order:	Gentianales
Family:	Rubiaceae

Genus:	*Vangueria*
Species:	*V. madagascariensis*

Vangueria is a genus of flowering plants in the family Rubiaceae. The genus contains over 50 species distributed in Africa[84]. The fruits of *Vangueria infausta*, are consumed by humans[85]. The roots and leaves are used by traditional healers. Leaves of *Vangueria spinosa* are used traditionally as antimicrobial[86]. Leaf extracts of *Vangueria spinosa* were screened for antibacterial activity. The ethyl acetate fraction was significantly active[86].

Vangueria madagascarinsis

Vangueria madagascarinsis – also known as Spanish tamarind and tamarind of Indies[87]. is a plant of many medicinal attributes. The plant is cultivated in some African and Asian countries for its medicinal and nutritional value[88-90]. Roots and bark are used in traditional medicine. In some African countries an extract

from the roots is used to treat worm infections[85]. It has been reported that the plant possesses antimicrobial properties[91]. The antioxidant and cytotoxic properties has also been documented[92]. However, Some *Vangueria* species - *V. latifolia*, *V. pygmaea*, *V. thamnus* - are known to be toxic[93].

1.7.5 *Trichyspermum ammi*

-Taxanomic profile

Kingdom:	Plantae
Family:	Apiaceae
Genus:	*Trachyspermum*
Species:	***T. ammi***

Trichyspermum ammi fruits

Trichyspermum ammi is a herb of multidimensional uses in Sudanese ethnomedicine. It is an annual herbaceous plant reaching 30-70cm in height . *Trichyspermum ammi* beongs to family Apiaceae which comprises around 2700 species.The

plant is indigenous to the middle east[94,95].Fruits are claimed to have antimicrobial, antiseptic and antithelmintic properties[96].A major constituent of this herb – thymol- is known to exhibit antifungal, germicidal and spasmolytic activities[97].Another bioactive constituent –carvacrol is associated with expectorant and antifungal properties[98].The fruits are used traditionally against nausea, vomiting and abdominal anorexia[99].Powdered fruits are inhaled to relief cold[100,101].It has been reported that the fruits exhibited antiulcer activity[100].*In vivo* studies demonstrated that supplemtation of *Trichyspermum ammi* enhanced the digestive enzymes and promoted secretion of bile acids[100].The antifungal activity of this plant against a panel of fungi has been reported[101]. The acitivity is probably due to the phenolics of *Trichyspermum ammi* .The antiinflammatory effect of seed extracts has been demonstrated in model animals[102].It has been shown that V exhibited in vivo hepatoprotective properties[103] . The hypotensive and broncho-dilating effect of *Trichyspermum ammi* has been reported[104].Also the hypolipidemic effect *Trichyspermum ammi* seeds has been testified[105].

Aim of this study

This study was designed to:

-Extract oils from five medicinal plants grown in Sudan.

-Characterization of constituents of the oils.

-Evaluation of the antimicrobial potential of the oils.

2-Materials and Methods

2.1 Materials

2.1.1 Plant material

Seeds of *Xemania aegyptiaca, Commiera Africana, Trachyspermum ammi, Vangueria madagascarinsis* and *Vigna subterrenea* were collected from around Damazin-Sudan. The plants were authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, Khartoum-Sudan.

2.1.2 Instruments

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

2.1.3 Test organisms

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

Table 1: Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungi

2.2- Methods

2.2.1 Extraction of oil

Powdered seeds of studied plant (500g) were exhaustively extracted with n-hexane by maceration. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

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

2.2.2 GC-MS analysis

The studied oils were analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas.Oven temperature program is presented in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)

-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions

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

2.2.3

Antimicrobial activity

i)-Bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto 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 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable

organism per ml of the stock suspension was determined by means of the surface viable counting technique.

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

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

Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

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

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

3-Results and Discussion

Five medicinal plants widely used in Sudanese system of traditional medicine have been investigated for constituents by GC-MS analysis.The oils from these plant species have been extracted by maceration and their antimicrobial potential has been evaluated.

3.1 *Xemania aegyptiaca*

Oil of *Xemania aegyptiaca* was extracted with hexane. The oil was investigated by GC-MS and the constituents of the oil have been characterized. The oil was also evaluated for its antimicrobial potential using the cup plate agar diffusion bioassay.

3.1.1 GC- MS analysis of *Xemania aegyptiaca* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of the *Xemania aegyptiaca* oil. The analysis revealed the presence of 34 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

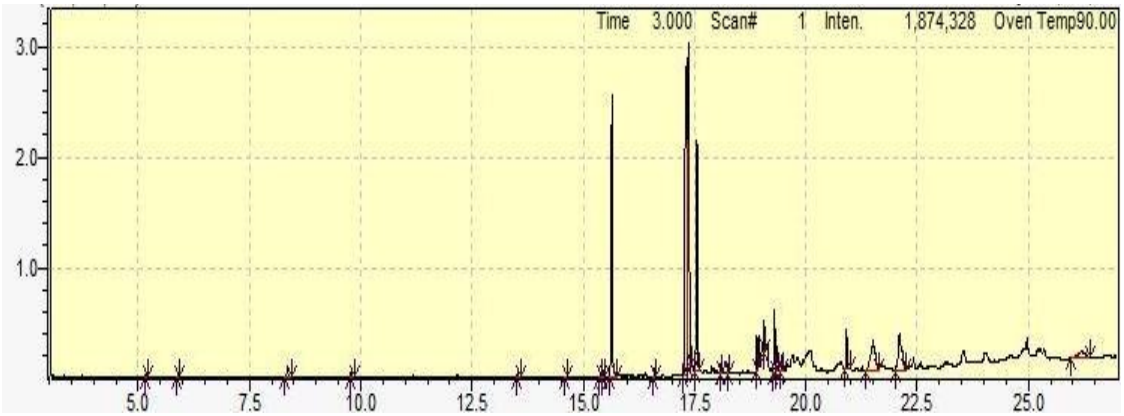


Fig. 3.1: Total ion chromatograms

Table 3.1: Constituents of *Xemania aegyptiaca* oil

No.	Name	Ret.Time	Area%
1.	Nonanoic acid, 9-oxo-, methyl ester	10.632	0.05
2.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.489	0.03
3.	Dodecanoic acid, methyl ester	11.735	0.05
4.	9-Octadecene, (E)-	13.439	0.02
5.	8-Heptadecene	13.617	0.05
6.	Methyl tetradecanoate	14.182	0.28
7.	Pentadecanoic acid, methyl ester	15.317	0.07
8.	7,10-Hexadecadienoic acid, methyl ester	16.092	0.12
9.	7,10,13-Hexadecatrienoic acid, methyl ester	16.165	0.27
10.	9-Hexadecenoic acid, methyl ester, (Z)-	16.200	0.60
11.	Hexadecanoic acid, methyl ester	16.410	7.62
12.	n-Hexadecanoic acid	16.841	0.19
13.	cis-10-Heptadecenoic acid, methyl ester	17.217	0.09
14.	Heptadecanoic acid, methyl ester	17.430	0.11
15.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.180	12.90
16.	9-Octadecenoic acid (Z)-, methyl ester	18.253	5.49
17.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.286	5.99
18.	Methyl stearate	18.425	3.51
19.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	18.628	1.97
20.	.gamma.-Linolenic acid, methyl ester	19.929	0.48
21.	cis-13-Eicosenoic acid, methyl ester	20.096	13.78
22.	cis-11-Eicosenoic acid, methyl ester	20.135	2.67
23.	Eicosanoic acid, methyl ester	20.270	2.82
24.	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	21.605	0.72

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

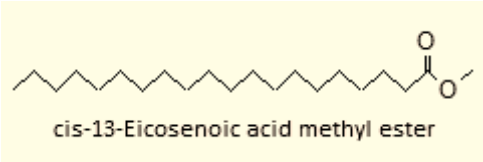
i)cis-13-Eicosanoic acid methyl ester(13.78%)

ii) 9 12-Octadecenoic acid methyl ester (12.90%)

ii) Hexdecanoic acid (7.26 %)

Fig. 3.2 presents the mass spectrum of cis-13-eicosanoic acid methyl ester.The signal at m/z324(RT.20.096) corresponds the molecular ion $[C_{21}H_{40}O_2]^{+\cdot}$. The mass spectrum of 9,12-octadecadienoic acid methyl ester is illustrated in Fig.3.3. The

peak at m/z 294(RT. 18.180)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.4.The peak at m/z 270 which appeared at (RT.16.410) is due to M^+ $[C_{17}H_{32}O_2]^+$.



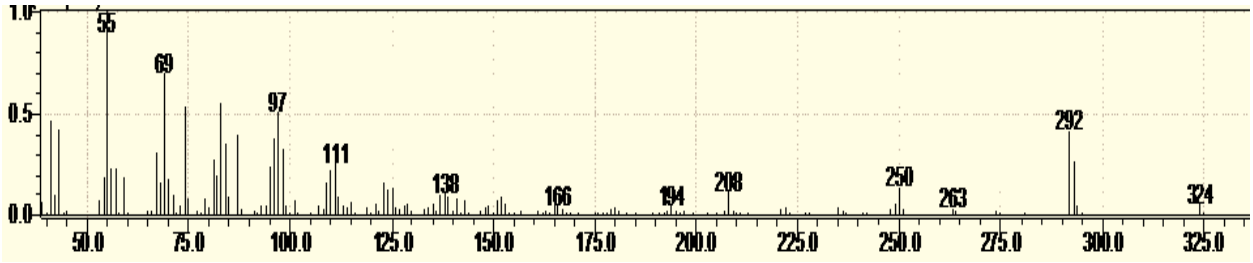
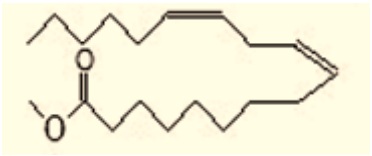


Fig. 3.2: Mass spectrum of cis-13-eicosanoic acid methyl ester



9,12-Octadecenoic acid methyl ester

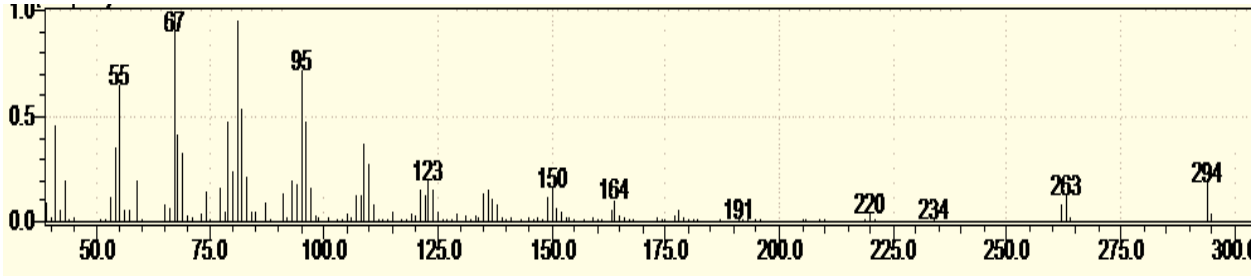
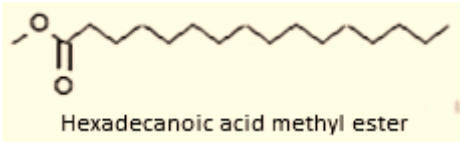


Fig.3.3: Mass spectrum of 9,12-octadecadienoic acid



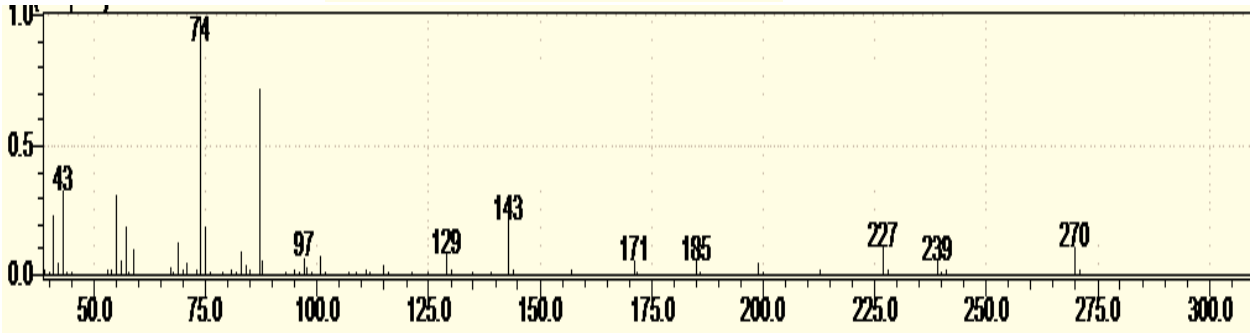


Fig. 3.4: Mass spectrum of
hexadecanoic acid methyl ester

3.1.2 Antimicrobial activity of *Xemania aegyptiaca* oil

Xemania aegyptiaca 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 (3.2).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* and *Escherichia coli* .

Table : 3.2 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	10	12	15	13	--
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*

3.2 *Commiera africana*

3.2.1-GC-MS analysis of *Commiera africana* oil

Commiera africana oil was studied by GC-MS. The GC-MS analysis showed 61 constituents(Table 3.3) which were confirmed by the retention times and mass spectra fragmentation pattern. Major components are:

i-(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol (20.62%)
ii-2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)- (20.50%)
iii- Acetic acid octyl ester (8.27%)

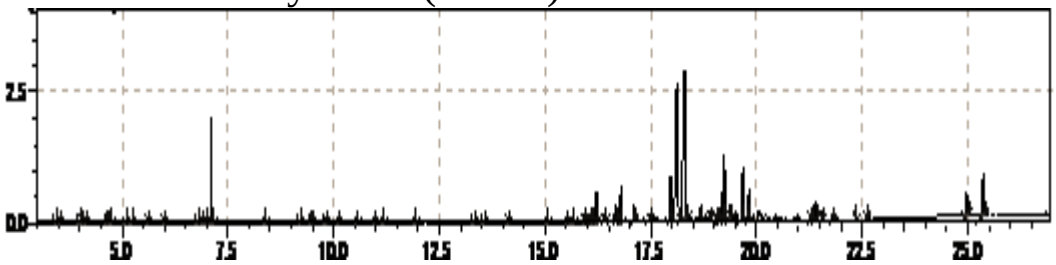


Fig.3.5 : Total ions chromatograms

Table 3.3: Constituents of *Commiera africana* oil

No.	Name	Ret.Time	Area%
1.	.alpha.-Phellandrene	3.415	0.02
2.	.alpha.-Pinene	3.511	0.06
3.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	3.974	0.03
4.	.beta.-Pinene	4.037	0.03
5.	Heptane, 2,2,4,6,6-pentamethyl-	4.127	0.03
6.	p-Cymene	4.612	0.66
7.	D-Limonene	4.669	0.07
8.	Eucalyptol	4.726	0.16
9.	.gamma.-Terpinene	5.064	0.02
10.	1-Octanol	5.176	0.37
11.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.596	0.07
12.	1,3-Dimethyl-1-cyclohexene	5.981	0.10
13.	Terpinen-4-ol	6.769	0.02
14.	Benzenemethanol, .alpha.,.alpha.,4-trimethyl-	6.870	0.02
15.	.alpha.-Terpineol	6.949	0.03
16.	Acetic acid, octyl ester	7.110	8.27
17.	Phenol, 2-methyl-5-(1-methylethyl)-	8.349	0.16
18.	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	9.204	0.05
19.	Geranyl acetate	9.455	0.10
20.	.alpha.-ylangene	9.491	0.02
21.	Methyleugenol	9.781	0.74
22.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	10.101	0.08
23.	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	10.540	0.02
24.	5-Decen-1-ol, acetate, (E)-	10.950	0.05
25.	4,6-di-tert-Butyl-m-cresol	11.174	0.03
26.	Octanoic acid, hexyl ester	11.910	0.09
27.	8-Dodecen-1-ol, acetate, (Z)-	13.279	0.07
28.	Methyl tetradecanoate	13.529	0.03
29.	Heptyl caprylate	14.110	0.08
30.	geranyl-.alpha.-terpinene	15.040	0.09

31.	Tetracyclo[5.2.1.0(2,6).0(3,5)]decane, 4,4-dimethyl-	15.501	0.04
32.	Hexadecanoic acid, methyl ester	15.630	0.21
33.	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	15.915	0.64
34.	Alloaromadendrene	16.053	0.11
35.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	16.109	0.15
36.	1,5,9-Cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methylethenyl)-	16.218	2.50
37.	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	16.384	0.58
38.	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	16.663	1.21
39.	(-)-Tricyclo[6.2.1.0(4,11)]undec-5-ene, 1,5,9,9-tetramethyl- (isocaryophyllene-I1)	16.794	3.04
40.	Thunbergol	17.104	1.48
41.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	17.465	0.42
42.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	17.966	4.06
43.	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	18.118	20.62
44.	2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)-	18.297	20.50
45.	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	18.660	1.32
46.	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	18.888	0.85
47.	22,23-Dibromostigmasterol acetate	19.041	0.37
48.	(-)-Globulol	19.103	1.06
49.	Widdrol hydroxyether	19.173	2.05
50.	Acetic acid, 1-methyl-3-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl ester	19.235	5.69
51.	Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester	19.381	1.50
52.	Nerolidol isobutyrate	19.680	5.50
53.	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	19.819	2.50
54.	Hexadecanoic acid, tetradecyl ester	21.262	0.47
55.	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-, (-)-	21.381	1.65
56.	(-)-Isolongifolol, acetate	21.551	0.75
57.	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	21.791	0.70
58.	.alpha.-Santalol	22.343	0.11
59.	Oleoyl chloride	22.581	0.21
60.	.beta.-Amyrin	24.964	2.62
61.	.alpha.-Amyrin	25.361	5.52

Fig.3.6 illustrates the mass spectrum of (1S,2E,4S,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol. The molecular ion $[C_{20}H_{34}O_2]^+$

appeared at m/z306(RT.18.118). The mass spectrum of 2,6,10-dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)- is presented in Fig.3.7. The peak at m/z278(RT.18.297) corresponds the molecular ion $[C_{17}H_{26}O_3]^+$. The mass spectrum of acetic acid, octyl ester is shown Fig. 3.8. The signal at m/z173(RT.7.110) is due to the molecular ion $[C_{10}H_{20}O_2]^+$.

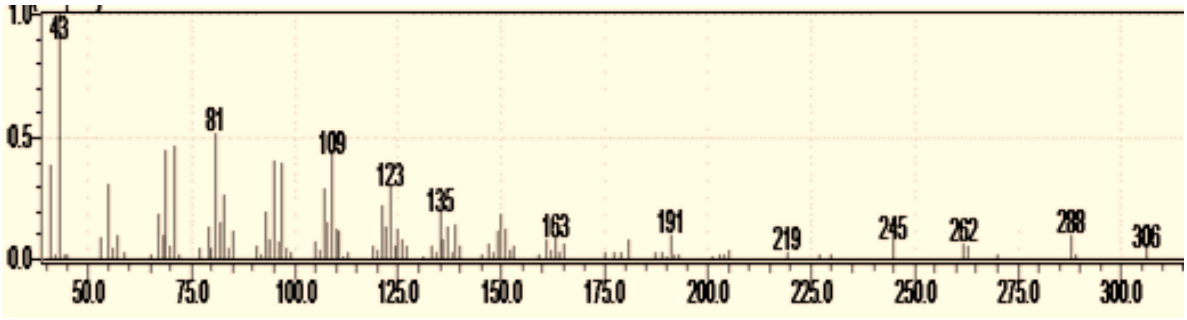


Fig. 3.6: Mass spectrum of (1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol

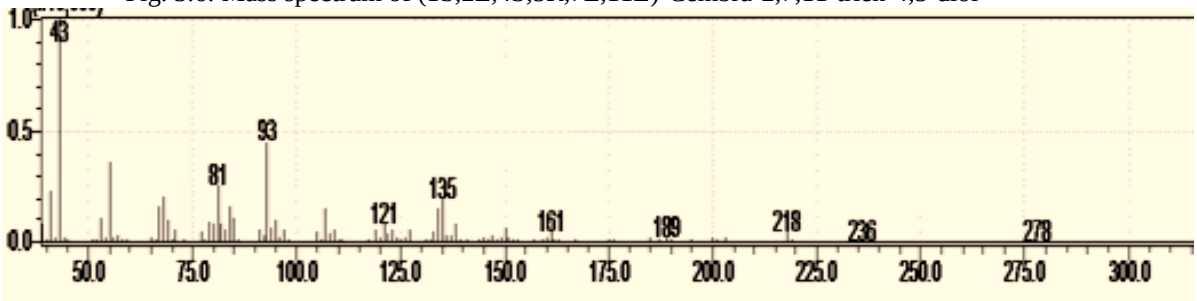


Fig. 3.7: Mass spectrum of 2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl-, (E,E,E)-

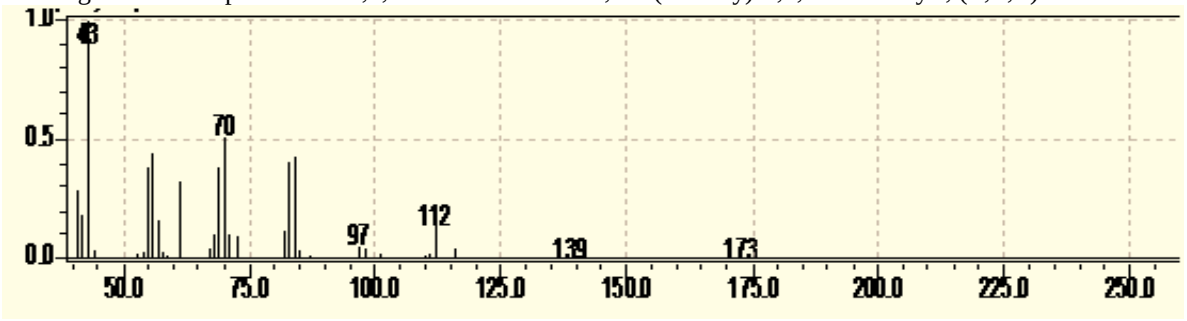


Fig. 3.8: Mass spectrum of acetic acid, octyl ester

3.2.2 Antimicrobial activity of *Commiera africana* oil

Commiera africana 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 (3.4).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 showed moderate activity against *Staphylococcus aureus*.

Table 3.4 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	14	--	12	10	10
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 *Trachyspermum ammi*

Oil of *Trachyspermum ammi* was extracted with hexane. The oil was investigated by GC-MS and the constituents of the oil have been characterized. The oil was also evaluated for its antimicrobial potential using the cup plate agar diffusion bioassay.

3.3.1 GC- MS analysis of *Trachyspermum ammi* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of *Trachyspermum ammi* oil. The analysis revealed the presence of 11 components - Table (3.5).The total ion chromatogram is presented in Fig.3.9.

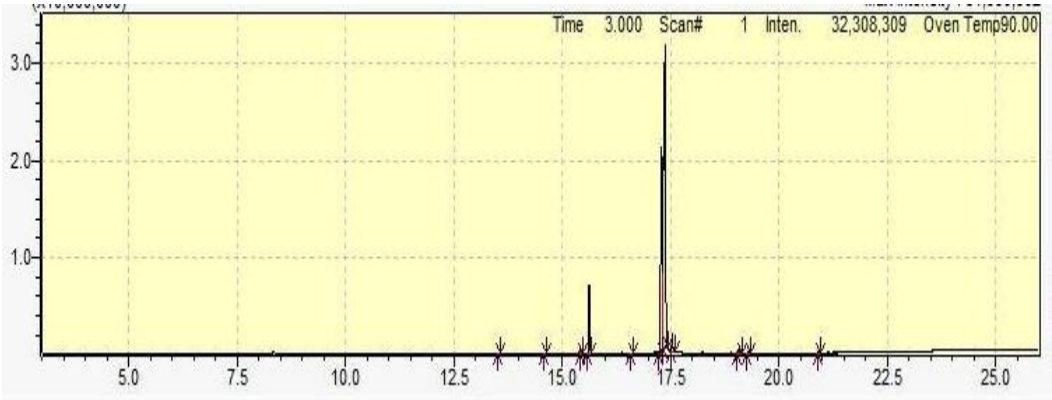


Fig. 3.9: Total ion chromatograms

Table 3.5: Constituents of *Trachyspermum ammi* oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.531	0.03
2.	Pentadecanoic acid, methyl ester	14.602	0.05
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.431	0.35
4.	Hexadecanoic acid, methyl ester	15.627	6.53
5.	Heptadecanoic acid, methyl ester	16.605	0.04
6.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.298	29.57
7.	9-Octadecenoic acid (Z)-, methyl ester	17.378	61.11
8.	Methyl stearate	17.542	1.75
9.	6-Octadecenoic acid, methyl ester, (Z)-	19.074	0.39
10	Eicosanoic acid, methyl ester	19.297	0.14
11	Docosanoic acid, methyl ester	20.920	0.04

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

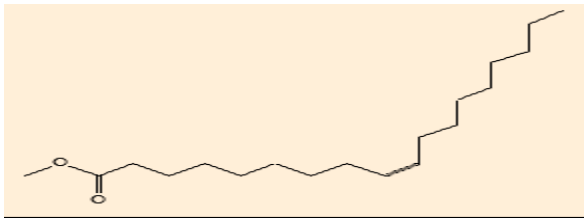
i) 9-Octadecenoic acid (Z)-, methyl ester(61.11%)

ii) 9 12-Octadecenoic acid methyl ester (29.57%)

ii) Hexdecanoic acid (6.53 %)

Fig. 3.10 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.The signal at m/z296(RT.17.378) corresponds the molecular ion $[C_{19}H_{36}O_2]^+$. The mass spectrum of 9,12-octadecadienoic acid methyl ester is illustrated in Fig.3.11. The peak at m/z 294(RT. 17.298)corresponds M^+

$[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.12.The peak at m/z 270 which appeared at (RT.15.627) is due to M^+ $[C_{17}H_{32}O_2]^+$.



9-Octadecenoic acid methyl ester

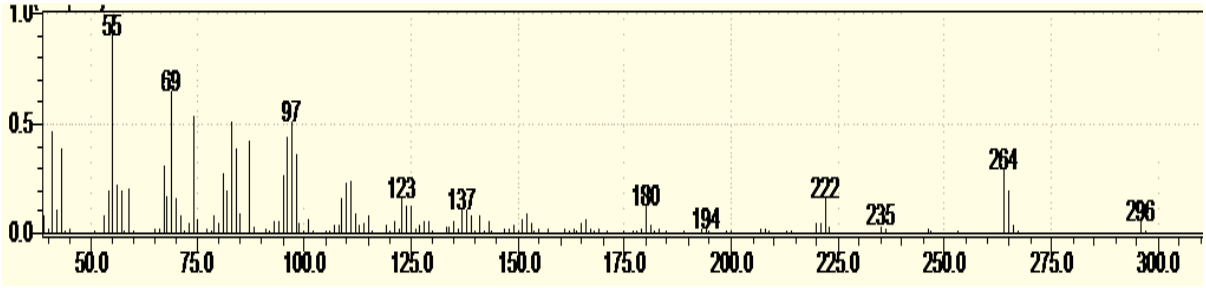
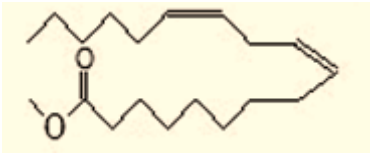


Fig. 3.10: Mass spectrum of 9-ctadecenoic acid methyl ester



9,12-Octadecadienoic acid methyl ester

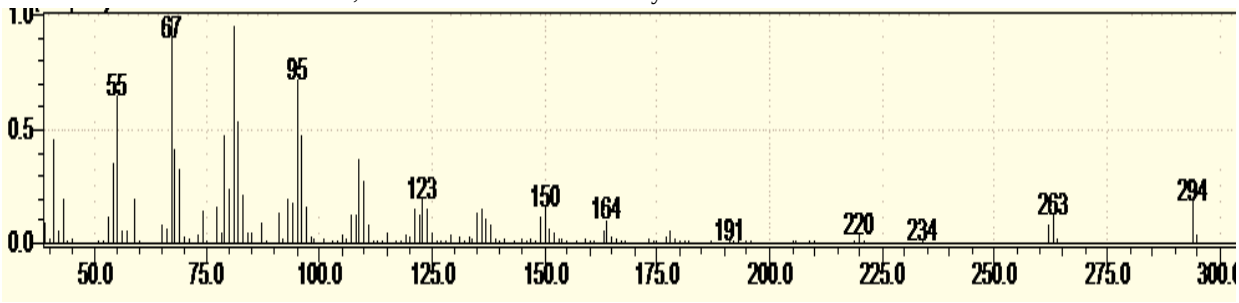
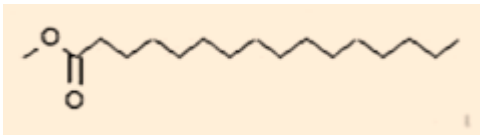


Fig.3.11: Mass spectrum of 9,12-octadecadienoic acid



Hexadecanoic acid methyl ester

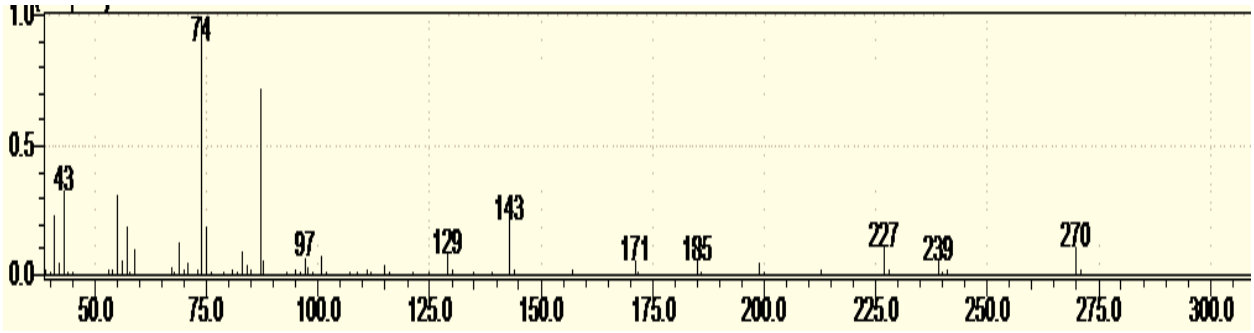


Fig. 3.12: Mass spectrum of
hexadecanoic acid methyl ester

3.3.2 Antimicrobial activity of *Trachyspermum ammi* oil

Trachyspermum ammi 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 showed significant activity against *Staphylococcus aureus* and *Candida albicans*.

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

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	22	--	--	11	24
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- *Vangueria madagascarinsis*

Oil of *Vangueria madagascarinsis* was extracted with hexane. The oil was investigated by GC-MS and the constituents of the oil have been characterized. The oil was also evaluated for its antimicrobial potential using the cup plate agar diffusion bioassay.

3.4.1 GC- MS analysis of *Vangueria madagascarinsis* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of *Vangueria madagascarinsis* oil. The analysis revealed the presence of 19 components - Table (3.7).The total ion chromatogram is presented in Fig.3.13.

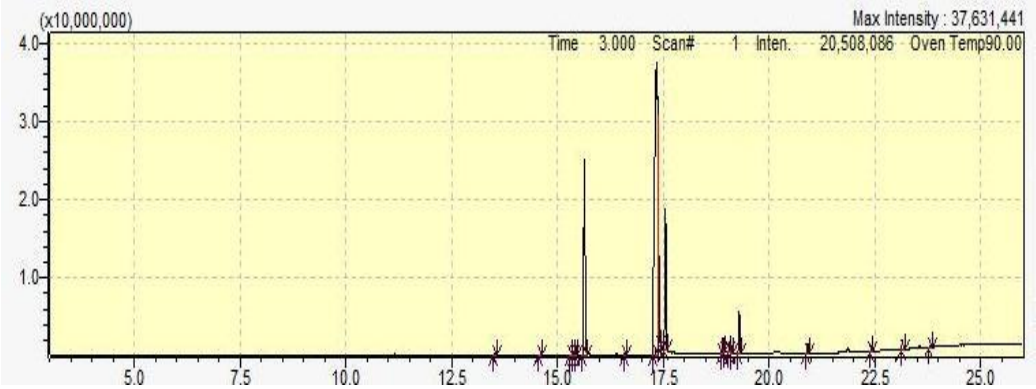


Fig. 3.13: Total ion chromatograms

Table 3.7: Constituents of *Vangueria madagascarinsis* oil

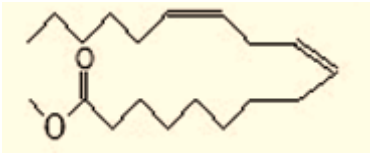
No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.531	0.11
2.	Pentadecanoic acid, methyl ester	14.603	0.05
3.	7,10-Hexadecadienoic acid, methyl ester	15.333	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	15.394	0.08
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.435	0.40
6.	Hexadecanoic acid, methyl ester	15.644	15.43
7.	Heptadecanoic acid, methyl ester	16.607	0.18
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.355	53.68
9.	9-Octadecenoic acid (Z)-, methyl ester	17.383	12.89

10.	Methyl stearate	17.556	10.23
11.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	18.900	0.46
12.	9,12-Octadecadienoyl chloride, (Z,Z)-	18.937	0.73
13.	cis-11,14-Eicosadienoic acid, methyl ester	19.066	0.45
14.	cis-11-Eicosenoic acid, methyl ester	19.099	0.71
15.	Eicosanoic acid, methyl ester	19.298	2.72
16.	Docosanoic acid, methyl ester	20.919	0.60
17.	Tetracosanoic acid, methyl ester	22.422	0.43
18.	Squalene	23.158	0.70
19.	Hexacosanoic acid, methyl ester	23.823	0.12

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

i) 9 12-Octadecenoic acid methyl ester (53.68%)
ii) ii) Hexdecanoic acid (15.43 %)
iii) 9-Octadecenoic acid (Z)-, methyl ester(12.89%)
iv) Methyl stearate(10.23%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is illustrated in Fig.3.14. The peak at m/z 294(RT. 17.355)corresponds M^+ $[C_{19}H_{34}O_2]^+$ · The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.15.The peak at m/z 270 which appeared at (RT.15.644) is due to M^+ $[C_{17}H_{32}O_2]^+$. Fig. 3.16 presents the mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.The signal at m/z296(RT.17.383) corresponds the molecular ion $[C_{19}H_{36}O_2]^+$.The mass spectrum of methyl stearate is shown in Fig.3.17. The signal at m/z298(RT>17.556) due to the molecular ion $[C_{19}H_{38}O_2]^+$.



9,12-Octadecadienoic acid methyl ester

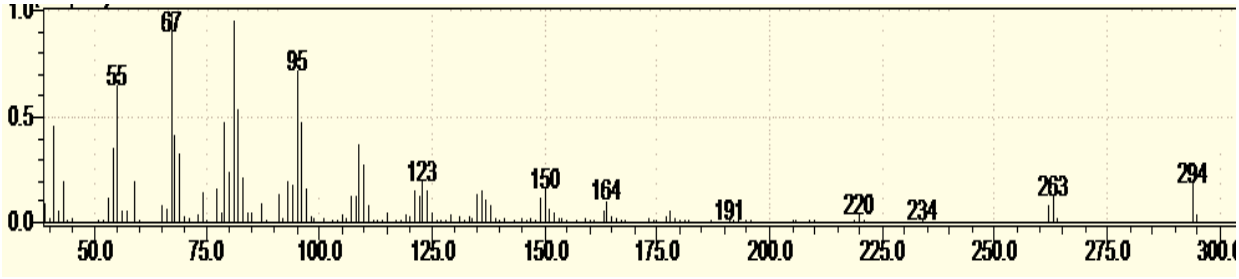
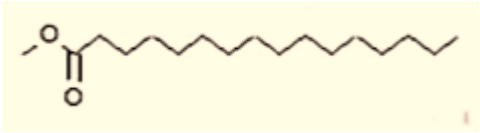


Fig.3.14: Mass spectrum of 9,12-octadecadienoic acid



Hexadecanoic acid methyl ester

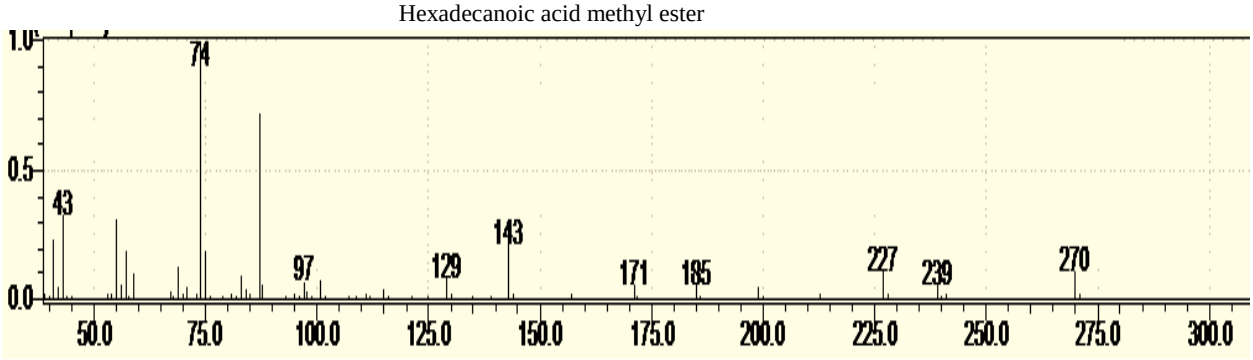
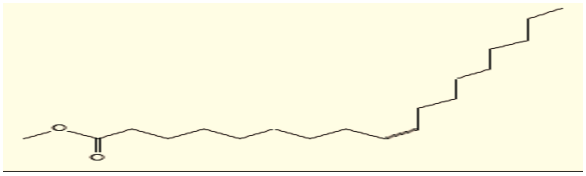


Fig. 3.15: Mass spectrum of hexadecanoic acid methyl ester



9-Octadecenoic acid methyl ester

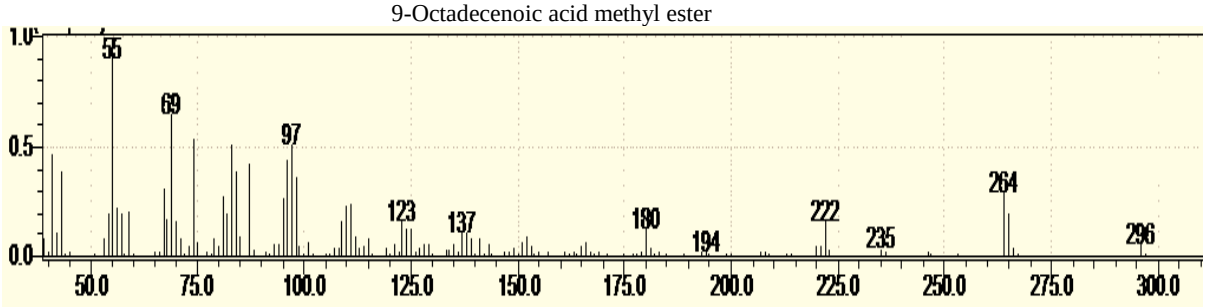
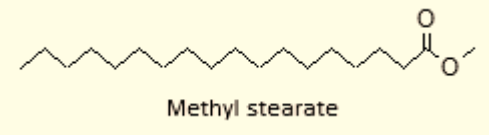


Fig. 3.16: Mass spectrum of 9-ctadecenoic acid methyl ester



Methyl stearate

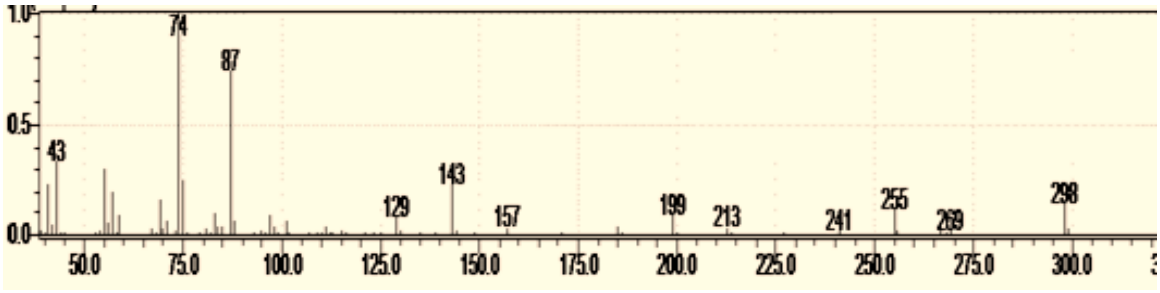


Fig. 3.17: Mass spectrum of methyl stearate

3.4.2 Antimicrobial activity of *Vangueria madagascarinsis* oil

Vangueria madagascarinsis 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 showed activity sagnificant against *Pseudomonas aeruginosa* and moderate activity against other test organism except *Bacillus subtilis*.

Table : 3.8 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	14	--	15	19	14
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 *Vigna subterrenea*

Oil of *Vigna subterrenea* was extracted with hexane. The oil was investigated by GC-MS and the constituents of the oil have been characterized. The oil was also evaluated for its antimicrobial potential using the cup plate agar diffusion bioassay.

3.5.1 GC- MS analysis of *Vigna subterrenea* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of *Vigna subterrenea* oil. The analysis revealed the presence of 30 components - Table (3.9).The total ion chromatogram is presented in Fig.3.18.

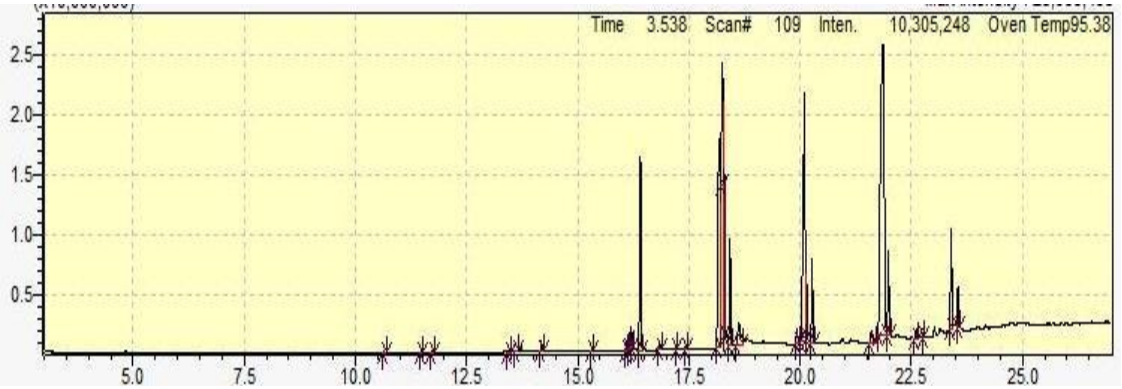


Fig. 3.18: Total ion chromatograms

Table 3.9: Constituents of *Vigna subterrenea* oil

No.	Name	Ret.Time	Area%
1.	Nonanoic acid, 9-oxo-, methyl ester	10.632	0.05
2.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.489	0.03
3.	Dodecanoic acid, methyl ester	11.735	0.05
4.	9-Octadecene, (E)-	13.439	0.02
5.	8-Heptadecene	13.617	0.05
6.	Methyl tetradecanoate	14.182	0.28

7.	Pentadecanoic acid, methyl ester	15.317	0.07
8.	7,10-Hexadecadienoic acid, methyl ester	16.092	0.12
9.	7,10,13-Hexadecatrienoic acid, methyl ester	16.165	0.27
10.	9-Hexadecenoic acid, methyl ester, (Z)-	16.200	0.60
11.	Hexadecanoic acid, methyl ester	16.410	7.62
12.	n-Hexadecanoic acid	16.841	0.19
13.	cis-10-Heptadecenoic acid, methyl ester	17.217	0.09
14.	Heptadecanoic acid, methyl ester	17.430	0.11
15.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.180	12.90
16.	9-Octadecenoic acid (Z)-, methyl ester	18.253	5.49
17.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.286	5.99
18.	Methyl stearate	18.425	3.51
19.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	18.628	1.97
20.	.gamma.-Linolenic acid, methyl ester	19.929	0.48
21.	cis-13-Eicosenoic acid, methyl ester	20.096	13.78
22.	cis-11-Eicosenoic acid, methyl ester	20.135	2.67
23.	Eicosanoic acid, methyl ester	20.270	2.82
24.	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	21.605	0.72
25.	13-Docosenoic acid, methyl ester, (Z)-	21.881	31.47
26.	Docosanoic acid, methyl ester	21.984	2.96
27.	cis-10-Nonadecenoic acid, methyl ester	22.607	0.36
28.	Tricosanoic acid, methyl ester	22.774	0.15
29.	15-Tetracosenoic acid, methyl ester, (Z)-	23.399	3.92
30.	Tetracosanoic acid, methyl ester	23.548	1.26

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

i) 13-Docosenoic acid methyl ester(31.47%)
ii) Cis-13-Eicosenoic acid methyl ester (13.78 %)
iii) 9,12-Octadecenoic acid (Z)-, methyl ester(12.90%)
iv) Hexadecanoic acid methyl ester(7.62%)

The mass spectrum of 13-deocosenoic acid methyl ester is illustrated in Fig.3.19. The peak at m/z 352(RT. 21.881)corresponds M^+ $[C_{23}H_{44}O_2]^+$ Fig.3.20 shows the mass spectrum of cis-13-ecosenoic acid methyl ester. The signal at

m/z324(RT.20.096) corresponds the molecular ion $[C_{21}H_{40}O_2]^+$. The mass spectrum of 9,12-octadecadienoic acid methyl ester is illustrated in Fig.3.21. The peak at m/z 294(RT. 18.180)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.22.The peak at m/z 270 which appeared at (RT.16.410) is due to M^+ $[C_{17}H_{32}O_2]^+$.

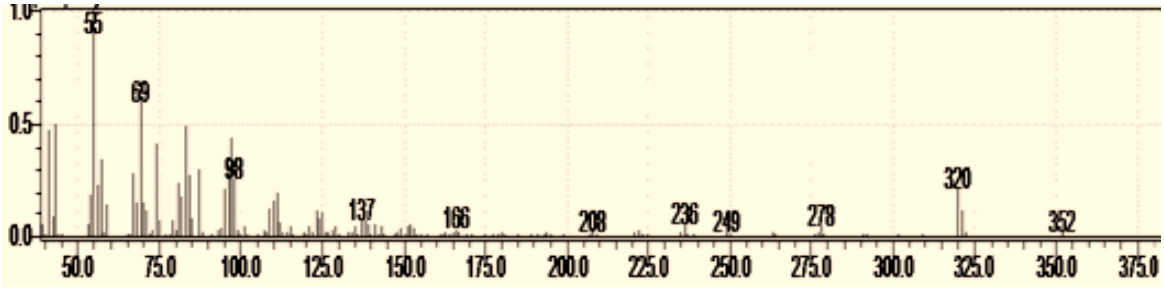


Fig. 3.19: mass spectrum of 13-docosenoic acid methyl ester

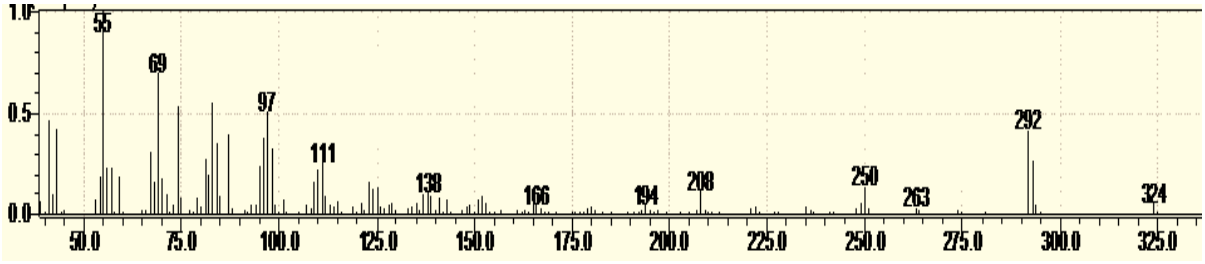
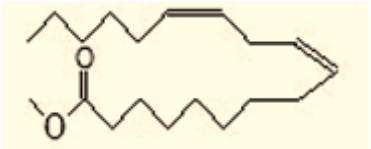


Fig. 3.20: Mass spectrum of cis-13-eicosanoic acid methyl ester



9,12-Octadecadienoic acid methyl ester

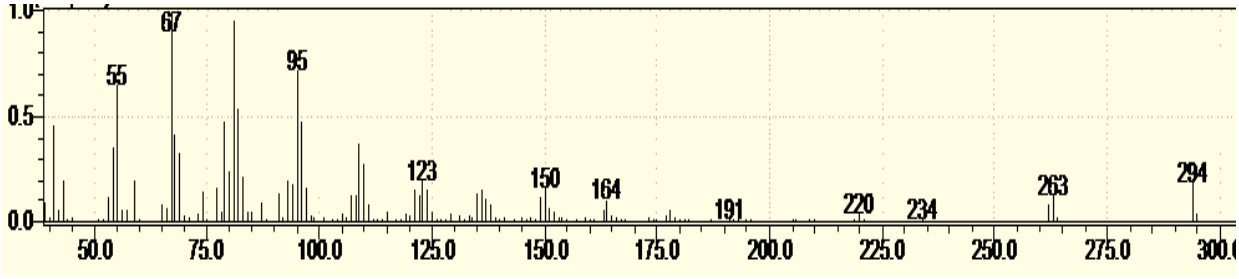
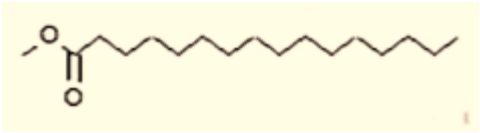


Fig.3.21: Mass spectrum of 9,12-octadecadienoic acid



Hexadecanoic acid methyl ester

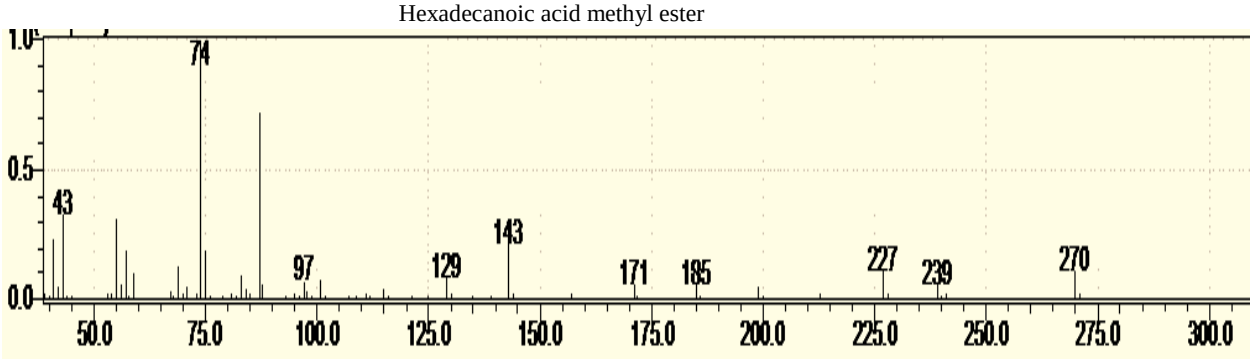


Fig. 3.22: Mass spectrum of
hexadecanoic acid methyl ester

3.5.2 Antimicrobial activity *Vigna subterrenea* oil

Vigna subterrenea 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 significant activity against *Pseudomonas aeruginosa* beside moderate activity against other test organisms except *Bacillus sabtilis.*

Table 3.10 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	14	--	15	19	14
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*

Conclusion

The oils from five medicinal plants(*Xemania aegyptiaca, Commiera Africana, Trachyspermum ammi, Vangueria madagascarinsis* and *Vigna subterrenea*) have been analyzed by GC-MS. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the GC-MS library. The oils were also screened for antimicrobial potential via the cup plate agar diffusion bioassay.

Recommendations

The following is highly recommended:

-The biologically active constituents of the oil may be isolated by HPLC and evaluated for their biological potential.

-The secondary metabolites of the studied plants may be isolated , characterized by spectral tools and evaluated for their biological activity.

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