Sudan University of Science and Technology

College of Postgraduate Studies

Investigation of Constituents and Biological Activity of Oils From Some Medicinal Plants Grown in Sudan

دراسة المكونات والفعالية البيولوجية لزيوت بعض النباتات الطبية المزروعة في السودان

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

By

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March,2022

الاســـــتهـــــلال

قال تعالي :

(وَإِذْ قُلْتُمْ يَا مُوسَىٰ لَنْ نَصْبِرَ عَلَىٰ طَعَامٍ وَاحِدٍ فَادْعُ لَنَا رَبَّكَ يُخْرِجْ لَنَا مِمَّا تُنْبِتُ الْأَرْضُ مِنْ بَقْلِهَا وَقِثَّائِهَا وَفُومِهَا وَعَدَسِهَا وَبَصَلِهَا ۖ قَالَ أَتَسْتَبْدِلُونَ الَّذِي هُوَ أَدْنَىٰ بِالَّذِي هُوَ خَيْرٌ ۚ اهْبِطُوا مِصْرًا فَإِنَّ لَكُمْ مَا سَأَلْتُمْ ۗ وَضُرِبَتْ عَلَيْهِمُ الذِّلَّةُ وَالْمَسْكَنَةُ وَبَاءُوا بِغَضَبٍ مِنَ اللَّهِ ۗ ذَٰلِكَ بِأَنَّهُمْ كَانُوا يَكْفُرُونَ بِآيَاتِ اللَّهِ وَيَقْتُلُونَ النَّبِيِّينَ بِغَيْرِ الْحَقِّ ۗ ذَٰلِكَ بِمَا عَصَوْا وَكَانُوا يَعْتَدُونَ)

سورة البقرة الآية: (61)

صدق الله العظيم

Dedication

To,

My parents,

husband, children

and brothers.

Acknowledgement

I would like to thank **Almighty Allah** for giving me strength to complete this work.

I would like to express my gratitude to my supervisor Prof. Mohamed Abdel Karim for his careful supervision, valuable advice, kind treatment and effort during this work.

I would like to express my gratitude to all those who helped me during this work, also I would like to express my heartfull gratitude to my family. Thanks for the Staff and Technician of the Chemistry Dept. – Sudan University of Science and Technology for all facilities.Also thanks are extended to University of Medical Sciences and Technology, for GC-MS measurements.

Thanks to The Medicinal and Aromatic Plants Research Institute for all facilities.

Abstract

In this study the oils from five plants of medicinal attributes **(***Croton cordofana, Cissus quadrangularis, Syzygium aromaticum* ,*Croton zambesicus* and *Hibiscus sabdariffa*) have been investigated by GC-MS and the antimicrobial activity has been screened. Gas chromatography - mass spectrometry has been used for the identification and quantification of the constituents of *Syzgium aromaticum* oil. The analysis revealed the presence of 13 components dominated by: eugenol (60.35%). GC-MS analysis of *Croton zambesicus* oil revealed the presence of 22 components dominated by :9-octadecenoic acid methyl ester(12.98%). *Hibiscus sabdarifa* oil gave 24 constituents. Major component being :9-octadecenoic acid (Z)-, methyl ester (30.23%). *Cissus quadrangularis* oil was studied by GC-MS. The analysis showed the presence of 37 constituents dominated by:E,E,Z-1,3,12-nonadecatriene-5,14-diol(22.11%). *Croton cordofana* oil revealed the presence of 19 components. Major component is: 9 , 12-octadecadienoic acid methyl ester (44.32%).The target oils have been assessed for their antimicrobial potency and different antimicrobial responses has been observed.

المستخلص

فى هذا البحث تمت دراسة خمسة نباتات طبيه لها استخدامات عديده فى الطب الشعبى بالسودان , هذه النباتات هى : الكركدى , السلعلع , ام قليقله ,القرنفل والدنقل. تم تحديد مكونات زيوت هذه النباتات بتقنيه الكروموتوغرافيا الغازيه – طيف الكتله كما وتم تحديد نشاط مضاد الميكروبات لهذه الزيوت باستخدام تقنيه انتشار الاقار .اعطى تحليل الكروموتوغرافيا الغازيه – طيف الكتله لزيت نبات القرنفل 13 مكونا اهمها : اوقينول (60.35 %) . اما زيت ام قليقله فقد اثبت تحليله وجود 22 مكونا اهمها : حمض 9 ,12- اوكتاديكادايونويك(31.21%).اثبت تحليل الكروموتوغرافيا الغازيه – طيف الكتله لزيت نبات الكركدى وجود 24 مكونا اهمها:حمض 9- اوكتاديكينويك(30.23%).اما زيت نبات السلعلع فقد احتوى على 37 مكونا تصدرها :E,E,Z -1,3,12- نونـاديكاترايين بنسبة (22.11%).احتوى زيت نبات الدنقل على 19 مكونا اهمها : حمض 9 ,12- اوكتاديكادايونويك(44.32%) .اجرى اختبار مضاد الميكروبات لهذه الزيوت حيث اعطت فعاليه متفاوته.

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CHAPTER ONE

Introduction

1. Introduction

1.1-Oils,fats and waxes

An oil is any nonpolar chemical substance that is a viscous liquid atambient temperatures and is both hydrophobic (immiscible with water, literally"water fearing")and lipophilic (miscible with other oils, literally "fat loving"). Oils have a highcarbon and hydrogen content and are usually flammable and surface active.

The general definition of oil includes classes of chemical compoundsthat may be otherwise unrelated in structure, properties, and uses. Oils may be animal, vegetable, or petrochemical in origin, and may be volatile or non-volatile. They are used for food (e.g.,olive oil), fuel (e.g.,heating oil), medical purposes (¢.g., mineral oil), lubrication (e.g. motoroil), and the manufacture of many types of paints, plastics, and othermaterials. Specially prepared oils are used in some religious ceremoniesand rituals as purifying agents.

Organic oils are produced in remarkable diversity by plants, animals, and other organisms through natural metabolic processes. Lipid is the scientific term for the fatty acids, steroids and similar chemicals often found in the oils produced by living things, while oil refers to an overall mixture of chemicals. Organic oils may also contain chemicals otherthan lipids, including proteins, waxes (class of compounds with oil-like properties that are solid at common temperatures) and alkaloids.

Lipids can be classified by the way that they are made by an organism, their chemical structure and their limited solubility in water compared tooils. They have a high carbon and hydrogen content and areconsiderably lacking in oxygen compared to other organic compounds and minerals; they tend to be relatively nonpolar molecules, but mayinclude both polar and nonpolar regions as in the case of phospholipids and steroids(1)

Several edible vegetable and animal oils, and also fats, are used forvarious purposes in cooking and food preparation. In particular, many foods are fried in oil much hotter than boiling water. Oils are also used for flavoring and for modifying the texture of foods .

Cooking oils are derived either from animal fat, as butter, lard and other types, or plant oils from the olive, maize, sunflower and many other species (1)

Butter is a water-in-oil emulsion resulting from an inversion of thecream; in a water-in-oil emulsion, the milk proteins are the emulsifiers. Butter remains a solid when refrigerated, but softens to a spreadable consistency at room temperature, and melts to a thin liquid consistency at 32-35 °C (90-95 °F). The density of butter is 911 g/L. (0.950 Ib perUS pint). It generally has a pale yellow color, but varies from deep yellow to nearly white. Its unmodified color is dependent on the animals' feed and genetics but is commonly manipulated with

food colorings in the commercial manufacturing process, most commonly carotene [2]

Fat is one of the three main macro-nutrients, along with carbohydrate andprotein. Fats, also known as triglycerides, are esters of three fatty acid chains and the alcohol glycerol. The terms "lipid", "oil" and "fat" are often confused. "Lipid" is the general term, though a lipid is not necessarily a triglyceride. "Oil" normally refers to a lipid with short or unsaturated fatty acid chains that is liquid at room temperature, while "fat" (in the strict sense) may specifically refer to lipids that are solids atroom temperature - however, “fat" (in the broad sense) may be used in food science as a synonym for lipid. Fats, like other lipids, are generally hydrophobic, and are soluble in organic solvents and insoluble in water.

Fat is an important foodstuff for many forms of life and fats serve both structural and metabolic functions. They are a necessary part of the diet of most heterotrophs (including humans). Some fatty acids that are set free by the digestion of fats are called essential because they cannot be synthesized in the body from simpler constituents. There are two essential fatty acids (EFAs) in human nutrition: alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid (an omega-6 fatty acid). Otherlipids needed by the body can be synthesized from these and other fats. Fats and other lipids are broken down in the body by enzymes called lipases produced in the pancreas.

Fats and oils are categorized according to the number and bonding of the carbon atoms in the aliphatic chain. Fats that are saturated fats have

nodouble bonds between the carbons in the chain. Unsaturated fats have one or more double bonded carbons in the chain. The nomenclature isbased on the non-acid (non-carbonyl) end of the chain. This end is calledthe omega end or the n-end. Thus alpha-linolenic acid is called anomega-3 fatty acid because the 3rd carbon from that end is the first double bonded carbon in the chain counting from that end. Some oilsand fats have multiple double bonds and are therefore called polyunsaturated fats. Unsaturated fats can be further divided into cis-fats, which are the most common in nature, and trans- fats, which are rarein nature. Unsaturated fats can be altered by reaction with hydrogen affected by a catalyst. This action, called hydrogenation, tends to break all the double bonds and makes a fully saturated fat. Liquid cis-unsaturated fats such as vegetable oils are hydrogenated to produce saturated fats, which have more desirable physical properties e.g., they melt at a desirable temperature (30-40°C),and store well, whereas polyunsaturated oils. go rancid when they react with oxygen in the air. However, trans fats are generated during hydrogenation as contaminants created by an unwanted side reaction on the catalyst during partial hydrogenation [(4).]

Waxes are a diverse class of organic compounds that are lipophilic,malleable solids near ambient temperatures. They include higher alkanes and lipids, typically with melting points above about 40°C (104 °F),melting to give low viscosity liquids. Waxes are insoluble in water but soluble in organic, nonpolar solvents. Natural

waxes of different types are produced by plants and animals and occur in petroleum.

Waxes are organic compounds that characteristically consist of long alkyl chains. They may also include various functional groups such asfatty acids, primary and secondary long chain alcohols, unsaturated bonds, aromatics, amides, ketones, and aldehydes. They frequently contain fatty acid esters as well. Synthetic waxes are often long-chain hydrocarbons (alkanes or paraffin) that lack functional groups.

Waxes are synthesized by many plants and animals. Those of animal origin typically consist of wax esters derived from a variety of carboxylic acids and fatty alcohols. In waxes of plant origin, characteristic mixtures of unesterified hydrocarbons may predominate over esters. The composition depends not only on species, but also on geographic location of the organism [5],

1.2-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.

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.

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.

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.

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.

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.

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.

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[(6),]

1.3-Essential oils

Many essential oils included in herbal pharmacopoeias are claimed to possess antimicrobial activity, with the oils of bay, cinnamon, clove and thyme reported to be the most potent in studies with food-borne bacterial pathogens. Active constituents include terpenoid chemicals and other secondary metabolites. Despite their prevalent use in alternativemedicine, essential oils have seen limited use in mainstream medicine.

While 25 to 50% of pharmaceutical compounds are plant-derived, none are used as antimicrobials, though there has been increased

research inthis direction. Barriers to increased usage in mainstream medicineinclude poor regulatory oversight and quality control, mislabeled ormisidentified products, and limited modes of delivery[7],

An essential oil is a concentrated hydrophobic liquid containing volatile (defined as "the tendency of a substance to vaporize") aroma compoundsfrom plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant fromwhichthey wereextracted, such as oil of clove.

An oil is "essential" in the sense that itcontains the "essence of" the plant's fragrance- the characteristic fragrance of the plant from which it is derived. The term essential usedhere does not mean indispensable as with the terms essential amino acidor essential fatty acid which are so called since they are nutritionally required by a given living organism.

Essential oils are generally extracted by distillation, often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, and cold pressing. They are used inperfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products.

Essential oils have revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that uses essential oils and other aromatic compounds. Oils are volatilized, diluted in carrier oil and used in massage, diffused in the air by a nebulizer, heated over a candle flame, or burned as incense.

Essential oils are usually lipophilic (“*oil-loving*") compounds. That usually are not miscible with water. They can be diluted in solvents like pure ethanol and polyethylene glycol. The most common way to safely dilute essential oils for topical use is in; carrier oil. This can be any vegetable oil readily available, the most popular for skin care beingjojoba, coconut, wheat germ, olive and avocado [8].

1.4-Gas chromatography-mass spectrometry

Gas chromatography—mass spectrometry (GC-MS) is an effective analytical tool that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples.

GC-MS can alsobe used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. GC-MS , like liquid chromatography-mass spectrometry, allows analysis and detection even of tiny amounts of a substance.

The GC-MS is composed of two major parts : the gas chromatograph and the mass spectrometer. The gas chromatographutilizes a capillary column which depends on the column's dimensions(length, diameter, film thickness) as well as the phase properties (e.g.5% phenyl polysiloxane). The difference in the chemical properties between

different molecules in a mixture-and their relative affinity forthe stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, anddetect the ionized molecules separately. The mass spectrometer does thisby breaking each molecule into ionized fragments and detecting thesefragments using their mass-to-charge ratio.

These two techniques, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometryprocess normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Ffame ionization detector) can not differentiate between multiple molecules that happen to take the same amount of time to travel through the column (ie. have the same retention time), which results in two or more molecules that co-elute.Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will be have in the same way inboth a gas chromatograph and a mass spectrometer.

Therefore, when an identifying mass spectrum appears at a characteristic retention time in aGC-MS analysis, it typically increases certainty that the analyte of interest is in the sample.

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sectormass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight(TOF) and tandem quadrupoles (MS-MS).

After the molecules travel the length of the column they pass through the transfer line and enter into the mass spectrometer where they are ionizedby various methods with typically only one method being used at anygiven time. Once the sample is fragmented it will then be detected,usually by an electron multiplier diode, which essentially turns theionized mass fragment into an electrical signal which is then detected.

A mass spectrometer is typically utilized in one of two ways: full scan or selective ion monitoring (SIM). The typical GC-MS instrument iscapable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

The primary goal of GC-MS is to identify and quantify an amount of substance. This is done by comparing the relative concentrations

among the atomic masses in the generated spectrum. Two kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately related data.

Another method of analysis measures the peaks in relation to oneanother. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All valuesabove 3% are assigned. The total mass of the unknown compound isnormally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elementswhich are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many natural isotopes,can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically; this identification is done automatically by programs which come with the instrument, given a listof the elements which could be present in the sample. A “full spectrum” analysis considers all the “peaks” within a spectrum.

Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption thatat a given retention time, a set of ions is characteristic of a certain compound, This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity ofthe analysis increases. So, SIM analysis allows for a smaller quantity ofa compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass.

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies.

GC-MS is the main tool used in sports anti-doping laboratories to test thletes' urine samples for prohibited performance-enhancing drugs, forexample anabolic steroids [8].

1-5-Solvent Extraction

Solvent extraction, also known as Liquid—liquid extraction orpartitioning, is a method to separate a compound based on the

solubility of its parts. This is done by using two liquids that don't mix, for example water and an organic solvent.

Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. It is also used to recover plutonium from irradiated nuclear fuel, a process which is usually called nuclear reprocessing. The recovered plutonium can then be re-used as nuclear fuel.

In this process one of the components of a mixture dissolves in a particular liquid and the other component is separated as a residue by filtration. Solvent extraction involves crushing of oil seeds and oil seed cakes.From ancient time, vegetable oils were obtained by crushing oil seeds .

Around this time many European countries and United States of America had established huge solvent extraction plants for recovering directly almost all the available oil in the oil seeds like cotton seed and soybean[9]

There is a net transfer of one or more species from one liquid into another liquid phase, generally from aqueous to organic. The transfer is driven by chemical potential, i.e. once the transfer is complete, the overall system of protons and electrons that make up the solutes and the solvents are in a more stable configuration (lower free energy). The solvent that is enriched in solute(s) is called extract. The feed solution that is depleted in solute(s) is called the raffinate. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to

countercurrent distribution equipment called as mixer settlers .This type of process is commonly performed after a chemical reaction as part of the work-up, oftenincluding an acidic work-up [10].

The term partitioning is commonly used to refer to the underlying chemical and physical processes involved in liquid-liquid extraction, but on another reading may be fully synonymous with it. The term solvent extraction can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix [11],

Solvent extraction is exclusively used in separation and purification ofuranium and plutonium, zirconium and hafnium, separation of cobaltand nickel, separation and purification of rare earth elements etc., its greatest advantage being its ability to selectively separate out even very similar metals. One obtains high-purity single metal streams on‘stripping’ out the metal value from the ‘loaded’ organic wherein one can precipitate or deposit the metal value. Stripping is the opposite ofextraction: Transfer of mass from organic to aqueous phase [12]

In solvent extraction, a distribution ratio is often quoted as a measure of how well-extracted a species is. The distribution ratio is equal to the concentration of a solute in the organic phase divided by its concentration in the aqueous phase. Depending on the system, the distribution ratio can be a function of temperature, the concentration of chemical species in the system, and a large number of other parameters.

Sometimes, the distribution ratio is referred to as the partition coefficient, which is often expressed as the logarithm, two immiscible liquids are shaken together. ‘The more polar solutes dissolve preferentially in the more polar solvent, and the less polar solutes in the less polar solvent.Although the distribution ratio and partition coefficient are often used synonymously, they are not necessarily so. Solutes may exist in more than one form in any particular phase, which would mean that the partition coefficient (Kd) and distribution ratio (D) will have different values. This is an important distinction to make as whilst the partition coefficient has a fixed value for the partitioning of a solute between two phases, the distribution ratio changes with differing conditions in thesolvent.

After performing liquid—liquid extraction, a quantitative measure must be taken to determine the ratio of the solution’s total concentration ineach phase of the extraction. This quantitative measure is known as the distribution ratio or distribution coefficient [13].

1.6 – The target species

1.6.1.*Hibiscus Sabdariffa*

*Hibiscus Sabdariffa*seeds

-Taxanomy:

Order :Mavales

Family :Mavaceae

Subfamily. Malvoideae

Genus :*Hibiscus*

Species: H- *Sabdariffa*

Common Name : Roselle

Native Range : Asia (India – Malaysia) and tropical Africa.

Hibiscus sabdariffa . (Malvaceae) is otherwise known as Roselle or red Sorrel (English). It is anative to Asia (India), Malaysia, and tropical Africa including Sudan where it is indiscriminately consumed by the

populace as beverages because of its aphrodisiac and other pharmacological properties [14].

Hibiscus sabdariffa (Roselle) has been used traditionally as a food, in herbal drinks, in hot and cold beverages, as flavoring agent in the food industry and as herbal medicine[14],

The seeds contain crude fatty oil (21.85%), crude protein (27.78%), carbohydrate (21.25%), crude fiber (16.44%), and ash (6.2%). Roselle seeds are a good source of lipid – soluble antioxidants, particularly gamma tocopherol[14].

Sterols of *Hibiscus sabdariffa* include beta – sitosterol (71.9%), compesterol (13.6%), delta-5- avenasterol(5.9%), cholesterol (1.35%), and clerosterol (0.6%). Total tocopherols were detected at an average concentration of 2000mg/kg, including alpha – tocoperol (25%), gamma – tocopherol(74.5%), and delta tocopherol(0.5%) [15].

The plant is around 3-5m tall with alternate leaves, yellow flowers, red calyces and red fruits after they mature and light brow kidney- shaped seeds. The red tea from the calyces is also called as karkade.

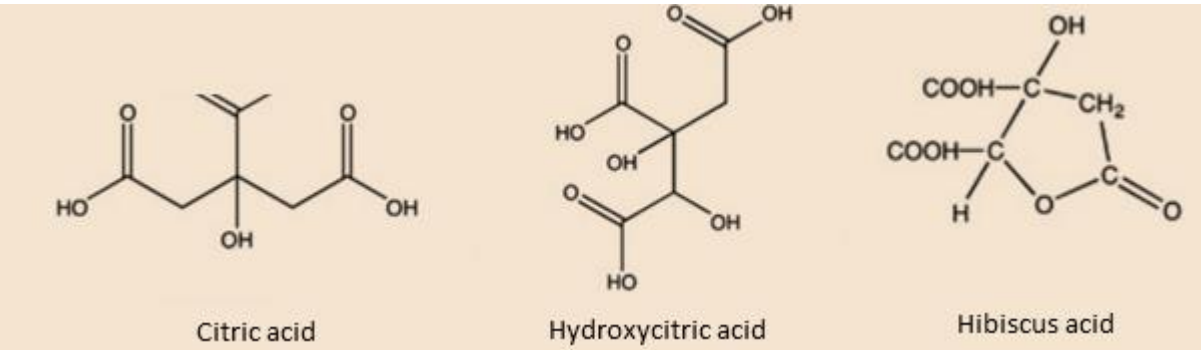
According to the Ayurvedic literature, the whole plant has medicinal values. The pharmacology evidences of this plant such as antioxidant, antihyperlipidemic, antihypertensive, hepatoprotective, and anti-cancer, antipyleetic activities have been established[16].

The fresh calyx (the outer whorl of the flower) is eaten raw or cooked. It is rich in pectin and citric acid. It is eaten in salads, or cooked and used as flavoring in cakes etc .It is also used in making jellies, soups, sauces pickles . It is used to add a red color and flavor to herb teas, and can also be roasted and used as acoffee substitute. The flowers are produced all year around . There are two types of calys- green and red. The green is more commonly used as a vegetable, while the red types are make an acid flavored drink[17].

Hibiscus sabdariffa is easy to grower in most well drained soils but can tolerate poor soils. It requires 4-8 months growth night – time temperatures with amininmum of 20^{o}c. as well as 13 hours of sun light and monthly rainfall ranging from (130-250mm) during the first four months to prevent premature flowering. Rain or high humidity during the harvest time and drying process can downgrade the quality of the calyces and reduce the yield. The quality of of this plant is determined by seed stock, localgrowing conditions, time of harvest, post harvest handling and mainly the drying steps. Most of the time it grows as supplement crop and it is susceptible to fungi , viral and bacterial attack and also to insects. Asingle plant produces about 1.5kg of fruit[18].

Hibiscus Sabdarifa extracts contain a high percentage of organic acids, including citric acid, hydroxy citric acid, hibiscus acid, malic and tartaric acids as major compounds. Based on previous studies, the

percentage of organic acids in (hibiscus flowers) is : citric acid 12-20%, malic acids 2-9%, tartaric acid 8% and 0.02-0.5% of ascorbic acid (vitamin C)[19].



The leave are used for animal fodder and fibre . The seeds can be used to feed poultry as well as sheep and the residue from the seeds oil extraction can also be used to feed cattle and chicks[20].

1.6.2-*Croton Zambesicus*

-Taxanomy

Order :Malpighiales

Family :Euphorbiaceae

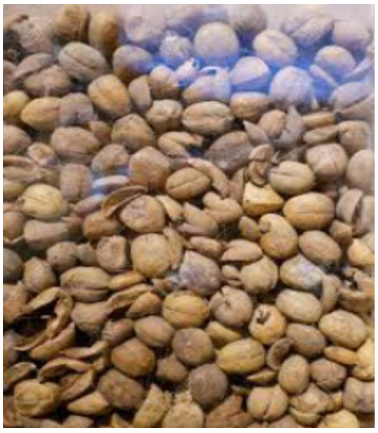
Subfamily: Crotonoideae

Genus :*Croton, L*

Species: *Croton zambesicus* Mull

Common Name : Si Erraleone

Native Range : Tropical Africa (Nigeria)



Croton Zambesicus

Croton zambesicus belongs to the family Euphorbiaceae. It is a shrub or small tree up to 16m high, of fringing forest and savannah, from the Gambia to South Nigeria and widely distributed elsewhere in tropical Africa. The tree has silvery leaves, rusty – scaly below, and has an attractive appearance. It is often planted in towns and villages . In time past it was planted as a fetish tree, wood is pale yellow, fine grained, bard and gives a good polish. The stems are used in parts of western Africa for hut. The bark slash emits an aromatic smell and an infusion of bark is used in some African countries in cases of malaria.

The leaves are considered as strengthening. A soup made of them is given to dysentery cases in Sudan, a leaf decoction is used as a wash in fever, convulsions, etc. Leaf is also used traditionally for headache and as a vermifuge.

The shoots and root are used in Sudan as tonic, febrifuge and for menstrual pain. The fruits, like the bark, are aromatic. They are used by some African communities to spice food and prepare a sort of scent.The seeds are said to have medicinal uses in Togo. In kordofan– Sudan-they are used to flavor tea .An unspecified part of the plant is used in Zambia for nose- troubles[21,22].

Croton zambesicus is a medicinal plant grown in Africa particularly in Sudan. It is widely spread in tropical Africa. It has antimicrobial and antihypertensive potentials. The genus Croton is well known for its diterpenoid content and different types of diterpenoids (phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane, ets) have been isolated from this genus[23].Recently it has been found that the ethanolic extract from the leaves of *C. zambesicus* have profertility property [24],

Ethanolic extract of *Croton zambesicus* has the highest antimicrobial effect against *staphyloccus aureus* with the 0.6 g/ml concentration giving the highest zone of inhibition. However, the effect of the aqueous extract of *C.zambesicus* on s*treptococcus* species was low . phytochemical screening showed the presence of many constituents such as cyanogenic glucoside, steroids, anthraquinones, phenols, saponins and flavonoids. Quantitative analyses of mineral elements showed the presence of sodium, potassium, calcium , magnesium , zinc , Iron , lead , cupper , manganese and potassium . Magnesium

has the highest composition and lead was not detected . Anti- nutrients present in *C-Zambesicus* were also determined and it includes tannins, phenols, phylates, oxalates, saponins, flavonoids and alkaloids while the proximate nutirents have highest composition of carbohydrate[25].

1.6.3. *Syzygium aromaticum,*

- **Taxanomy:**

Order :Myrtales
Family :Myrtaceae
Subfamily. Myrtaceae
Genus :Syzygium – P-Br
Species: Syzygiumaromaticum
Common Name : Clove
Native Range : Indonesia

Syzygiumaromaticum

Syzygium aromaticum is a tree in the family Myrtaceae, native to Indonesia with the aromatic flower buds known as cloves, and

commonly used as a spice. The plant is commercially harvested in Indonesia, as well as in India, Pakistan, Srilanka, Comoro islands, Madagascar, Seychelles, and Tanzania.

Syzygium *aromaticum* oil can be used as an antibacterial , antifungal, antiseptic agent, antioxidant and antithrombtic agent, as well as other biological activities of its constituents. Due to its numerous pharmacological activites, *S.aromaticum* can be considered as potential drug candidate for many ailments[26].

Syzygium aromaticum essential oil has been used as atopical anesthetic and flavoring for years. It is known to have antimicrobial activity, mostly related to its content of eugenol and other polyphenolic compounds. Other uses of colve have also arisen, like insect -repellent or growth promoter agent. Clove essential oil has a controlling effect over native microflora and over pathogenic microorganisms such as *Escherichia coli.* Clove has been successfully employed as surface sanitizer agent in lettuce seeds and as a bio-preservative in leafy green vegetables (applied either pre-harvest or post-harvest). The application of clove on leafy vegetables also reduced peroxidase activity. Clove essential oil should be considered as viable alternative to chlorine as sanitizer agent due to its low toxicity and low environmental impact[27].

Clove is widely used as a spice all over the world for the strong aromatic smell . Clove oil is a popular remedy for toothache due to its

potent antiseptic and analgesic activity. It also has strong antioxidant and antiviral activities[28].

Eugenol is the main component of clove oil . The lipid profiles, the contents of tocols, and total phenolics in cold pressed clove oil are well documented[28]. Cold pressed clove oil was reported to possess stronger radical scavenging and antimicrobial activity compared to virgin oil. Similarly, the cold pressed clove oil was also reported to possess hepatoprotective activity in experimental animals along with other biological activities[28].

Cloves are dried flowers buds of *Syzygium aromaticum* (L.) **Merrill** and **Perry** (Myrtaceae) . Cloves are like small, round – headed nails, about 10-17.5mm long, and blackish brown in color. The stalk consists of a cylindrical bypanthium, above which is a bilocular ovary containing numerous ovules on axile placentas, the head consists of four, slightly projecting , clayx teeth, four membranous petals, and numerous in curved stames surrounding large style[29].

The most precious spice, is derived from an ever green tree having a height of 15m. It is known as the clove tree . It has been used around the globe for centuries in preservation of food items and formulation of medicinal items. Buds are produced by the tree, which are used whole, or ground as aspice,

The principle phenolic components of clove are eugenol, terpenoids, tannins, and gallic acid, which have great potential for pharmaceutical, food, and agricultural applications[30].

Clove trees start flowering from the fourth year after planting under good management conditions. Full bearing stage is reached only after 15 years. Flowering season ranges from October to December. Flower buds are harvested when they turn pink. The harvested flower buds are separated from the clusters by hand and are dried for 4-5 days. On an average 3-4 kg of dried buds can be harvested from 15-year old clove trees. The dried buds yield 14-21 % oil, which contains 70-90% eugenol and 5-12% eugenol acetate[31].

1.6.4.*Cissus quadrangularis,*

-Taxanomy:

Superorder :Rosanae

Order :Vitales

Family :Vitaceaem- grape

Genus :Cissus

Species :*Cissus quadrangularis* **L.**

Cissus quadrangularis

*Cissus quadrangularis*is a perennial climber widely used in Sudanese ethnomedicine.This plant grows in warm tropics and may propagate through stem cutting[32,33].*Cissus quadrangularis* possesses digestive, anthelmintic and tonic properties[3]. The plant is rich in ascorbic acid, calcium and carotene[34,35].It also contains flavonoids, steroids and terpenoids[36-39].*Cissusquadrangularis*exhibited significant analgesic activity[40,41]. It also showed beneficial effect on recovery of bone mineral density in postmenopausal osteoperosis[42-46]. Some *in vitro* studies demonstrated the antioxidant activity of this plant [47,48].It has been reported that *Cissus quadrangularis* posseses anti-inflammatory effect[49-51].

1.6.5.*Croton cordofana,*

-Taxanomy:

Kingdom : Plantae

Order :Malpighiales

Family :Euphorbiaceae

Genus : Croton

Species :*Croton cordofana*

Croton cordofana

The genus *Croton* comprise a diverse group of plants including trees, herbs and shrubs. *Croton* species are used against a wide array of diseases including cancer,diabetes, dysentery , worms ,ulcers, inflammations and weight loss[52-57].The medicinally important species: *Croton sylvaticas* is used traditionally against tuberculosis, inflammatory conditions and infections[58].The acetylcholinesterase inhibitory action of this species has been reported[59].Also the

antiplasmodial effect has been demonstrated[60]. Another *Croton*species , namely: *Croton gratissirnas* showed anticancer ,antioxidant and anti-inflammatory properties[61]. The medicinally important *Crotonmenyherthi* possesses antimicrobial and selected enzyme inhibitory effect[62]. Another Croton species :*Croton limae* exhibited antimicrobial activty[63]

Aim of this study

This study was designed to :

-Extract the oils from five potential medicinal plants (*Hibiscus Sabdariffa Croton Zambesicus Syzygium aromaticum Cissus quadrangularis* L. *Croton cordofana*).

-Identify and quantify the constituents of the oils by GC-MS analysis.

-Evaluate the antimicrobial activity of the targeted oils.

CHAPTER TWO

Materials and Methods

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Hibiscus sabdariffa, Croton zambesicus, Cissus quadrangularis* and *Croton cordofana* were collected from a forest reserve around Damazin-Sudan. Seeds of *Syzygium aromaticum* were purchased from the local market-Khartoum-Sudan.The plant materials were authenticated by direct comparison with a reference herbarium sample.

2.1.2-GC-MS analysis

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

2.1.3-Test organisms

The studied oils were screened for antimicrobial activity using the standard microorganisms : *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa, Escherichia coli* and the fungal species *Candida albicans.*

2.2-Methods

2.2.1-Extraction of oil

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

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

2.2.2-GC/MS analysis

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

- *Oven temperature program*

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

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

Column oven temperature	150.0°C
Injection temperature	300.0°C
Rate	4/min
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

2.2.3-Antimicrobial assay

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

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

Chapter Three

Results and Discussion

3-Results and Discussion

In this study the oils from five plants ofmedicinal attributes **(***ton cordofana ,Cissusquadrangularis, Syzygiumaromaticum, Croton zambesicus***,** *Hibiscus sabdariffa*)have been investigated by GC.MS and the antimicrobial activity has been screened.

3.1-*Syzgium aromaticum*

3.1.1-GC-MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of *Syzgium aromaticum* oil. The analysis revealed the presence of 13 components - Table (3.1).The total ion chromatogram is presented in Fig.3.1.

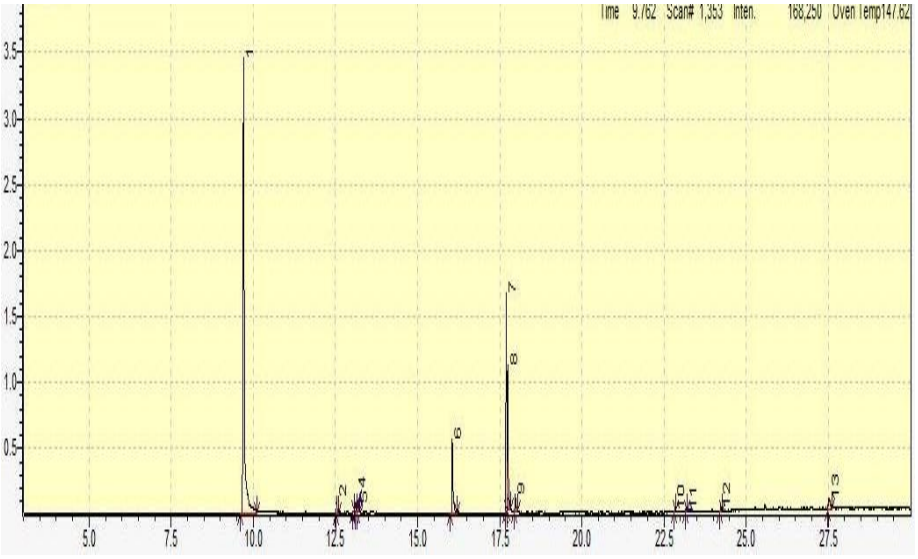


Fig. 3.1: Total ion chromatograms

Table 3.1: Constituents of the oil

No.	Name	Ret.Time	Area%
1.	Eugenol	9.709	60.35
2.	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, trans	12.544	1.05
3.	Caryophyllene oxide	13.041	0.12
4.	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	13.135	0.15
5.	cis-p-mentha-1(7),8-dien-2-ol	13.176	0.78
6.	Hexadecanoic acid, methyl ester	16.056	7.54
7.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.700	14.75
8.	9-Octadecenoic acid (Z)-, methyl ester	17.744	11.37
9.	Methyl stearate	17.974	1.56
10.	Heneicosanoic acid, methyl ester	22.832	0.19
11.	3-Oxabicyclo[4.2.0]oct-5-ene, endo-8-methyl-exo-8-(2-propenyl)-	23.166	0.28
12.	Tetracosanoic acid, methyl ester	24.230	0.34
13.	.beta.-Sitosterol	27.528	1.52

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

i) Eugenol (60.35%)
ii) 9, 12-octadecadienoic acid methyl ester (31.21 %)
iii) 9-Octadecenoic acid methyl ester(29.55%)
iv) Hexdecanoic acid methyl ester(15.95 %)

The mass spectrum of eugenol is presented in Fig.3.2. The molecular ion M^+ $[C_{10}\ H_{12}O_2]^+$ appeared at m/z164. The GC-MS analysis showed a mass spectrum(Fig.3.3) identical with 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT.17.057)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The analysis showed a mass spectrum(Fig.3.4) identical with that of 9-octadecenoic acid methyl ester.The signal at m/z 296 (RT.17.105) corresponds : $M^+[C_{19}H_{36}O_2]^+$.

The GC-MS analysis also gave a spectrum(Fig.3.5) characteristic of hexadecanoic acid methyl ester .The peak at m/z 270(RT.15.392) accounts for: M^+ $[C_{17}H_{34}O_2]^+$.

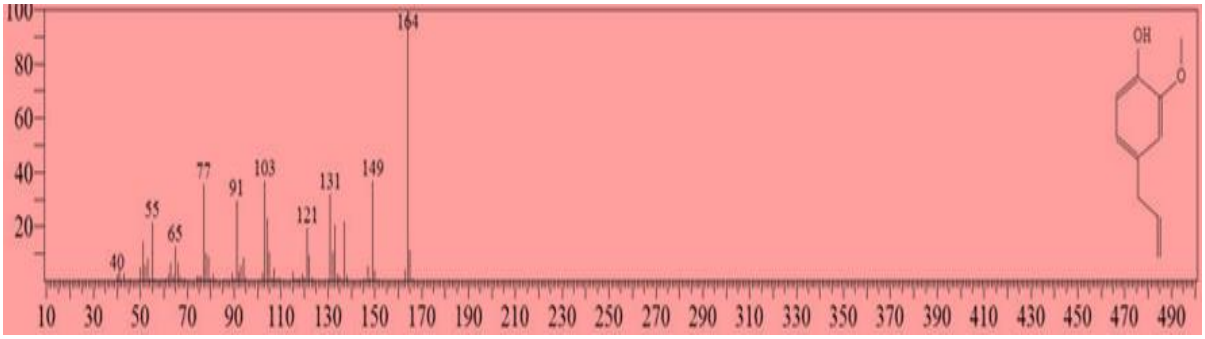


Fig.3.2: Mass spectrum of eugenol

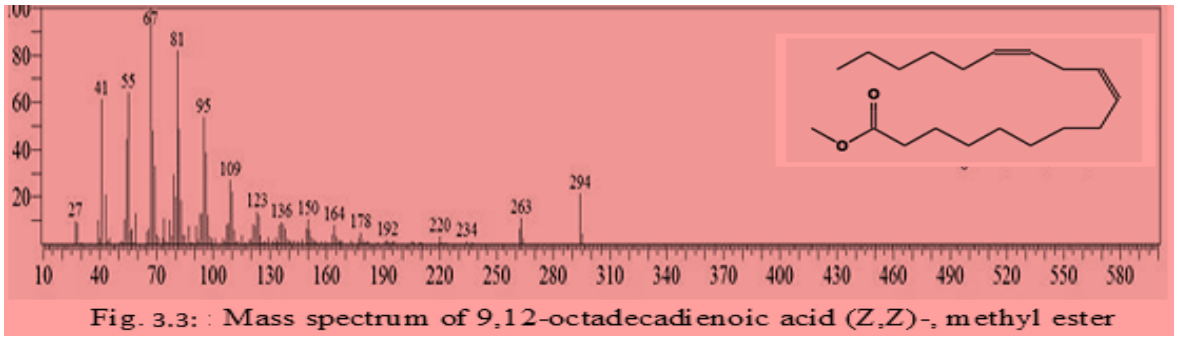


Fig.3.3:Mass spectrum of 9,12-octadecadienoic acid (Z)-, methyl ester

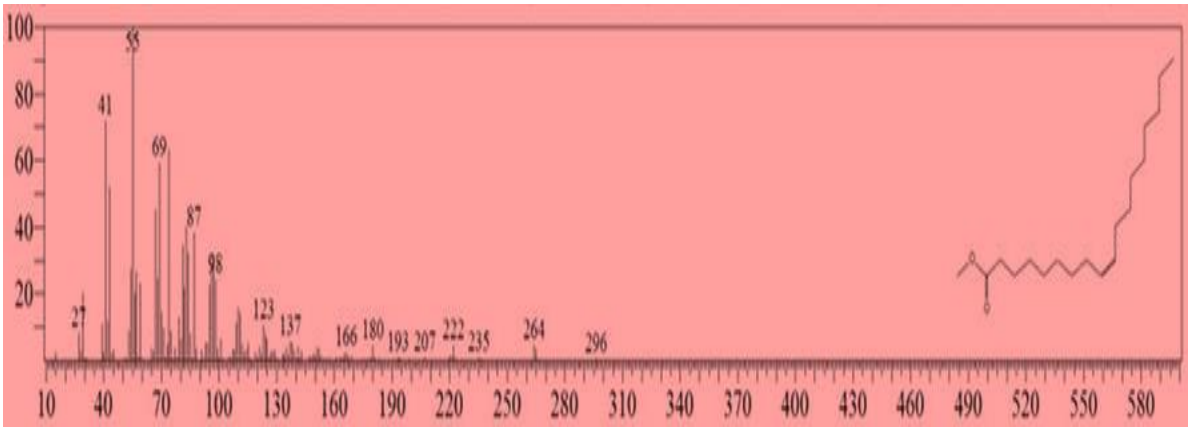


Fig.3.4:Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

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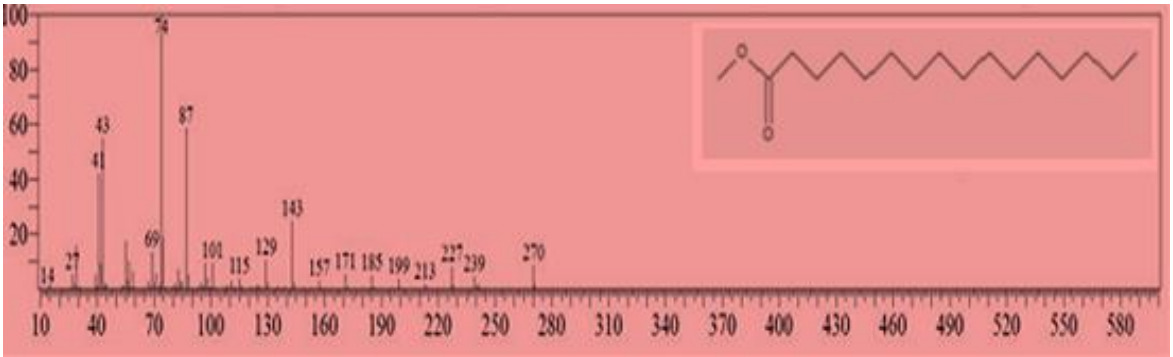

Fig 3.5: Mass spectrum of hexadecanoic acid, methyl ester

3.2.2.Antimicrobial activity

*Syzgium aromaticum*oil was assessed for antimicrobial activity against five standard microorganisms . The average of the diameters of the growth inhibition zones are presented in Table (3.4).Results were interpreted in the following terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The oil showedsignificant activity against*Staphylococcus aureus* and *Escherichia coli*moderate activity against *Bacillus subtilis* and the fungal species *Candida albicans*.

Table 3.4 : Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	19	15	22	13	14
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus* (G +ve)
Bs.: *Bacillus subtilis*(G +ve)
Ec.: *Escherichia coli* (G-ve)
Pa.: *Pseudomonas aeruginosa* (G –ve)
Ca.: *Candida albicans*(Fungus)

3.2-*Croton zambesicus*

3.2.1-GC/MS analysis

Gas chromatography - mass spectrometry has been used for the identification and quantification of *Croton zambesicus*oil. The analysis revealed the presence of 22 components - Table (3.3).The total ion chromatogram is presented in Fig.3.6.

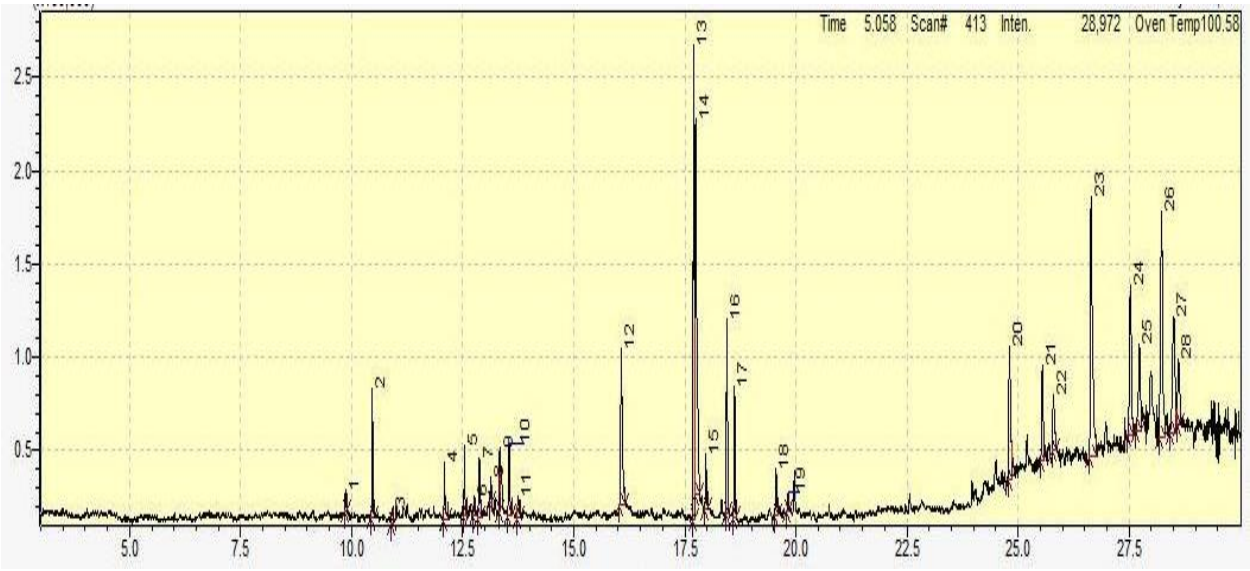

Fig. 3.6: Total ion chromatograms

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

i)9-Octadecenoic acid methyl ester(12.98%)
ii) cholest-5-en-3-ol, 24-propylidene-, (3.beta.)- (11.41%)

iii)9, 12-octadecadienoic acid methyl ester (10.26 %)

iv)5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-(10.10%).

The mass spectrum of that of 9-octadecenoic acid methyl esteris shown in Fig. 3.7.The signal at m/z 296 (RT.17.743) corresponds : $M^+[C_{19}H_{36}O_2]^+$.The mass spectrum of cholest-5-en-3-ol, 24-propylidene-, (3.beta.)- is presented in Fig.3.8 .The signal at m/z 426(RT.26.649) corresponds the molecular ion $[C_{30}H_{50}O]^+$

Table 3.1: Constituent of the oil

No.	Name	Ret.Time	Area%
1.	.alpha.-ylangene	9.862	0.43
2.	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	10.465	2.47
3.	Santolinatriene	10.907	0.38
4.	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	12.089	1.30
5.	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl, trans	12.540	1.29
6.	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.beta.,8.beta.)]-	12.760	0.35
7.	Cubenol	12.876	1.65
8.	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	13.126	0.38
9.	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	13.336	1.18
10.	5-Hydroxy-4-hydroxymethyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene	13.544	2.09
11.	5-(5-ethenyltricyclo[5.2.1.0(2,6)]decyl-3)pent-4-enoic acid, methyl ester	13.747	0.64
12.	Hexadecanoic acid, methyl ester	16.070	5.34
13.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.699	10.26
14.	9-Octadecenoic acid (Z)-, methyl ester	17.743	12.98
15.	Methyl stearate	17.973	1.41
16.	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	18.448	4.33
17.	Nerolidolisobutyrate	18.617	2.74
18.	Dihydro-isosteviol methyl ester	19.555	0.97
19.	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	19.830	0.41

20	5(S),9(S),10(S)-15,16-Epoxycleroda-3,8,13(16),14-tetraene-19,18:20,12(S)-diolactone (swassin)	24.810	4.51
21	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	25.546	3.01
22	Campesterol	25.797	1.85
23	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	26.647	11.41
24	.beta.-Sitosterol	27.529	5.69
25	.beta.-Amyrin	27.733	3.82
26	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]	28.230	10.10
27	.alpha.-Amyrin	28.510	6.43
28	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-	28.616	2.58

Fig. 3.9 illustrates the mass spectrum of 9, 12-octadecadienoic acid methyl ester. The peak at m/z 294(RT.17.699)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of 5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]- is presented in Fig.3.10. The signal at m/z

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

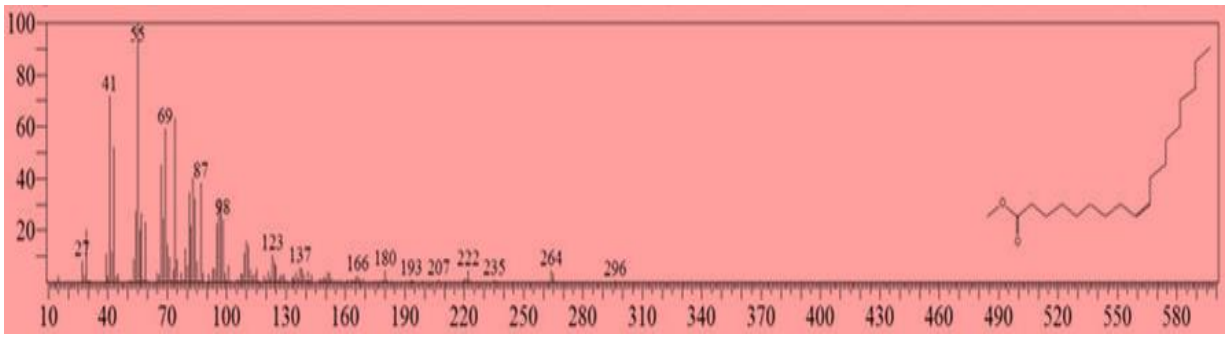

Fig.3.7:Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

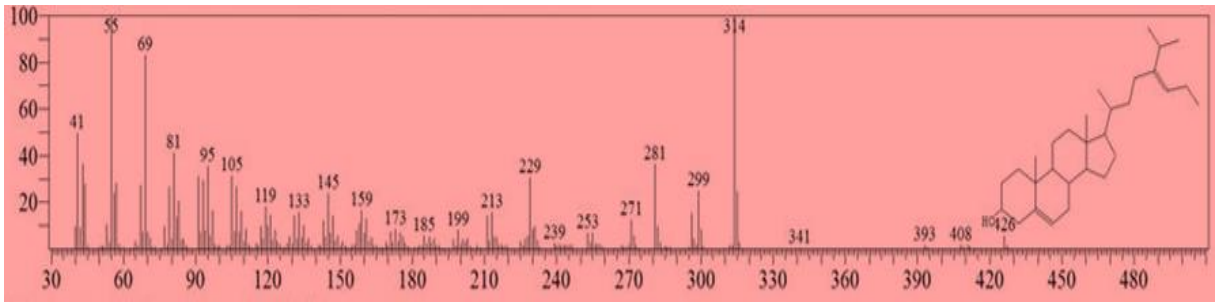

Fig. 3.8 : Mass spectrum of cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-

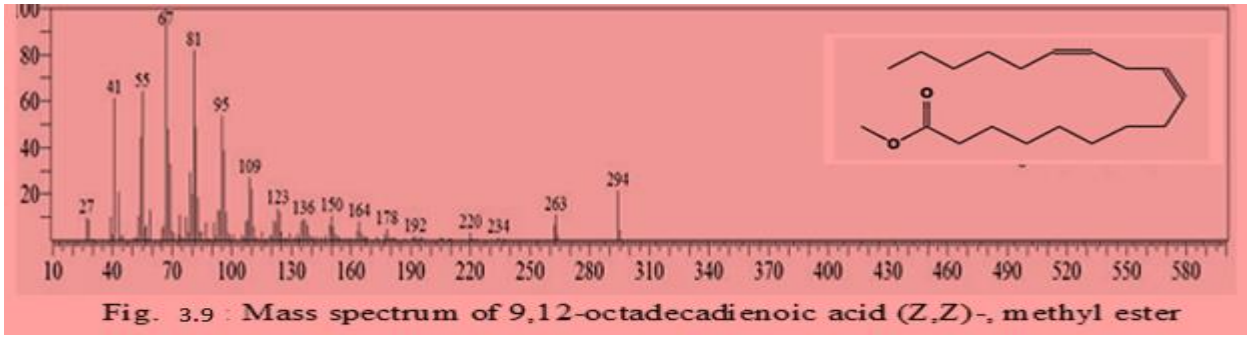

Fig. 3.9 : Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

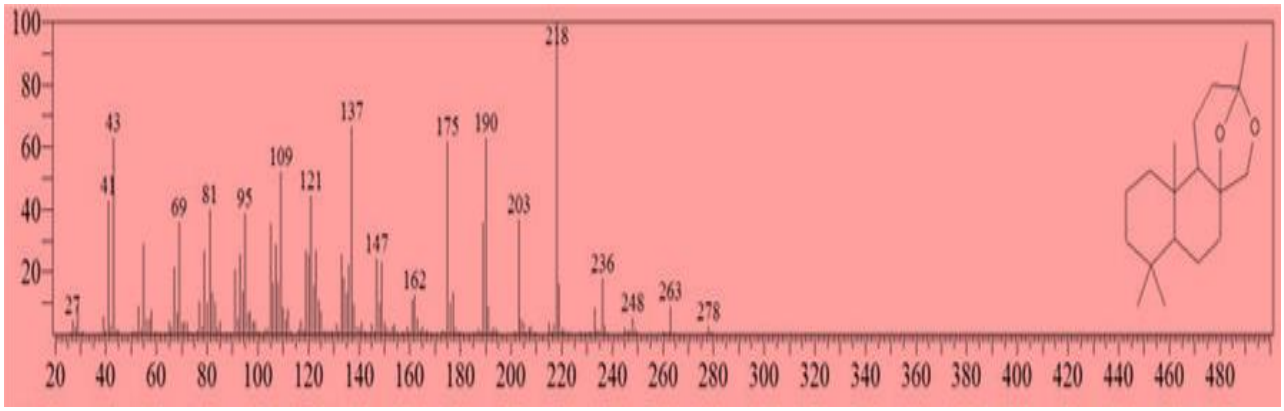

Fig.10 ; Mass spectrum of 5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-

3.2.2.Antimicrobial activity

*Croton zambesicus*oil was assessed for antimicrobial activity against five standard microorganisms . The average of the diameters of the growth inhibition zones are presented in Table (3.4).Results were interpreted in the following terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Ampicilin , gentamicin and clotrimazole were used as positive controls. The oil showedmoderate activity against*Bacillus subtilis* beside weak activity against other test organisms.

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

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	10	15	9	12	11
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(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. *Hibiscus sabdariffa* L.

3.3.1- GC-MS analysis

GC-MS analysis of*Hibiscus sabdariffa* L.oil was conducted. Twenty-four constituents were identified by GC-MS. The total ion chromatogram (TIC) is given in Fig. (3.11) while the constituents of the oil are outlined in Table 3.5.

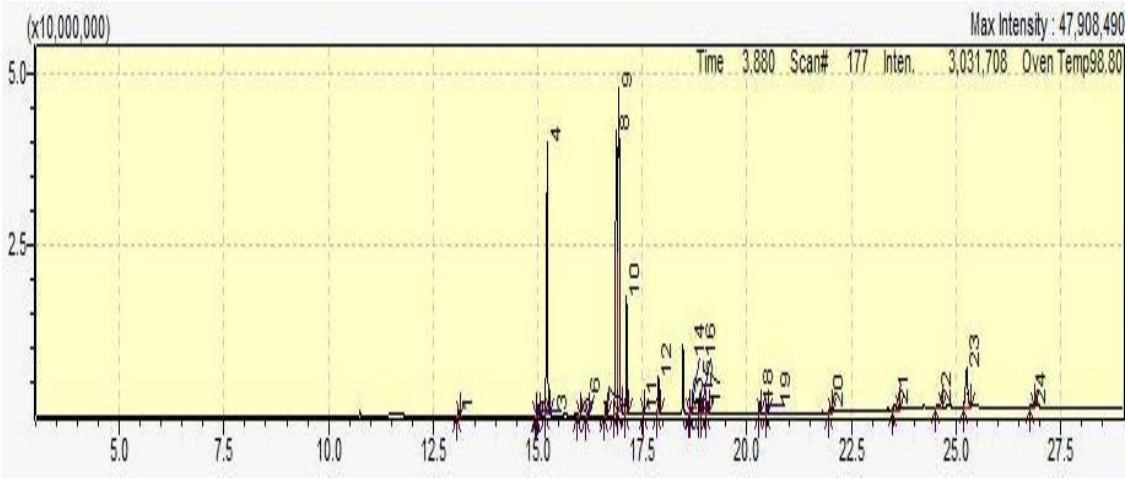

Fig .3.11: Total ions chromatograms

Table 3.5: Constituents of the oil

ID#	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.109	0.27
2.	7-Hexadecenoic acid, methyl ester, (Z)-	14.970	0.03
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.009	0.27
4.	Hexadecanoic acid, methyl ester	15.228	19.37
5.	cis-10-Heptadecenoic acid, methyl ester	15.956	0.18
6.	Heptadecanoic acid, methyl ester	16.180	0.13
7.	8,11-Octadecadienoic acid, methyl ester	16.626	0.69
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.888	28.33
9.	9-Octadecenoic acid (Z)-, methyl ester	16.944	30.23
10.	Methyl stearate	17.124	6.66
11.	Methyl 2-octylcyclopropene-1-octanoate	17.533	0.23
12.	10-Nonadecenoic acid, methyl ester	17.886	2.08
13.	11-Octadecenoic acid, methyl ester	18.645	0.17
14.	cis-11-Eicosenoic acid, methyl ester	18.662	0.25
15.	Eicosanoic acid, methyl ester	18.864	1.13

16.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.942	0.70
17.	8,11,14-Docosatrienoic acid, methyl ester	19.055	0.62
18.	13-Docosenoic acid, methyl ester, (Z)-	20.301	0.70
19.	Docosanoic acid, methyl ester	20.479	0.45
20.	Tetracosanoic acid, methyl ester	21.977	0.36
21.	.gamma.-Sitosterol	23.545	0.78
22.	Stigmasterol	24.568	0.52
23.	.beta.-Sitosterol	25.254	5.50
24.	Campesterol	26.813	0.35

Major components of the oil are discussed below:

9-Octadecenoic acid (Z)-, methyl ester (30.23%)

The EI mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester is shown in Fig. 3.12. The peak at m/z 296, which appeared at R.T. 16.944, in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 265 corresponds to loss of a methoxyl function.

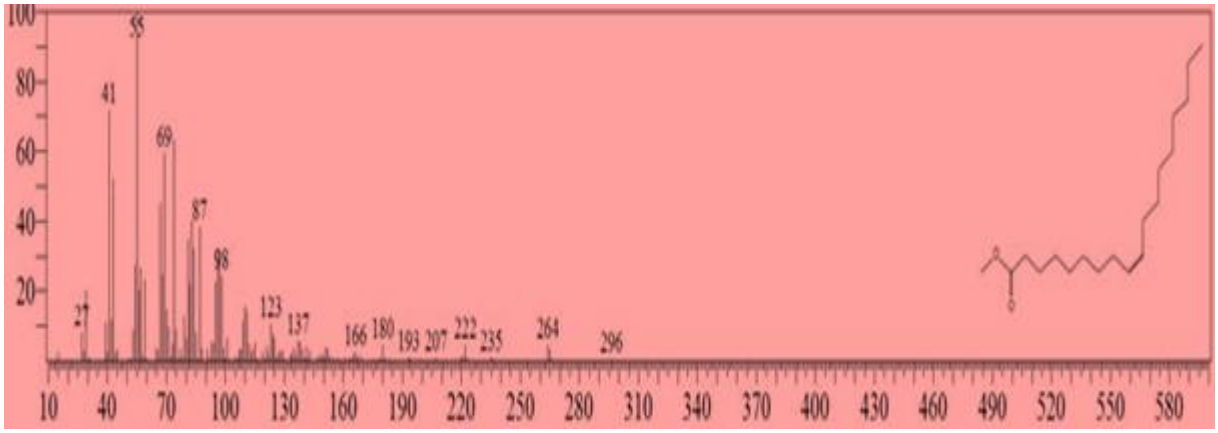

Fig. 3-11: Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (28.33%)

Fig (3-12) demonstrates mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester**.** The peak at m/z 294, which appeared at R.T. 16.888, in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl function.

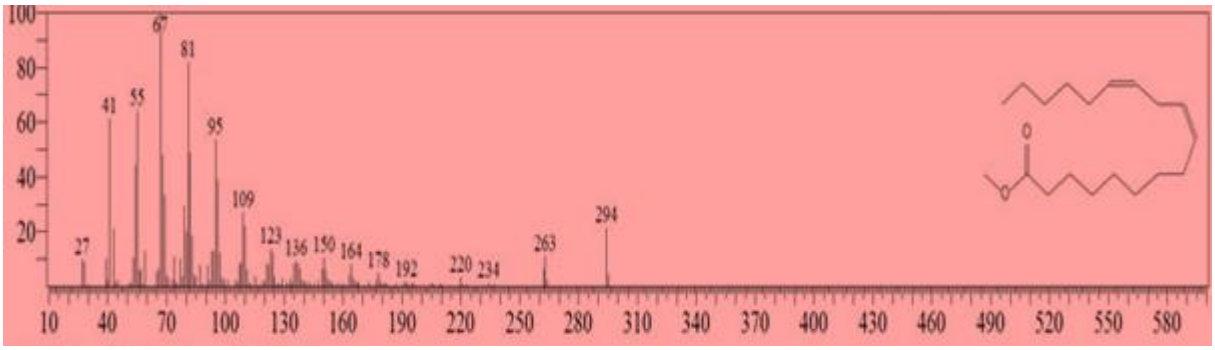

Fig. 3-12: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

Hexadecanoic acid, methyl ester (19.37%)

Mass spectrum of Hexadecanoic acid, methyl ester Methyl stearate is shown in Fig. 3-13. The peak at m/z 270, which appeared at R.T. 15.228, in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 corresponds to loss of a methoxyl function.

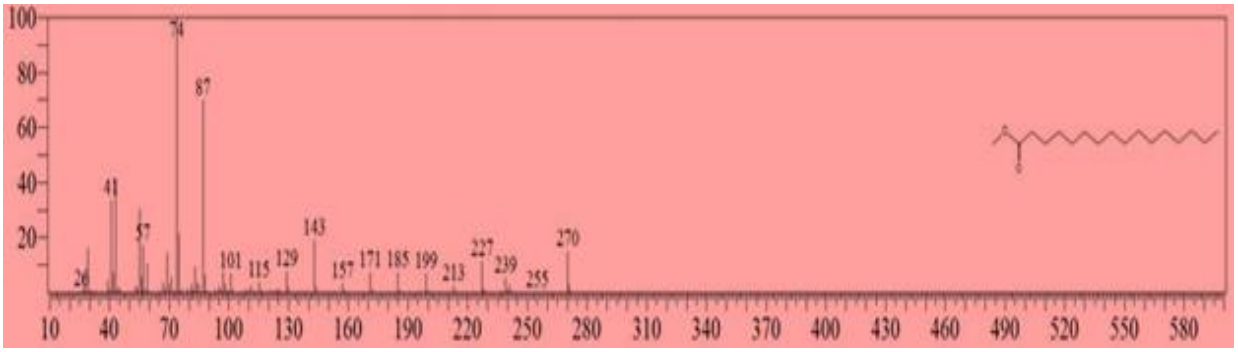

Fig. 3-13: Mass spectrum of Hexadecanoic acid, methyl ester

Methyl stearate (6.66%)

Mass spectrum of Methyl stearate is shown in Fig. 3-14. The peak at m/z 298, which appeared at R.T. 17.124, in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267 corresponds to loss of a methoxyl function.

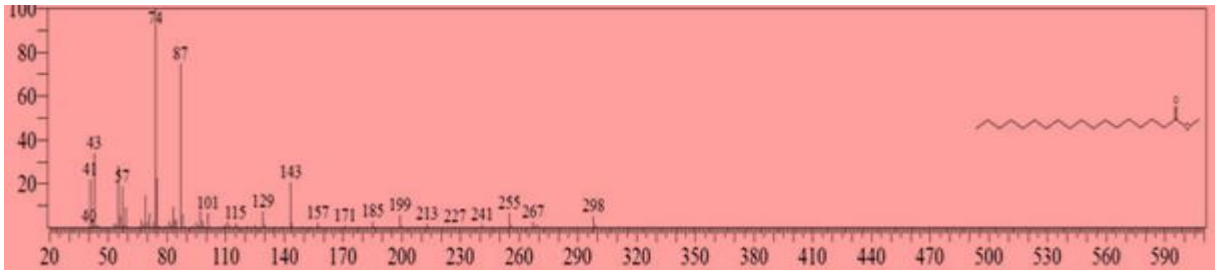

Fig. 3-14: Mass spectrum of Methyl stearate

3.3.2- Antibacterial activity

The oil was screened for antimicrobial activity against five standard organisms.The averages of diameters of the growth inhibition zones are shown in Table 3.6.

Table 3.6: Antimicrobial activity of *Hibiscus sabdariffa* L.oil

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	12	13	12	14	14
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

The oil showed moderate antibacterial activity against *Pseudomonas aeruginosa* and *Candida albicans*, it also shown moderate anticandidal activity. However, it exhibited partial activity against *Staphylococcus aureus*and *Escherichia coli*.

6.4. *Cissus quadrangularis*

6.4.1-GC-MS analysis

Cissus quadrangularis oil was studied by GC-MS.. The analysis showed the presence of 37 consitiuents(Table 3.7).The total ions chromatogram is depicted in Fig. 3.15.

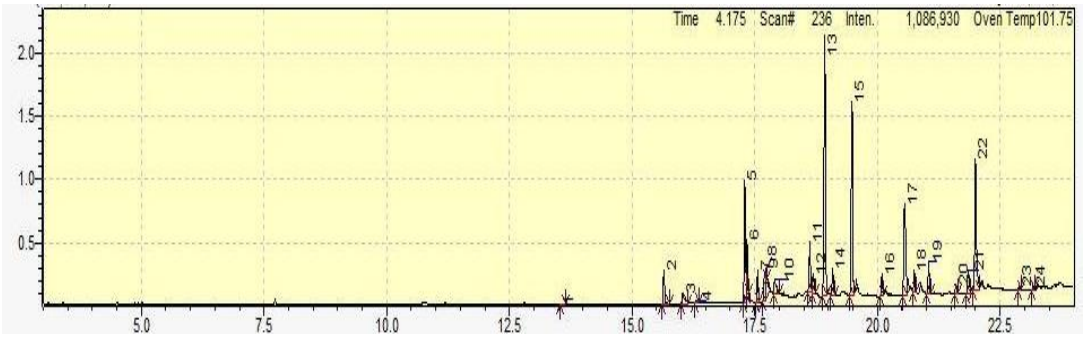

Fig. 3.15: Total ions chromatograms

Table 3.7 : Constituents of the oil

No.	Name	Ret.Time	Area%
14.	Methyl tetradecanoate	13.564	0.04
15.	Hexadecanoic acid, methyl ester	15.650	2.45
16.	n-Hexadecanoic acid	16.034	1.55
17.	Hexadecanoic acid, ethyl ester	16.310	0.19
18.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.300	8.67
19.	9-Octadecenoic acid (Z)-, methyl ester	17.343	3.17
20.	Methyl stearate	17.560	2.10
21.	Linoleic acid ethyl ester	17.704	2.24
22.	Oleic Acid	17.734	0.57
23.	9,12-Octadecadienoic acid, ethyl ester	17.908	1.82
24.	13-Hexyloxacyclotridec-10-en-2-one	18.623	4.09
25.	cis-9-Hexadecenal	18.699	1.47
26.	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.930	22.11

27.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.088	2.05
28.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	19.484	15.44
29.	Heptanoic acid, tert-butyl dimethyl silanyl ester	20.094	1.68
30.	9,12-Octadecadienoyl chloride, (Z,Z)-	20.552	11.02
31.	Glycidol stearate	20.753	1.55
32.	Z,Z-8,10-Hexadecadien-1-ol	21.046	2.30
33.	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	21.601	0.70
34.	cis,cis-7,10,-Hexadecadienal	21.854	2.13
35.	Trilinolein	22.000	12.17
36.	13-Docosenamide, (Z)-	22.892	0.33
37.	Squalene	23.174	0.16

The GC-MS analysis showed the presence of steroids , aldehydes and ketones as minor constituents. The oil was dominated by:

i- E,E,Z-1,3,12-Nonadecatriene-5,14-diol(22.11%)

ii- (R)-(-)-14-Methyl-8-hexadecyn-1-ol (15.44%)

iii- Trilinolein(12.17%)

iv- 9,12-Octadecadienoyl chloride, (Z,Z)- (11.02%)

v- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester(8.67%).

The mass spectrum of E,E,Z-1,3,12-nonadecatriene-5,14-diol [$C_{19}H_{34}O_2$] is shown in Fig.3.16. The peak at m/z292 (R.T. 18.930 in total ion chromatogram), corresponds :M^+ - 2H.The mass spectrum of (R)-(-)-14-Methyl-8-hexadecyn-1-ol is illustrated in Fig.3.17 .The signal at m/z252 (RT,19.484) accounts for the molecular ion : $M^+[C_{17}H_{32}O]^+$.Fig.3.18 shows the mass spectrum of trilinolein. The

peak at m/z878(RT,22.000) accounts for : $M^+[C_{57}H_{98}O_6]^+$. The mass spectrum of 9,12-Octadecadienoyl chloride, (Z,Z)- is illustrated in Fig.3.19. The molecular ion : $M^+[C_{18}H_{31}ClO]^+$ appeared at m/z298.

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.3.20. The peak at m/z294(R.T.17.300) in total ion chromatogram), corresponds : $M^+[C_{19}H_{34}O_2]^+$.The peak at m/z263 is due to loss of a methoxyl function.

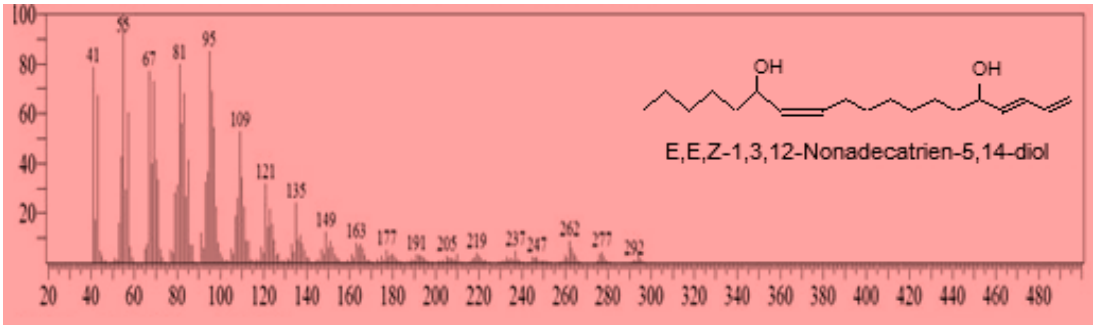

Fig.3.16 : Mass spectrum of E,E,Z,1,3,12-Nonadecatrien-5,14-diol

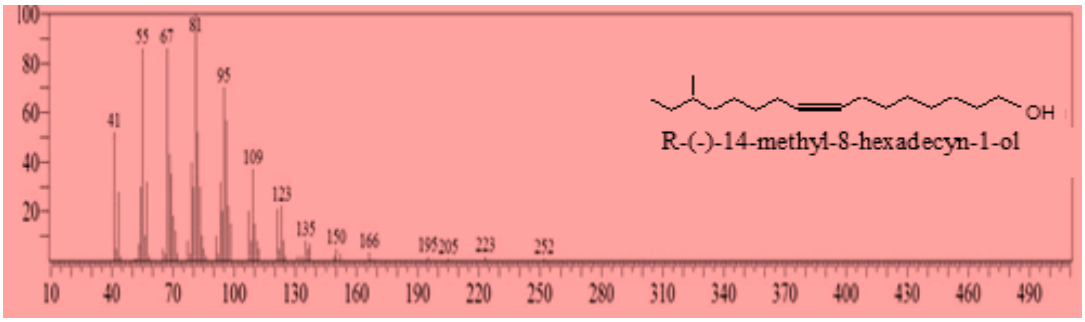

Fig.3.17 : Mass spectrum of R-(-)-14-methyl-8-hexadecyn-1-ol

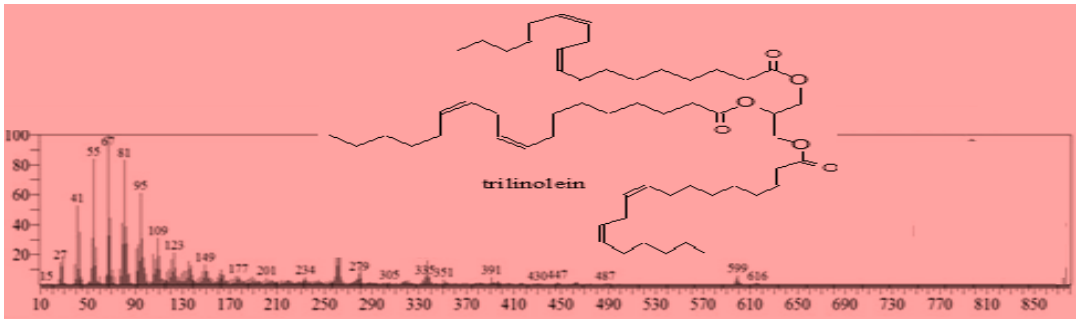

Fig.3.18: Mass spectrum of trilinolein

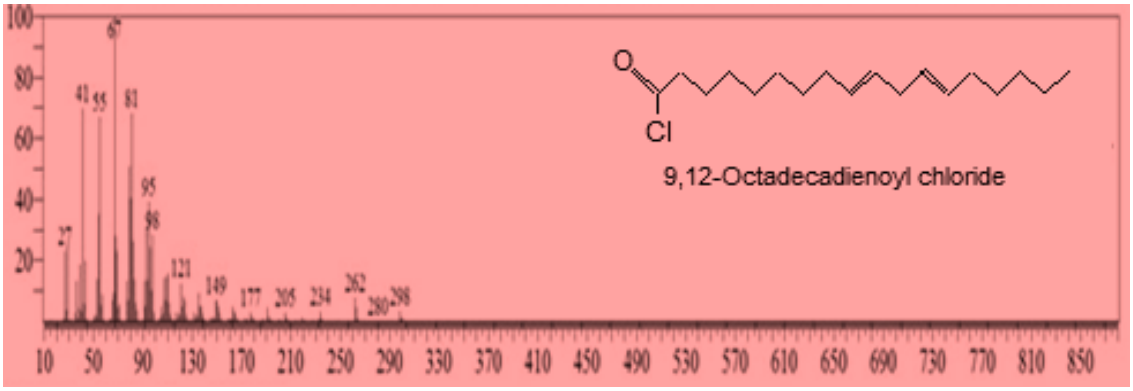

Fig. 3.19 : Mass spectrum of 9,12-octadecadienoyl chloride

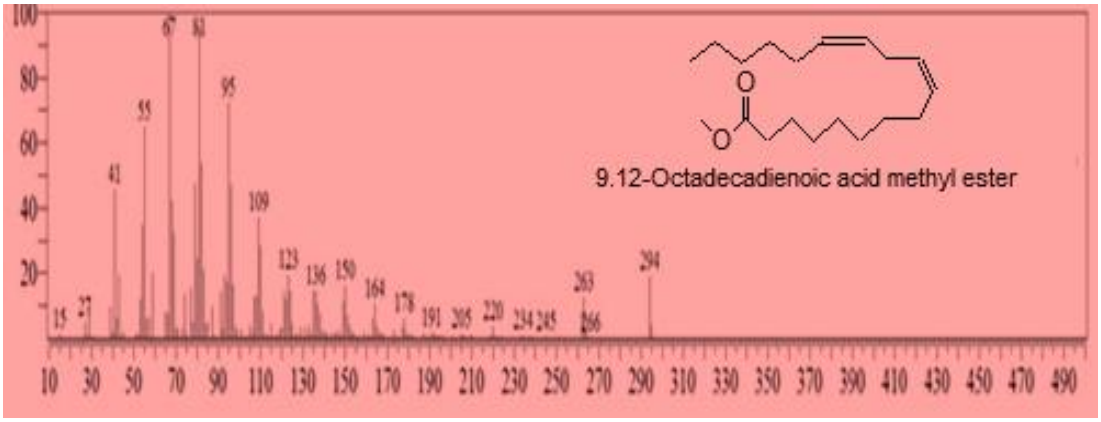

Fig.3.20: Mass spectrum of 9,12-octadecadienoic acid methyl ester

6.4.2.Antimicrobial assay

Cissus quadrangularis oil was evaluated for its antimicrobial activity against five pathogenic microbes(*Bacillus subtilis (*G+ve) ,*Staphylococcus aureus(*G+ve), *Pseudomonas aeroginosa* (G-ve) ,*Escherichia coli* (G-ve) and *Candida albicans* -fungus). The oil showed moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 3.8 : Inhibition zones(mm/mg sample)

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

3.5. *Croton cordofana*

3.5.1.GC-MS analysis

Croton cordofana oil was studied by GC-MS. The analysis revealed the presence of 19 components as shown in Table 3.9. The total ions chromatogram is presented in Fig. 3.21.

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

i) 9 12-Octadecenoic acid methyl ester (44.32%)

ii) 9-Octadecenoic acid methyl ester(23.42%).

iii) Hexdecanoic acid (15.18% %)

iv) Methyl stearate(12.99%).

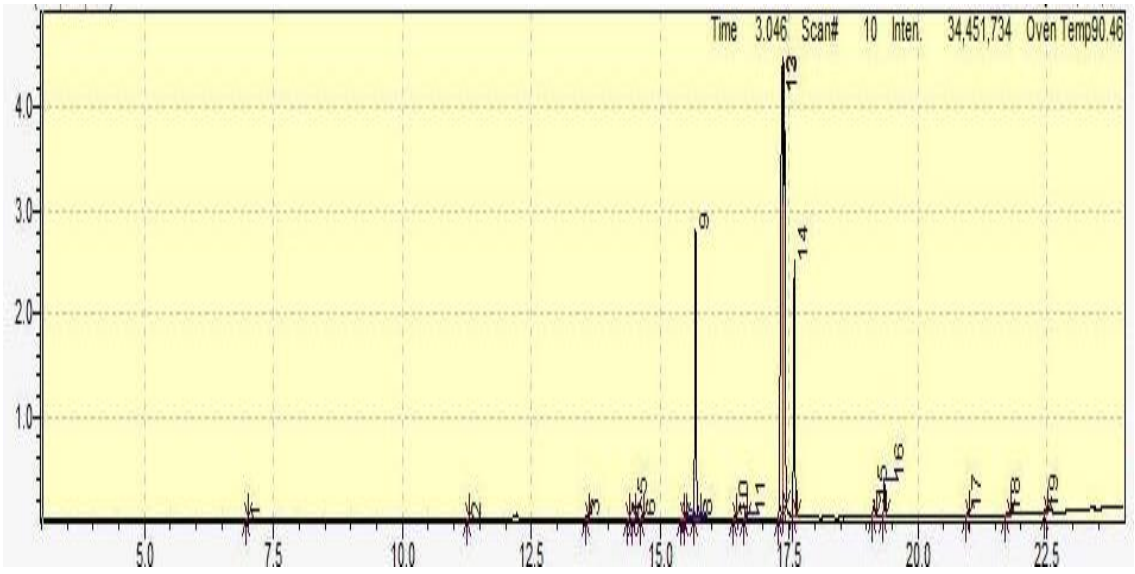

Fig. 3.21 : Total ions chromatogram

Table 3.9: Constituents of the oil

No.	Name	Ret.Time	Area%
1.	L-.alpha.-Terpineol	6.988	0.04
2.	Dodecanoic acid, methyl ester	11.263	0.01
3.	Methyl tetradecanoate	13.579	0.15
4.	6-Octadecenoic acid, methyl ester, (Z)-	14.386	0.02
5.	5-Octadecenoic acid, methyl ester	14.492	0.03
6.	Pentadecanoic acid, methyl ester	14.652	0.09
7.	7-Hexadecenoic acid, methyl ester, (Z)-	15.443	0.05
8.	9-Hexadecenoic acid, methyl ester, (Z)-	15.486	0.05
9.	Hexadecanoic acid, methyl ester	15.687	15.18
10	cis-10-Heptadecenoic acid, methyl ester	16.448	0.08
11	Heptadecanoic acid, methyl ester	16.656	0.24
12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.371	44.32
13	9-Octadecenoic acid (Z)-, methyl ester	17.410	23.42
14	Methyl stearate	17.602	12.99
15	cis-11-Eicosenoic acid, methyl ester	19.150	0.93
16	Eicosanoic acid, methyl ester	19.349	1.74
17	Docosanoic acid, methyl ester	20.972	0.33
18	Tricosanoic acid, methyl ester	21.736	0.08
19	Tetracosanoic acid, methyl ester	22.477	0.25

Fig. 3.22 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 17.371)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.17.410) 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 (RT.15.687) is due to M^+ $[C_{17}H_{32}O_2]^+$. Fig. 3.25 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.602) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 accounts for loss of a methoxyl.

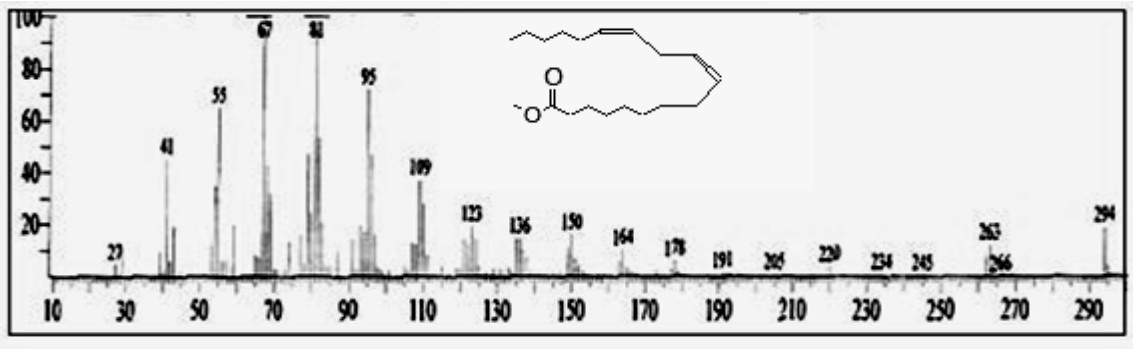

Fig.3.22: Mass spectrum of 9,12-octadecadienoic acid

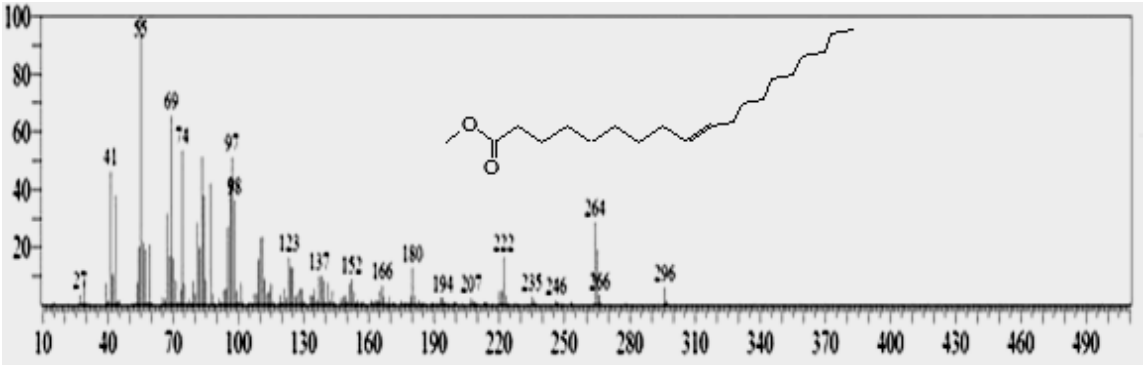

Fig.3.23 : Mass spectrum for 9-octadecenoic acid[z]-,methyl ester

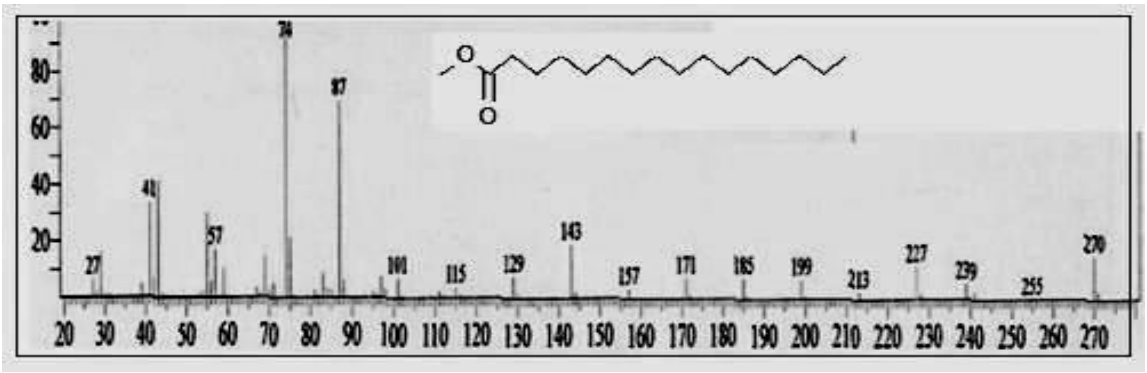

Fig.3.2 4: Mass spectrum of hexadecanoic acid methyl ester

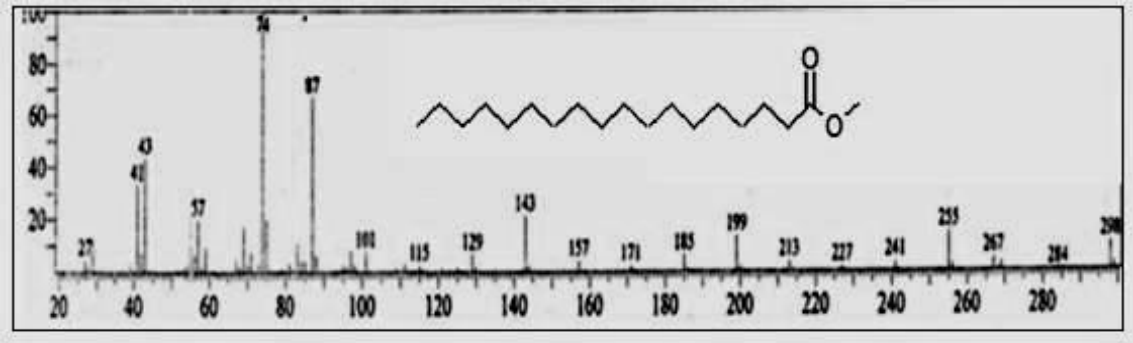

Fig.3.25: Mass spectrum of methyl stearate

3.5.2.Antimicrobial assay

Croton cordofana oil was assessed for antimicrobial activity against five standard microorganisms . 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 oil failed to show activity against any of the test organisms.

Table 3 .10: Inhibition zones(mm/mg sample)

Type	**Sa**	Bs	Ec	Ps	Ca
Oil(100mg/ml)	--	--	--	--	--
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(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

In this study , the oils from five plants **(***Cassia siama, Cordia Africana, Poliostiyma themigii* **,** *Detarium microcarpum* and *Aristolochia bracteolate*) of medicinal attributes have been extracted and the constituents of the oils have been characterized by the technique of GC/MS. The target oils have been assessed for their antimicrobial activity using the cup plate agar diffusion bioassay and all the oils samples exhibited different antimicrobial responses towards the test organisms.

Recommendations

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

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

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