Sudan University of Science and Technology

College of Postgraduate Studies

AStudy of oils from some Medicinal Plants

Grown in Sudan

دراسة الزيوت فى بعض النباتات الطبية السودانية

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

By

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قال تعالى :

﴿ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡحَيُّ ٱلۡقَيُّومُۚ لَا تَأۡخُذُهُۥ سِنَةٞ وَلَا نَوۡمٞۚ لَّهُۥ مَا فِي ٱلسَّمَٰوَٰتِ وَمَا فِي ٱلۡأَرۡضِۗ مَن ذَا ٱلَّذِي يَشۡفَعُ عِندَهُۥٓ إِلَّا بِإِذۡنِهِۦۚ يَعۡلَمُ مَا بَيۡنَ أَيۡدِيهِمۡ وَمَا خَلۡفَهُمۡۖ وَلَا يُحِيطُونَ بِشَيۡءٖ مِّنۡ عِلۡمِهِۦٓ إِلَّا بِمَا شَآءَۚ وَسِعَ كُرۡسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضَۖ وَلَا يَـُٔودُهُۥ حِفۡظُهُمَاۚ وَهُوَ ٱلۡعَلِيُّ ٱلۡعَظِيمُ ٢٥٥﴾

صدق الله العظيم

سورة البقرة

Dedication

To

My father and mother,

Brothers and sisters,

Sons (*Ahmed, Omer and Abu-Bakr*),

Daughter (*Ayah*)

To my soul Daughter *wifag*

Friends,

And to all my love

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First and foremost I would like to thank **Almighty ALLAH** for all blessing, giving, and helping me to accomplish this study.

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Abstract

The oils from Solanum *dubium, Martynia annua, Physalis angulate, Abutilon pannosum, Argemone Mexicana and Leonotis nepetifolia* were subjected to GC-MS analysis. Eighteen components, Eighteen constituents, Twenty-three components, Twenty-six constituents and nine components have been detected by GC-MS analysis respectively.

Solanum *dubium* oil showed significant activity against *Pseudomonas aeroginosa.*

Martynia annua and Physalis angulate oils were evaluated for antimicrobial activity it failed to exhibit inhibitory effect against five standard human pathogens.

Abutilon pannosum oil showed significant activity against *Candida albicans*.

Argemone mexicana oil showed showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Staphylococcus aureus*.

The studied *Leonotis nepetifolia* oil showed good activity against *Pseudomonas aeruginosa* and partial activity against *Staphylococcus aureus.*

المستخلص

في هذه الدراسة تم تحليل زيت نبات الجبين الصغير بواسطة الكروموتوغرافيا الغازية –طيف الكتلة حيث اتضح وجود 18 مكونا اهمها 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (51.14%), Hexadecanoic acid, methyl ester (19.27%), Methyl stearate (12.45%), 9-octadecenoic acid (Z)-, methyl ester (10.17%) أيضا تم تحليل زيت بذورعرق العقرب اكد وجود 18 مكونا أهمها: Octadecadienoic acid (Z, Z)-, methyl ester (43.01%), 9-Octadecenoic acid (Z)-, methyl ester (25.26%), Hexadecanoic acid, methyl ester (13.96%). اعطى تحليل زيت نبات كرم كرم 23 مكونا أهمها:-9, 12 Octadecadienoic acid (Z, Z)-, methyl ester (25.93%), 13-Docosenoic acid, methyl ester, (Z) – (25.34%), 9-Octadecenoic acid (Z)-, methyl ester (13.95%),. اما زيت القرقدان اتضح وجود 26 مركبا أهمها 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (39.67%), Hexadecanoic acid, methyl ester (28.44%), Methyl stearate (6.45%). عند تحليل زيت االخشخاش المكسيكى اتضح وجود 9 مركبات أهمها : Oleic Acid (35 .76 %), 9,12-Octadecadienoic acid (Z,Z)-(22.97%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester(14.23%), . نبات ام كشوكشو كشف عن وجود 21 مركبا أهمها : 9-octadecenoic acid (Z)-, methyl ester (35.40%), 6-octadecynoic acid, methyl ester (22.68%), 9, 12-octadecadienoic acid (Z, Z , methyl ester)hexadecanoic acid, methyl ester (14.44%),(15.59%) ثم اجرى اختبار مضاد الميكروبات للزيوت قيد الدراسة حيث ابدى الجبين الصغير فعالية ضد : *Pseudomonas aeroginosa*. اما زيت عرق العقرب فقد ابدى فعالية متوسطة ضد مضادات الاكسدة ولم يوثر على الميكروبات وزيت كرم كرم لم يبدى فعالية عالية ضد الميكروبات.وابدى فعالية عالية ضد مضادات الاكسدة. اما القرقدان فقد كانت فعاليته عالية ضد : *Candida albicans*. كذلك اعطى الخشخاش المكسيكى ابدى فعالية عالية ضد مضادات الاكسدة وفعالية كبيرة ضد *Pseudomonas aeruginosa* وفعالية معتدلة ضد *Staphylococcus aureus* .

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Chapter One

1-Introduction

1.1General approach

Natural products are defined as chemical substances produced by life. Such products can also be synthesized. Natural products have played a pivotal role in the development of the field of organic chemistry by providing challenging synthetic targets [1] .

Medicinal plants and their extracts are very frequently used in Sudan and also are widely consumed in Africa and all over the world. Ethnomedicine has been used for the treatment of human disorders since long time and immemorial. About 80% of the world population depend on traditional medicine for their primary health care.[2]

In Sudan, 90% of Sudan's population-specially in rural areas depends largely on phytotherapy since admission to hospitals and obtaining modern synthetic drugs are limited and a high percentage of the population is nomads.[2,3] Sustainability of the use of medicinal plants is an important concern.

Recently the demand for medicinal herbs is increasing in Africa as the population grows and pressure on medicinal plant resources will become greater than ever.

Interest in plant-derived medicines has also increased in the developed countries among the pharmaceutical companies.[4] In contrast, due to their minor side effects, the medicinal plants are widely used to treat many human diseases.[5] The increasing cost of health care and the failure of allopathic medicine to treat some diseases have also participated to the increasing consumption of traditional medicine to fight disease. Until now, there is no pharmacopoeia or formal training for the traditional medical healers in Sudan, and their knowledge is completely based on acquired folklore and local traditions.

Medicinal plants with a long history of safe and efficient use are likely to have a pharmaceutical outcome.[6] However, almost all of the medicinal herbal products are unlicensed. Unknown consequences of some of medicinal plants have been detected. Examples of toxic reactions, allergic reactions, drug interactions, drug contamination, and mistaken plant identities are provided.[7]

1.2 Essential oils

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile, ethereal oils or aetherolea. Essential oils are obtained by steam distillation or by mechanical processes or ‘’dry’’ distillation.

The term -essential oil - dates back to the sixteenth century and has been coined by Paracelsus von Hohenheim of Switzerland[8].

Essential oils or “essences” owe their name to their flammability.[9 10]. Essential oils are soluble in alcohol, ether, and fixed oils, but insoluble in water. These volatile oils are generally liquid and colorless at room temperature. They have a characteristic odor and are usually liquid at room temperature and have a density less than unity, with the exception of a few cases (cinnamon oil for example). They have a refractive index and a very high optical activity.

Volatile oils are responsible for different scents that plants emit. They are widely used in the cosmetics industry, perfumery, and also aromatherapy. The latter is intended as a therapeutic technique including massage, inhalations, or baths using these volatile oils. In plants essential oils serve as chemical signals allowing the plant to control or regulate its environment (ecological role). They also play a role in attraction of pollinating insects, repelling predators, inhibition of seed germination.They are also associated in communication between plants (emission signals chemically signaling the presence of herbivores, for example). Moreover, essential oils also possesses antifungal and deterrent activities.

All parts of aromatic plants may contain essential oils as follows: Flowers, of course, including: orange, pink, lavender, and the

(clove) flower bud or (ylang-ylang) bracts. Leaves, most often, including eucalyptus, mint, thyme, bay leaf, savory, sage, pine needles, and tree underground organs, e.g., roots (vetiver) Rhizomes (ginger, sweet flag). Seeds (carvi, coriander), Fruits, including: fennel, anise, citrus epicarps; wood and bark, including cinnamon, sandalwood, rosewood. [10]

1.3 Chemistry of essential oils

Essential oils are produced by various differentiated structures, especially the number and characteristics of which are highly variable. Essential oils are localized in the cytoplasm of certain plant cell secretions, which lies in one or more organs of the plant; namely, the secretory hairs or trichomes, epidermal cells, internal secretory cells, and the secretory pockets. These oils are complex mixtures that may contain over 300 different compounds[11]. They consist of organic volatile compounds, generally of low molecular weight below 300. The vapor pressure of essential oils at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in the vapor phase[12,13]. Essential oils are known to belong to various chemical classes including : alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. Alcohols, aldehydes, and ketones offer a wide variety of aromatic notes, such as fruity ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal

(γ-selinene), etc. Components of essential oils belong to the vast majority of the terpene family. A Large number of compounds belonging to terpenoids have so far been identified in essential oils[14] including functionalized derivatives of alcohols (geraniol, α-bisabolol), ketones (menthone, p-vetivone) of aldehydes (citronellal, sinensal), esters (γ-tepinyl acetate, cedryl acetate), and phenols (thymol).

On the other hand, volatile oils may contain non-terpenoids generated by the *in vivo* by phenylpropanoids pathway, such as eugenol, cinnamaldehyde, and safrole. Biogenetically, terpenoids and phenylpropanoids have different primary metabolic precursors and are generated through different biosynthetic routes The pathways involved in terpenoids are the mevalonate and mevalonate-independent (deoxyxylulose phosphate) pathways, whereas phenylpropanoids originate through the shikimate pathway[15,16]. Some authors have reviewed the biosynthetic pathways of terpenoids and phenylpropanoids, respectively, the enzymes and enzyme mechanisms involved, and informations about genes encoding for these enzymes have been reported.[15,16] As far as composition is concerned , essential oils have a very high variability of their composition, both in qualitative and quantitative terms. Various factors are responsible for this variability and can be grouped into two categories

- Intrinsic factors related to the plant, and interaction with the environment (soil type and climate, etc.) and the maturity of the plant concerned, even at harvest time during the day,
- Extrinsic factors related to the extraction method and the environment.

The factors that determine essential oil yield and composition are numerous. In some cases, it is difficult to isolate these factors from each other as they are interrelated and influence each other. These parameters include the seasonal variations, plant organ, and degree of maturity of the plant, geographic origin, and genetics[17,18,19]. Several techniques are used for the trapping of volatiles from aromatic plants. The most often used device is the circulatory distillation apparatus described by Cocking and Middleton[20] introduced in the European Pharmacopoeia and several other pharmacopoeias. This device consists of a heated round-bottom flask into which the chopped plant material and water are placed and which is connected to a vertical condenser and a graduated tube, for the volumetric determination of the oil. At the end of the distillation process, the essential oil is separated from the water phase for further investigations. The length of distillation depends on the plant material to be investigated. It is usually fixed to 3–4 h. A further improvement was the development of a simultaneous distillation–solvent extraction device by Likens and Nickerson in[21]. The device

permits continuous concentration of volatiles during hydrodistillation in one step using a closed-circuit distillation system.

1.3.1 Chemical constituent of essential oils

Essential oils consist of chemical compounds which have hydrogen, carbon and oxygen as their building blocks.[22] They can be essentially classified into two groups:

-Volatile fraction of essential oil: these constitutes 90–95% of the oil in weight, and consists of the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

-Nonvolatile residue : 1–10% by weight of the oil and containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids. However, the properties of these components can change. For example, the components from the oils extracted from plants can change according to how when and where these plants are grown and harvested. [23]

Essential oil constituents can be also be subdivided into 2 groups including: hydrocarbons which are made up of mostly terpenes and the oxygenated compounds which are mainly alcohols, aldehydes, esters, ketones, phenols and oxides.

a- Alcohols

Alcohols are present either as a free compound or combined with a terpene or ester and are found is found in many plants.Examples including linalool, geraniol in geranium and palmarosa and citronellol found in rose, lemon and eucalyptus.

Alcohols are generally considered safe and have a very low or totally absent toxic reaction in the body or on the skin and so can be used on children. They are extremely useful due to their antiviral, antibacterial and antiseptic properties.

b- Aldehydes

Aldehydes are present as citral in lemon, citronellal in lemongrass, lemon balm and citrus eucalyptus. Other known aldehydes include benzaldehyde, cinnamic. aldehydes and peril aldehyde. Essential oils containing aldehydes are helpful in treating inflammation, *Candida* and viral infections. Some aldehydes are anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, and sedative. [23]

c- Hydrocarbon

Building blocks of essential oils are hydrogen and carbon. Basic Hydrocarbon found in plants is isoprene.

d- Terpenes

Some examples of terpenes are : limonene, pinene, piperene, camphene . Terpenes may possess antiinflammatory, antiseptic, antiviral and anti-bactericidal properties.

Terpenes are grouped into: monoterpene, sesquiterpene, diterpenes, triterpenes and polyterpenes. When two of the isoprene units are joined head to tail, the result is a monoterpene, when three are joined, it's a sesquiterpene and similarly four linked isoprene units are diterpenes. [24]

i-Monoterpenes

The majority of monoterpenes are unsaturated hydrocarbons (C_{10}). Oxygenated derivatives of monoterpenes such as alcohols, ketones, and carboxylic acids are known as monoterpenoids. Limonene which is consisting of two isoprene units is an example of a monoterpene. These C_{10} hydrocarbons are widely distributed in nature with more than 400 naturally occurring monoterpenes. Moreover, besides being linear derivatives (geraniol, citronellol), the monoterpenes can be cyclic molecules (menthol – monocyclic; camphor – bicyclic; pinenes .

The monoterpene thujone is the toxic agent found in *Artemisia absinthium*(wormwood) . Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil is used as a disinfectant and deodorant. Camphor is used as a counter -irritant, anesthetic, expectorant, and antipruritic, among many other uses.

ii- Sesquiterpenes

Sesquiterpenes constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury. These are having properties like anti-inflammatory, anti-septic, analgesic and anti-allergic.

The C_{15} sesquiterpenes are biogenetically derived from farnesyl pyrophosphate and in they could be linear, monocyclic or bicyclic.

iii- Sesquiterpene Lactones

Sesquiterpene lactones have proved to be of interest from chemical and chemotaxonomic point of view, but also possess some biological potential including: antitumor, anti-leukemia, cytotoxic and antimicrobial activities.

Sesquiterpene lactones may be classified according to their carboxylic skeletons thus, guaianolides, pseudoguaian olides, eudesmanolides, xanthan olides etc. can be derived from the germacran olides. Structural features of all these compounds are associated with much of the biologic activity. For example, beta-caryophyllene in basil and black pepper

iv- Diterpenes

Isoprene has been an integral part in most of the components as there are four isoprene units in diterpenes. These molecules are too heavy to allow for evaporation, so they are rarely found in distilled volatile oil. Almost all plant families contain diterpenes. There are about

2500 known diterpenes that belong to 20 major structural types. Derivatives of diterpenes are plant hormones gibberellins and phytol occurring as a side chain on chlorophyll. Biosynthesis of these phytochemicals occurs in plastids and mixtures of monoterpenes and diterpenes are the major constituents of plant resins. Diterpenes arise from metabolism of geranyl pyrophosphate (GGPP). Diterpenes are used in certain sedatives (coughs) as well as in antispasmodics and anxiolytics.

v - Acids

Generally Organic acids are found in very small quantities in their free state within. Essential Oils Plant acids act as components or buffer systems to control acidity. These also act anti-inflammatory. Examples are cinnamic and benzoic acid in benzoin, Citric and lactic.

vi- Esters

Natural esters are used medicinally for their soothing, balancing effects. They are known to possess antimicrobial potential. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system.

Natural esters generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic.[25]

vii- Ketones

Essential oils containing ketones are used for promoting wound healing .Ketones found in plants are also used for upper respiratory complaints. They assist the flow of mucus and ease congestion. However some ketones are toxic. The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil.

viii- Lactones

Lactones-containg essential oils possess anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis . They also exert expectorant action. Lactones have an even stronger expectorant action than ketones. [25]

1.3.2 Methods of extraction of essential oils

1.3.2.1 Steam distillation

Steam distillation is widely used for extraction of essential oils especially for temperature- sensitive materials. For a long time, it has been a popular laboratory method for purification of organic compounds, but has become obsolete after emergence of vacuum distillation. However, steam distillation remains important in certain industrial sectors [26] During the process of steam distillation, water or steam is introduced into the distillation apparatus. The water

vapor carries small amounts of the vaporized compounds to the condensation flask, where the condensed liquid phase separate, allowing for easy collection. This process effectively allows for distillation at lower temperature, reducing the deterioration of the desire product, if the substances to be distilled are very sensitive to heat, steam distillation may be applied under reducing the operating temperature further. After distillation, the vapors are condensed. Usually the immediate product is two phase system of water and organic distillate allowing for separation of the compounds by decantation, partitioning or other suitable methods [27]. Steam distillation is also widely used in petroleum refineries and petrochemical plant where it is commonly referred to as steam stripping [28,29]. Also steam distillation is an important process for the separating fatty acids from a matrix and for treating crude products such as tall oils to extract and separate soaps and other commercially important organic compounds [30].

1.3.2.2 Hydrodistillation

Hydro distillation is another technique used for oil extraction. It is simple and oil quality is directly related to the skill of the operator, not only in managing the still but in selecting or preparing the raw material.

1.3.2.3 Vacuum distillation

Vacuum distillation is also used in extraction of essential oils. This process allows very accurate control of distillate since it can be adjusted according to the boiling points of various oil constituents

1.3.2.4 Enfleurage

Enfleurage is suitable for extracting flower oils. In this process the essential oil is absorbed on wax or fat and then recovering the oil by solvent extraction. Layers of flowers are laid on trays of specially prepared fat and the flower layers removed and renewed until fat is saturated. However, this process is highly labor intensive, but products are of extremely high quality.

1.3.2.5 Solvent extraction

During the process of solvent extraction, a solvent is passed through the plant material and the oil is obtained by evaporation of the solvent. It can take place under normal atmospheric condition, in a partial vacuum or in the presence of gas. Commercial plants used batch, battery or continuous flow system, single or multi-solvent techniques, and include solvent recovery and oil refining equipment. These plants are generally expensive to construct and operate and are frequently located in developed countries using dried material. Since solvent extraction removes volatile and non-volatile constituents, composition of the oil obtained can differ significantly from distilled oil, and may contain undesirable components requiring removal. The solvent used frequently influences the oil

obtained as a residue or odor moderate, but solvent extracted oils are generally considered to reflect a plants natural odor more accurately than distilled oils. Commonly used is petroleum ether, hexane, toluene or other binary solvents.

1.3.2.6 Gaseous extraction

Extraction by liquid carbon dioxide has been used successfully in extraction of essential oils. In this process CO_2 which is under pressure and regulated temperature, is passed through the raw material, then via a separator to recover oil. This method of extraction is considered superior to liquid solvent, since it preserves important heat -sensitive components and requires less energy. Besides that, carbon dioxide is safe, non-combustible, odorless, tasteless, inexpensive and readily available which are ideal properties for an extraction solvent, while its low viscosity enables it to penetrate the material being extracted and its latent heat of evaporation allows it to be removed without residue [31].

1.4 Biological activity of essential oils

1.4.1 Antimicrobial and antioxidant activity

It has been reported that essential oils and their constituents can exert antimicrobial potential [32,33] . Some mechanism of antimicrobial potency have been studied in detail[34]. One of the features of essential oils is their hydrophobicity.This property allow

essential oils to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable [35]. This can then cause leakage of ions and other cellular molecules [36,37,38,39]. Greater loss of cell contents or critical output of molecules and ions can lead to cell death [40]. It is well established now that essential oils or their constituents can have a single target or multiple targets of their activity. For instance, trans-cinnamaldehyde can inhibit the growth of *Escherichia coli* and *Salmonella typhimirium* without disintegrating the OM or depleting intracellular ATP. Similar to thymol and carvacrol, trans-cinnamaldehyde likely gains access to the periplasm and deeper portions of the cell [41]. Carvone is also ineffective against the OM and does not affect the cellular ATP pool. [42] It has been reported that the essential oils : cinnamaldehyde, citral, carvacrol, eugenol and thymol are characterized by the highest antibacterial activity, then comes essential oils containing terpene alcohols. Those essential oils which are containing ketones or esters, such as β-myrcene, α-thujone, or geranyl acetate, had much weaker antibacterial activity, while volatile oils containing terpene hydrocarbons were usually inactive [43,44]. Essential oils usually contain a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, having important antibacterial activities. [4,26,28] These compounds are responsible for the disruption of the cytoplasmic membrane, the

driving force of protons, electron flow, active transport, and also coagulation of cell contents. [3540,46] The mode of action of essential oil as antibacterial is largely dependant on the chemical structure of the essential oil. [45] The vital role of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, was confirmed. [39,45,47] It has been established that the relative position of the phenolic hydroxyl group on the ring does not appear to influence the intensity of the antibacterial activity. The inhibitory effect of thymol against *bacillus cereus*, *staphylococcus aureus,* and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol, for example.[34,39] However, carvacrol and thymol act differently against Gram-positive and Gram-negative species. [45] Thymol, eugenol, and carvacrol have an antimicrobial effect against : *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica*, Clostridium *jejuni, Lactobacillus sake, Staphylococcus aureus, and Helicobacter pyroli*. [48,49] Valuable antibacterial constituents of essential oils include: certain alcohols, aldehydes, and ketones, monoterpene (geraniol, linalol, menthol, terpineol, thujanol, myrcenol, citronelîaî, neral,thujone, camphor, carvone,etc.) ,phenylpropanes (cinnamaldehyde), and monoterpenes (γ-terpinene, p-cymene). Among these compounds, carvacrol is the most active. Known to be non-toxic, it is used as a preservative and food flavoring in drinks, sweets, and other preparations.

It has been noted that essential oils are more active against Gram-positive than Gram-negative bacteria. [50,51,52,54 ,55] The latter are less susceptible to the action of essential oils with the outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide film. [53] Furthermore, the antibacterial activity of essential oils is related to their chemical composition, the proportions of volatile molecules, and their interactions.[45,50,56] .A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects. [56] Some studies have concluded that the use of the whole essential oil provides an effect which is greater than that of the major components used together. [57 58] The combined effects of plant volatile oils and benzoic acid derivatives against *L. monocytogenes* and *S. enteritidis* are considered as synergistic since the combined components allowed ≥log10 higher inhibition than the sum of the inhibitory effects of the components used separately. [59] Increased antifungal effects were caused by combinations (1:5, 1:7, and 1:9) of essential oils of *S. aromaticum* (clove) and *Rosmarinus ficinalis* against C. albicans. [60]It has been reported that [34] combined, carvacrol and thymol showed additive effects against *S. aureus* and *P. aeruginosa* by using half-fold dilutions within the Bioscreen plat. Synergistic effects of cinnamaldehyde/thymol or cinnamaldehyde/carvacrol against *S. typhimurium* has been

explained as follows: thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell, and, on the other hand, that thymol or carvacrol could increase the number, size, or duration of the existence of the pores created by the binding of cinnamaldehyde to proteins in the cell membrane. [61] These facts justify a synergistic effect achieved when these two components are used in combination.However mechanisms of interaction that produced antagonistic effects were less studied. [62] It has been documented that essential oils are effective on the inhibition of growth and reduction in numbers of the more serious foodborne pathogens, such as *Salmonella* spp., *E. coli* , and *Listeria monocytogenes* .[59]Another important property of essential oils is their antioxidant potential.Numerous numerous have demonstrated the antioxidant properties of essential oils. It seems that the antioxidant activity of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties. [63] Most of the essential oils are dominated by oxygenated monoterpenes such as alcohols (*Achillea filipendulina*), aldehydes (*Galagania fragrantissima*), ketones (*Anethum graveolens, Artemisia rutifolia, Hyssopus seravschanicus, Mentha longifolia, and Ziziphora clinopodioides*), and esters (*Salvia*

sclarea). *Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons, whereas phenolic terpenoids, such as thymol or carvacrol, characterize *Origanum tyttanthum* and *Mentha longifolia* essential oils, which would explain why both plants exhibited generally the strongest antioxidant activity. It has been shown that thymol and carvacrol, which are predominant in *Origanum tyttanthum*, are also responsible for the antioxidant activity of several other essential oils, such as that of *Mentha longifolia* and *Thymus serpyllus* .[64] Cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme essential oils are characterized by the most important antioxidant properties. [43] Thymol and carvacrol are endowed with significant free radical scwvenging property. Their activity is related to their phenolic structure. Essential oils phenolic compounds have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition. [57] It has been reported that the antioxidant activity of essential oils could be due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes like linalool, 1,8-cineoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: α-terpinene, β-terpinene and α-Terpinolene. [60]

It has been noted that essential oils with significant scavenging capacity of free radicals may play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and

immune system decline. In fact, these diseases may result from cellular damage caused by free radicals.[60,61] Essential oils also act as hepatoprotective agents in ageing mammals and it has been proved that they possess a beneficial impact upon the PUFAs, in particular the long chain C20 and C22 acids. [65] Moreover, essential oils being able to scavenge free radicals may also play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and immune system decline. [66] .The antioxidant activity of of *Zataria multiflora* Boiss. (Lamiaceae) essential oil in rats [67]. Antioxidant activity was measured by the test of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition .The authors reported that all *Zataria multiflora* oils ZMO tested doses were able to scavenge DPPH radical ($p < 0.05$). Moreover, ZMO decreased TBARs in a dose-dependent manner. No alteration in liver function test LFT enzymes or changes in histopathology of the liver was considered in ZMO treated groups. The results indicated that ZMO might be used in human healthy and food industry. [67] It has been demonstrated that [68] essential oil of *Wedelia chinensis* (Osbeck) increases both the level of catalase and glutathione peroxidase in the lung and liver tissues.

1.5 Methods of analysis of essential oils

1.5.1 Chromatography

The separation technique - chromatography is a very specific and selective separation technique, utilizing the small differences in the distribution of each component between two phases: the stationary and the mobile phase. It is thus used for the separation of closely related compounds in mixture and also to separate widely different compounds [69].

Separation techniques combined with mass spectrometry are important enhancement to the mass resolving and mass determining capabilities of mass spectrometry where it is used in tandem with chromatographic, and other separation techniques[70].

1.5.2- Liquid chromatography

Liquid chromatography- mass spectrometry (LC\MS) separates compounds chromatically before they are introduced to the ion source and mass spectrometer. It differs from (GC\MS) in that the mobile phase is liquid which is usually a mixture of water and an organic solvent, instead of gas.

Most commonly electrospray ionization source is used in (LC\MS).

Other popular and commercially available (LC\MS) ion sources are atmospheric pressure chemical ionization and atmospheric pressure photoionization.

There are also some newly developed ionization techniques like laser spray [71]

1.5.3 Capillary electrophoresis – mass spectrometry

The technique of capillary electrophoresis-mass spectrometry (CE-MS) combines the liquid separation process of capillary electrophoresis with mass spectrometry [71]. (CE\MS) is typically copied to electrospray ionization [72].

1.5.4 Gas chromatography-mass spectrometry

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

- drug detection

-fire investigation

-environment analysis

-explosive investigation

-identification of unknown samples

- airport security

This powerful analytical tool can identify trace elements in materials that were previously thought to have disintegrated beyond identification. This technique allows analysis and detection even of tiny amount of substance. Since (GC-MS) is used to perform 100% specific test which positively identifies the presence of particular substance, then it is used as a gold standard for forensic substance identification [70]. The need to unequivocally identify the constituents of complex matrix was the motivation for the development of different instrumental coupling techniques (tandem), including the widely and successfully used gas chromatography (GC) coupled with mass- spectrometry (MS). This technique is an extremely favorable, synergistic union, as the compounds susceptible to be analyzed by GC (low –molecular weight, medium or low polarity in ppm concentration) are also compatible with the MS requirements. Besides both analyses proceed in the same aggregation state (vapor phase). However, the only conflict (short term and already resolved) between GC and MS were the different working pressure, for example atmospheric at the GC column exit and low in the ionization chamber, respectively. This drawback was overcome by technically introducing an efficient vacuum pump (turbo molecular and gas-jet pumps) and above all due to the introduction of gas chromatography capillary columns (internal diameter 0.18 to 0.32 mm) [73] [74].

1.5.5 Mass spectrometry

Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions on the basis of their mass to charge ratio in simpler terms, a mass spectrum measures the masses within sample. The technique is used in many different fields and is applied to pure samples as well as complex mixtures. Mass spectrum can be defined as a plot of the ion signals as a function of the mass –to-charge-ratio. These spectra are used to determine the elemental or isotopic signature of a sample as well as the masses of particles and of molecules, and to elucidate the chemical structure of molecules, such as peptides and other chemical compounds. In a typical MS procedure, a sample, which may be solid, liquid or gas, is ionized, for example by bombarding it with electrons. This may cause some samples molecules to break into charged fragments. The fragmented ions are then separated according to their mass-to-change ratio, typically by accelerating them and subjecting them to an electric or magnetic field. [75]

1.6 Examples of essential oils different Sudanese plants

2.6.1 *Zingiber officinale*

Ginger (Zingiber officinale Rosco) possesses various medicinal properties including antioxidant, anti-inflammatory, anticancer and antimicrobial activities and could also be used as a spice in food Processing, [76.77] which has been widely used in food, medical and

cosmetic industries [78]. Its diethyl ether extract contains 95% of terpenes, including zingiberene, sesquiphellandrene or geranial. However, gingerols and shogaols are the main constituents of hydrophilic extract. **[79]** As reported, GEO has exhibit strong antimicrobial, antifungal and antioxidant activities. [80,81] Although the medical value of GEO was recognized long time ago, the study of ginger is still at the primary stage and the deeply processed products of ginger are still not available in market. Chemical constituents of essential oil of fresh ginger are: zingiberene (20%) which was followed by geraniol (7.3%), beta-bisabolene (5.5%), ar-curcumene (4%), nerol, betasesquiphellandrene, trans beta-bisabolene (3.5%), limonene (2.3%) (primacy fragment among the monoterpenes), beta-farnesene and cis-nerolidol (2%) (foremost in the class of oxygenated sesquiterpenes) The beta- farnesene were identified better (2%) than methanolic leaves extract of *Elettaria cardamomum* (cardamom) (0.08%), the queen of spices for cooking which belongs to the same Zingiberaceae family.**[82]** The results of Aziz et al., [83] were implied that 12, 11 and 10 chemical constituents were fractioned in the GC - MS essential oil of Japan, Bangladeshi and China gingercategorically.Thmajor components of the essential oil of ginger from Ghaziabad region were zingiberene (46.71%), citronellyl n-butyrate (19.34%), valencene (7.61%) β-phellandrene (3.70%), β-funebrene (3.09%), camphene (2.59%), α-

pinene (1.09%) and selina-4(14),7(11)-diene (1.03%). Zingiberene could be employed as natural food preservatives, preventing lipid peroxidation (at concentration levels 20-100 μg/ml), which could cause food spoilage.

1.6.2 Jatropha curcas

Jatropha curcas is a species of flowering plant species in the spurge family, Euphorbiaceae, that is native American tropics, most likely Mexico and Central America.[84] It is originally native to the tropical areas of the Americas from Mexico to Argentina, and has been spread throughout the world in tropical and subtropical Regions, the world becoming naturalized or invasive in many areas.[97] The specific epithet, "curcas", was first used by Portuguese doctor Garcia de Orta more than 400 years ago.[86] Common names in English include physic nut, Barbados nut, poison nut, bubble bush or purging nut.[85] In parts of Africa and areas in Asia such as India it is often known as "castor oil plant" or "hedge castor oil plant",[85] but it is not the same as the usual castor oil plant, Ricinus communis (they are in the same family but different subfamilies). J. curcas is a semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft.) or more.[84] It is resistant to a high degree of aridity, allowing it to grow in deserts.[5][6] It contains phorbol esters, which are considered toxic[87 88]. The seeds contain 27–40% oil[89] (average: 34.4%[90])

that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine. Edible (non-toxic) provenances can be used for animal feed and food [91] [87] .The oil contains 43 (61.43%). Among the identified compounds, 16 had a content of more than 1%, and the total contents of these 16 compounds reached 81.36%. The four most abundant components were 22,23-dihydro-stigmasterol (16.14%), alpha-tocopherol (15.18%), beta-amylin (7.73%) and dotriacontanol (7.02%). The content of gamma-tocopherol reached 2.88% and vitamin E reached 18.06% in the extract. [92]

1.7 The target plant species

1.7.1 Solanum dobium

Solanum dobium one of the most popular plant known and wildly spread.Worldwide in the eastern Europe and western Asia. Small S. dubium is found in east and west of Sudan. Solanum dobium belonging to the family Solanaceae, locally known as Gubbain, and used in rural aeas for milk coagulation. the second species of the gubbain is bigger in size similar to peace of citrus (lemon)in the same properties It is a bushy pubescent herb grown widely in northern, central and western Sudan along with other species such as S. innacum, S. esculentum, S. macrocarpon and S. melongena Research on S. dubium was focused mainly on obtaining Solanum

crude enzyme (Renen) from the seeds in pure form and commercial production of the enzyme for cheese making [93]

Herbal medicines have been extensively used in developed countries hence they are natural and relatively safe. [94] They contain plant materials as their pharmacologically active components Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants According to the World Health Organization about 80% of the world's population living in developing countries relies essentially on plants for primary health care. [95] World Health Organization (WHO) defines traditional medicine as the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being [96] (WHO, 2008). Medicinal plant used for cheese coagulating in Sudan The species of the family Solanaceae are medicinal herbs and contain unique alkaloids and other biochemical constituents used for the treatment of diverse ailments such as diabetes, cholera, bronchitis, high blood pressure and as laxatives. [97]Scientific classification of *Solanum dubium* in table (1).

table 1:Taxanpmy of s.dobium

Kingdom	Plantae

Family	Solanaceae
Genus	Solanum
	

Solanum dubium plant

1.7.2 *Martynia annua*

Martynia annua is a monotypic genus in the Martyniaceae family consisting of a single species, *Martynia annua* L., which is commonly known as cat's claw, tiger's claw, or ice plant.[98 However, the name 'ice plant' may also refer to members of the unrelated plant family Aizoaceae. It is native to Mexico, central America and the Caribbean, and has been introduced throughout the tropics.[98][99] It is now quite common in rural areas of Africa. *Martynia annua* is used for making beads and ornaments,[98] and has a history of folks medicine uses in the Indian subcontinent. It is an erect, somewhat shrubby annual plant about 1 metre (3.3 ft.) tall, covered with glandular hairs, and has ovate, mucilaginous leaves 8–20 centimetres (3.1–7.9 in) wide and 6–19 centimetres (2.4–7.5 in) long. The leaves are opposite, and have red petioles. They resemble

sticky rhubarb. Its flowers are pale pink and tubular, and have nectar guides and purple spots. Fruits become blackened when ripe and have hooked spines at the tip, lending its name "cat's claw" or "tiger's claw".[100] They stick to animal fur and eventually the seeds fall out as the fruit gets crushed by the animal's feet. Scientific classification of *Martynia annuais* given in in Table (2)

Martynia annua

Table1.2: Scientific classification of *Martynia annuais*

Kingdom	Plantae
Order	Lamiales
Family	Martyniaceae
Species	M. annua
Binomial name	*Martynia annua* L

*M. annua i*s often grown as a medicinal herb. In South-eastern Asia and India, it has been present long enough to become part of the traditional pharmacopoeia.

Traditional healers use it for treating epilepsy, inflammation, sore throat, skin affections and tuberculosis.[101].

1.7.3 *Physalis angulata* L

Physalis angulata L. belongs to the family of Solanaceae, and it is frequently used as traditional medicine in China. It generally grows in valleys or country roadsides at an altitude of 500–5000 m. [102] The whole herb of *Physalis angulata* L. has been widely used to relieve inflammatory conditions, diabetes, anemia and cancer. [103] Its main constituents include withanolides, terpenoids, carotenoids, flavonoids and polysaccharides [104]. The medicinal plants of the genus *Physalis* are known to produce withanolides, which structurally have an ergostane skeleton. [105] Most withanolides are polyoxygenated and their structures can be divided into two types of lactone, lactol and -lactone based on the differences between their substituted groups at C-17 side chain.

Most withanolides isolated from the genus *Physalis* belong to the -lactone/lactol type, which have different modified skeletons, such as physalins, neophysalins and withaphysalins. These modified withanolides have the characteristic groups of 5-6 epoxides, 5-ene withanolides and 6-7 epoxide [106]. More than100 withanolides containing the modified skeletons were isolated from *P. alkengi, P. pubescens* and *P. angulata.* [107–108] Withanolides have multiple pharmacological effects, such as antitumor, antistress,

immunosuppressive, anti-microbial and anti-inflammatory activities.

Physalis is a widely used for dealing with hepatitis, malaria, rheumatism, cancer, dermatitis and asthma. [109 - 110] the species are used for treating asthma, urinary problems, rheumatism and tumor. [111] the scientific classification *of Physalis angulate* in table (3)

Table (3) Physalis angulata

Kingdom	Plantae
Order	Solanales
Family	Solanaceae
Genus	*Physalis* L.

Physalis angulate

1.7.4 Abutilon Pannosum

Abutilon pannosum (family Malvaeae) is commonly known as Kanghi or khapat [112] Its extract is used for getting rid of thirst, curing bronchitis, dysentery, gonorrhea, diarrhea, inflammation of the bladder, reducing fever and various other diseases. [113.114] Abutilon pannosum play a vital role in medicine and essential and integral part in complementary and alternative medicine and due to this they develop the ability for the formation of secondary metabotite like flavonoid alkaloids, steroids and phenolic substance which are in turn used to restore health and heal many diseases. Natural product of plant and animal origin after vast resource of newer medicinal agent with potential in clinical use plant used for traditional medicine contain a wide range of substances that can be used to treat chronie as well as infections disease (Duraipandiyan et al., 2006). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against various diseases. Today, the commercially available antibiotics are becoming ineffective against the pathogens as they develop resistance to it. [115] Abutilon is used in local medicines for the treatment of various ailments. Among this, Abutilon pannosum, is an under shrub and is distributed in India, Pakistan, Tropical Africa, China and Arabia. We were focused in kachchh district region

species. The leaves of pannosum were used as adjunct to medicines used for stack complaints. The plant contains mucilage, tannins asparagines, Gallic acid and sequiterpens. Its extract is also used in relieving thirst, in treating bronchitis, diarrhea, gonorrhea and inflammation of the bladder and in reducing fever. In addition, it is used in cleaning wound and ulcer treating vaginal infection, diabetics, hemorrhoids and can also use as an anemia. [116] the scientific classification *Abutilon pannosum* in table (4).

table 4:Taxanpmy of *Abutilon pannosum*

Kingdom	Plantae
Family	Malvaceae
Genus	Abutilon
Species	*Abutilon pannosum*

Abutilon pannosum

1.7.5 *Argemone Mexicana*

Argemone Mexicana is herbal drug with various applications[117] Majority of seeds normally do not germinate during their first season after shedding, but instead enter into the seed bank, thus producing seedlings, even in a well- maintained field, probably for several years.[118] Argemone Mexicana (family: Papaveraceae) is one of the important medicinal plant which naturally occurs in various countries like India, Australia, South Africa and other parts of the world. They are adapted to a wide variety of habitat and tend to grow best in soil of low fertility. It has different chemical constituents like oxysanguinarine, reticuline, columbamine etc. and have several pharmacological activities, among these the anticancer activity have facilitated the extent of further research for finding out better treatment of disease.

Medicinal properties have been attributed to the sap and oil from the seed. In the Guianas the whole plant is used as an infusion against asthma. The root is taken for stomach pain. Sap from the cut end of the stem is applied to cavities as a treatment for toothache. Children having difficulty with urination are given infusions of petals. In India (Madhya Pradesh) it is reported to be a homeopathic drug. In

West Africa it is used as a cosmetic. The traditional use of the plant in management of cancer [119] also contribute to the development of successful immune therapies of some carcinomas due to their apoptotic potential. [120] the plant has very good peripheral activity and significant analgesic activity in comparison to the Aspirin. [121] In India, *A. Mexicana* seeds are added to mustard oil in very small quantities, to increase its pungency. [122] *A. Mexicana* has wound healing activity in rats. [123]Scientific classification is shown below.

Argemone Mexicana

Table (5) scientific classification of *A. Mexicana*

Order	Ranunculales
Family	Papaveraceae

Genus	Argemone
Species	*A. Mexicana*

1.7.6 Leonotis nepetifolia (L)

Leonotis nepetifolia (L) R. Br commonly known as Lion's ear, has number of therapeutic properties and is also known as Christmas candlestic. It grows ahight of 3 meters and has whorls of striking lipped flowers, that are most commonly orange, but can vary to red, white, and purple. It has very soft serrated leaves that can grow up to 4 inches wide. Leonotis nepetifolia generally grows in patches along roadside or barren unused agriculture waste land during rainy season. The mature plant attains the height up to 2 meter. The orange yellow coroneted verticilaster inflorescence and distinct odour are amongst the unique characters of this plant. [124] The scientific classification of *Leonotis nepetifolia* in table (1.6)

Table1.6: scientific classification of *Leonotis nepetifolia*

Kingdom	Plantae
Family	Lamiaceae
Genus	Leonotis
Species	L. nepetifolia

The genus Leonotis has 12 species widely distributed in Pan Tropics and is represented by one species, Leonotis nepetifolia in India. It belongs to family Lamiaceae Leonotis nepetifolia is an economically important It has many therapeutic properties and proved in Madagascar, Brazil, Canada, Kenya and many African Countries to treat diseases, rheumatism, dysmenorrhea, bronchial asthma, fever, diarrhoea influenza and malaria and is also an analgesic. The decoction of the leaves is used to treat coughs, burns and skin ailments. The whole plant is used for menstrual pain and unspecified female complaints. This plant exhibited various pharmacological activities such as activity, antidiabetic, anticancer, anti-inflammatory, anticonvulsant, wound healing, hepatoprotective activity and Phytochemical examination of this plant indicated the presence of alkaloids (leonurine and stachydrene), iridoid glycoside (leonuride), iridoid glycosides (leonurin and leonuridine), diterpenoids (leocardin), flavonoids (rutin, quercetin, hyperoside, apigenin), volatile oil, tannins and vitamin A. Leonotis nepetifolia is highly therapeutic and is used in various Ayurvedic formulations. This article briefly reviews the pharmacological and various therapeutic aspect of Leonotis nepetifolia. [125] Leonotis nepetiifolia is also used as an analgesic, to treat fever, diarrhoea, bronchial asthma, malaria, influenza etc., The indigenous system of Medicine

in India is mainly practiced based on plants and India is not remorse in utilizing the plants in its medical system. [126]

Leonotis nepetifolia tree

Leonotis nepetifolia furit

Aim of this study

This study was designed to:

- Extract of oils from six medicinal plants.
- Conduct a GC-MS analysis to specify constituents of the oils.
- Evaluate the antimicrobial potential of the targeted plant species.

Chapter two

2- Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of salanum dubium Martynia annua Physalis angulata Abutilon pannosum candida albicans Argemone Mexicana were collected from the Damazin (Sudan) and identified by direct comparison with a herbarium sample.

2.1.2- Instruments

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

2.1.3-Test organisms

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

2.2-Methods

2.2.1-Extraaction of oils

Powdered plant material (400g)was exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil.

2.2.2-GC-MS analysis

The targeted oils were analyzed by GC-MS.A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length;0.25mm diameter;0.25um, thickness) was used.. Oven temperature program and other chromatographic conditions are shown below:

Table 2.1: Oven temperature program

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

Table 2.2 : Chromatographic conditions

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

2.2.3 Antimicrobial assay

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 ^{O}C for 24 hour.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended is 100 ml of normal saline to product a suspension containing about 10^8 - 10^9 Colony forming units per ml. The suspension was stored in the refrigerator at 4 ^{O}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 ^{O}C for 24 hours.

ii) Fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25 ^{O}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) - Antimicrobial test

The cup-plate agar diffusion method was adopted with some minor modification, to assess the antimicrobial 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 ^{O}C in a water bath. 920 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 o.1ml samples of each compound using adjustable volume micrometer pipette and allowed to diffuse at room temperature for two hours. The plate were then incubated in the upright position at 37^{o}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.

Chapter three

3.Results and Discussion

In this study six medicinal plants of potential medicinal attributes grown in Sudan has been investigated. The oils from these plant species have been analyzed by GC-MS and the antimicrobial potential of the oils has been evaluated *in vitro.*

3.1 *Solanum dubium*

Salanum dubium oil was 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.

Eighteen components have been detected by GC-MS analysis (Table 3.1).The typical total ion chromatograms (TIC) is presented in (Fig: 1***)***.

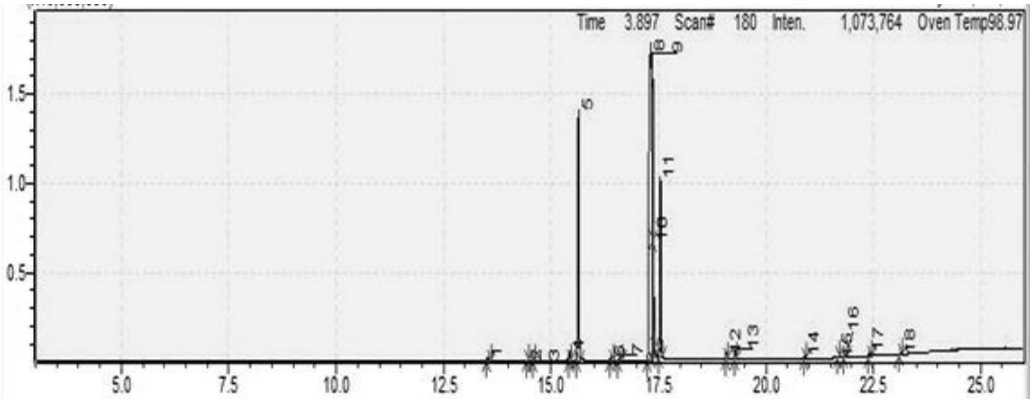


Fig.1: Total ions chromatograms

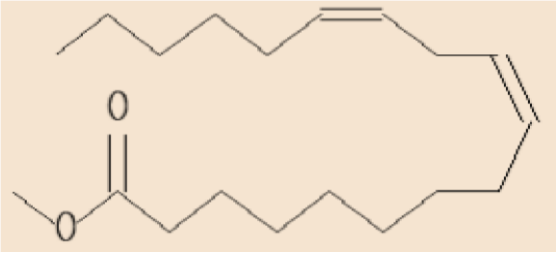
Table 3.1: Constituents of the oil

NO	Name	Ret.Time	Area%	Formula
1.	Methyl tetradecanoate	13.528	0.34	C15H30O2
2.	5-Octadecenoic acid, methyl ester	14.442	0.03	C19H36O
3.	Pentadecanoic acid, methyl ester	14.601	0.05	C16H32O2
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.432	0.61	C17H32O2
5.	Hexadecanoic acid, methyl ester	15.636	19.27	C17H34O2
6.	17-Octadecynoic acid	16.394	0.21	C19H34O2
7.	Heptadecanoic acid, methyl ester	16.602	0.32	C18H36O2
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.321	51.14	C19H34O2
9.	9-Octadecenoic acid (Z)-, methyl ester	17.361	10.17	C19H36O2
10	9-Octadecenoic acid, methyl ester, (E)-	17.385	3.31	C19H36O2
11	Methyl stearate	17.546	12.41	C19H38O2
12	cis-13-Eicosenoic acid, methyl ester	19.092	0.36	C21H40O2
13	Eicosanoic acid, methyl ester	19.293	0.70	C21H42O2
14	Docosanoic acid, methyl ester	20.914	0.24	C21H42O2
15	Tricosanoic acid, methyl ester	21.680	0.07	C24H48O2
16	beta.-Sitosterol	21.839	0.11	C29H50O
17	Tetracosanoic acid, methyl ester	22.418	0.48	C25H50O2
18	Squalene	23.151	0.18	C30H50

The mass spectra of major constituents of the oil are briefly discussed below:

i) 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(51.14%)

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.2. The peak at m/z294 with retention time 17.321 corresponds to the molecular ion M^+ $[C19\,H_{34}O_2]^+$ while the signal at m/z263 is due to loss of a methoxyl group.



9, 12-octadecadienoic acid (Z, Z)-, methyl ester

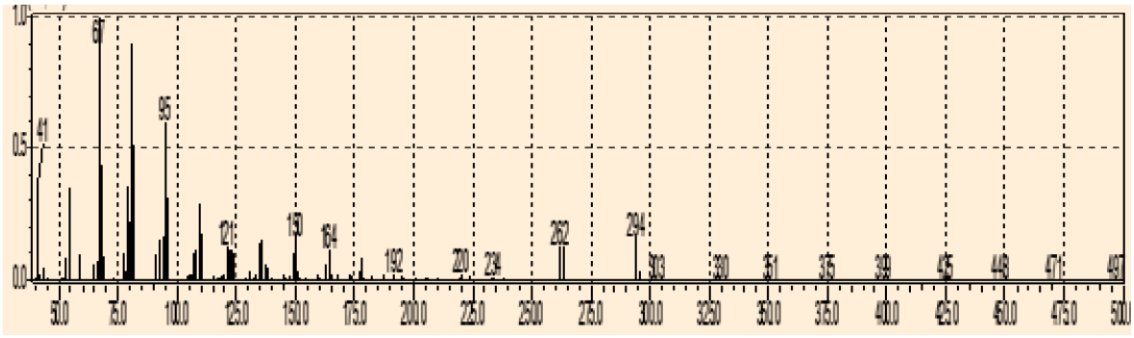
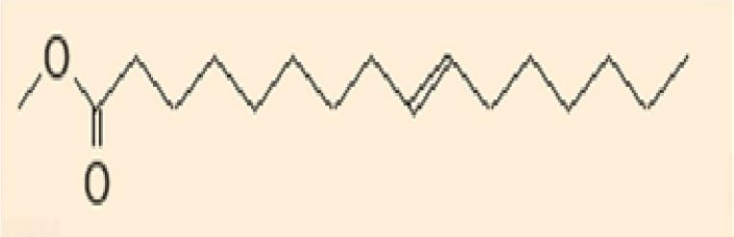


Fig.2: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

ii)- Hexadecanoic acid, methyl ester(19.27)%

Fig. 3 presents the mass spectrum of hexadecanoic acid, methyl ester. The peak at m/z270 with retention time 15.636 is due to the molecular ion M^+ $[C_{17}H_{34}O_2]^+$.



hexadecanoic acid, methyl ester

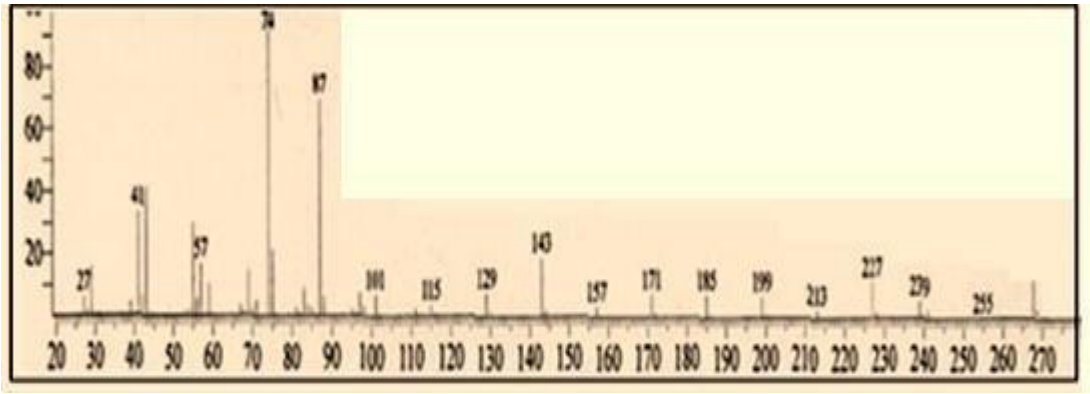
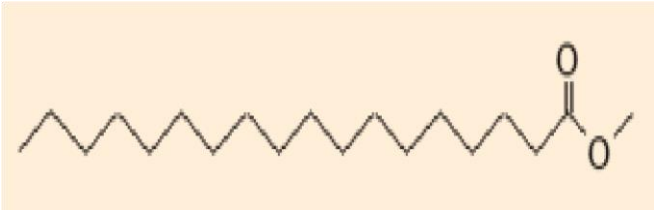


Fig. 3 :Mass spectrum of hexadecanoic acid, methyl ester

iii- Methyl stearate 12.45%

Fig.4 shows the mass spectrum of methyl stearate. The signal at m/z298 (retention time:17.546) is due to the molecular ion M^+ $[C_{19}H_{38}O_2]^+$.The peak at m/z267 is due to loss of a methoxyl .



Methyl stearate

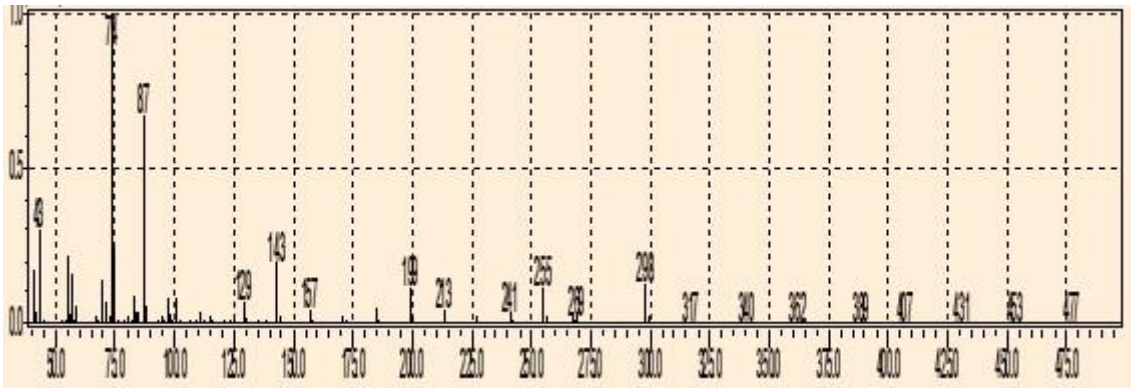
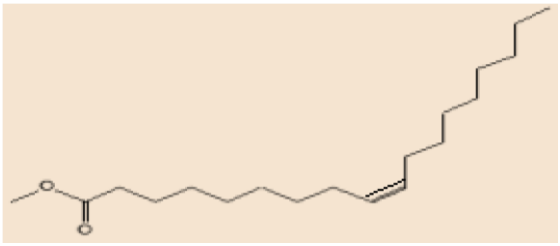


Fig.4 : Mass spectrum of methyl stearate

iv- 9-octadecenoic acid (Z)-, methyl ester (10.17%)

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.5. The peak at m/z 296 with retention time 17.361 accounts for the molecular ion M^+ $[C_{19}H_{36}O_2]^+$.



9-octadecenoic acid (Z)-, methyl ester

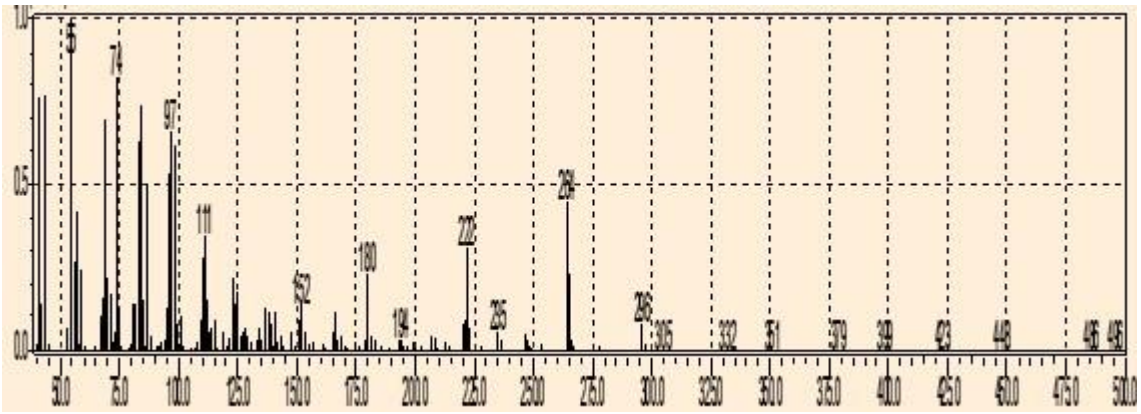


Fig. 5. 9-octadecenoic acid (Z)-, methyl ester

3.1.1 Antimicrobial activity

Salanum dubium seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table(3.2).The results were interpreted in the following manner:

(>9mm: inactive;9-12mm:partially active; 13-18mm: active; < 18mm:very active) .Tables(3.3) and (3.4) represent the

antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed significant activity against *Pseudomonas aeroginosa* . However, at the same concentration, it failed to exhibit inhibitory effect against other test organisms.

Table 3.2 Antimicrobial Activity of *Salanum dubium* seed oil

Oil	Antibacterial activity				
	Gram positive		Gram negative		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	--	6		18	--

Table 3.3 : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 3.4 : Antifungal activity of standard chemotherapeutic agent

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

3.2 *Martynia annua*

Martynia annua oil was 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.

3.2.1 Constituents of oil

Eighteen constituents have been detected by GC-MS analysis (Table 4.5).The typical total ion chromatograms (TIC) is presented in Fig.6.

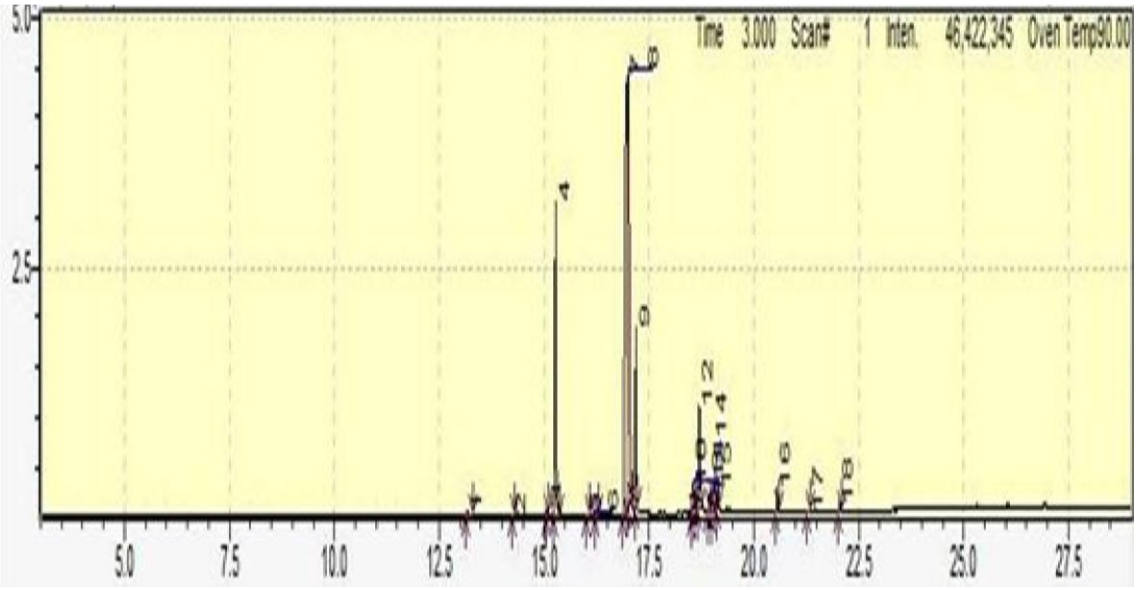


Fig. 6: Total ions chromatograms

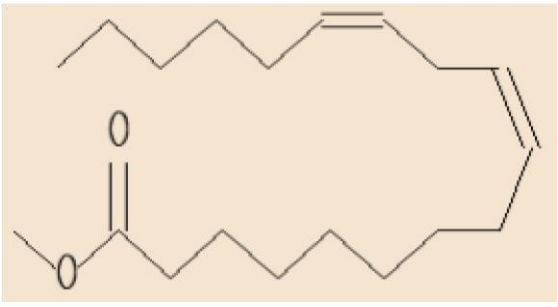
Table 3.5: Constituents of the oil

NO	Name	Rent time	Area%	Formula
1.	Methyl tetradecanoate	13.175	0.13	C15H30O2
2.	Pentadecanoic acid, methyl ester	14.246	0.03	C16H32O2
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.072	0.17	C17H32O2
4.	**Hexadecanoic acid, methyl ester**	**15.277**	**13.96**	C17H34O2
5.	cis-10-Heptadecenoic acid, methyl ester	16.033	0.06	C18H34O2
6.	Heptadecanoic acid, methyl ester	16.243	0.09	C18H36O2
7.	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**16.973**	**43.01**	C19H34O2

8.	**9-Octadecenoic acid (Z)-, methyl ester**	**17.014**	**25.26**	C19H36O2
9.	Methyl stearate	17.186	6.80	C19H38O2
10	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.535	1.23	C22H38O2
11	11,14-Eicosadienoic acid, methyl ester	18.574	1.04	C21H38O2
12	11,13-Eicosadienoic acid, methyl ester	18.701	4.39	C21H38O2
13	Eicosanoic acid, methyl ester	18.926	0.59	C21H42O2
14	2-Ethylbutyric acid, dodec-9ynyl ester	19.009	1.07	C18H32O2
15	8,11,14-Docosatrienoic acid, methyl ester	19.122	0.79	C23H40O2
16	Docosanoic acid, methyl ester	20.545	0.96	C21H42O2
17	Tricosanoic acid, methyl ester	21.308	0.05	C24H48O2
18	Tetracosanoic acid, methyl ester	22.045	0.37	C25H50O2

The mass spectra of the major components are discussed below:

i)-Octadecadienoic acid (Z, Z)-, methyl ester (43.01%)



9, 12-octadecadienoic acid (Z, Z)-, methyl ester

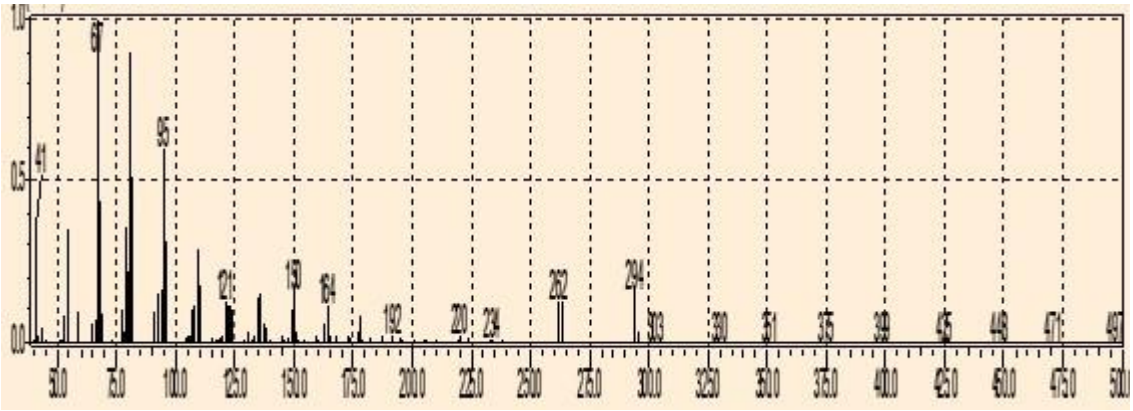
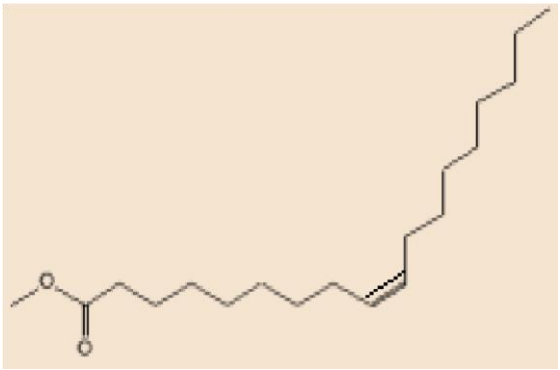


Fig.7: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

Fig. 7 shows the mass spectrum 9, 12-octadecadienoic acid (Z, Z)-, methyl ester. The signal at m/z 294(retention time 16.973) is attributed to the molecular ion $M^+ [C_{19} H_{34} O_2]^+$. **ii) 9-Octadecenoic acid (Z)-, methyl ester 25.26%**

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is depicted in Fig.8. The signal at m/z 296 with retention time 17.014 is due to the molecular ion $M^+ [C19 H_{36} O_2]^+$.



9-octadecenoic acid (Z)-, methyl ester

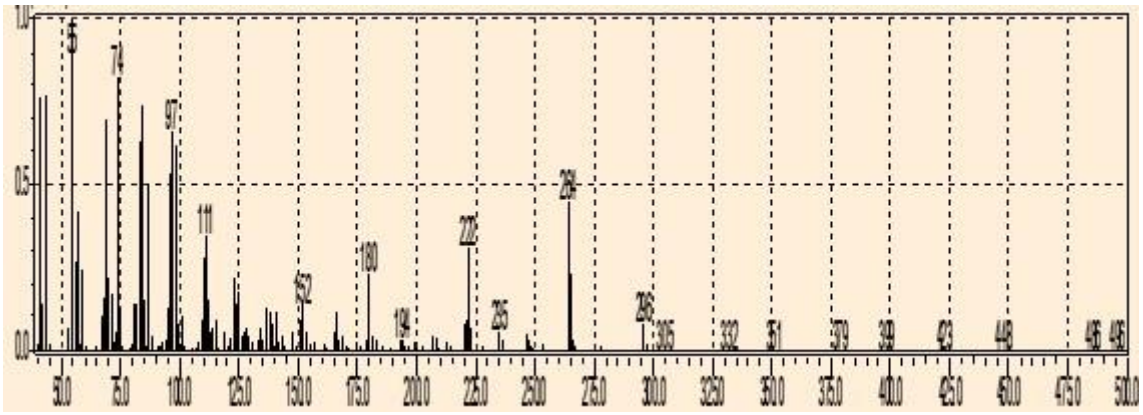
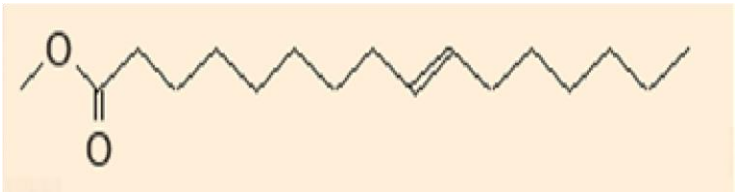


Fig.8 :. 9-octadecenoic acid (Z)-, methyl ester

iii)- Hexadecanoic acid, methyl ester (13.96%)

The mass spectrum hexadecanoic acid, methyl ester is illustrated in Fig.9.The signal at m/z 270 with retention time 15.277 is due to the molecular ion $M^+[C_{17}H_{34}O_2]^+$.



hexadecanoic acid, methyl ester

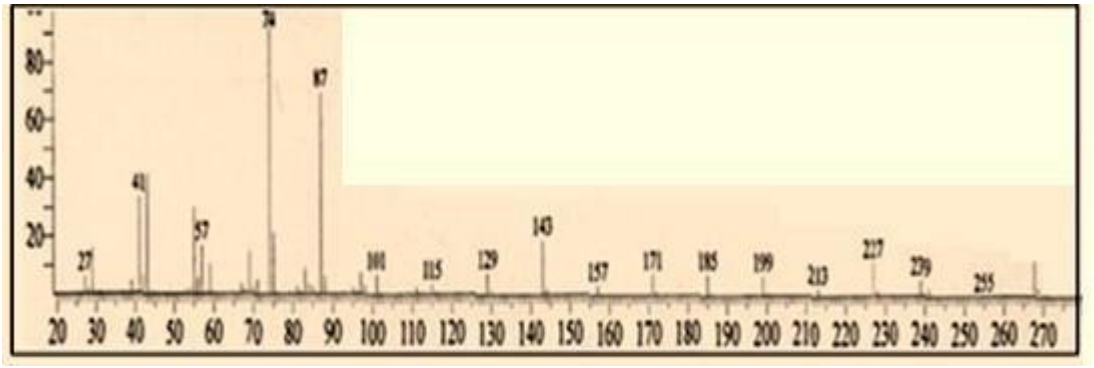


Fig.9 :Mass spectrum of hexadecanoic acid, methyl ester

3.3 *Physalis angulata*

The oil from *Physalis angulata* was subjected to GC-MS analysis. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the library.

3.3.1 Constituents of oil

Twenty-three components have been detected by GC-MS analysis (Table 3.6).The typical total ion chromatograms (TIC) is presented in Fig.10.

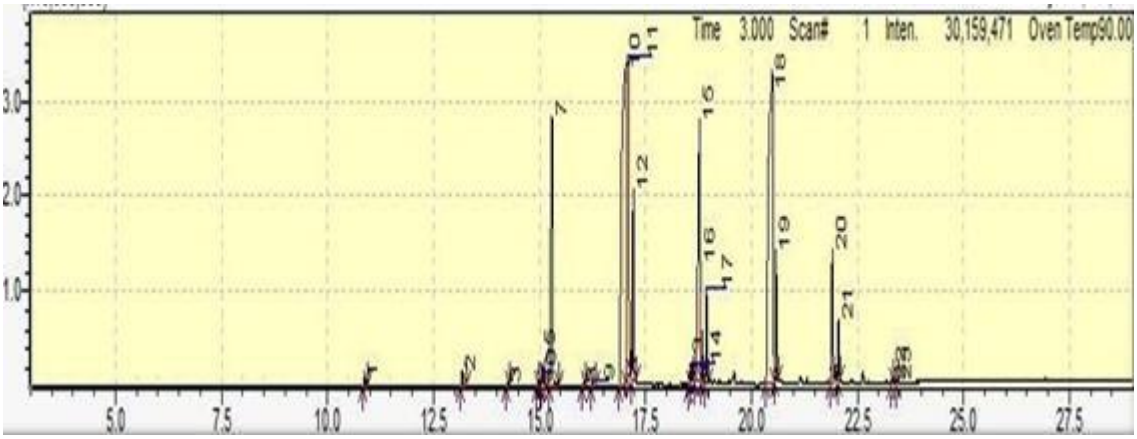


Fig.10 : Total ions chromatograms

Table 3.6: Constituents of the oil

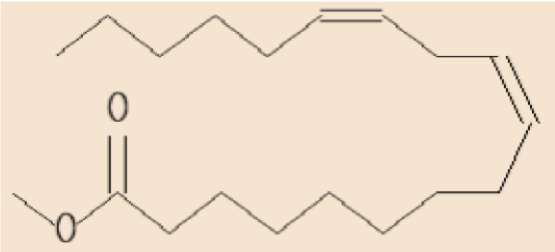
NO	Name	R.T	Area %	Formula
1.	Dodecanoic acid, methyl ester	10.865	0.24	C13H26O2
2.	Methyl tetradecanoate	13.172	0.31	C15H30O2
3.	Pentadecanoic acid, methyl ester	14.246	0.03	C16H32O2
4.	7,10-Hexadecadienoic acid, methyl ester	14.969	0.04	C17H30O2
5.	cis,cis,cis-7,10,13Hexadecatrienal	15.035	0.07	C16H26O2
6.	9-Hexadecenoic acid, methyl ester, (Z)-	15.071	0.33	C17H32O2

7.	**Hexadecanoic acid, methyl ester**	**15.296**	**8.24**	C17H34O2
8.	cis-10-Heptadecenoic acid, methyl ester	16.037	0.09	C18H34O2
9.	Heptadecanoic acid, methyl ester	16.245	0.11	C18H36O2
10	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**17.029**	**25.93**	C19H34O2
11	**9-Octadecenoic acid (Z)-, methyl ester**	**17.081**	**13.95**	C19H36O2
12	Methyl stearate	17.214	4.22	C19H38O2
13	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.541	0.32	C22H38O2
14	11,14-Eicosadienoic acid, methyl ester	18.581	0.38	C121H38O2
15	**cis-11-Eicosenoic acid, methyl ester**	**18.778**	**10.12**	C21H40O2
16	cis-13-Eicosenoic acid, methyl ester	18.810	1.59	C121H40O2
17	Eicosanoic acid, methyl ester	18.939	1.83	C21H42O2
18	**13-Docosenoic acid, methyl ester, (Z)-**	**20.489**	**25.34**	C23H44O2
19	Docosanoic acid, methyl ester	20.576	2.50	C21H42O2
20	15-Tetracosenoic acid, methyl ester, (Z)-	21.905	3.19	C25H48O2
21	Tetracosanoic acid, methyl ester	22.050	1.06	C25H50O2
22	cis-10-Nonadecenoic acid, methyl ester	23.311	0.06	C20H38O2
23	Hexacosanoic acid, methyl ester	23.443	0.05	C27H54O2

The mass spectra of the major constituents of *Physalis angulata* oil are discussed hereafter:

i) 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(25.93%)

Fig. 11 shows the mass spectrum 9, 12-octadecadienoic acid (Z, Z)-, methyl ester. The signal at m/z 294(retention time 17.029) is due to the molecular ion M^+ $[C_{19}H_{34}O_2]^+$.The peak at m/z263 accounts for loss of a methoxyl function.



9, 12-octadecadienoic acid (Z, Z)-, methyl ester

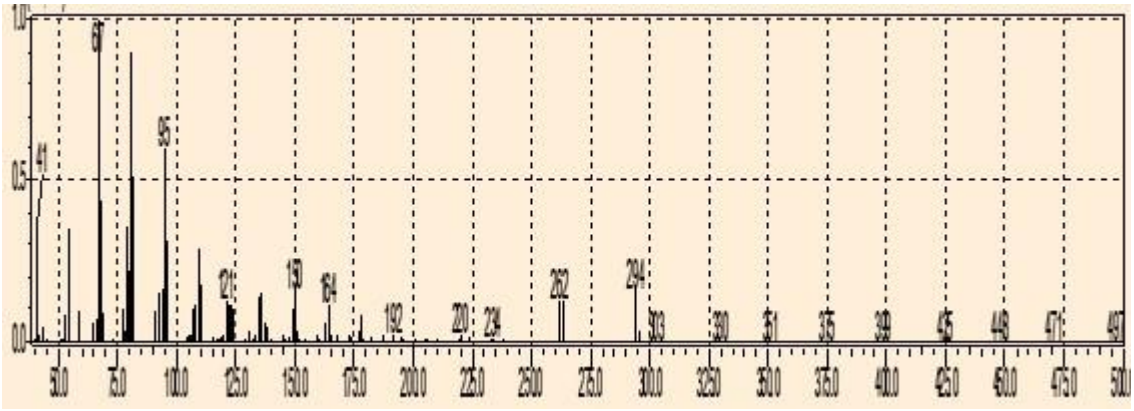
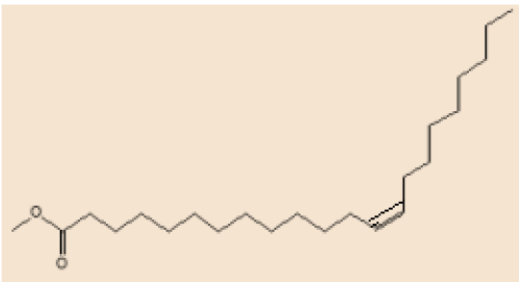


Fig.11: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

ii)13-Docosenoic acid, methyl ester, (Z) – (25.34%)

The mass spectrum of 13-docosenoic acid, methyl ester, (Z)-is shown in Fig.12 .The peak at m/z 352 (R.T. 20.489) is attributed to the molecular ion $M^+[\ C_{23}H_{44}O_2]^+$.



13-docosenoic acid, methyl ester

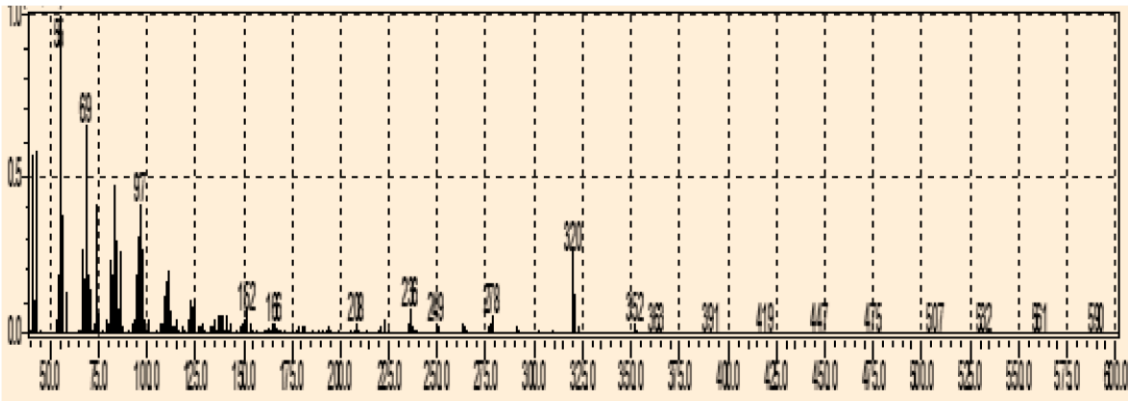
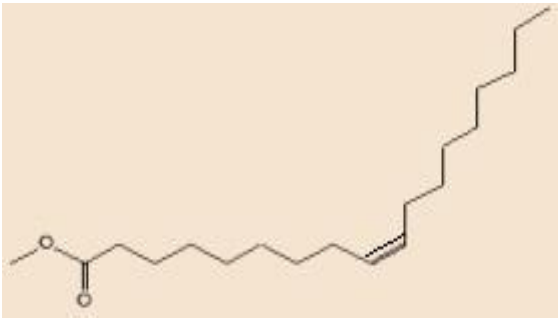


Fig.12: Mass spectrum of 13-docosenoic acid, methyl ester

iii) 9-Octadecenoic acid (Z)-, methyl ester (13.95%)

The mass spectrum 9-octadecenoic acid (Z)-, methyl ester is illustrated in Fig.13. The signal at m/z 296 with retention time 17.081 is due to the molecular ion. $M^+[C_{19}H_{36}O_2]^+$.



9-octadecenoic acid (Z)-, methyl ester

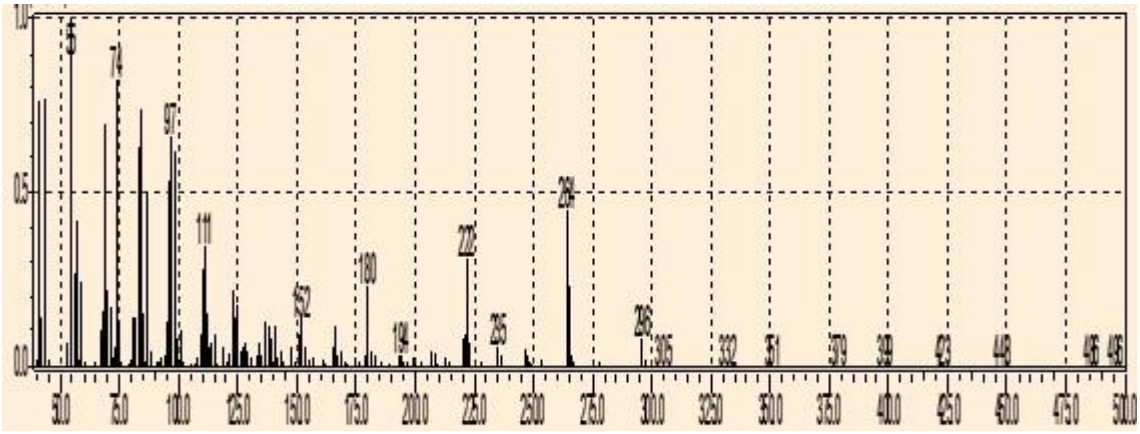
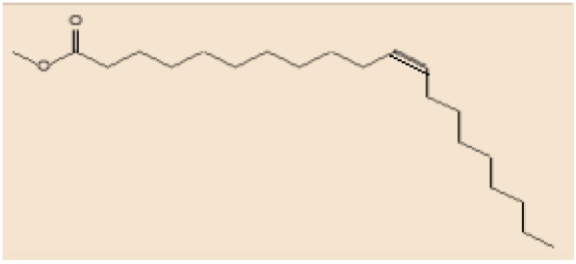


Fig. 13. 9-octadecenoic acid (Z)-, methyl ester

iv) cis-11-Eicosenoic acid, methyl ester (10.12%)

Fig. 14 shows the mass spectrum cis-11-eicosenoic acid, methyl ester. The signal at m/z 324 with retention time 18.778 is attributed to the molecular ion M^+ $[C_{21}H_{40}O_2]^+$.



cis-11-eicosenoic acid, methyl ester

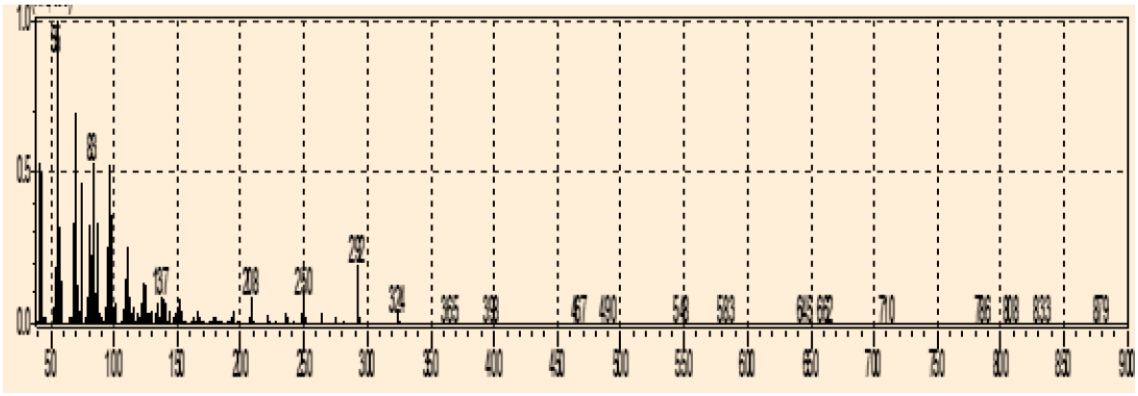


Fig.14 Mass spectrum of cis-11-eicosenoic acid, methyl ester

v) Hexadecanoic acid, methyl ester 8.24%

The mass spectrum hexadecanoic acid, methyl ester is illustrated in Fig.15. The signal at m/z 270 (RT.15.296) is due to the molecular ion $M^+[C_{17}H_{34}O_2]^+$.The signal at m/z239 accounts for loss of a methoxyl group.

hexadecanoic acid, methyl ester

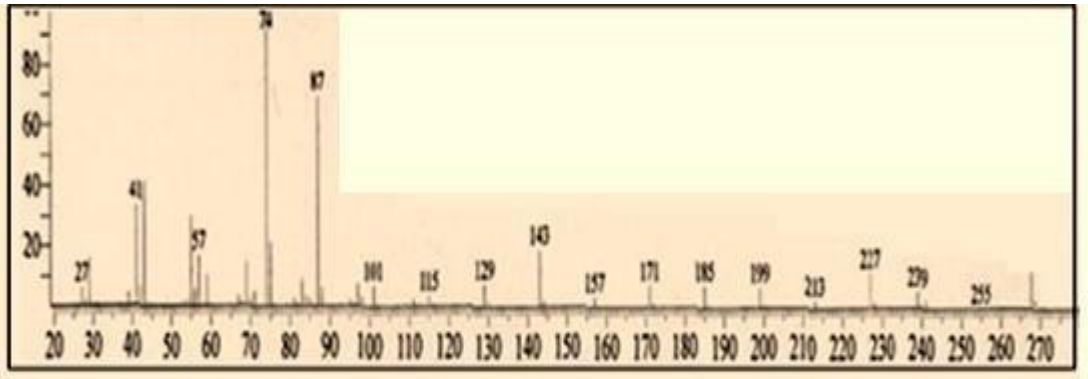


Fig 15 :Mass spectrum of hexadecanoic acid, methyl ester

3.4- *Abutilon pannosum*

The oil from *Abutilon pannosum* was subjected to GC-MS analysis. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the library.

3.4.1 Constituents of oil

Twenty-six components have been detected by GC-MS analysis (Table 3.7).The typical total ion chromatograms (TIC) is presented in Fig 16.

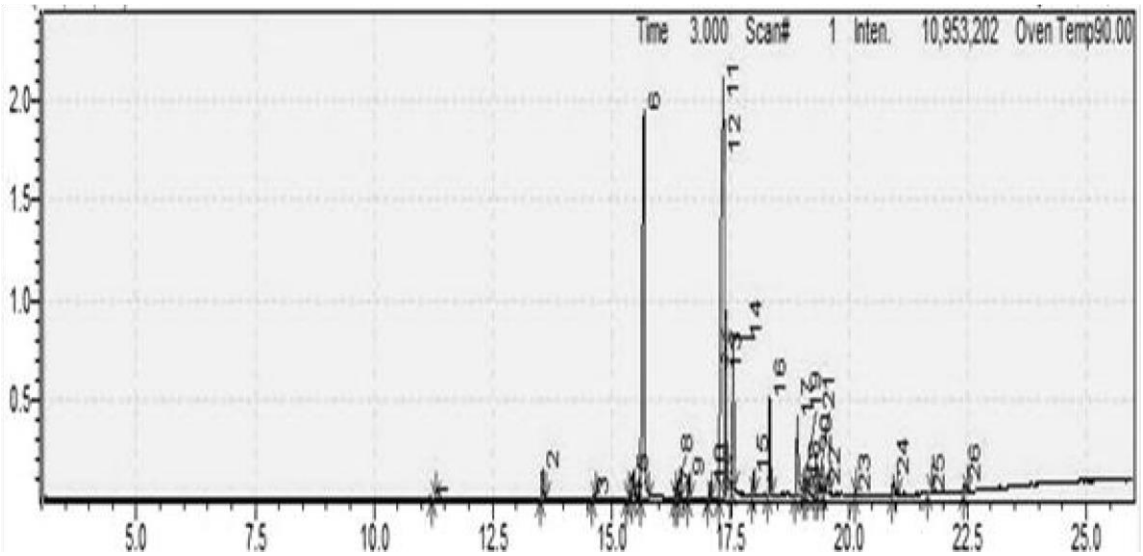


Fig.16: Total ions chromatograms

Table 3.7: Constituents of the oil

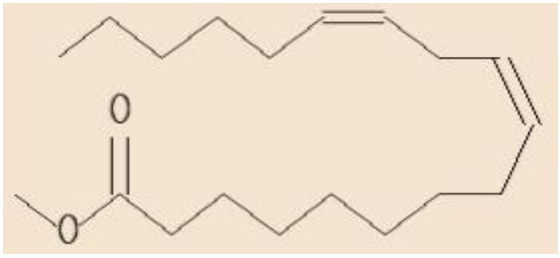
N0	Name	R. T	Area%	Formula
1.	Dodecanoic acid, methyl ester	11.255	0.02	C13H26O2
2.	Methyl tetradecanoate	13.551	1.27	C15H30O2
3.	Pentadecanoic acid, methyl ester	14.625	0.12	C16H32O2
4.	7,10-Hexadecadienoic acid, methyl ester	15.352	0.03	C17H30O2
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.455	0.88	C17H32O2
6.	**Hexadecanoic acid, methyl ester**	**15.689**	**28.44**	C17H34O2
7.	n-Propyl 9,12hexadecadienoate	16.352	0.35	C19H34O2

8.	cis-10-Heptadecenoic acid, methyl ester	16.406	0.70	C18H34O2
9.	Heptadecanoic acid, methyl ester	16.625	0.75	C18H36O2
10	Methyl 2-octylcyclopropene1-heptanoate	17.078	0.61	C19H34O2
11	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**17.364**	**39.67**	C19H34O2
12	9-Octadecenoic acid (Z)-, methyl ester	17.389	3.90	C19H36O2
13	9-Octadecenoic acid, methyl ester, (E)-	17.415	1.85	C19H36O2
14	**Methyl stearate**	**17.569**	**6.45**	C19H38O2
15	cis-11,14-Eicosadienoic acid, methyl ester	17.985	0.77	C21H38O2
16	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	18.337	4.01	C20H38O2
17	9-Octadecynoic acid, methyl ester	18.919	4.73	C19H34O2
18	9-Octadecenoic acid, 12hydroxy-, methyl ester, [R(Z)]-	19.079	0.58	C19H36O3
19	cis-11-Eicosenoic acid, methyl ester	19.114	0.70	C21H40O2
20	Eicosanoic acid, methyl ester	19.313	1.63	C21H42O2
21	PGH1, methyl ester	19.385	0.44	C22H38O2
22	8,11,14-Docosatrienoic acid, methyl ester	19.497	0.57	C23H40O2
23	Heneicosanoic acid, methyl ester	20.139	0.07	C22H44O2
24	Docosanoic acid, methyl ester	20.935	0.89	C21H42O2
25	Tricosanoic acid, methyl ester	21.702	0.13	C24H48O2
26	Tetracosanoic acid, methyl ester	22.439	0.44	C25H50O2

The mass spectra of the main constituents of the oil are discussed below:

i) 9, 12-Octadecadienoic acid (Z, Z)-,methyl ester(39.67%) Fig. 17 represents the mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester. The peak at m/z 294 with retention time

17.364 is due to the molecular ion $M^+[C_{19}H_{34}O_2]^+$.The peak at m/z263 accounts for loss of a methoxyl function.



9, 12-octadecadienoic acid (Z, Z)-, methyl ester

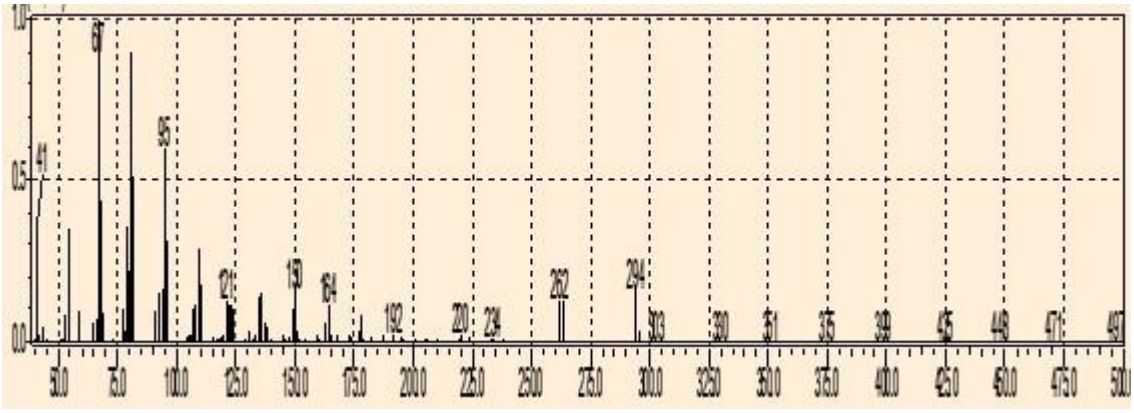
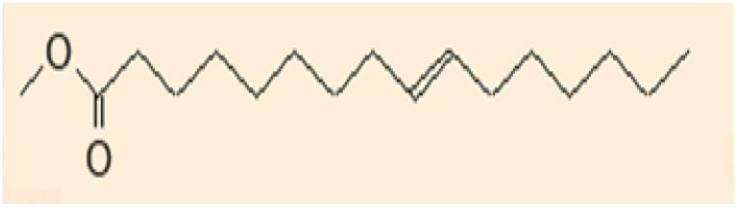


Fig.17: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

ii) Hexadecanoic acid, methyl ester (28.44%)

The mass spectrum of Hexadecanoic acid, methyl ester is depicted in Fig.18. The signal at m/z 270 (retention time 15.689) is due to the molecular ion $M^+[C_{17}H_{34}O_2]^+$.



hexadecanoic acid, methyl ester

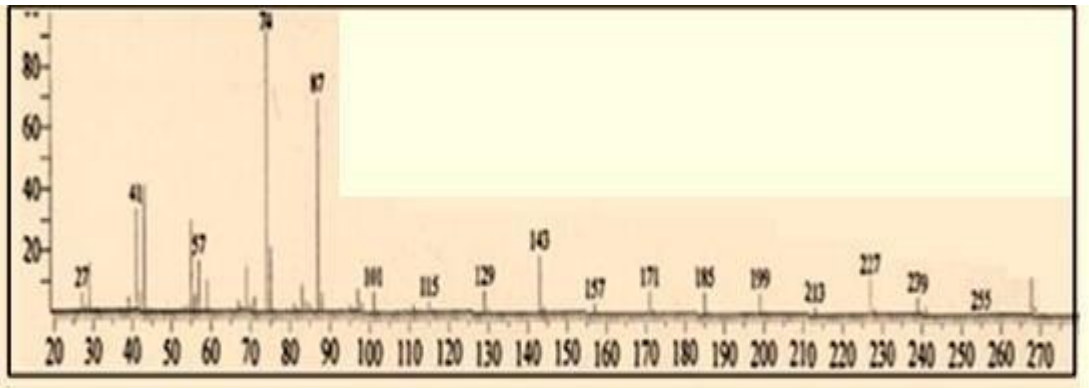
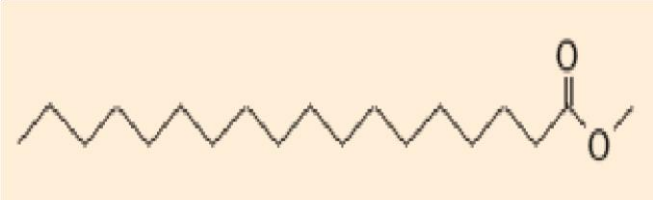


Fig 18 :Mass spectrum of hexadecanoic acid, methyl ester

iii) Methyl stearate 6.45%

The mass spectrum methyl stearate is illustrated in Fig.19. The signal at m/z 298 (retention time17.569) is due to the molecular ion M^+ $[C_{19}H_{38}O_2]^+$.The peak at m/z267 is due to loss of a methoxyl .



methyl stearate

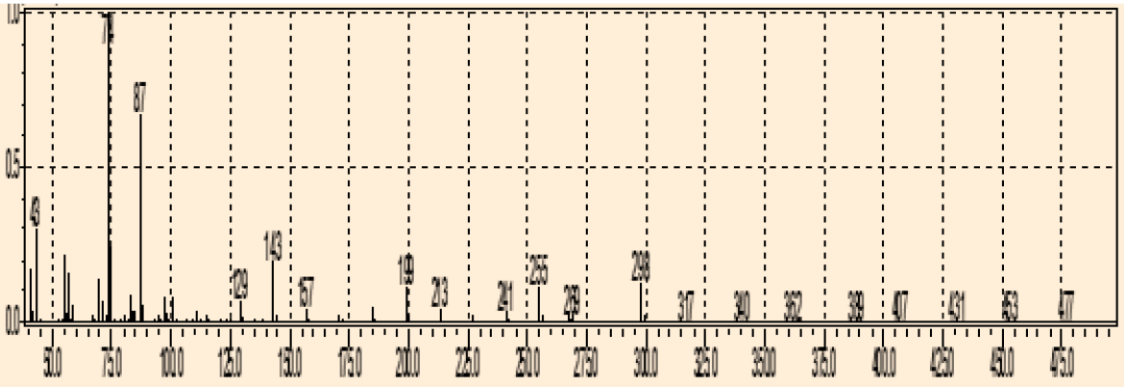


Fig.19 : Mass spectrum of methyl stearate

3.4.2-Antimicrobial activity

Abutilon pannosum seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table(3.8).The results were interpreted in the following manner:

(>9mm: inactive;9-12mm:partially active; 13-18mm: active; < 18mm:very active) .Tables(3.9) and (3.10) represent the antimicrobial activity of standard antibacterial and antifungal drugs respectively.

At a concentration of 100mg/ml the oil showed significant activity against *Candida albicans*. However, at the same concentration, it failed to exhibit inhibitory effect against other test organisms.

Table 3.8: Antimicrobial Activity of *Abutilon pannosum* seed oil

Oil	Antibacterial activity				
	Gram positive		Gram negative		
mg/ml	*Bs.*	*Sa.*	*Ec.*	*Pa.*	*Ca.*
100	--	--	--	--	18

Table 3.9 : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-

Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table 3.10 : Antifungal activity of standard chemotherapeutic agent

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

3.5- *Argemone mexicana*

GC-MS analysis of *Argemone mexicana* oil was conducted and the identification of the constituents was based on retention times and computer matching of the MS data with the (NIST) mass spectrum library.

3.5.1 The GC-MS analysis

The GC-MS analysis of the studied oil revealed the presence of 9 components (Table 3.11).The typical total ion chromatograms(TIC) is depicted in Fig.20.

Table 3.11: Constituents of *Argemone Mexicana* oil

NO	Name	Ret.Time	Formula	Area%
1	Hexadecanoic acid,	15.621	C17H34O2	2.66
	methyl ester			
2	n-Hexadecanoic acid	16.015	C16H32O2	4.97
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.272	C19H34O	14.23
4	9-Octadecenoic acid (Z)-, methyl ester	17.316	C 19H36O2	5.99
5	Methyl stearate	17.535	C19H38O2	1.32
6	9,12-Octadecadienoic acid (Z,Z)-	17.705	C18H32O2	22.97
7	Oleic Acid	17.731	C 18H34O2	35.76
8	9-Octadecenoic acid, 12hydroxy-, methyl ester, [R-(Z)]-	19.050	C19H36O3	3.61
9	Isopropyl linoleate	20.528	C19H36O3	8.49

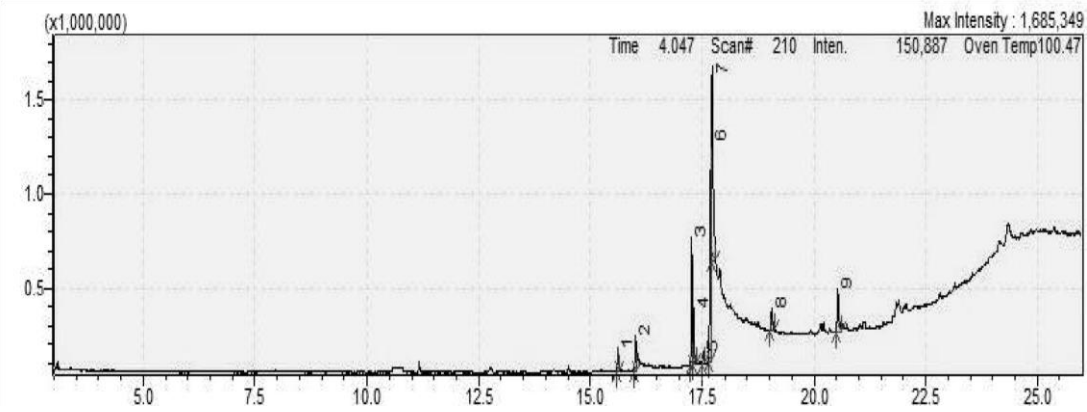
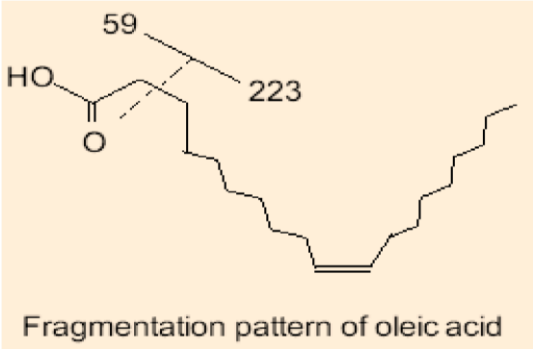


Fig.20: Total ions chromatograms

The mass spectra of the major constituents of the oil are discussed below:

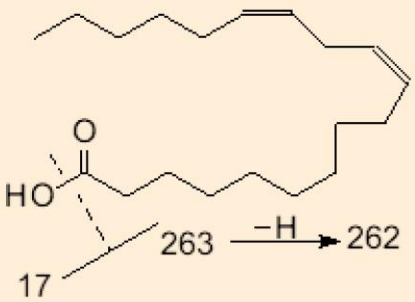
i)Oleic Acid (35 .76 %)



Fragmentation pattern of oleic acid

The mass spectrum of oleic acid is shown in Fig. 21.The peak at m/z 282, which appeared at R.T. 17.731 in total ion chromatogram, corresponds M+[$C_{18}H_{34}O_2$]+ .The signal at m/z 223 is due to loss of - CH_2CO_2H group.

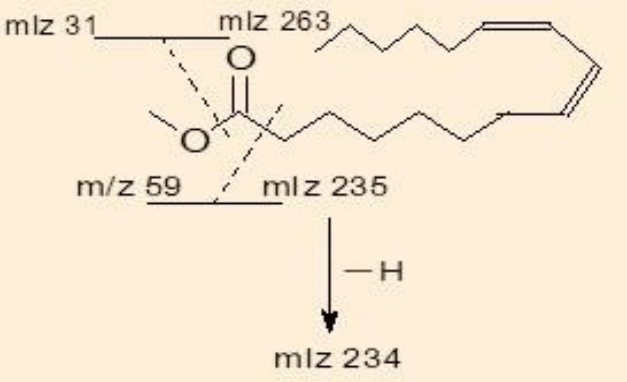
ii) 9,12-Octadecadienoic acid (Z,Z)-(22.97%)



Fragmentation pattern of 9 12 -octadecdienoic acid

Figure 22 displays the mass spectrum of 9, 12-octadecadienoic acid (Z,Z) . The peak at m/z 280(R.T 17.705) is due to M^+ [$C_{18}H_{32}O_2$]$^+$, The signal at m/z 262 is due to loss of a hydroxyl function.

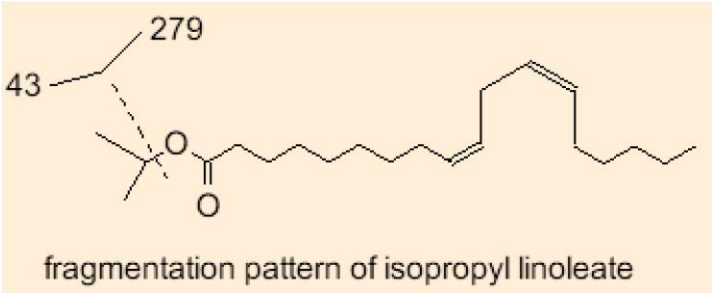
iii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester(14.23%)



Major fragmentation of 9,12-octadecadienoic acid methyl ester

The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is shown in Fig. **23**.The peak at m/z 294(R.T. 17.272) corresponds M^+ $[C_{19}H_{34}O_2]^+$

iv) Isopropyl linoleate (8.49%)



fragmentation pattern of isopropyl linoleate

In Fig. 24 (mass spectrum of isopropyl linoleate) , the molecular ion: M^+[$C_{19}H_{36}O_3]^+$ appeared at m/z 312 (R.T. 20.528) **.**

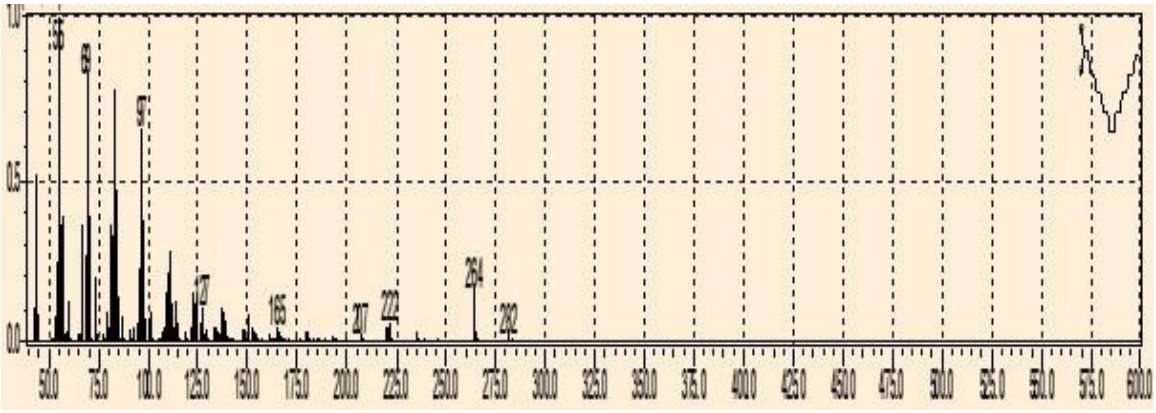


Fig.21:Mass spectrum of oleic acid

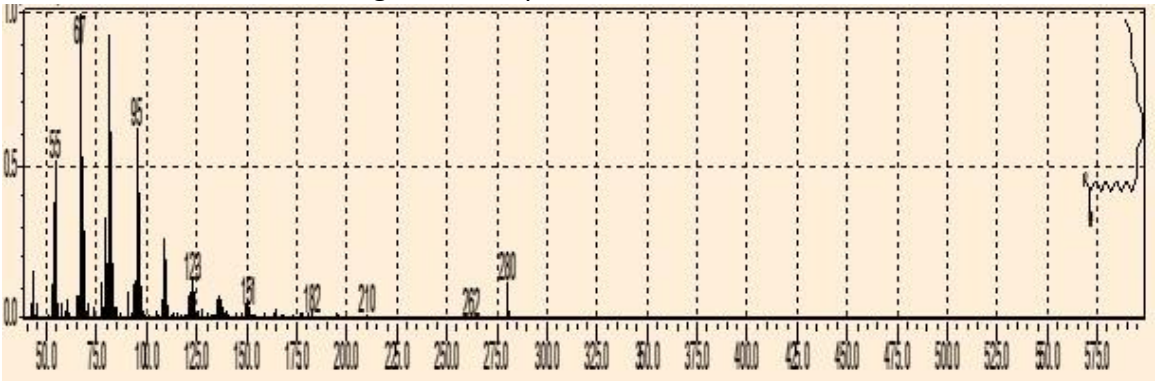


Fig.22:Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-

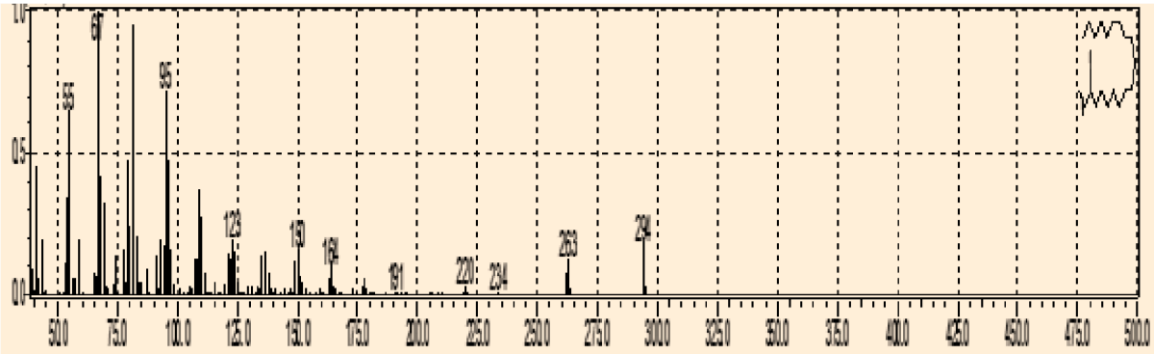


Fig.23: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

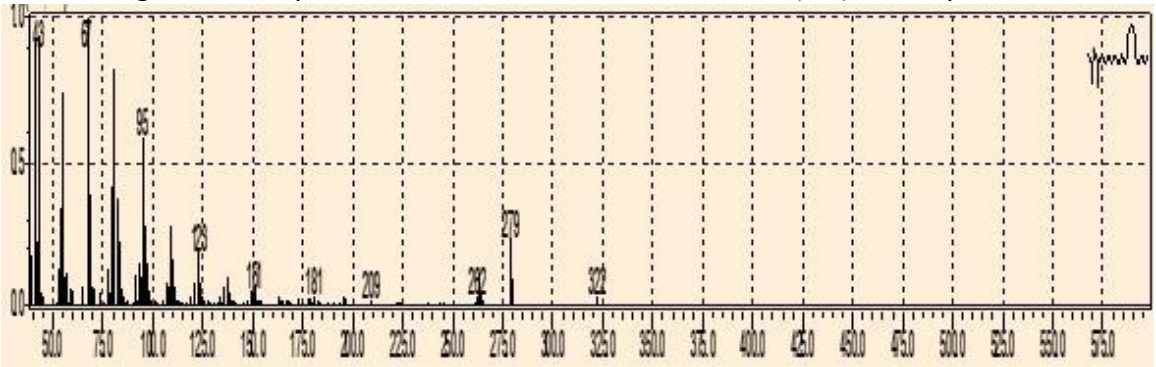


Fig 24.: Mass spectrum of Isopropyl linoleate

3.5.2 Antibacterial activity

In cup plate agar diffusion assay, the oil was assayed for antimicrobial activity. The averages of the diameters of the growth inhibition zones are listed in Table (3.12) Ampicilin, gentamycin and clotrimazole were used as positive controls(Tables 3.13 and 3.14).

At a concentration of 100mg/ml. the oil showed showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Staphylococcus aureus*.

Table 3.12 : Inhibition diameters(mm) of the oil

Drug	Conc.(mg/ml)	Ec	Pa	Sa	Bs	Ca
corochorus olitorius oil	100	--	22	14	--	--

Table 3.13 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40 20 10	15 14 11	30 25 15	- - -	- - -
Gentamycin	40 20 10	25 22 17	19 18 14	22 18 15	21 15 12

Table 3.14 : Antifungal activity of standard chemotherapeutic agent

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

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

3.5.3 Antioxidant activity

The antioxidant potential of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties.[20]

The phenolic compounds have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition.[21]

It has been reported that *A. mexicana* **Linn** root could be a potential source of natural antioxidant, that could have greater importance as

therapeutic agent in preventing or slowing oxidative stress related to degenerative diseases[22]

Argemone mexicana oil showed significant radical scavenging activity(Table 3.15).

Table 3.15: Antioxidant activity of *Argemone mexicana* oil

sample	%RSA± SD(DPPH)	IC_{50}± SDmg/ml(DPPH)
A. Mexicana oil	85.5 ± 0.06	0.131±0.01
Standard(propyl gallate)	92.2 ±0.01	0.077 µg ±0.01

3.6 *Leonotis nepetifolia*

The GC-MS analysis of *Leonotis nepetifolia* oil showed 21 components(Table 3.16).The total ions chromatogram is given in Fig.25.

Table 3.16 : Constituents of oil

No	Name	Ret.Time	Area%	Formula
1	Dodecanoic acid, methyl ester	11.227	0.02	$C_{13}H_{26}O_2$
2	cis-5-Dodecenoic acid, methyl ester	13.264	0.04	C13H24O2
3	Methyl tetradecanoate	13.538	0.14	C15H30O2
4	4-Octadecenoic acid, methyl ester	14.347	0.02	C19H36O2
5	Pentadecanoic acid, methyl ester	14.612	0.03	C16H32O2
6	Cyclododecyne	15.341	0.01	C17H32O2
7	7-Hexadecenoic acid, methyl ester, (Z)-	15.399	0.22	C17H32O2
8	9-Hexadecenoic acid, methyl ester, (Z)-	15.444	0.96	C17H32O2

9	Hexadecanoic acid, methyl ester	15.656	14.44	C17H34O2
10	cis-10-Heptadecenoic acid, methyl ester	16.408	0.05	C18H34O2
11	Heptadecanoic acid, methyl ester	16.617	0.09	C18H36O2
12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.312	15.59	C19H34O2
13	9-Octadecenoic acid (Z)-, methyl ester	17.395	35.40	C19H36O2
14	6-Octadecynoic acid, methyl ester	17.609	22.68	C19H34O2
15	cis-11-Eicosenoic acid, methyl ester	19.087	1.46	C21H40O2
16	Eicosanoic acid, methyl ester	19.305	2.15	C21H42O2
17	6,9-Octadecadienoic acid, methyl ester	19.441	0.53	C19H34O2
18	Docosanoic acid, methyl ester	20.927	0.69	C21H42O2
19	.gamma.-Sitosterol	21.890	4.40	C29H50O
20	Tetracosanoic acid, methyl ester	22.431	0.29	C25H50O2
21	.alpha.-Amyrin	22.704	0.64	C30H50O

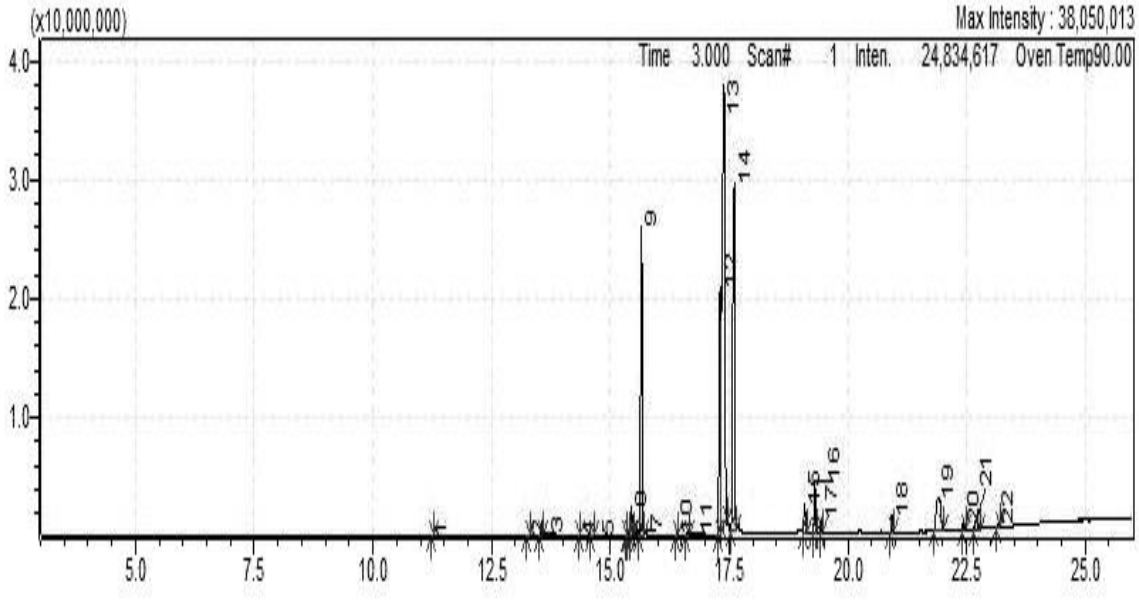


Fig. 25: Total ion chromatograms

The mass spectra of the major constituents are discussed below.

i) 9-octadecenoic acid (Z)-, methyl ester

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is displayed in Fig. 26. The molecular ion M^+ $[C_{19}H_{36}O_2]^+$ appeared at m/z 296(R.T. 17.395).

Fig.27 displays the mass spectrum of 6-octadecynoic acid, methyl ester. The peak at m/z 294(RT.17.609) represents M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is illustrated in Fig. 28. The molecular ion M^+ $[C_{19}H_{34}O_2]^+$ appeared at 294 m/z (RT. 17.312)

The mass spectrum of hexadecanoic acid, methyl ester(Fig.29) showed the molecular ion $[C_{17}H_{34}O_2]^+$ at m/z 270(R.T 15.656).

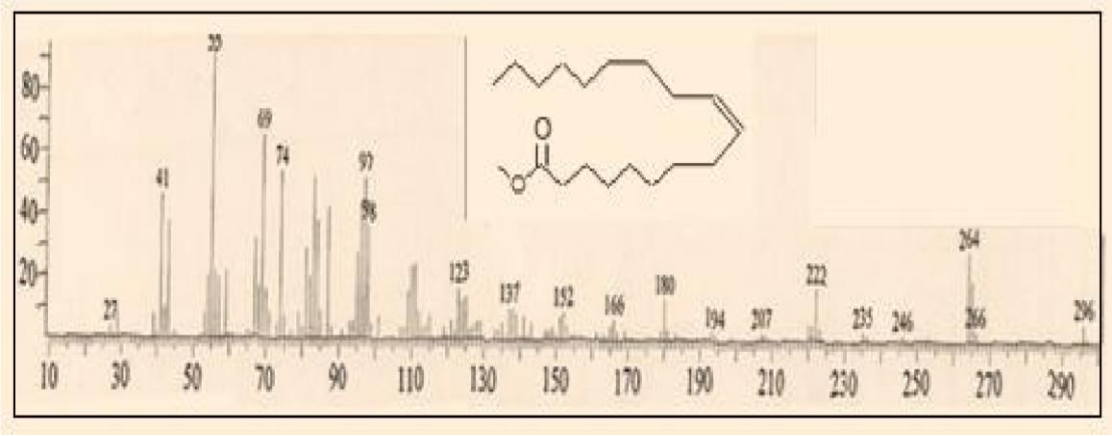


Fig.26 : Mass spectrum of 9-octadecenoic acid methyl ester

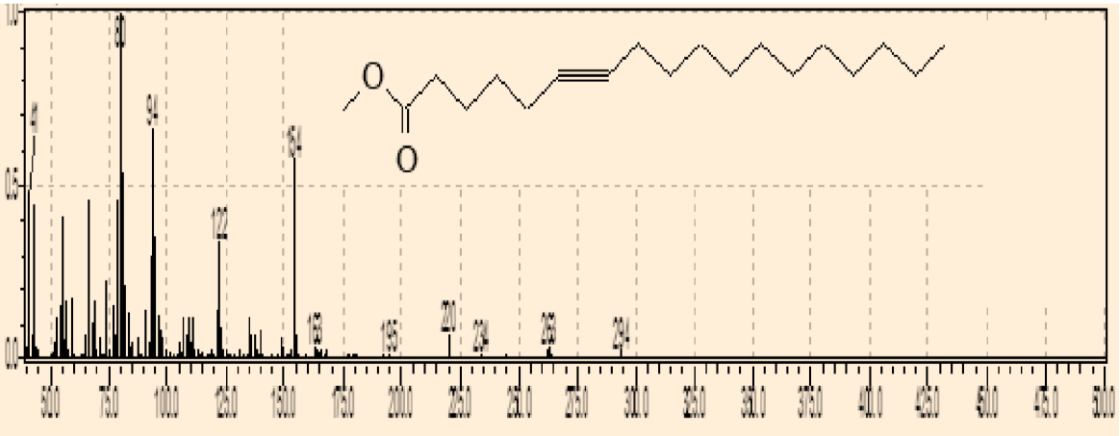


Fig.27: 6-Octadecynoic acid, methyl ester

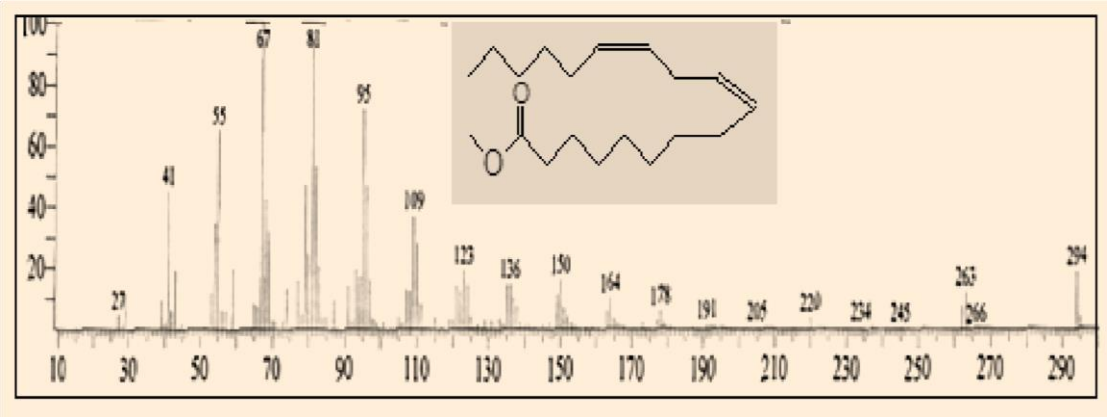


Fig. 28 Mass spectrum of 9, 12 octadecanoic acid methyl ester

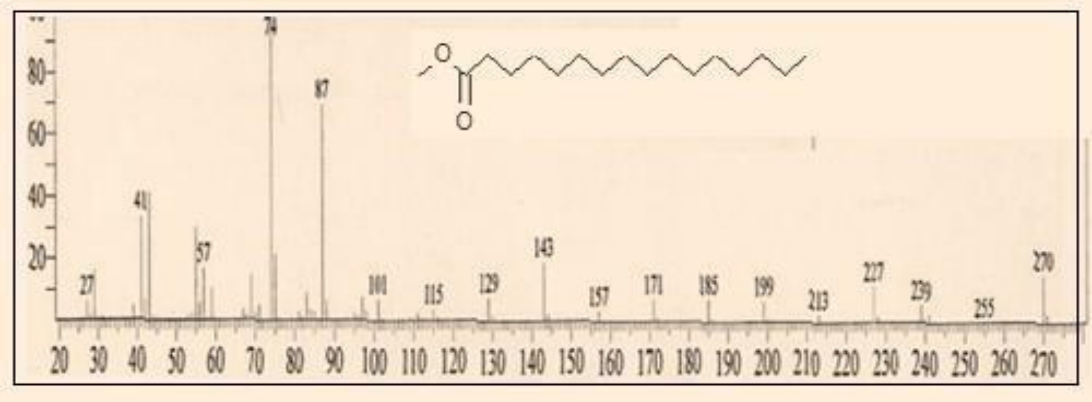


Fig. 29: Mass spectrum of hexadecanoic acid methyl ester

3.6.1 Antimicrobial activity of *Leonotis nepetifolia*

The averages of the diameters of the growth inhibition zones are listed in Table 3.17 The studied oil showed good activity against *Pseudomonas aeruginosa* and partial activity against

Staphylococcus aureus.

Table 3.17: Diameters of inhibition zones (mm

Sample	Ps	Sa
Leonotis nepetifolia oil concentration of 100mg/ml.	16	12

Conclusion

The 0ils from six potential medicinal plants grown in Sudan (Solanum *dubium, Martynia annua, Physalis angulate, Abutilon pannosum, Argemone Mexicana and Leonotis nepetifolia* have been investigated . The targeted oils have been analyzed by GC-MS and the different constituents have been characterized by this technique. Furthermore the oils have been evaluated for their antimicrobial activity and different antimicrobial responses have been observed.

Recommendation

The following is recommended:

i-The extracted oils may be evaluated for other biological activities like antimalarial anti-viral anti-inflammatory and antileishmenial.
ii- Other phytochemicals in studied plants may be isolated and their biological activity investigated.
iii- Biological activity- guided fractionation may be attempted.

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