1-Introduction

1.1-The targeted plant species

1.1.1- *Acacia nubica* Benth.

Acacia genus is a large genus- about 1350 species- in the family Fabaceae[1]. These species are considered as substantial source of gallic and ellagic acids[2] . Most of these species contain flavonoids beside other phenolics[3]. In ethnomedicine some Acacia species are used as antidiabetic, hypotensive, antiamoebic, antidiarrhoeic, anti- inflammatory[4].

Acacia nubica

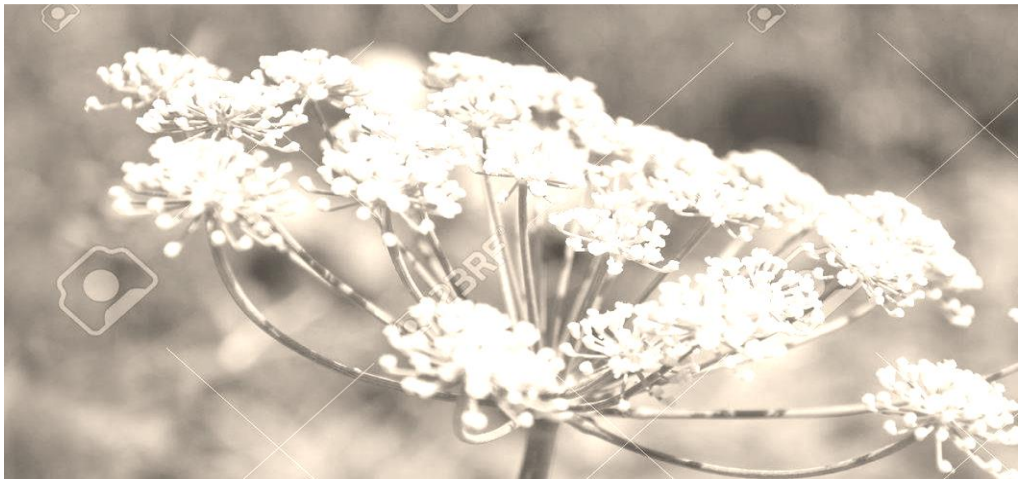
The antimicrobial potential of many *Acacia* species has been documented[5]. In Sudanese system of medicine *Acacia nilotica* is used against cough, sore throat, malaria, intestinal worms and wounds[6-9]. Pods of *Acacia nilotica* are used commercially in Sudan for leather tanning[10]. The effect of the aqueous extract of *Acacia nilotica* pods on diabetic models has been studied. Diabetic rats exhibited hypoglycemia,significant increase in lipid peroxidation and elevated serum urea and creatinine[11] . The gum from *Acacia seyal*- Gum Arabic- finds many traditional uses including kidney disorders. Though the pharmhacological effects of Gum Arabic were extensively investigated in animal models, there is paucity of data regarding quantified use in humans[12] . Acacia gum has been used as demulcent in pharmaceutical preparations. The gum has been used traditionally for healing wounds and has been shown to inhibit early deposition of plaque[13]. The antioxidant capacity of the medicinally important species *Acacia auricoliformis* has been evaluated[14]. The ethanol extract of *Acacia aroma* showed significant activity against Gram positive bacteria[15] .

Acacia nubica **Benth**. is a herb reaching a height of 1-5m. It is distributed in Egypt, Sudan, Saudi Arabia and Iran. However , Little information is available about *Acacia nubica*. The present

study deals with the characterization of seed oil constituents and the antimicrobial potency of *Acacia nubica*.

1.1.2- *Foeniculum vulgare* Mill.

Foeniculum vulgare Mill. (fennel) is a perennial herb in the family Apiaceae. Fennel is widely cultivated in many countries where it is used as flavouring agent in baked foods[16,17]. Fennel is rich in some minerals like calcium, sodium, iron, potassium and phosphorus. The plant also contains fibre (18.5%), protein (9.5%); fats (10%) beside niacin, riboflavin and thiamine[18] . Seeds of fennel which are hypotensive and diuretic are claimed to improve eyesight, while seed extract has been tested against glaucoma in experimental models[19] . Fennel essential oil contains some bioactive molecules like anethole, fenchone, estragol, p-anisaldehyde and α-phellandrene [20,21].



Foeniculum vulgare

Sterols, acetylated kaempferol and some benzoisofuranone derivatives have been reported from fennel[22,23]. Also some flavonoids have been isolated from fennel[24-26]. These phenolics seem to be responsible for the antioxidant properties of fennel. The antispasmodic , diuretic, antinflammatory, analgesic, hepatoprotective properties of fennel essential oil have been documented [27-30]. It has been reported that fennel essential oil exhibited antimicrobial activity[31,32].

However, beside its health promoting properties, a constituent of fennel- leugenol- has become a cause of concern since the structurally related, methylleugenol has been listed as a potential carcinogenic agent[33]

1.1.3-*Cordia africana* Lam.

Cordia africana **Lam.** (Synonym: Cordia abyssinica) is a small to medium-sized tree in the family Boraginaceae which comprises about 21 genera and 110 species[34]. Cordia africana grows up to 4-15m in height. The plant is widely distributed in east and south Africa[35]. *Cordia Africana* has a common occurrence in western Sudan where it is locally known as "Teak or Gombail" [36-37].

Cordia africana

Wood is moderately hard and durable wood and serves as raw material for making high quality furniture and household materials[38]. Cordia Africana is considered as a good source of herbal medicine, food (fruit is edible), firewood and bee forage [39-41]. The plant is used traditionally against stomach-ache, tooth-ache, wounds and cough[42].

1.1.4-*Acacia polyacantha* Willd

Acacia (Fabaceae) is a large genus comprising around 1350 species. Most Acacia species are rich in bioactive molecules including flavonoids and other phenolics[43]. Some Acacia species are used traditionally as antiinflammmatory, antidiabetic, antidiarrhoeic antimicrobial and as

hypotensive[44,45]]. The medicinally important species – *Acacia nilotica* – is used in Sudanese system of medicine against malaria, diabetes, wounds and intestinal worms[46-49] . Another Acacia species-*Acacia seyal* – is used against kidney disorders[50]. The antioxidant properties of *Acacia auricoliformis* has been reported[51].

Acacia polycantha

Acacia polyacantha Willd. is an erect, deciduous tree distributed along tropical Africa extending from Gambia to Ethiopia , Kenya and Zimbabwe [52]. The plant is used traditionally against gastrointestinal disorders[53].

1.1.5-*Lens culinaris* L

Lens culinaris **L.** (Lentil) is an annual edible legume reaching 40cm in height. Lens is a small genus in the family Fabaceae consisting of the cultivated variety- *Lens culinaris* and six other wild taxa[54,55]. In 2018 the global production of Lentil

reached 6.3 million tones mainly produced by Canada (33%) and India(25%)[56,57]. Lentil contains proteins beside some amino acids[58] . It also contains starch and insoluble dietary fibers [59,60]. The plant is a good source of prebiotics[61] beside considerable amount of prebiotic carbohydrates thus preventing gut-associated diseases[62,63].

Lens culinaris

Lentil is rich in potassium and has low fat and sodium content[64] . It is a good source of iron[65]. This legume herb contains several important minerals including boron, copper, zinc, manganese, selenium and molybdenum. It also contains vitamins, niacin, riboflavin, pyridoxine, pantothenic acid, folate, α, β and γ- tocopherols [66,67]. Beside these vitamins , the plant contains polyphenolics which act as free radical

scavengers[68,69]. It seems that Lantil is a diet with health promoting effects.

1.2-Natural products

A large number of modern drugs have been isolated or derived from plant material[70-72] and many examples are known including : atropine, morphin,cocain..etc. More than 80% of the world population now depend on medicinal plants which contribute to the primary healthcare of different communities[73-75]. This is mainly due to the side effects of several synthetic drugs and the unaffordable cost of modern drugs . Medicinal plants include bioactive constituents(steroids, alkaloids,flavonoids..etc) which are very helpful in treating various ailments[76] and may serve as leads for drug discovery and drug development.

Natural products are defined as chemical substances produced by plants or animals. Natural products include any substance produced by life. Natural products can also be prepared by chemical synthesis (both semi-synthesis and total synthesis) and have played a central role in the development of the field of organic chemistry by providing challenging synthetic targets. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients[70].

1.2.1--Saponins

Saponins are constituting an important class of natural product being found in abundance in a wide array of plant species.They are glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative[70].

The aglycone (glycoside-free) portions of the saponins are termed sapogenins. The number of saccharide chains attached to the sapogenin/aglycone core can vary – giving rise to another dimension of nomenclature (monodesmosidic, bidesmosidic, etc.) – as can the length of each chain. A somewhat dated compilation has the range of saccharide chain lengths being 1–11, with the numbers 2-5 being the most frequent, and with both linear and branched chain saccharides being represented. Dietary monosaccharides such as D-glucose and D-galactose are among the most common components of the attached chains[70].

1.2.2--Tannins

Tannins which are important class of natural products are widely distributed in the plant kingdom, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what

causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or tea. Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruits.Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 (proanthocyanidins)[70].

1.2.3--Steroids

Steroids are widely distributed in nature where they could be synthesized by both plants and animals.They are cyclic compounds characterized by an arrangement of seventeen carbon atoms in a four-ring structure, where the rings are three composed of 6-carbons (rings A, B, and C) followed by one with 5-carbons (ring D). Further common features are an 8-carbon side chain attached to a carbon on ring D, and two or more methyl groups at the points where adjacent rings are "fused". Hundreds of distinct steroids are found in animals, fungi, plants, and elsewhere, and specific steroids underlie proper structure and function in many biological processes. Their core tetracyclic ring structure is synthesized in each organism by biochemical pathways that involve cyclization of a thirty-carbon chain, squalene, into an intermediate, either lanosterol or cycloartenol. From such intermediates, organisms then derive critical steroids such as cholesterol, the sex hormones estradiol and testosterone and bile

acids. Based on such structures, synthetic and medicinal chemists synthesize novel steroids for use as drugs such as the anti-inflammatory agent dexamethasone[70].

1.2.4-Glycoside

In chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body[71].

1.2.5-Alkaloids

Alkaloids are defined as natural basic nitrogen-containing compounds with pronounced physiological activity. Some synthetic compounds of similar structure are also termed alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and, more rarely, other elements such as chlorine, bromine, and phosphorus[70].

1.2.6-Flavonoids

Flavonoids are defined as polyphenolic secondary metabolites which are universally distributed in green plant kingdom, located in cell vacuoles. These polyphenolics are associated with a variety of biological activities in plants, animals, and bacteria. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for color and aroma of flowers, fruit to attract pollinators consequently fruit dispersion; help in seed germination, growth and development of seedling. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, Function as signal molecules, allelopathic compounds, phytoalexins, detoxifying agents, antimicrobial defensive compounds. Flavonoids have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimation and freezing tolerance[70].

More than 8000 flavonoids have been isolated and identified from plants to date. Flavonoids are associated with a wide range of biological and pharmacological activities, including: antioxidant activity, anticancer activity, protection against cancer formation (chemo-protection), , cardiovascular and hepatic protection, antibacterial, antifungal and antiviral activity.

Flavonoids also play a vital role in hormone-related female diseases, such as breast cancer and menopausal syndrome. Due to their diverse physiological activities, flavonoids have therefore

been subjected to extensive research in order to improve their activity [71].

1.3- Essential oils

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers)[77]. Essential oils are odiferous bodies of an oily nature, obtained almost exclusively from plant organs: flowers and buds, leaves, bark or wood, roots, stems, rhizomes, fruits and seeds[78,79]. Essential oils are generally liquid, and their pleasant odour and essence is responsible for the strong characteristic smell or fragrance of aromatic plants[80].

Essential oils can be found in various plant organs, being produced and stored in secretary structures that differ in morphology, structure, function, and distribution. These specialized structures minimize the risk of auto toxicity and can be found on the surface of the plant organs or within the plant tissues, being classified as external or internal secretary structures, respectively. In nature, essential oils play very important roles in plant defense and signaling processes . For example, essential oils are involved in plant defense against microorganisms, insects, and herbivores, attraction of pollinating insects and fruit-dispersing

animals, water regulation and all elopathic interactions[81] . Essential oils are hydrophobic, are soluble in alcohol, non polar or weakly polar solvents, waxes and oils, but only slightly soluble in water and most are colourless or pale yellow, with exception of the blue essential oil of chamomile (Matricaria chamomilla) and most are liquid and of lower density than water (sassafras, vetiver, cinnamon and clove essential oils being exceptions) [82].

1.3.1- Classification of essential oil

Essential oils may be classified using different criteria: consistency, origin, and chemical nature of the main components [83-86].

Essential oils depending on their consistency are classified as :

i)Essences

Fluid essences are liquids which are volatile at room temperature.

ii)Balsams

Balsams are natural extracts obtained from a bush or tree. They usually have a high benzoic and cynamic acid content with their corresponding esthers. They are thicker, not very volatile, and less likely to react by polymerising. Examples of balsams are copaiba balsam, Peruvian balsam, Banguy balsam, Tolu balsam, Liquid amber[86].

iii)Resins

Within the resin group we find a number of possible combinations and mixes:

a)Resins

These are amorphous solid or semi-solid products of a complex chemical nature. They are physiological or physio-pathological in origin. Colophony, for example, is obtained by separating trementine an oleoresin. It contains abietic acid and derivates[86].

b)Oleoresins

These are homogeneous mixes of resins and essential oils. Trementine, for example, is obtained by making incisions in the trunk of different pine species. It contains resin (colophony) and essential oil (trementine essence) which are separated by steam drag distillation. The term oleoresin is also used to refer to vegetable extracts obtained using solvents, which should be virtually free of said solvents. They are frequently used instead of spices in foodstuffs and pharmacy because of their advantages (stability, microbiotic and chemical uniformity, and easy to add)[86].

c)Gum-resins

These are natural plant or tree extracts. They are a mix of gums and resins.

Essential oils depending on origin are classified as:

i)Natural oils

Natural oils are obtained straight from the plant and are not modified physically or chemically afterwards. However, they are expensive because of their limited yield[86].

ii)Artificial oils

Artificial oils are obtained using processes of enriching the essence with one or several of its components. For example, essences of rose, geranium, and jasmine are enriched with linalool, and aniseed essence with athenol[86].

iii)Synthetic oils

As the name suggests, are usually produced by combining their chemically synthesised components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences…)[86]

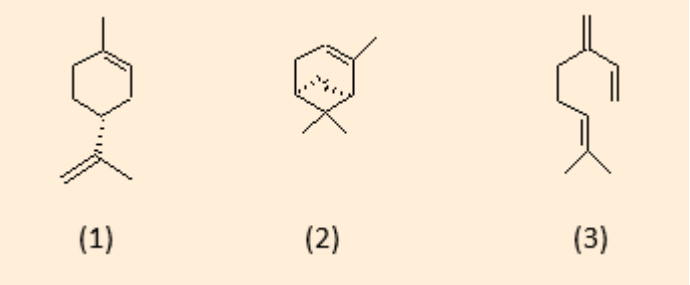
1.3.2-Constituents of essential oils

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers)[87].

1.3.2.1. Hydrocarbons

The majority of essential oils fall into this category these contain molecules of hydrogen and carbon only [88,89] and are classified into terpenes (monoterpenes: C10, sesquiterpenes: C15, and diterpenes: C20). These hydrocarbons may be acyclic, alicyclic

(monocyclic, bicyclic or tricyclic) or aromatic. Limonene(1), α-pinene (2) , myrcene (3)[90-92] .

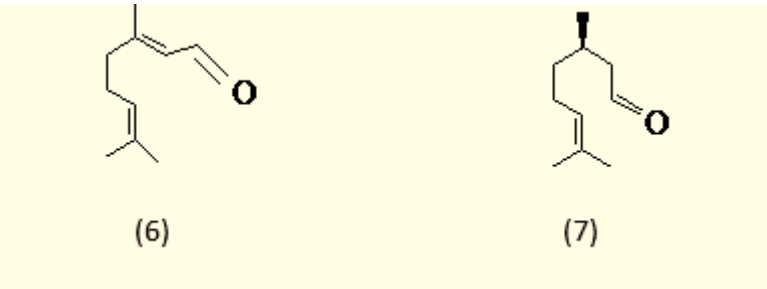


1.3.2.2 -Alcohols

Alcohols have the hydroxile group (OH) bonded to a C_{10} skeleton. Their names end in –ol. They are highly sought after for their aroma. In addition to their pleasant fragrance, alcohols are the most therapeutically beneficial of essential oil components. Linalool (4) gives tea taste , menthol(5) , another compound found in this group, is responsible for the smell and taste of mint[90-94].

1.3.2.3- Aldehydes

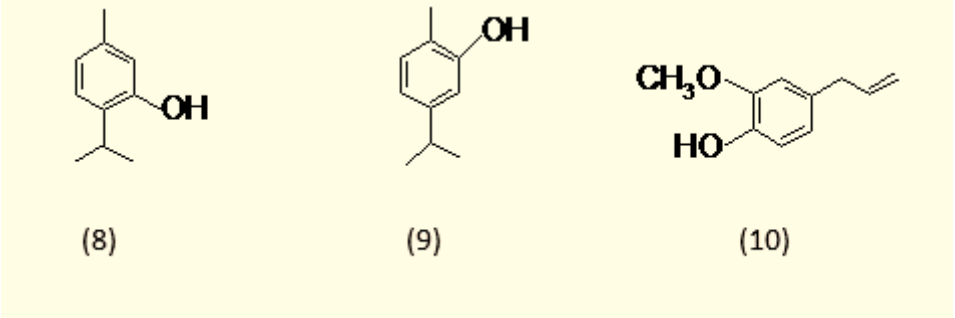
Aldehydes are highly reactive compounds. Their names end in –al. Many of them, such as those found in citrus fruits, match their respective alcohol. For example: geranial (6), and citronelal(7) [90,95].



(6) (7)

1.3.2.4- Phenols

They are only found in a few species These aromatic components are among the most reactive, potentially toxic and irritant, especially for the skin and the mucous membranes. The most important are thymol (8) and carvacrol (9) , eugenol(10)[90,91].



(8) (9) (10)

1.3.2.5-Ketones

These are produce by the oxidation of alcohol and fairly stable molecules they end-one example Carvone (11). Ketones are not very common in the majority of essential oils are not particularly

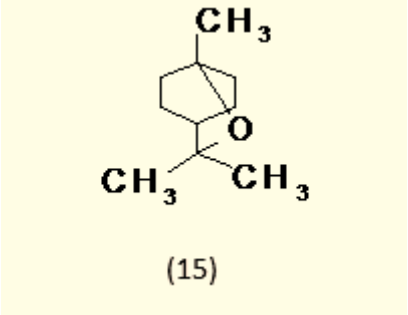
important as fragrances or flavor substances. In some cases, ketones are neurotoxic and abortifacients such as camphor (12)[90,94,96] .

1.3.2.6- Esters

Esters are sweet smelling and give a pleasant smell to the oils and are very commonly found in a large number of essential oils. They include for example, linalyl acetate (13), geranyl acetate (14)[90,93,94,97].

1.3.2.7- Ethers

Ethers or monoterpenic oxides are reactive and unstable. One example common ether is 1.8 –cineol (also known as eucalyptol (15)[83,86].



(15)

1.3.3- -Biological potential of essential oils

Essential oils are natural volatile substances obtained from a variety of plants. Approximately 3000 essential oils are known, and 10% of them have commercial importance in the cosmetic, food, and pharmaceutical industries [98,99]. In general, essential oils have a nice smell, that is why they are used in different industries, especially in odoants in perfumes (fragancias and lotions), in foodstuff (like flavoring and preservatives) and in pharmaceutical products (therapeutic

Essential oils exhibit a wide spectrum of pharmacological activities such as infection control, wound healing, pain relief, nausea, inflammation and anxiety [100,101]. Also, particular emphasis has been placed on the antibacterial, antifungal and insecticidal activities of essential oil from plants [102,103].

Traditional medicines containing essential oils have been scientifically proven to be effective in treating various ailments like malaria and others of microbial origin [104-105]. It has been

observed that different essential oils overlap in their actions although they may differ in their chemical composition[105]. The biological activities of essential oils have been attributed to the composition or specific essential oil constituent.

1.3.3.1- Antmicrobial potential of essential oils

Many reviews discussing the antimicrobial properties of many essential oils have been published[106;107] and the mechanism of action has been thoroughly discussed[108]. Hydrophobicity is an important feature of essential oils. This property allows essential oils to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable[109;110].This can then cause leakage of ions and other cellular molecules[111;112] .Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death[113].

It has been reported that essential oils can have a single target or many targets for their activity . Thymol and Carvacrol oils gain access to the periplasm and deeper portions of the cell [114]. Carvone oil can also be intereffective against the OM and does not affect the cellular ATP pool [115]. It has been reported that essential oils containing mainly aldehydes or phenols, such as citral, carvacrol, eugenol, or thymol do possess the highest antibacterial activity, followed by essential oils containing terpene

alcohol. Other essential oils containing ketones or esters such as geranyl acetate show much weaker activity, while volatile oil containing terpenes hydrocarbons are usually inactive[116;117]. Essential oils characterized by a high level of phenolic compound, such as carvacrol, eugenol, and thymol, have important antibacterial activities [118]. Such compounds are responsible for the disruption of the cytoplasm membrane [119]. It has been shown that the chemical structure of essential oils effects their mode of action concerning their antibacterial activity [118].

The vital role of hydroxyl groups in the antimicrobial activity of oils, such as carvarol and thymol, was confirmed[118]. However the relative position of the phenolic hydroxyl group on the ring does not appear influence the antibacterial activity.

It has been shown that the action of thymol against *Bacillus cereus, Staphylococcus aureus,* and *Pesudomons aeruginosa* appears to be comparable to that of carvarol [112].Also it has been shown that carvarol and thymol act differently against Gram positive and Gram negative species [112]. Thymol, carvarol have an antimicrobial effect against a broad spectrum of bacterial strains including: *Escherichia coli, Bacillus cereus, Liserimano cytogenes, Samonella enteric, Clostridium jejuni, Lactobacillus sake, Staphylococcus* and *Helicobacter pyroli*[120-122]

Other essential oils also have valuable antibacterial properties like those containing certain alcohols, aldehydes , ketones and monoterpenes. Among these compounds, carvarol is the most active. Carvarol is used as a presevative and food flavoring in drink, sweats, and other preparation. It has been shown that essentials are more active against Gram positive than Gram negative bacteria [123]. 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 the lipopolysaccharids film[124-126]. Furthermore, the antibacterial activity of essential oils is related to their chemical composition, the properties of volatile molecules, and their interactions[127]. An additive effect is observed when the combination is equal to the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is less important when they are tasted together than when used individually [123.]

It has been reported that a synergistic effect was observed when the combination of substance is greater than the sum of the individual effects [124]. Some studies have shown that the use of the whole essential oils provides an effect which is greater than that of the major components used together[125.] This suggests that minor components are essential for activity and may have synergistic effect. The additive and synergistic effects of the combination of

1,8-cineole and aromadendrene against methicillin _ resistant *Staphylococcus aureus (*MRSA) and thancomycin –resistant enterococci(VRE) has been demonstrated[37]. In addition, essential oils have also revealed to be effective on the inhibition of growth and reduction in numbers of the more serious food borne pathogens, such as *Salmonella.spp.* and *E. coli* [127].

1.3.3.2- Antioxidant activity

It has been demonstrated that the antioxidant activity of an essential oil is associated with its composition. Secondary metabolites with conjugated double bonds usually show substantial antioxidative properties [128-130].

Thymol and carvacrol are potentially active antioxidants. The activity of these phytochemicals is related to their phenolic compounds having redox properties and thus play an important role in neutralizing harmful free radicals [125].

It has been demonstrated that antioxidant propeties of essential oils is also due to certain alcohol, esters, ketones, aldehyde, and monoterpenes [128].

It is known that essential oils with important scavenging capacity of free radicals may play an important role in some diseases prevention, such as, brain dysfunction, cancer, heart disease, and

immune system decline. In fact these disease may result from cellular damage caused by free radicals [129].

Essential oils have shown their action as hepatoproective as well as against ageing . Also it has been proved that they possess a beneficial impact upon the(PUFAs), in particular the long chain C_{20} and C_{22} acids [131].

1.3.3.3- Cancer chemoprotective activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researcher for their potential activity against cancer. Essential oils and their constituents target the discovery of new anticancer natural products [126].

Essential oils would act in the prevention of cancer as well as, at its removal. It is well known that certain food, such as garlic and turmeric, are good sources of anticancer agents [132.] Garlic essential oil is source of sulfur compounds recongnized for their preventive effect against cancer [133;134]. Daily sulfide, daily disulfide, and daily tri sulfide are examples.

These compounds activates, in rates, the enzymes involved in the detoxification process of hepatic phase1(disintegraction of chemical bonds that link carcinorganictoxins to each other) and phase 2(bonds to toxins released detoxifying enzymes, such as glutathione s- transferase)[125].

Metabolism happens mainly in the liver - the body largest internal organ. The portal vein carries blood from the small intestine directly to the liver. Sixty percent of liver tissue is made up of hepatic cells. More chemical processes happen in these than in any other group of cells in the body. Phase 1 metabolism involves chemical reactions, such as oxidation (most common) reduction and hydrolysis[125].

There are three possible results of phase 1 metabolism (i) the drug becomes completely inactive i.e. the metabolites are pharmacologically in active (ii)the metabolites are pharmacologically active, but less so than the original drug (iii) the original substance is not pharmacologically active, but one of its metabolites[135].

Phase 2 metabolism involves reaction that chemically change the drug or phase1 metabolites into compounds that are soluble enough to be excreted via urine. In the reaction, the metabolites which are attached to an ion is capable of grouping. This is called conjugation and the products called a conjugate [136].

It has been demonstrated that many essential oils have a cytotoxin activity namely *Melissa fficinalis*[137].*Melaleuca alternifolia. Artemisia annua* and *comptonia peregrine* [138].

1.3.4- Methods of extraction of essential oils

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[139].

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[140].

Steam distillation is also widely used in petroleum refineries and petrochemical plant where it is commonly referred to as steam stripping[141,142].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[143].

1.3.4.1-Hydrodistillation

Hydrodistillation 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[143].

1.3.4.2- 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[143].

1.3.4.3-- 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[143].

1.3.4.4- 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[143].

1.3.4.5- 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. Beside 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 enable it to penetrate the material being extracted and its latent heat of evaporation allows it to be removed without residue [144].

Aim of this study

This research was aimed to:

-Extract oils from five medicinal plants.

-Investigate the constituents of the oils by GC-MS analysis.

-Evaluate the extracted oils for their antimicrobial activity.

2-Materials and Methods

2.1 Materials

2.1.1 Plant material

Seeds of *Acacia nubica, Foeniculum vulgare, Cordia Africana, Lens culaniris* and *Acacia polycantha* were collected from around Damazin-Sudan. The plants were authenticated by the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants Research Institute, Khartoum-Sudan.

2.1.2 Instruments

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

2.1.3 Test organisms

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

Table 1: Test organisms

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

2.2- Methods

2.2.1 Extraction of oils

Powdered seeds of studied plant (400g) were exhaustively extracted with n-hexane by maceration.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes .The

hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

2.2.2 GC-MS analysis

The studied oils were analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier

gas.Oven temperature program is presented in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

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

Table 3: Chromatographic conditions

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

2.2.3 Antimicrobial activity

i)-Bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

ii)-Fungal suspensions

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

iii)-Testing for antimicrobial activity

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

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

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

3-Results and Discussion

In this research five plants used in Sudanese system of medicine have been investigated.The oils of the targeted species have been extracted by maceration and the constituents of the oils have been characterized by GC-MS. Furthermore the antimicrobial potential of the oils has been evaluated.

3.1- *Acacia nubica*

3.1.1-The GC-MS analysis of *Acacia nubica* oil

GC-MS analysis of *Acacia nubica* oil was conducted and the identification of the constituents was accomplished by comparison of retention times and through the MS library (NIST). The GC-MS analysis of the studied oil revealed the presence of 24 constituents (Table 3.1).The following constituents were detected in the chromatogram as major constituents:

9,12-Octadecanoic acid methyl ester (31.54%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 3.1. The peak at m/z 294, which appeared at R.T. 17.528 in total ion chromatogram, corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$. The signal at m/z 263 corresponds to loss of a methoxyl function.

9-Octadecanoic acid methyl ester (15.86%)

The mass spectrum of 9-octadecanoic acid methyl ester is displayed in Fig. 3.2.The peak at m/z 296, which appeared at R.T. 17.587 corresponds M+[C19H36O2]+ .The signal at m/z 265 accounts for loss of a methoxyl function.

Hexadecanoic acid methyl ester (15.50%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.3. The signal at m/z 270 (R.T. 15.865) corresponds M+[C17H34O2]+ . The signal at m/z 239 is due to loss of a methoxyl.

Methyl stearate (12.11%)

Fig.3.4 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T. 17.784) corresponds M+[C19H38O2]+, while the peak at m/z 267 corresponds to loss of a methoxyl .

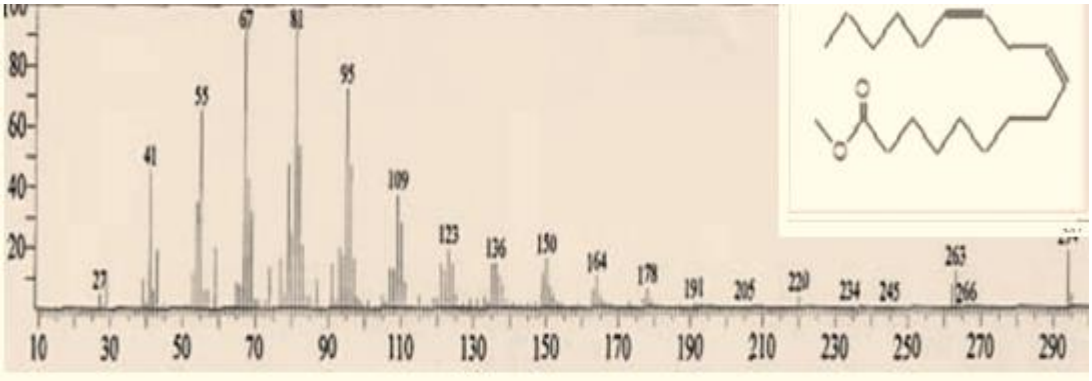


Figure 3.1: Mass spectrum of 9,12-octadecanoic acid methyl ester

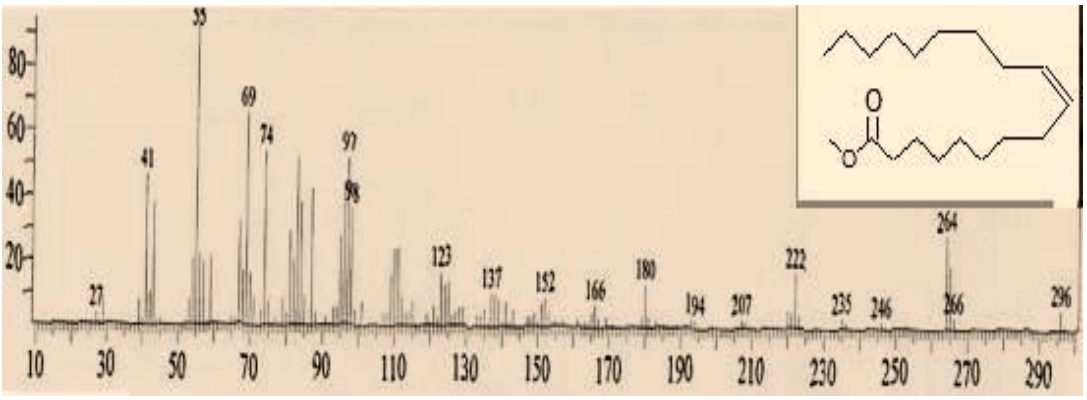


Figure 3.2: Mass spectrum of 9-octadecanoic acid methyl ester

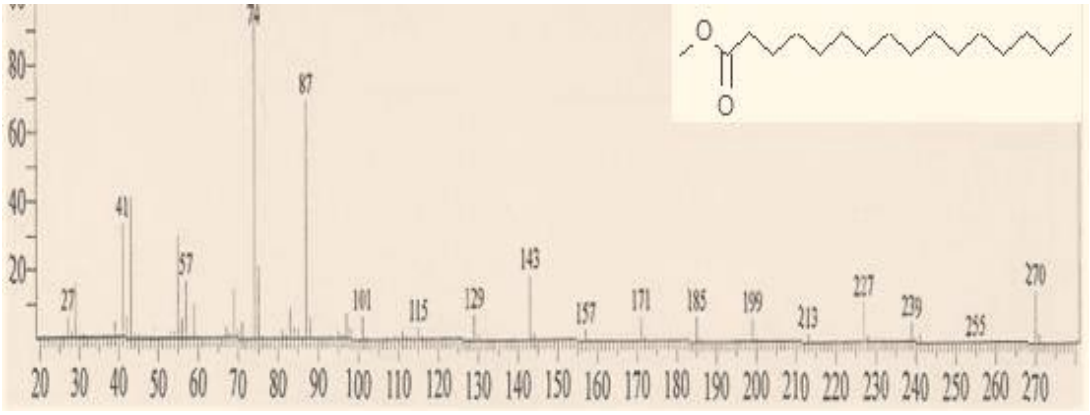


Figure 3.3: Mass spectrum of hexadecanoic methyl ester

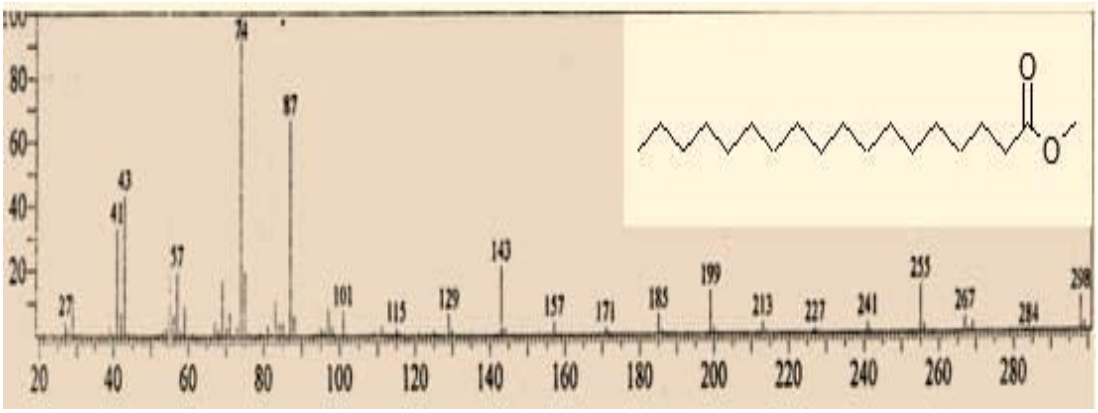


Figure3. 4: Mass spectrum of methyl stearate

Table 3.1: Constituents of *Acacia nubica* oil

No.o.	R.T.	Area	Name
1	11.160	0.10	Beta-curcumene
2	11.261	0.04	Alpha-Farnesene
3	11.326	0.03	Beta-Bisabolene
4	11.419	0.02	Dodecanoic acid, methyl ester
5	11.532	0.06	Cyclhexene,3-(1,5-dimethyl-4-hexenyl)-
6	13.742	0.19	Methyl tetradecanoate
7	14.819	0.06	Pentadecanoic acid, methyl ester
8	15.656	0.55	9-Hexadecenoic acid, methyl ester
9	15.865	15.50	Hexadecanoic acid, methyl ester
10	16.242	2.01	Pentadecanoic acid
11	16.620	0.23	Cis-10--Heptadecenoic acid, methyl ester
12	16.829	0.32	Heptadecanoic acid, methyl ester
13	17.528	31.54	9,12-Octadecadienoic acid(Z,Z)- , methyl
14	17.587	15.86	9-Octadecenoic acid(Z)-, methyl ester
15	17.784	12.11	Methyl stearate
16	19.171	3.63	Tridecanedial
17	19.295	1.54	Oxiraneoctanoic acid ,3-octyl-, methyl
18	19.329	0.71	11-Eicosenoic acid, methyl ester
19	19.531	5.12	Eicosenoic acid, methyl ester
20	19.5889	1.18	BGHI , methyl ester
21	19.698	1.33	9,12,15-Octadecatrienoic acid ,-2,3-
22	20.975	0.35	13-Docosenoic acid ,methyl ester
23	21.154	4.59	Docosenoic acid ,methyl ester
24	21.916	0.43	Tricosanoic acid , methyl ester

3.1.2-Antimicrobial activity of the oil

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (3.2). The results were interpreted in terms of the commonly used terms (>9mm: inative; 9-12mm: partially active; 13-18mm: active; <18mm: very active) . The oil showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and moderate inhibitory

effect against *Pseudomonas aeruginosa*. However, it failed to exhibit anticandidal properties.

Table 3.2: Antimicrobial activity of *Acacia nubica* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	20	22	17	14	--
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

3.2- *Foeniculum vulgare*

3.2.1-GC-MS analysis of *Foeniculum vulgare* oil

The oil of *Foeniculum vulgare* was investigated by GC-MS. The analysis revealed detection of 53 components. The retentions times and percentages of these constituents are illustrated in Table 3.3. Fig.3.5 shows the total ion chromatograms. The hexane fraction was dominated by fatty acids (87.83%) followed by aldehydes (5.80%), ketones (2.90%), alcohols (1.44%), mono-and sesquiterpenes (1.23%) and hydrocarbons (0.80%)-see Fig.3.6.

Major constituents of the hexane fraction are discussed below:

a-9-Octadecenoic acid methyl ester(40.24%)

Fig. 3.7 shows the mass spectrum of 9-octadecanoic acid methyl ester .The peak at m/z 296(R.T. 17.600) accounts for :

$M^{+}[C_{19}H_{36}O_2]^{+}$, while the signal at m/z265 corresponds to loss of a methoxyl .

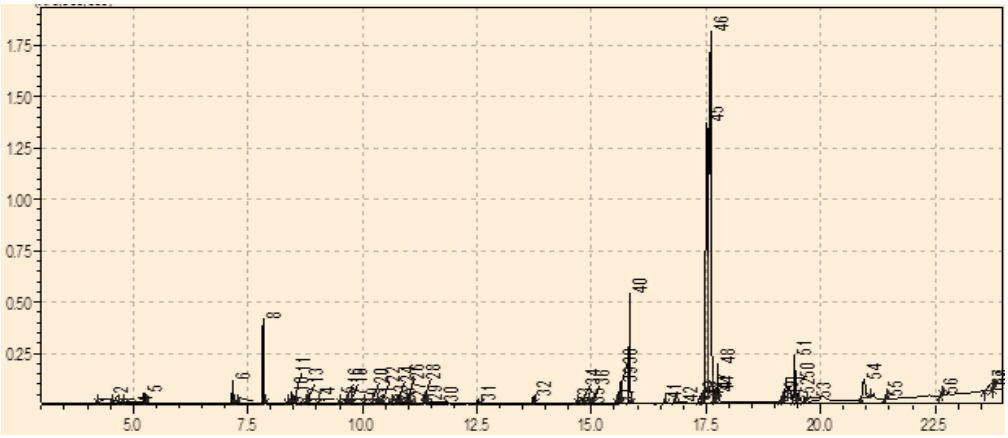


Figure 3.5: Total ions chromatograms

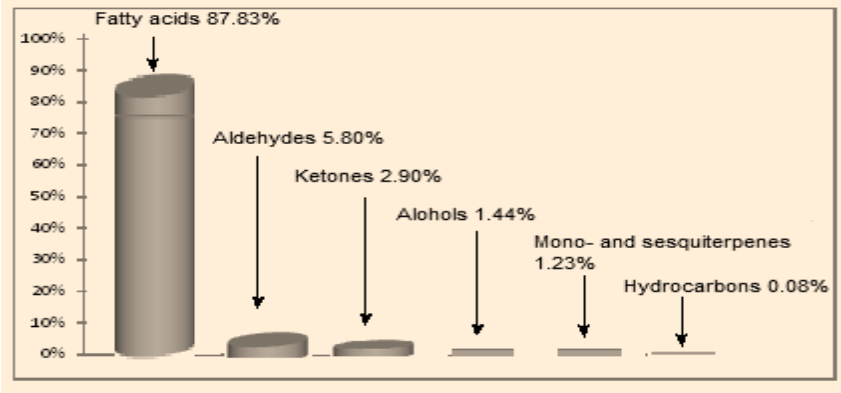


Figure 3. 6: Abundance of oil constituents

b-9,12-Octadecadienoic acid methyl ester(27.38%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 3.8.The peak at m/z 294, which appeared at R.T. 17.519 in total ion chromatogram,is due to the molecular ion : $M+[C_{19}H_{34}O_2]+$ .The peak at m/z263 corresponds to loss of a methoxyl group.

c-Hexadecanoic acid methyl ester(6.69%)

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 3.9.The peak at m/z 270, which appeared at R.T. 15.834 in total ion chromatogram, corresponds to $M^{+}[C_{17}H_{34}O_2]^{+}$ .The peak at m/z239 corresponds to loss of a methoxyl function.

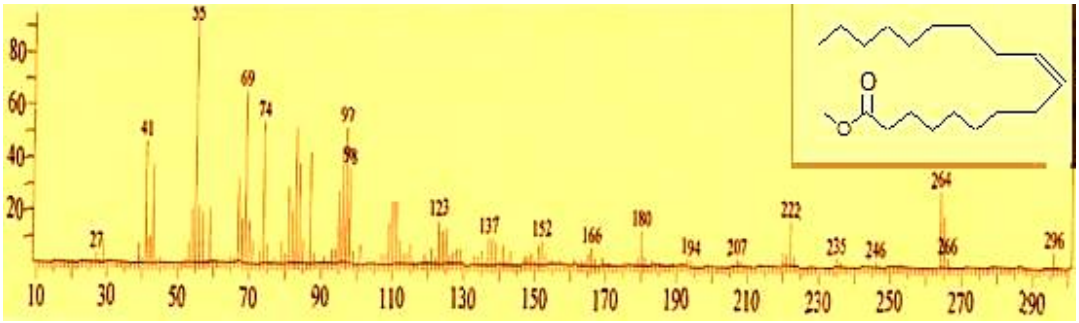


Figure 3.7: Mass spectrum of 9-octadecanoic acid methyl ester

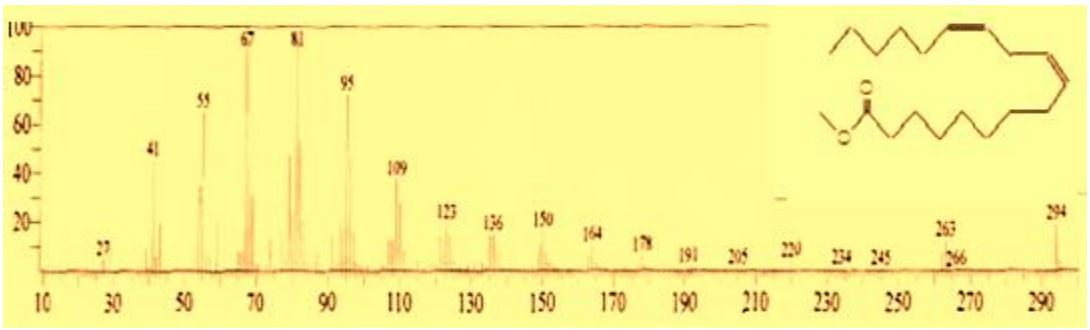


Figure 3.8: Mass spectrum of 9,12-octadecanoic acid methyl ester

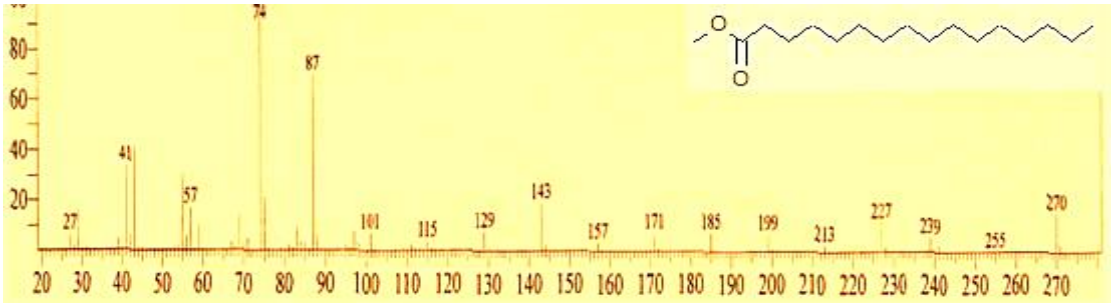


Figure 3.9: Mass spectrum of hexadecanoic methyl ester

Table 3.3: Constituents of the oil

Peak#	R.Time	Area	Area%	Name
1	4.203	84570	0.06	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth
2	4.525	30413	0.02	.alpha.-Phellandrene
3	4.680	11635	0.01	1,3-Cyclohexadiene, 1-methyl-4-(1-methyle
4	4.781	117328	0.08	o-Cymene
5	5.239	790094	0.53	.gamma.-Terpinene
6	7.169	1770355	1.20	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
7	7.254	32642	0.02	Ethanol, 2-(3,3-dimethylcyclohexylidene)-,
8	7.838	7377623	4.98	Benzaldehyde, 4-(1-methylethyl)-
9	8.340	105596	0.07	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
10	8.461	978631	0.66	2-Caren-10-al
11	8.500	84529	0.06	p-Cymen-7-ol
12	8.736	65607	0.04	3-Cyclopenten-1-one, 2-hydroxy-3-(3-meth
13	8.786	151033	0.10	Bicyclo[2.2.1]heptan-2-ol, 7,7-dimethyl-, ac
14	9.014	107999	0.07	1,4-Cyclohexadiene-1-methanol, 4-(1-meth
15	9.496	139965	0.09	Silane, (4-ethylphenyl)trimethyl-
16	9.625	73030	0.05	Benzoic acid, 4-(1-methylethyl)-, methyl es
17	9.685	88198	0.06	2,4-Pentadienoic acid, 3,4-dimethyl-, isopro
18	9.738	172439	0.12	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7
19	9.998	98576	0.07	Benzaldehyde dimethyl acetal
20	10.188	29159	0.02	2,5-Dimethylbenzenethiol, S-pentafluoropr
21	10.310	177806	0.12	Caryophyllene
22	10.421	65563	0.04	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4
23	10.605	255455	0.17	(E)-.beta.-Famesene
24	10.750	30200	0.02	1,4,7,-Cycloundecatriene, 1,5,9,9-tetrameth
25	10.804	24453	0.02	.beta.-copaene
26	10.964	129754	0.09	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene
27	10.999	453512	0.31	Di-epi-.alpha.-cedrene
28	11.320	94523	0.06	.beta.-Bisabolene
29	11.374	209527	0.14	Butylated Hydroxytoluene
30	11.408	113605	0.08	Dodecanoic acid, methyl ester
31	12.520	377438	0.25	Carotol
32	13.726	605076	0.41	Methyl tetradecanoate
33	14.642	54816	0.04	5-Octadecenoic acid, methyl ester
34	14.802	246515	0.17	Pentadecanoic acid, methyl ester
35	14.922	100059	0.07	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodeca
36	15.023	44048	0.03	2-Pentadecanone, 6,10,14-trimethyl-
37	15.534	42935	0.03	7,10-Hexadecadienoic acid, methyl ester
38	15.607	1058379	0.71	7,10,13-Hexadecatrienoic acid, methyl este
39	15.637	1395519	0.94	Methyl hexadec-9-enoate
40	15.834	9906577	6.69	Hexadecanoic acid, methyl ester
41	16.598	463306	0.31	Methyl 18-fluoro-octadec-9-enoate
42	16.809	180927	0.12	Heptadecanoic acid, methyl ester
43	17.361	817919	0.55	Methyl 5,11,14-eicosatrienoate
44	17.411	860594	0.58	Methyl 6,11-octadecadienoate
45	17.519	40550698	27.38	9,12-Octadecadienoic acid (Z,Z)-, methyl e
46	17.600	59585782	40.24	9-Octadecenoic acid (Z)-, methyl ester
47	17.671	817221	0.55	Phytol
48	17.750	2720097	1.84	Methyl stearate
49	19.141	399938	0.27	Methyl 5,13-docosadienoate
50	19.243	2970620	2.01	3-Hydroxy-2,6,6-trimethyl-hept-4-enoic aci
51	19.430	4046234	2.73	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4
52	19.501	533479	0.36	Methyl 18-methylnonadecanoate
53	19.659	417377	0.28	6,9,12,15-Docosatetraenoic acid, methyl est

Antimirobial activity

Foeniculum vulgare hexane fraction was evaluated for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (3.4). Ampicillin, gentamicin and clotrimazole were used as positive control(Table 3.5). *Foeniculum vulgare* hexane fraction exhibited significant activity against *Staphylococcus aureus* in the concentration range: 100-50mg/ml. It also exhibited excellent anticandidal activity at 100mg/ml.

Table 3.4: Antimicrobial activity of the oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	20	14	15	15	17
	50	18	-	14	14	15
	25	17	-	13	13	10
	12.5	15	-	12	12	9
	6.25	11	-	10	7	-

Table3. 5: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamicin	40	25	19	22	21

3.3-*Cordia africana*

3.3.1-GC-MS analysis of *Cordia africana* oil

The total ion chromatograms of *Cordia Africana* oil is shown in Fig. 3.10 and the constituents of the oil are depicted in Table 3.6. The GC-MS analysis revealed the presence of 27 components.

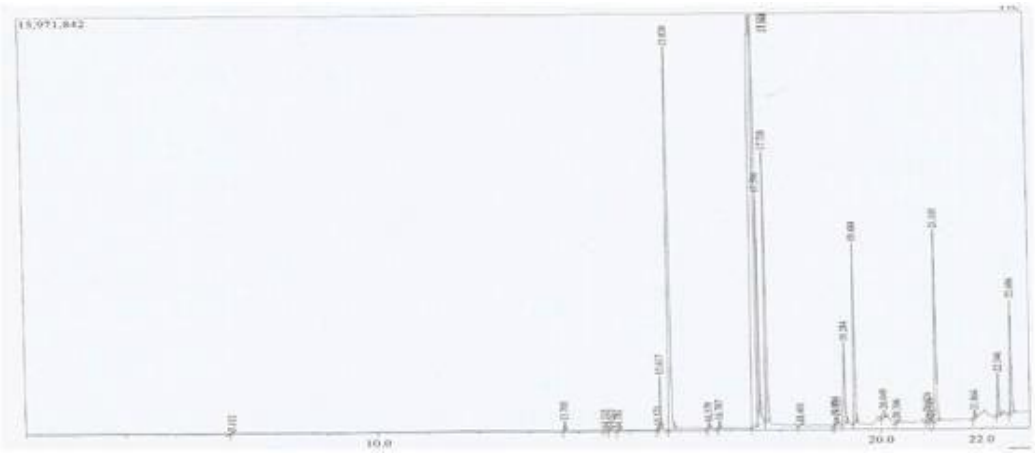


Figure 3.10: Total ion chromatograms of *Cordia Africana*

The following components were detected as major constituents:

a) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (37.02%)

Fig.3.11 shows the mass spectrum of the 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z294, with retention time 17.523, corresponds $M^{+}[C19H34O2]^{+}$. The signal at m/z 263 is due to loss of a methoxyl.

b)-Hexadecanoic acid, methyl ester (18.02%)

Fig. 3.12 shows the mass spectrum of the hexadecanoic acid methyl ester. The peak at m/z 270 (RT: 15.830) accounts for the molecular ion: $M^+[C17H34O2]^+$, while the signal at m/z 239 is due to loss of a methoxyl group.

c)-Methyl stearate (10.01%)

The mass spectrum of methyl stearate is shown in Fig. 3.13.The signal at m/z 298(RT: 17.738) is due to**:** $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 is due to loss of a methoxyl.

d)- Docosanoic acid, methyl ester (6.83%)

The mass spectrum of docosanoic acid methyl ester is presented in Fig. 3.14. The signal at m/z 354 (RT: 21.105) is due to: $M^+[C_{23}H_{46}O_2]^+$. The signal at m/z 323 is due to loss of a methoxyl group.

e)- 9-Octadecenoic acid (Z)-, methyl ester (6.18%)

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is displayed in Fig. 3.15. The peak at m/z 296 (RT: 17.568) is due to the molecular ion: $M^+[C_{19}H_{36}O_2]^+$.The signal at m/z 265 is attributed to loss of a methoxyl function.

(f) Eicosanoic acid, methyl ester (5.77%)

Fig. 3.16 shows the mass spectrum of the eicosanoic acid methyl ester. The peak at m/z 326 (RT: 19.484) corresponds

$M^+[C_{21}H_{42}O_2]^+$, while the signal at m/z 295 accounts for loss of a methoxyl.

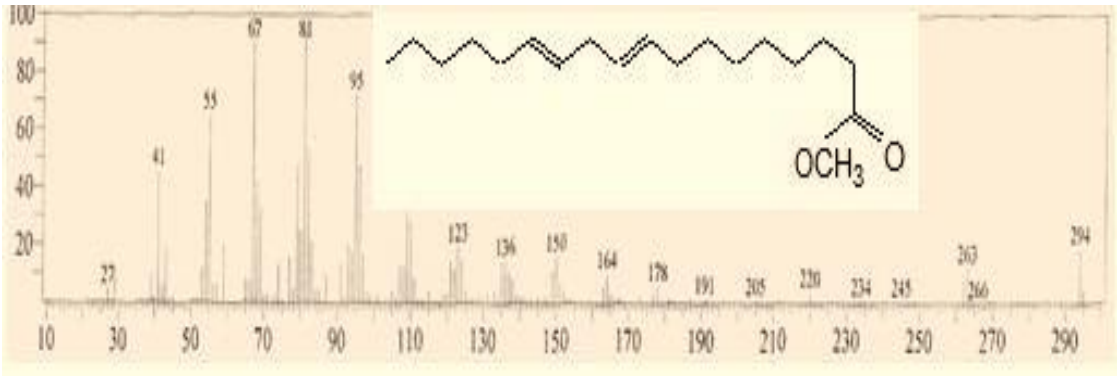


Figure 3.11: Mass spectrum of 9,12-octadecadienoic acid methyl ester

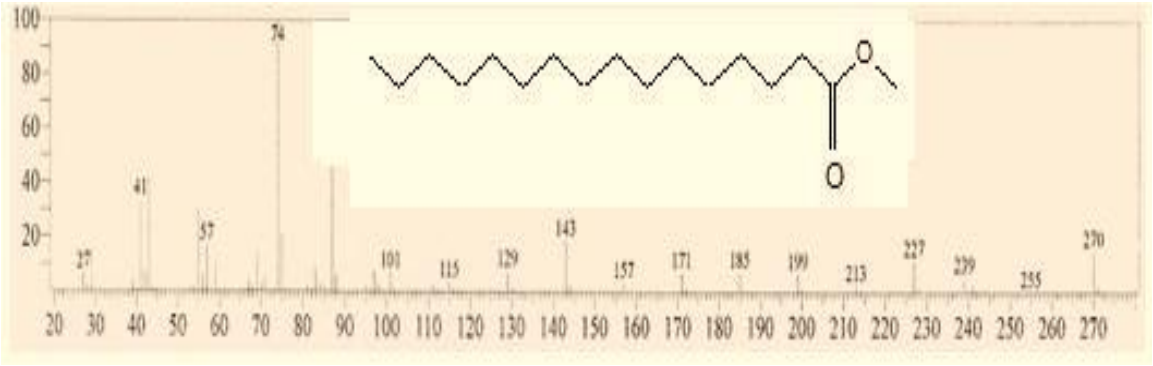


Figure 3.12: Mass spectrum of hexadecanoic acid methyl ester

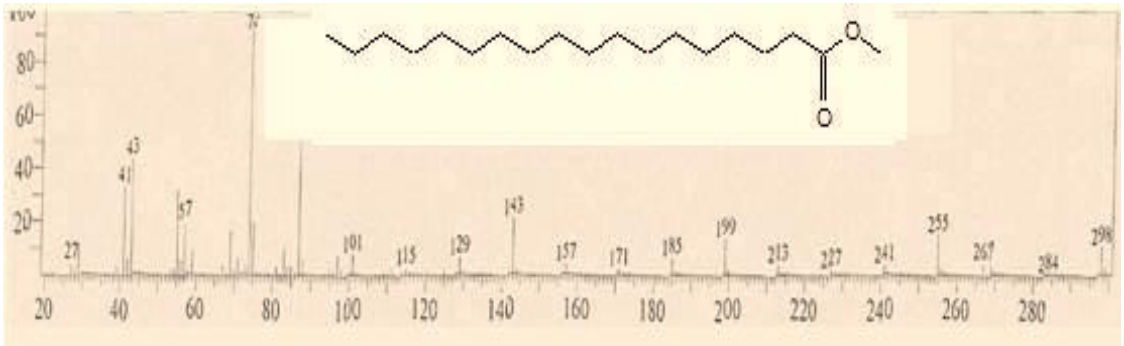


Figure3. 13: Mass spectrum of methyl stearate

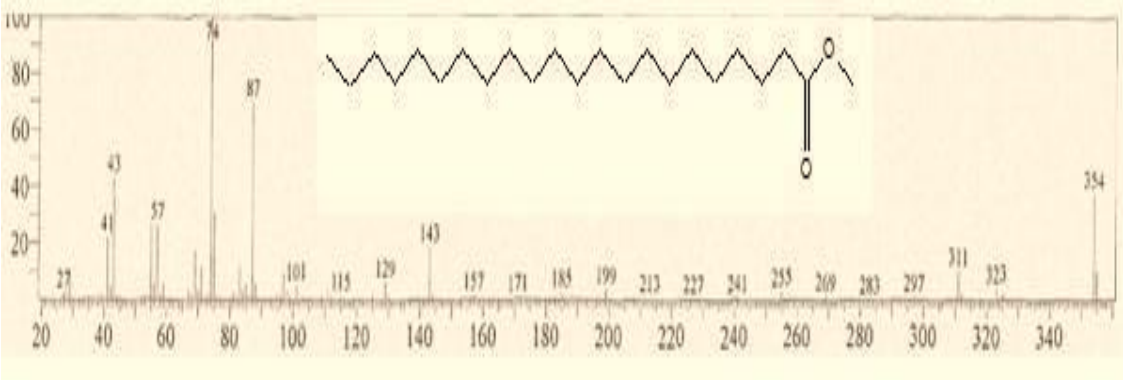


Figure 3.14: Mass spectrum docosanoic acid, methyl ester

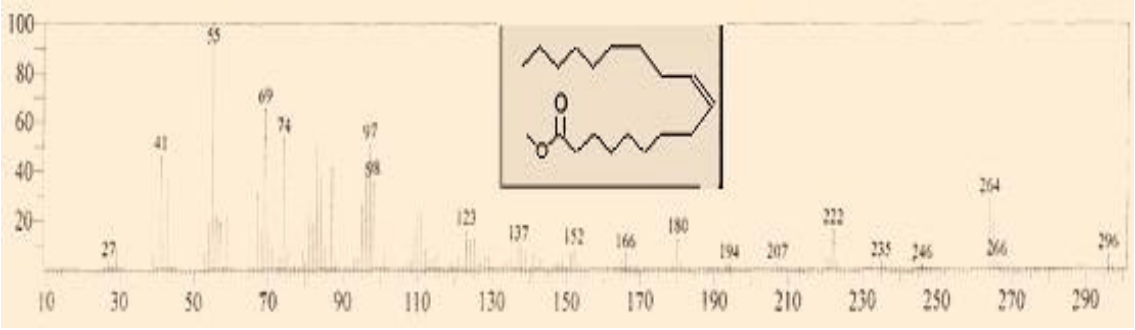


Figure 3.15: mass spectrum of 9-octadecenoic acid (Z)-, methyl ester

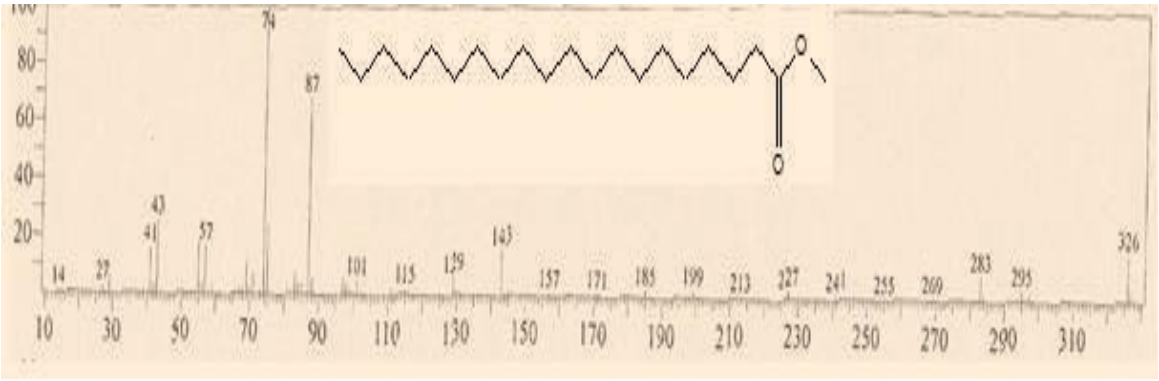


Figure3. 16: Mass spectrum eicosanoic acid methyl ester

Table 3.6: Constituents of *Cordia Africana* oil

No.	R. Time	Area%	Name
1	7.112	0.05	Alpha-Terpineol
2	13.705	0.24	Methyl tetradecanoate
3	14.515	0.03	4-Octadecenoic acid methyl ester
4	14.621	0.04	Cis-5-Dodecenoic acid methyl ester
5	14.781	0.03	Pentadecanoic acid methyl ester
6	15.571	0.11	Methyl hexadec-9-enoate
7	15.617	1.47	9-Hexadecenoic acid methyl ester
8	15.830	18.02	Hexadecanoic acid methyl ester
9	16.579	0.19	Cis-10-Heptadecenoic acid methyl ester
10	16.787	0.22	Heptadecanoic acid methyl ester
11	17.523	37.02	9,12-Octadecadienoic acid methyl ester
12	17.568	6.18	9-Octadecenoic acid (Z)-methyl ester
13	17.590	3.63	9-Octadecenoic acid methyl ester(E)-
14	17.738	10.01	Methyl stearate
15	18.401	0.12	Cis-10-Nonadecenoic acid methyl ester
16	19.084	0.27	Cyclopropaneoctanoic acid methyl ester
17	19.284	0.21	9,12-Octadecdienyl chloride
18	19.284	3.24	Cis-11-Eicosenoic acid methyl ester
19	19.484	5.77	Eicosanoic acid methyl ester
20	20.049	0.40	Stigmast-7-en-3-ol
21	20.306	0.11	Heneicosanoic acid methyl ester
22	20.926	0.24	13-Docosenoic acid methyl ester
23	2.981	0.10	Methyl 11-docosenoate
24	21.105	6.83	Docosanoic acid methyl ester
25	21.866	0.27	Tricosanoic acid methyl ester
26	22.346	1.36	hexatricontane
27	22.606	3.86	Tetrcosanoic acid methyl ester
		100.00	

3.3.2-Antimicrobial activity of the oil

The oil was screened for antimicrobial activity against five standard microorganisms (Table 3.7). The results are depicted in Table 3.8. The oil showed moderate activity against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa.* Ampicilin, gentamicin and

clotrimazole were used as positive controls (Table 3.9).

Table 3.7: Test organisms

No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC* 2836
2	*Staphylococcus aureus*	G+ve	ATCC* 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC* 27853
4	*Escherichia coli*	G-ve	ATCC* 25922
5	*Candida albicans*	fungi	ATCC* 7596

* NCTC. National collection of type culture, Colindale, England
*ATCC. American type culture collection, Maryland, USA

Table 3.8: Inhibitory effect of the oil

Sample	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	15	7	15	12	14

* B.S. = *Bacillus subtilis*, S.a. =*Staphylococcus aureus*, E.c. = *Escherichia coli*, P.a. = *Pseudomonas aeruginosa*, C.a.= *Candida albicans* ; Result: >18 mm: Sensitive, 13 to 18 mm: moderate: 9-12 ,partially active: : > 9 , inactive.

Table 3.9: Inhibitory effect of standard drugs

Drug	Sa	Bs	Ec	Ps	Ca
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

3.4- *Lens culinaris*

3.4.1-GC-MS analysis of *Lens culinaris* oil

GC-MS analysis of *Lens culinaris* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library.

The GC-MS spectrum of the studied oil revealed the presence of 26 constituents (Table 3.10). The typical total ion chromatograms (TIC) is depicted in Figure 3.17.

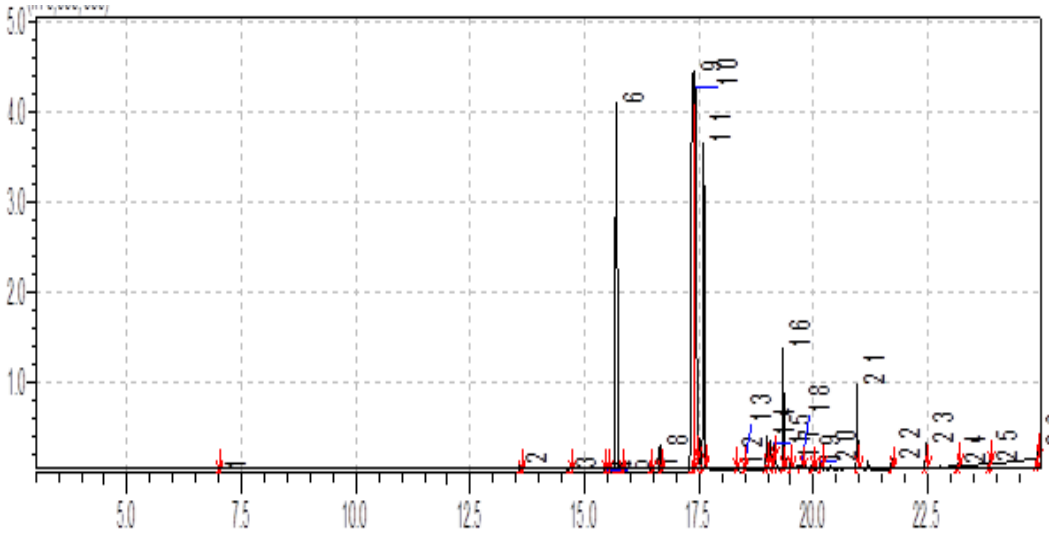


Figure3. 17: Total ions chromatogram

Table 3.10 : Constituents of the oil

No.	Name	RT.	Area%
1.	L-.alpha.-Terpineol	6.981	0.06
2.	Methyl tetradecanoate	13.565	0.25
3.	Pentadecanoic acid, methyl ester	14.639	0.17
4.	6-Octadecenoic acid, methyl ester, (Z)-	15.435	0.07
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.471	0.07
6.	Hexadecanoic acid, methyl ester	15.698	20.28
7.	7,10-Hexadecadienoic acid, methyl ester	16.433	0.19
8.	Heptadecanoic acid, methyl ester	16.643	0.83
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.383	37.49
10.	9-Octadecenoic acid (Z)-, methyl ester	17.415	17.19
11.	Methyl stearate	17.598	12.45
12.	cis-10-Nonadecenoic acid, methyl ester	18.288	0.13
13.	Nonadecanoic acid, methyl ester	18.473	0.07
14.	Z,Z-3,13-Octadecadien-1-ol	18.973	0.85
15.	cis-13-Eicosenoic acid, methyl ester	19.135	0.38
16.	Eicosanoic acid, methyl ester	19.335	3.70
17.	8,11,14-Docosatrienoic acid, methyl ester	19.501	0.42
18.	Methyl 2-octylcyclopropene-1-octanoate	19.738	0.31
19.	2-Furanpentanoic acid, tetrahydro-5-nonyl-,	19.976	0.24
20.	Heneicosanoic acid, methyl ester	20.158	0.28
21.	Docosanoic acid, methyl ester	20.954	2.74
22.	Tricosanoic acid, methyl ester	21.717	0.39
23.	Tetracosanoic acid, methyl ester	22.455	0.87
24.	Pentacosanoic acid, methyl ester	23.168	0.12
25.	Hexacosanoic acid, methyl ester	23.858	0.07
26.	Hexatriacontane	24.916	0.38

Major constituents are briefly discussed below:

i)-9,12-Octadecadienoic acid methyl ester (37.49%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Figure 3.18. The signal which was observed at m/z 294 (R.T. 17.383) is due to M+$[C_{19}H_{34}O_2]$+ , while the signal at m/z 263 corresponds to loss of a methoxyl.

ii) Hexadecanoic acid methyl ester (20.28%)

Figure 3.19 shows the mass spectrum of hexadecanoic acid

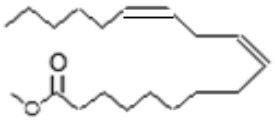
methyl. The peak m/z 270 (R.T. 15.698) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 is due to loss of a methoxyl .

iii)9-Octadecenoic acid methyl ester (17.19%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Figure 3.20. The peak at m/z 296 (R.T. 17.415) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 266 is attributed to loss of a methoxyl.

iv)-Methyl stearate(12.45%)

The EI mass spectrum of methyl stearate is displayed in Figure 3.21. The peak at m/z 298 with R.T. 17.598 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl group.



9,12-Octacedienoic acid methyl ester

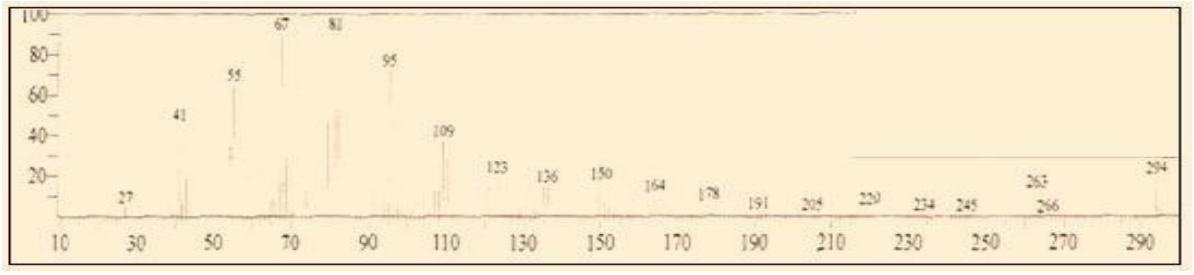
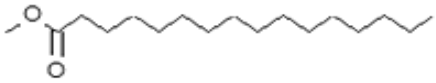


Figure3. 18: Mass spectrum of 9,12-octadecadienoic acid methyl ester



Hexadecanoic acid methyl ester

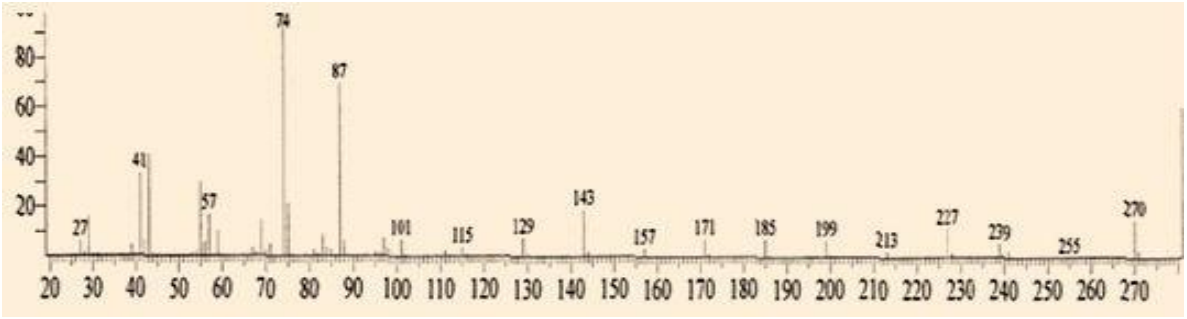
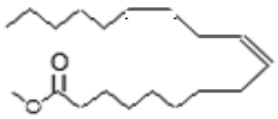


Figure3. 19: Mass spectrum of hexadecanoic acid methyl ester



9-Octadecenoic acid methyl ester

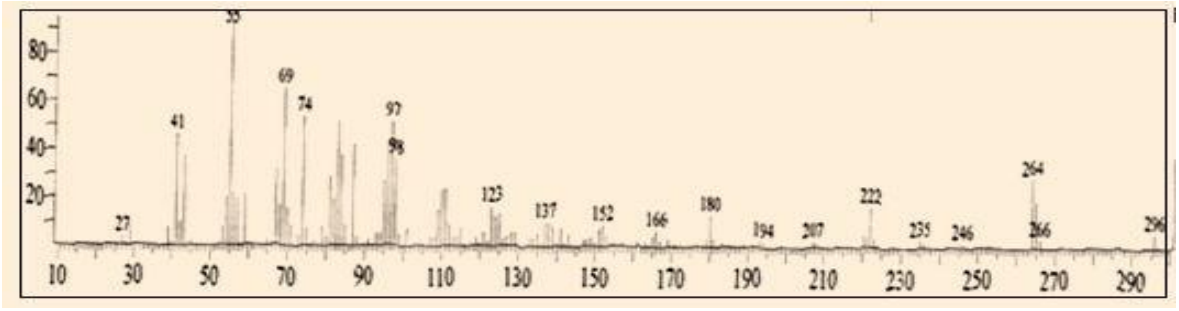
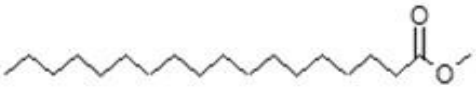


Figure 3.20: Mass spectrum of 9-octadecenoic acid methyl ester



Methyl stearate

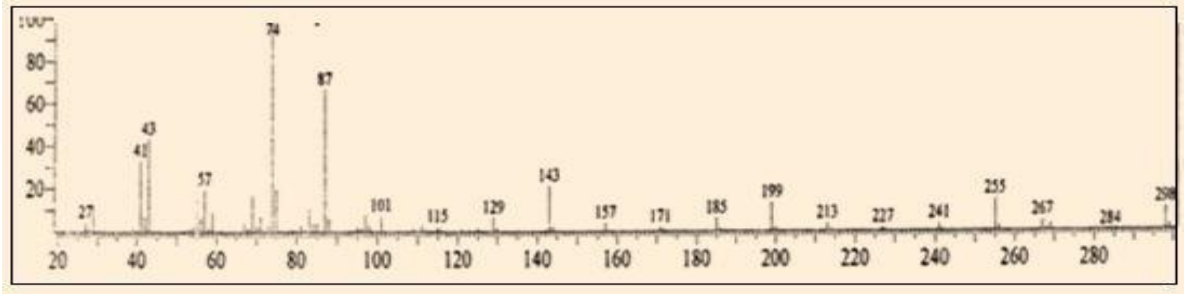


Fig.3.21 : Mass spectrum of methyl stearate

Antimicrobial activity

Lens culinaris 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 3.11.The results were interpreted in commonly used terms (>9mm: inative;9-12mm:partially active; 13-18mm: active. <18mm:very active). The oil showed good antibacterial activity against *Escherichia coli*. However, it exhibited partial activity against *Bacillus subtilis.*

Table 3.11: Inhibition zones (mm/mg sample)

Sample	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	--	10	14	--	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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

3.5- *Acacia polycantha*

3.5.1-The GC-MS analysis *Acacia polycantha* oil

The studied oil was analyzed by GC-MS and the identification of the constituents was accomplished by comparison of retention times and through the MS library (NIST). The GC-MS analysis of the studied oil revealed the presence of 32 constituents (Table 3.13). The following constituents were detected in the chromatogram as major constituents:

9,12-Octadecadienoic acid methyl ester(27.95%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3.22. The peak at m/z 294, which appeared at R.T. 17.514 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z263 is due to loss of a methoxyl function.

Methyl stearate(17.13%)

Fig. 3.23 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T. 17.757) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 accounts for loss of a methoxyl.

Hexadecanoic acid methyl ester(13.30%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 3.24. The signal at m/z 270 (R.T. 15.827) corresponds $M+[C_{17}H_{34}O_2]+$. The signal at m/z239 is due to loss of a methoxyl.

9-Octadecenoic acid(Z) methyl ester (9.57%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 3.25. The peak at m/z 296, which appeared at R.T. 17.564 corresponds $M^{+}[C_{19}H_{36}O_2]^{+}$. The signal at m/z265 accounts for loss of a methoxyl function.

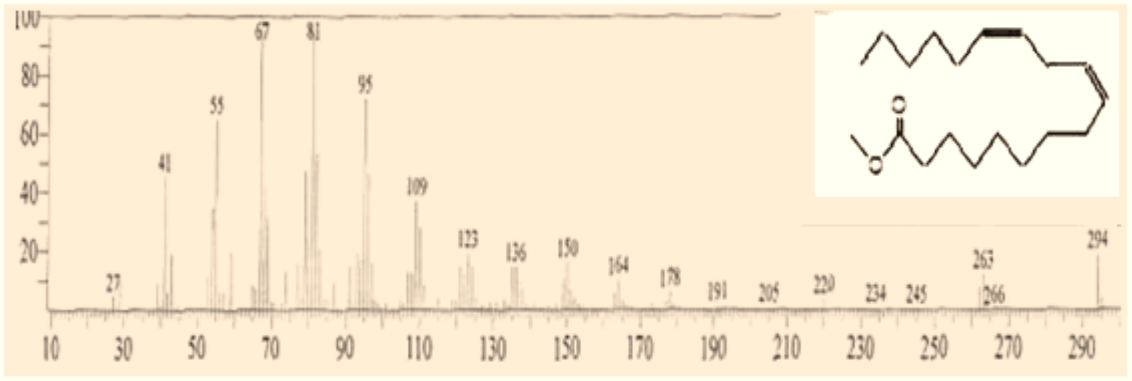


Figure 3.22: Mass spectrum of 9,12-octadecadienoic acid methyl ester

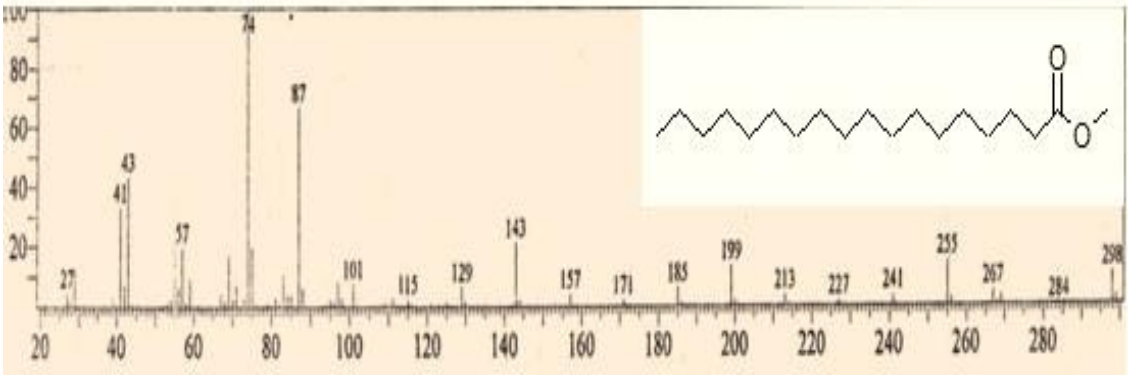


Figure 3.23: Mass spectrum of methyl stearate

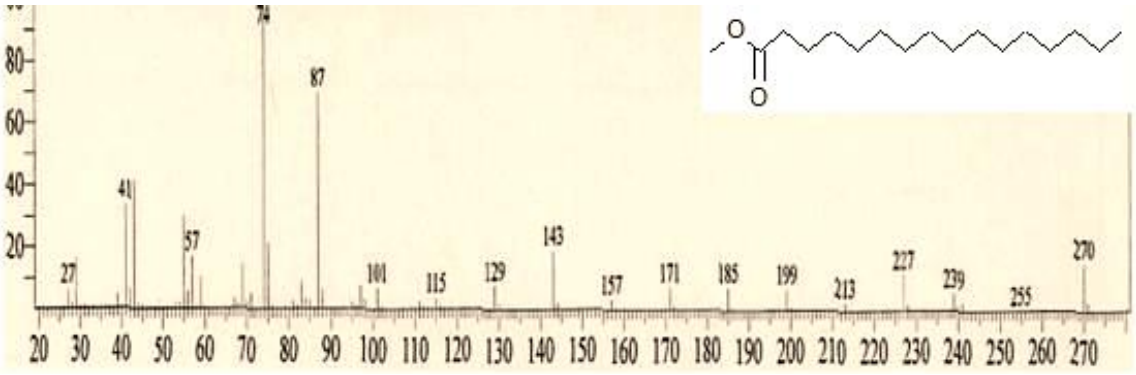


Figure 3.24: Mass spectrum of hexadecanoic acid methyl ester

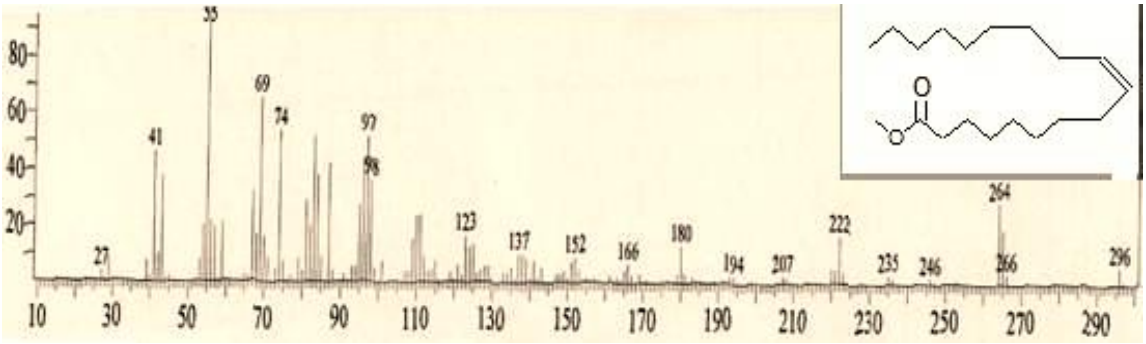


Figure 3.25: Mass spectrum of 9-octadecenoic acid methyl ester

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (3.12). The results were interpreted in terms of the commonly used terms (>9mm: inative; 9-12mm:partially active; 13-18mm: active; <18mm: very active). The oil showed moderate activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* However, it failed to exhibit activity against other test organisms.

Table3. 12 : Antimicrobial activity of the oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	7	8	15	--
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

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

Table 3.13: Constituents of the oil

No.	Name	Ret. time	Area %
1	Alpha-Terpeneol	7.112	0.04
2	Dodecanoic acid	11.386	0.01
3	Methyl tetradecaanoate	13.705	0.08
4	Cis-5-Docenenoic acid ,methyl ester	14.517	0.01
5	Pentadecanoic methyl ester	14.780	0.03
6	Cis-10-Nonadecenoic acid methyl ester	15.616	1.48
7	9-Hexadecenoic acid methyl ester	15.710	0.08
8	Hexadecenoic acid methyl ester	15.827	13.30
9	Cis-10-Heptadecenoic acid Hexadecenoic acid	16.579	0.24
10	Heptadecanoic acid Hexadecenoic acid methyl	16.787	0.37
11	9,12-Octadecadienoic acid methyl ester	17.514	27.95
12	9-Octadecenoic acid (Z) methyl ester	17.564	9.57
13	9-Octadecenoic acid (E) methyl ester	17.590	3.08
14	Phytol	17.651	0.36
15	Methyl stearate	17.757	17.13
16	Trans-Geranylgeraniol	18.392	0.15
17	Nonadecanoic acid methyl ester	18.622	0.15
18	Gama-Linolenic acid methyl ester	18.764	0.07
19	Methyl-5,11,14-eicosatrienoic acid methyl ester	19.086	0.60
20	Tridecanedial	19.127	4.71
21	Oxiraneoctanoic acid methyl ester	19.248	2.86
22	Cie-11-Eicosenoic acid methyl ester	19.285	0.72
23	Eicosanoic acid PGHI , methyl ester	19.491	7.91
24	PGHI , methyl ester	19.540	1.19
25	1-Naphthalenol decahydro-4a- , PGHI , methyl	19.648	1.49
26	Tricyclo[20.8..0.(7.16)]triacontane	19.982	0.23

27	Stigmast-7-en-3-ol,(3-beta-,5-alpha.,24S)	20.046	0.29
28	Heneicosanoic acid PGHI , methyl ester	20.307	0.23
29	Phenol,2,2`-methylene-bis[6-(1,1-dimethyl)	20.405	0.06
30	Methyl 20-methyl-heneicosanoate	21.105	3.52
31	Tricosanoic acid PGHI , methyl ester	21.868	0.46
32	Tetracosanoic acid PGHI , methyl ester	22.608	2.63

Conclusion

In this research five plants(*Acacia nubica*, *Foeniculum vulgare, Cordia Africana, Lens culaniris* and *Acacia polycantha)* used in Sudanese system of medicine have been investigated. The oils of the targeted species have been extracted by maceration and the constituents of the oils have been characterized by GC-MS. Furthermore the antimicrobial potential of the oils has been evaluated using the cup plate agar diffusion bioassay where different antimicrobial responses were detected.

Recommendations

1- The extracted oils may be evaluated for other biological activities like antimalarial , antiviral …etc.

2- Other phytochemicals of the targeted plant species may be isolated and identified. Furthemore they be evaluated for their biological activity.

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