1-Introduction

1.1-The targeted plant species

1.1.1- *Hyphaene thebaica* L.

Hyphaene thebaica L. (also known as Doum) is a type of palm tree in the family Arecaceae. In Sudan, the plant grows along Nile river banks [1,2]. Doum fruit pulp contains nutritional trace minerals,high quality protein beside the nutritionally essential linoleic acid [3]. The fruit also contains flavonoids, steroids, glycosides, terpenes and tannins[4,5]. Fruits are used traditionally against bilharzias and hypertension[6]. Also it could be of great merit for use as hypolipidemic agent [7]. It has been reported that the fruit possesses hypoglycemic properties[8]. Significant decrease in cholesterol, blood glucose, triglycerides and total lipids has been reported after administration of fruit decoction for a period of 1-2 months [9,10].

Hyphaene thebaica

Analysis of fruit aqueous extract revealed the presence of some bioactive flavonoids including quercetin, hesperetin and naringin[5]. The free radical scavenging capacity of fruit extracts has been documented[11,12]. The accumulation of free radicals in the body is a risk factor in many harmful oxidative processes including degenerative diseases of ageing and cancer[13-15]. Previous studies demonstrated the antiinflammatory activity of Doum extracts[16]. Such activity has been associated with the presence of saponins, flavonoids and coumarins which are known for their anti-proliferative activity [17]. The antibacterial activity of Doum extracts has also been reported[12,18].

1.1.2-*Acacia meelifera*

The genus *Acacia* (Fabaceae) contains around 1350 species[19]. Some Acacia species are rich in bioactive secondary metabolites such as flavonoids which have been reported from different *Acacia* species [20] and many Acacia species find wide applications in traditional medicine [21]. For example *Acaia nilotica* is a pioneer species and considered as a source of gum, timber and fodder[22]. Various parts of *Acaia nilotica* are used in Sudanese system of medicine. Leaves are antipyretic, astringent, tonic and a remedy for dysentery and diarrhea [23-25]. Bark is used traditionally against leucorrhoea, piles and vaginitis [26]. The roots are used for wounds and leucorrhoea [27].

Pod is a remedy for cough, impotency and urino-genital disorder [28], while gum is used against diabetes [29]. *Acacia nilotica* is also used traditionally in Sudan as a remedy for malaria, sore throat and intestinal worms [30-33].

Acacia mellifera

Acacia seyal- is considered as a safe dietary fiber by the United States Food and Drug Administration and its therapeutic uses were extensively examined in animal models[34;35]. The antioxidant activity of the medicinally important *Acacia auricoliformis* has been documented [25] and some *Acacia* species were claimed to exhibit potent antimicrobial activity [36].

Data on the medicinally important *Acacia mellifera* is very scarce. However, a methylated dihydrochalcone has been reported from *Acacia mellifera* bark [37].

1.1.3- *Parkinsonia aculeata*

Parkinsonia aculeata is small spiny deciduous tree in the legume family (Leguminosae) [38]. The plant is native to tropical America. Now it is widely distributed in many African and Asian countries [39;40]. *Parkinsonia aculeata* grows up to 3-10 m in height and it is characterized by a green bark and smooth branches [41]. Alkaloids and steroids have been reported from the leaves, stems and flowers. The edible seeds contain some proteins. Seeds are mucilaginous and reported to contain sugars and fatty oil [42;43].

Parkinsonia aculeata

Parkinsonia aculeata is used traditionally as antipyretic, and leaves are said to be diaphoretic and abortifacient[44]. Leaves are used against fever, malaria and rheumatic pain [45;46]. The Leaves are also used in the treatment of bacterial infections, typhoid fever, diabetes, diabetes-related complications and trypanosomiasis [47].

1.1.4- *Leucaenia leucocephala* L

Leucaenia leucocephala L. is an evergreen shrub in the family Fabaceae. The plant can reach 5-20m in height[48;49]. *Leucaenia leucocephala* is native to southern Mexico and northern and central America. However, the plant is diffused through the continents [50]. *Eucaenia leucocephala* is a multipurpose tree providing food, medicine, shade and firewood[51-54] Young leaves and pods are edible, though some caution is advised since leaves contain mimosene- an amino acid- which can be harmful in large quantities [55;56], roasted seeds are emollient [57]. A decoction of bark and root is used as abortefacient[55] . *Leucaenia leucocephala* has been used traditionally as anthelmintic, antimicrobial, antidiabetic, antihistaminic, antitumor and hepatoprotective [58].

Leucaenia leucocephala

Phytochemical screening of leaves revealed the presence of cardiac glycosides, alkaloids, flavonoids, tannins and saponins [51]. Bioactivity- guided studies indicated anthelmintic, antidiabetic and antiproliferative activities [51]. It has been reported that the leaves and seed extracts possess antioxidant activity [59]. These extracts affected renal function by reducing total protein and albumin. Leaves and seed possess antidiabetic effect [60;61].

1.1.5-*Sorghum bicolor*

Sorghum is a genus of plants in the family Gramineae which is widely cultivated as cereal crop [62]. Sorghum is the fifth most grown crop worldwide. In Africa Sorghum bicolor has been cultivated long ago [62] .This plant is a cone- like grass reaching 6m in height with branched clusters of grains. *Sorghum bicolor* is rich in phenolics which are known for their diverse pharmacological properties [63].

Sorghum bicolor

The flavonoids of this plant species contains , among others, apiginidin and 3-deoxyanthocyanidin [64;65] In contrast to other grains, sorghum grains are endowed with higher flavonoid content ranking them as an important diet [65] .Many health promoting effects has been associated with sorghum intake. The *in vivo* antianemic properties of Sorghum bicolor has been reported [66;67] .It has been shown that the leaf extracts possess anti-inflammatory activity [68-70]. Leave extract also exhibited significant free radical scavenging capacity [70] .Moreover, the role of leave extract in neurocognitive disorder has been documented [71;72].

1.2- Essential Oils

The term essential oil dates back to the sixteenth century and derives from the drug *(Quinta essential*), named by Paracelsus von Hohenheim of Switzerland[1]. Numerous authors have attempted to provide a definition of essential oils. The French Agency for Normalization defines essential oils as follows: The essential oil is the product obtained from a vegetable raw material, either by steam distillation or by mechanical processes[73].

An essential oil is a liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as oil of clove. The advantages of essential oils are their flavor concentrations and their

similarity to their corresponding sources. The majorities of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits. All parts of aromatic plants may contain essential oils

1.2.1- Essential oils extraction methods

There are several methods of extraction of essential oils. The timid technologies about essential oils processing are of abundant significance and are still overused around the globe[74].

a. Hydrodistillation (HD)

The conventional method for the extraction of essential oils is hydrodistillation, in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser. The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively[74].

b. Solvent extraction

Solvent extraction, also known as liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done by using two liquids that don't mix, for example, water and an organic solvent. In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent[74].

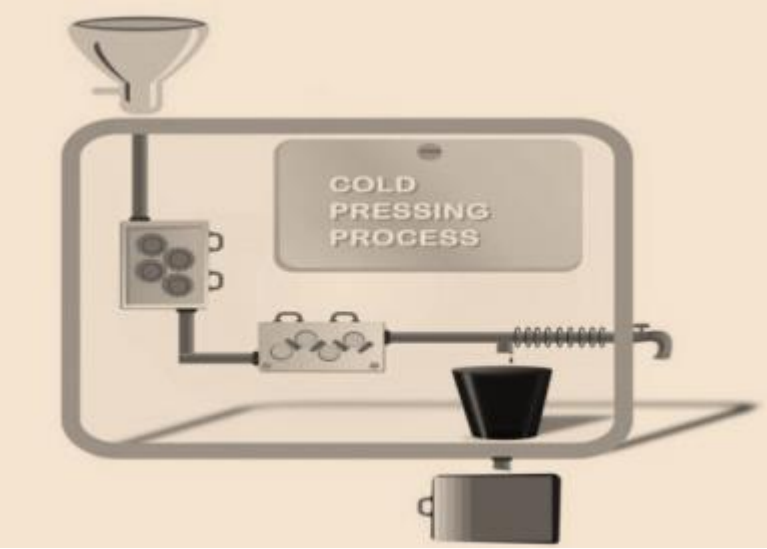
c. Soxhlet extraction

A Soxhlet extractor is a piece of laboratory apparatus, invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional Soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid[75].

d. Cold pressing method

The term cold pressed theoretically means that the oil is expeller-pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant .Cold pressed method is mainly used for extracting essential oils from plants, flower, seeds, lemon, tangerine oils[76] . In this process, the outer layer of the plants containing the oil is removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the

pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.

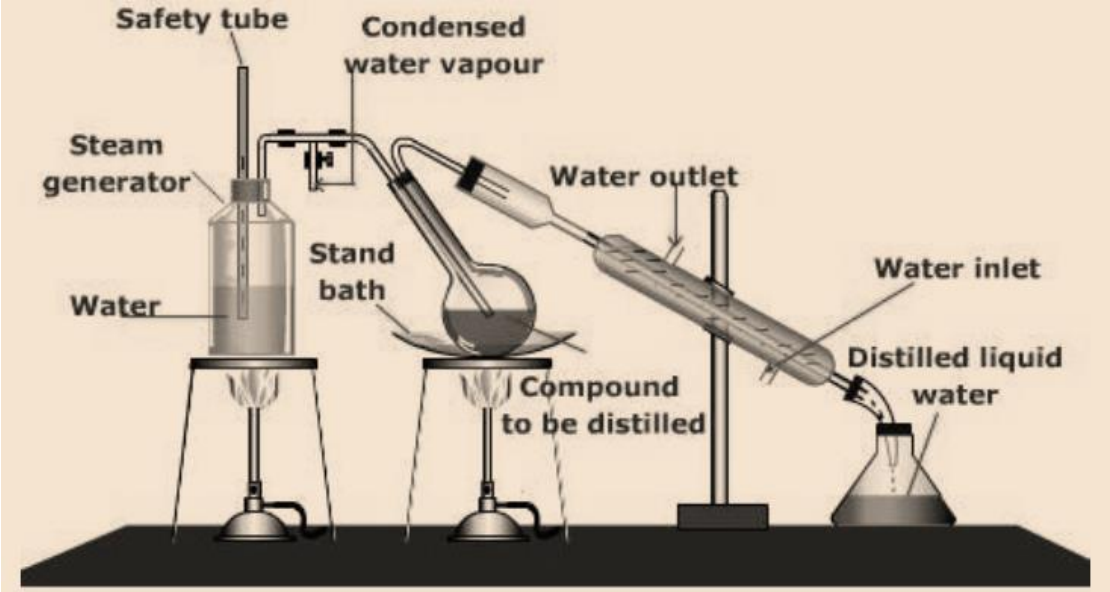


Cold Pressing Method

e. Steam Distillation

Steam distillation is a type of distillation (a separation or extraction process) for a temperature-sensitive plant such as natural aromatic compounds. Steam distillation is one of ancient and officially approved methods for isolation of essential oils from plant materials. The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material. This steam functions as agents that break up the pores of the raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then condensed further

and the essential oil is collected[77]. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.



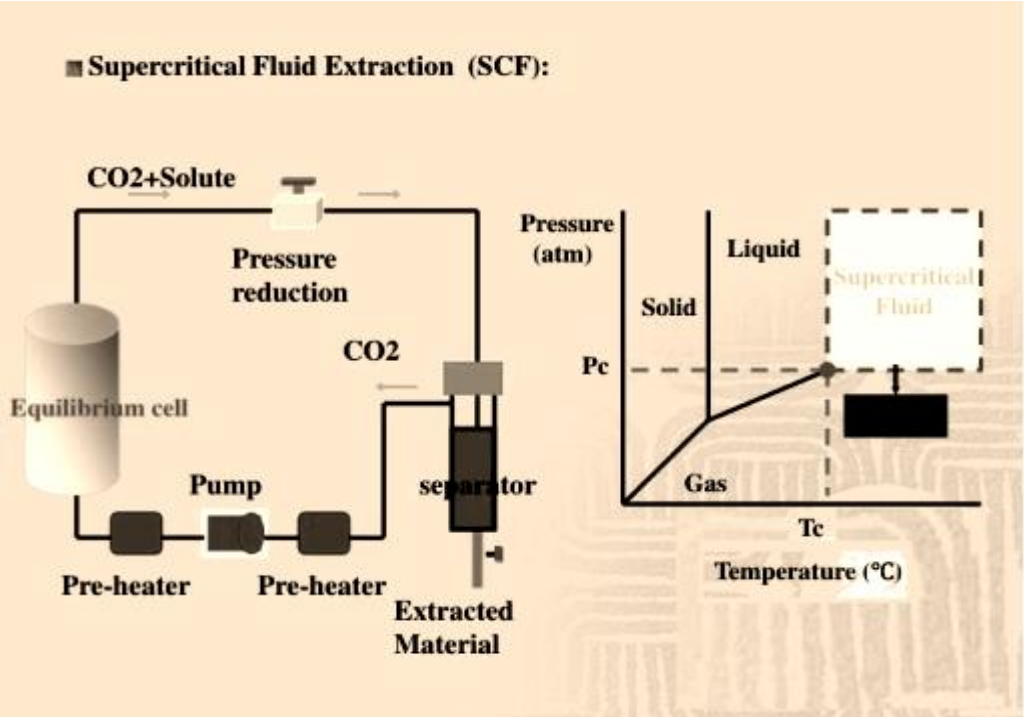
Apparatus for steam distillation

One of the disadvantages of conventional techniques is related with the thermolability of essential oils components which undergo chemical alterations (hydrolysis, isomerization, and oxidation) due to the high applied temperatures. New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO_2 emissions.

(i) Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Supercritical fluids (Jordan, 1991)such as essential oil extraction and metal cation extraction (Scheme 4). In practice, more than 90% of all analytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bars) and temperature (32^oC), CO_2 is relatively non-toxic, nonflammable, noncorrosive, safe, available in high purity at relatively low cost and is easily removed from the extract[78] .The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes. These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. It was found that extracts prepared by SFE yielded a higher antioxidant activity than extract prepared by other methods .This extraction method produces higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by steam distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical extracts

proved to be of superior quality, with better functional and biological activities[79]. Furthermore, some studies showed better antibacterial and antifungal properties for the supercritical product.

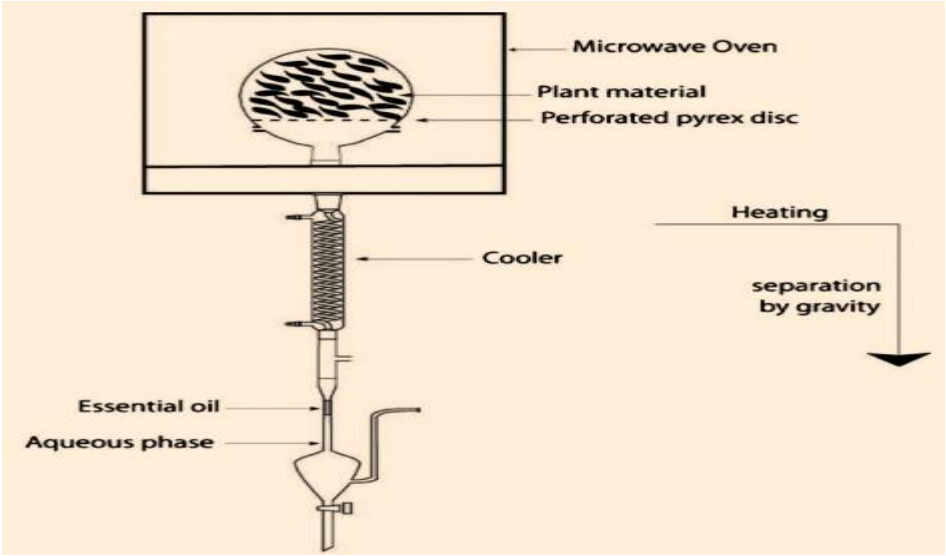


Supercritical fluid extraction set

(ii) Microwave hydrodiffusion and gravity (MHG):

(MHG) Is a new green technique for the extraction of essential oils. This green extraction technique is an original microwave blend microwave heating and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction of essential oils from different kind of plants (Scheme 5) (Guenther and Althausen, 1948). Microwave hydrodiffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require

solvent or water and as it does require less energy (Likibi et al., 2000). The performances and advantages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90 min or more but in this technique only 20 min) and reducing environmental impact and power saving[80].

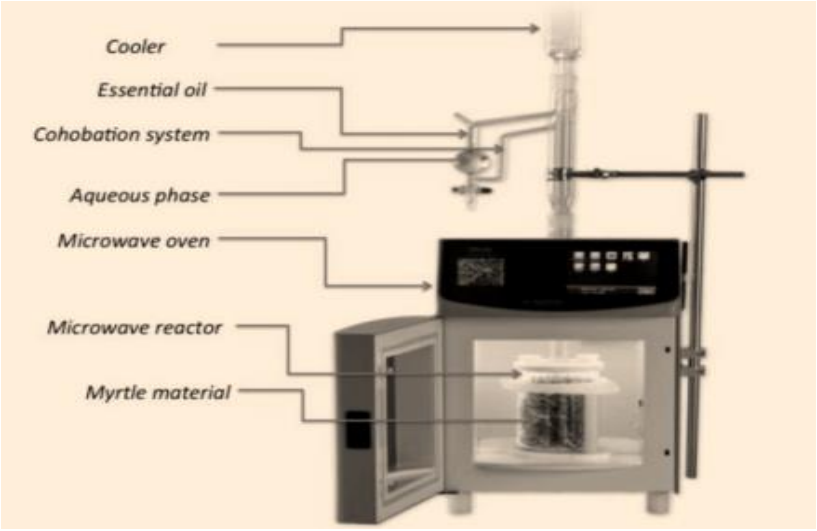


Microwave hydrodiffusion and gravity apparatus

(iii) Solvent-free microwave extraction

Solvent-free microwave extraction (SFME) is an extraction procedure of essential oil which is accomplished by the water of the plant material without added any solvent (Wang and Weller, 2006). Based on the integration of dry distillation and microwave heating energy. It consists on the microwave dry-distillation at atmospheric pressure of plant without adding water or any organic solvent. In a model SFME procedure, the plant material was moistened before to extraction by soaking in a certain amount of water for 1 to 2 h and

then draining off the excess water. After that, the moistened materials were subjected to the microwave oven cavity and a condenser was used to collect the extracted essential oils in a presetting procedure. The irradiation power, temperature, and extraction time were controlled by the panel in the instrument.

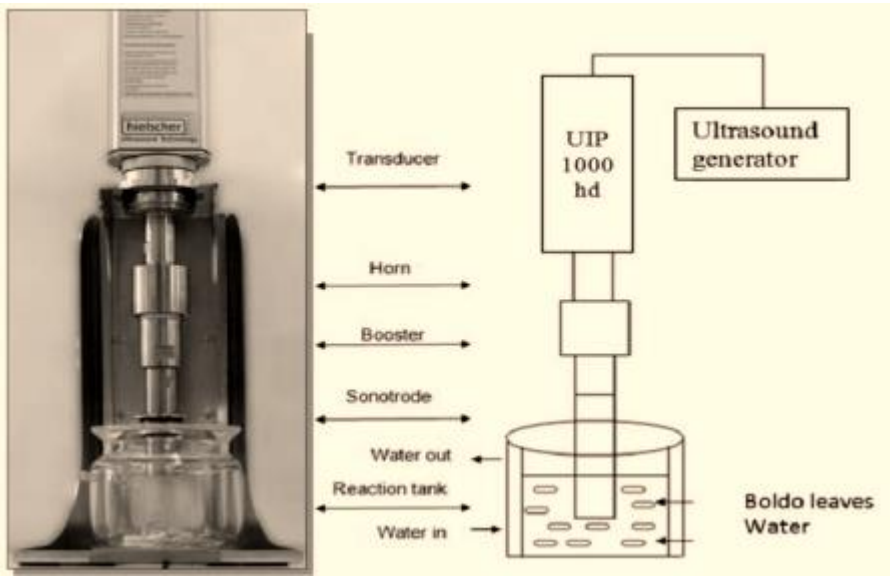


Solvent-free microwave extraction system

(iv) Ultrasonic-assisted extraction

Ultrasonic-assisted extraction (UAE) is a good process to achieve high valuable compounds and could be involved in increasing the estimate of some food by-products when used as sources of natural compounds or plant material[81] .The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive combinations. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination

with other techniques for example solvent extraction and hydrodistillation (Scheme 7). In these applications the power ultrasonic increases the surface wetness evaporation average and causes oscillating velocities at the interfaces, which may affect the diffusion boundary layer and generate rapid series of alternative expansions of the material, affecting cluster transfer . The plants raw material is immersed in water or another solvent (Methanol or ethanol or any one from the solvents) and at the same time, it is subjected to the work of ultrasound. This technique has been used for the extraction of many essential oils especially from the flower, leavesor seeds[82].



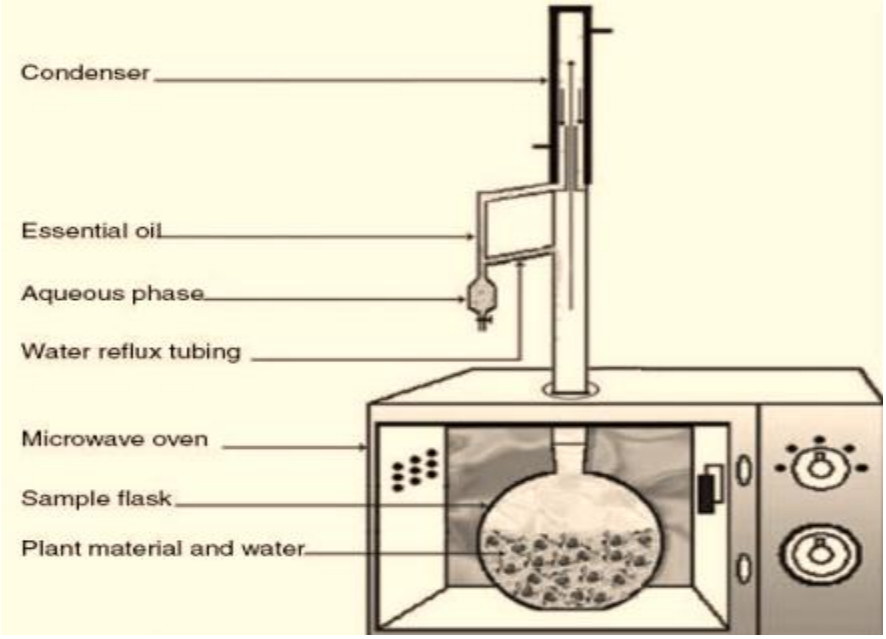
Ultrasonic-assisted extraction system

(v) Microwave-assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation is an advanced hydrodistillation technique utilizinga microwave oven in the

extraction process. The efficiency of Microwave assisted hydrodistillation is strongly dependent on the dielectric constant of water and the sample. High and fast extraction performance ability with less solvent consumption and protection offered to thermo labile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique (Scheme8). Application of Microwave-assisted hydrodistillation in separation and extraction processes has shown to reduce both extraction time and volume of solvent required, minimizing environmental impact by emitting less CO_2 in atmosphere and consuming only a fraction of the energy used in conventional extraction methods[83]. The use of Microwave-assisted hydrodistillation in industrial materials processing can provide a versatile tool to process many types of materials under a wide range of conditions. Microwave-assisted hydrodistillation is a current technology to extract biological materials and has been regarded as an important alternative in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heating and controllable heating process. The principle of heating using Microwave-assisted hydrodistillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic

conduction and dipole rotation, which in most cases occurs simultaneously.



Microwave-assisted hydrodistillation apparatus

1.2.2- Chemistry of essential oils

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.They consist of organic volatile compounds, generally of low molecular weight below 300. Their vapor pressure at atmospheric pressure and at room temperature is sufficiently high so that they are found partly in the vapor state[84]. These volatile compounds belong to various

chemical classes: 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).

1.2.3- Biological Activities of essential oils

a- Antibacterial activity

An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable. This can then cause leakage of ions and other cellular molecules. 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 Generally, essential oils are characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, with important antibacterial activities[85] .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 (Farhat et al., 2009, Ferhat et al., 2006, Lucchesi et al., 2004a). The chemical structure of essential oils affects their mode of action concerning their antibacterial activity. The action of thymol against *Bacillus cereus, Staphylococcus aureus*, and *Pseudomonas*

aeruginosa appears to be comparable to that of carvacrol, for example. However, carvacrol and thymol act differently against Gram-positive and Gram-negative . Thymol, eugenol, and carvacrol have an antimicrobial effect against a broad spectrum of bacteria: *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus*, and *Helicobacter pyroli*[86] .

b- Antioxidant Activity

The antioxidant potential of an essential oil depends on its composition. The essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties . Thymol and carvacrol are the most active compounds. Their activity is related to their phenolic structure. These phenolic compounds have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition .The antioxidant activity of essential oils is also due to certain alcohols, ethers, ketones, aldehydes, and monoterpenes: linalool, 1,8-cineoIe, geranial, citronellal, isomenthone, menthone, and some monoterpenes: α-terpinene, β-terpinene and α-terpinolene[87] .Essential oils with important 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 .

c- Anti-Inflammatory activity

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self-cells) and to remove dead or damaged host cells. Linalool and linalyl acetate showed anti-inflammatory activity on pawcarrageenan -induced mouse oedema[88]. The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. Essential oils, therefore, represent a new option in the treatment of inflammatory diseases.

d- Cancer chemoprotective activity

Essential oils would act in the prevention of cancer, as well as at its removal. It is well known that certain foods, such as garlic and turmeric, are good sources of anticancer agents (999). Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer. In addition, anticancer activity of D-limonene, the main component of Citrus essential oil has been proven, especially at the level of stomach cancer and liver [89].

e- Cytotoxicity

Essential oil cytotoxicity mainly correlates to the presence of phenols, alcohols, and monoterpene aldehydes. The cytotoxic properties of essential oils are of great importance because they assume their use not only against certain human pathogens and animal parasites, but also in the preservation of agricultural and marine products against microbial attack. Indeed, some components of essential oils are effective against a variety of microorganisms as bacteria, and others. In addition, α-humulene shows cytotoxicity against breast cancer cells *in vitro*. α-Humulene was reported to be responsible for cytotoxicity[90] .

1.3- Gas chromatography-Mass Spectrometry

GC-MS is a combination of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS). It is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified and quantified, but it cannot readily separate them. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid-1950. Gas chromatography and mass spectrometry are, in many ways, highly compatible techniques. In both techniques, the sample

is in the vapor phase, and both techniques deal with about the same amount of sample (typically less than 1 mg). Gas Chromatography (GC), is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium or an un-reactive gas such as nitrogen, and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside glass or metal tubing, called a column. The capillary column contains a stationary phase; a fine solid support coated with a nonvolatile liquid. The solid can itself be the stationary phase. The sample is swept through the column by a stream of helium gas. Components in a sample are separated from each other because some take longer to pass through the column than others. Mass spectrometry (MS) is acting as the detector for the GC. As the sample exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given component of sample is unique and therefore is an identifying characteristic of that component. It is so specific that it is often referred to as the molecular fingerprint. Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide

detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them[91].

1.3.1- Gas Chromatography

All forms of chromatography involve the distribution, or partitioning, of a compound between a mobile phase and a stationary phase. In GC, the mobile phase is a gas and the stationary phase is an immobile, high molecular weight liquid which is deposited on or chemically bonded to the inner walls of long capillary tubing. The term GLC (gas-liquid chromatography) is also used to refer to this separation technique. The capillary tubing through which the sample moves is called the chromatographic or GC column. Presently, most GC columns used for this work are manufactured from fused silica. They are generally 30-60 m in length and have an internal diameter of about 0.2 mm . By covering the outside surface of these capillary columns with a polymeric coating, these flexible fused silica GC columns are made more durable. The analysis of effluents for organic compounds requires extraction of the organics from the water matrix, concentration of the extract, separation of individual components of the organic extract by a GC column and detection of the separated components as they are eluted from the GC column. Complex mixtures of organic compounds are extracted from effluents by using high-purity organic solvents. The low-volatility organic compounds

extracted from an effluent sample can be concentrated to a small volume (typically, 1.0 mL or less) by removing the extraction solvent through evaporation. This concentration step is necessary in order to obtain detection limits in the low part-per-billion (ppb: 10-6 g/L). Some compounds of concern may be more volatile than the extraction solvent and would be lost in this process. Such compounds are removed from the sample by directly purging the aqueous sample using an inert gas and collecting the purged volatile compounds on an adsorbent trap designed for this purpose. In either case, organic compounds from the sample are separated from the bulk aqueous matrix and concentrated for GC analysis. The organic compounds are introduced into the GC column by injecting a few microlitres (μL) of the concentrated solvent extract into an injection port (non-volatile organics) or by heating the sorbent trap (volatile organics). An inert carrier gas (He, N_2, H_2), is used to sweep the extracted organic compounds, which are now in the vapor state, through the GC column. Compounds that have different solubilities in the liquid phase of the GC column will take different times to traverse the length of the column. For a specific set of experimental conditions, the time it takes a compound to travel through a GC column is a physical property of that compound - called its retention time. Generally, higher molecular weight compounds will have greater retention times than lower molecular weight compounds.

Also, compounds that have a similar polarity to that of the liquid phase will be more soluble in the phase and will have greater retention times than compounds less soluble in the liquid phase. Therefore, organic compounds in a mixture can be separated from each other by using gas chromatography, and the retention times of these compounds can be used to assist in their identification. Some environmental samples are so complex that there are hundreds of compounds present in their concentrated organic extracts. There are currently no GC columns available that can completely separate all components of such complex mixtures from each other. However, in most cases the principal sample components can be detected individually[92] .

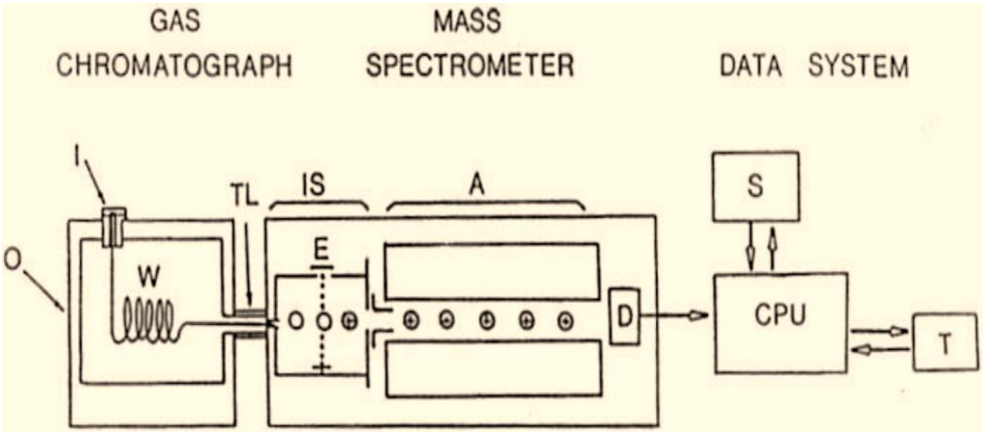


Diagram of a GC-MS System

In another design, ions travel through a magnetic field where their momentum is affected by the magnetic field strength. Conditions

can be controlled to allow the analyzer to scan across a range of m/z to form a mass spectrum. This design is called a magnetic sector mass analyzer. An important concept in GC-MS is resolution. In GC, resolution refers to the ability of the GC column to separate components in a mixture from each other. In mass spectrometry, mass resolution refers to the ability of an analyzer to separate ions that have similar m/z[93].

a- Gas supply

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure[94].

b- Injector

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.

c- Column

Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal[94] .

d- Oven

Gas chromatography has ovens that are temperature programmable.

1.3.2- Mass spectrometry

As the separated sample components elute from the GC column, they are monitored using any of a large number of detectors developed for this purpose. The most versatile of these detectors is the mass spectrometer (MS). When an MS detector is used to detect the compounds that elute from a GC column, the combined technique is called gas chromatography-mass spectrometry (GC-MS). Initially, molecules enter the source chamber of the mass spectrometer maintained under high vacuum, where they are bombarded by electrons. The energy transferred to molecules in this process causes them to ionize and dissociate into various fragment ions. Ions may be singly or multiply-charged. The positive ions formed are made to traverse an analyzer section. After the ions traverse the analyzer section where they are separated according to their mass-to-charge ratio (m/z), they are detected by an extremely sensitive device called an electron multiplier. By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. The mass spectrum of a compound is like a fingerprint that can be used to identify the original organic structure. It consists of a bar graph representation of the m/z of the ions and their abundances normalized to the most abundant ion (base peak). By

matching the GC retention time of a sample component and its mass spectrum with those of a standard reference compound analyzed under the same conditions, a positive identification of the sample component is obtained. Several different mass analyzers have been developed. One of the most common designs consists of a square array of four parallel metal rods. By controlling radio-frequency (RP and DC voltages) to these rods, an oscillating electric field is generated and this allows ions to be filtered according to their m/z. At a specific setting of voltages, only ions of the desired m/z will have a stable trajectory and will be able to reach the electron multiplier. By changing the applied voltages in a specified manner, the mass spectrum of a compound can be generated as the ions of various m/z are scanned. The entire process is performed in about one second. This design is called (QP) a quadruple mass analyzer.

a- Ion source

In the ion source, the products are ionized prior to analysis in the mass spectrometer

b- Mass analyzer

There are several very popular types of mass analyzer associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis.

c- Vacuum system

Mass analyzers require high levels of vacuum in order to operate in a predictable and efficient way.

d- Detector

The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal. The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges)[95].

Aim of this study

This research was designed to:

-Extract oils from five medicinal plants.

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

-Evaluate the targeted oils for their antimicrobial activity.

2-Materials and Methods

2.1 Materials

2.1.1 Plant material

Seeds of *Hyphane thebaica, Acacia mellifera, Parkinsonia aculeata , Lucaena leucocephala, Sorghum bicolor* were collected from Kordofan – western 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^{o}C for further manipulation.

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

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

2.2.2 GC-MS analysis

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

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

Table 2: Oven temperature program

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

Table 3: Chromatographic conditions

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

2.2.3 Antimicrobial activity

i)-Bacterial suspensions

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

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony 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 study the oils from five medicinal plants used in Sudanese traditional medicine have been investigated by GC-MS. The oils were also assessed for their antimicrobial activity by the agar diffusion method.

3.1- *Hyphane thebaica*

3.1.1- GC-MS analysis of *Hyphane thebaica* oil

GC-MS analysis of *Hyphane thebaica oil* was performed. Fifty constituents were detected. The constituents of the oil are presented in Table 3.1. Identification of the constituents was based on retention times and MS library data.

Major constituents are briefly discussed below:

The mass spectrum of 9,12-octadecadienoic acid-z,z- methyl ester(30.62%) is shown in Fig. 3.1. The peak at m/z294, which appeared at RT 17,303 in total ions chromatograms corresponds to $M^+[C_{19}H_{34}O_2]$. The signal at m/z263 is due to loss of a methoxyl.

Fig**. 3.**2 shows the mass spectrum of hexadecanoic acid methyl ester (23.19%). The molecular ion $M^+[C_{17}H_{34}O_2]$ appeared at m/z 270(RT,15.645). The signal at m/z 239 accounts for loss of a methoxyl.The mass spectrum of 9-octadecanoic acid methyl ester (16.12%) is presented in Fig. 3.3. The signal at m/z296 (RT,

17.339) is due to the molecular ion $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z266 is due to loss of a methoxyl.

Fig. 3.4 shows the mass spectrum of methyl stearate (5.84%).The peak at m/z 298 which appeared at RT 17.550 corresponds $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl.

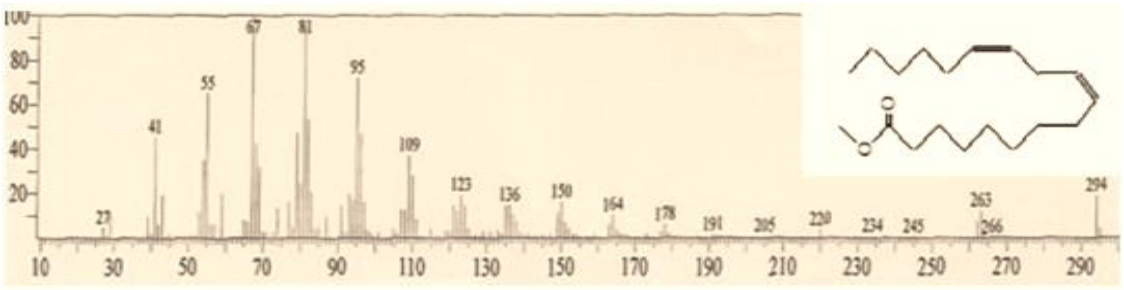


Figure 3.1: Mass spectrum of 9,12-octadecadienoic acid methyl ester

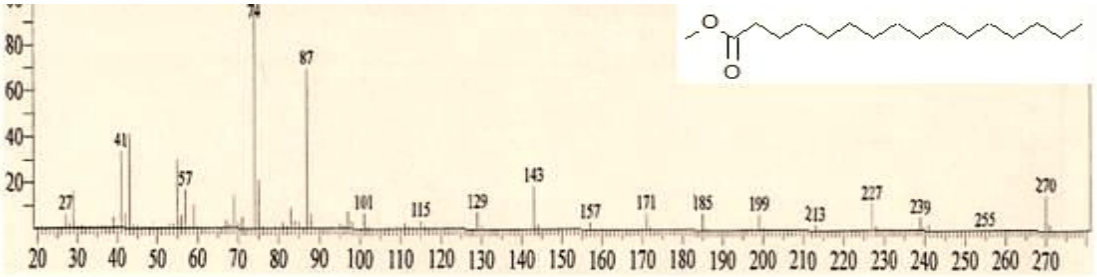


Figure 3.2: Mass spectrum of hexadecanoic acid methyl ester

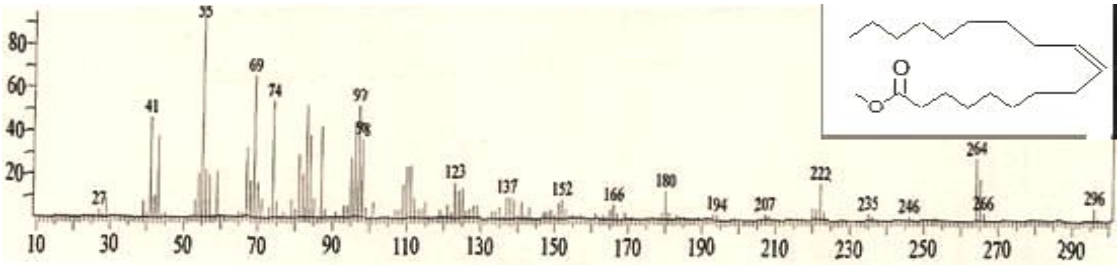


Figure 3.3: Mass spectrum of 9-octadecenoic acid methyl ester

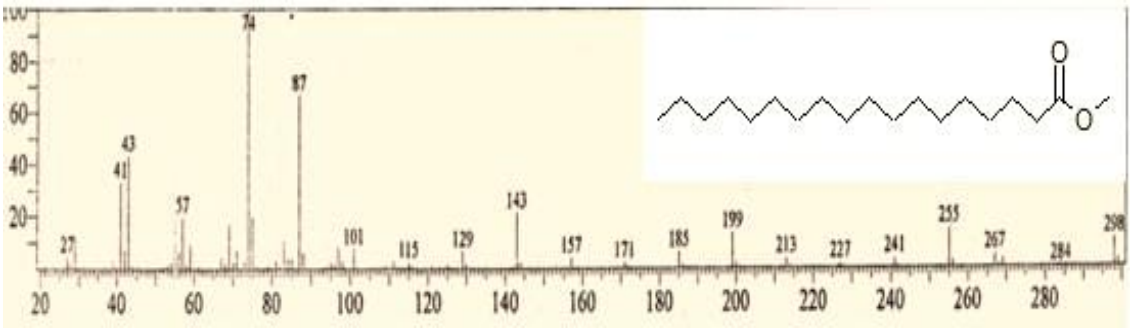


Figure 3.4: Mass spectrum of methyl stereate

3.1.2-Antimicrobial activity

The oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table 3.2. Results were interpreted as follows: (>9mm: inative; 9-12mm: partially active; 13-18mm: active ;<18mm: very active). Ampicilin, gentamycin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The oil also exhibited significant anticandidal activity.

Table 3.1: Constituents of *Hyphane theibica* oil

No.	R. Time	Area%	Name
1	6.981	0.14	Ethyl dioxovalerate
2	7.324	0.15	1-Pentanol, 2,2-dimethyl
3	7.432	0.08	Butanoic acid, 2-ethyl-2-methyl
4	7.930	0.12	2-Decanol, Methyl ether
5	7.997	0.22	Oxalic acid, cyclohexyl dodecyl ester
6	8.307	0.12	Dodecane
7	8.441	0.07	Bicycl[4.4.1]undeca-1,3,5,7,9-pentaene
8	8.659	0.08	Nonanoic acid, methyl ester

9	8.898	0.25	2-H-Pyranmethanol, tetrahydro-2,5-dimethyl
10	9.251	0.08	(1R,2R,3S,5R)-(-)-2,3-Pinanediol
11	9.560	0.07	Biphenyl
12	9.631	0.17	Tetradecane
13	9.806	0.02	Benzoic acid,3-methyl-4-nitro, methyl ester
14	9.905	0.05	Naphthalene,2,6-dimethyl
15	10.103	0.06	Naphthalene,2,3-dimethyl
16	10.145	0.08	Octadecanoic acid,9,10-dihydro, methyl ester
17	10.372	0.03	Naphthalene-1,3-dimethyl
18	10.419	0.06	Dodecane,2,6,10-trimethyl
19	10.896	0.27	Heptadecane,7-methyl
20	11.219	0.34	Dodecanoic acid , methyl ester
21	11.530	0.21	Nonanedioc acid , methyl ester
22	12.877	0.56	4-Fluoro , alpha, -methylbenzyl alcohol, methyl ester
23	12.986	0.07	Carbamic acid, N-[1-(4-methylphenyl)ethyl-]
24	13.230	0.19	Heptadecane,2,6,10,15-tetramethyl-
25	13.290	0.12	Hexadecane,2,6,10,14-tetramethyl-
26	13.360	0.33	2-Fluoro-,alpha,-methylbenzyl alcohol, methyl ester
27	13.534	1.42	Methyl tetradecanoate
28	14.053	0.08	2-(1H)-Quinoline, 4-hydroxy-6-(1-methylethyl)-3-
29	14.313	0.20	Heneicosane
30	14.608	0.36	Pentadecanoic acid, methyl ester
31	15.342	0.22	2-methyltetracosane
32	15.441	0.85	9-Hexadecenoic acid , methyl ester
33	15.645	23.19	Hexadecenoic acid , methyl ester
34	16.295	1.00	Hexadecenoic acid , ethyl ester
35	16.399	0.34	9,12-Octadecadienoic acid , methyl ester
36	16.613	1.10	Heptadecanoic acid , methyl ester
37	17.303	30.62	9,12-Octadecadienoic acid(Z,Z)- , methyl ester
38	17.339	16.21	9-Octadecenoic acid(Z)- , methyl ester
39	17.550	5.84	Methyl stearate
40	17.890	1.54	9,12-Octadecadienoic acid , ethyl ester
41	19.107	0.85	Cis-11-Eicosenoic ,methyl ester
42	19.305	1.49	Eicosenoic ,methyl ester
43	19.356	0.60	7,10,13-Eicosatrienoic acid , Eicosenoic ,methyl ester
44	20.924	2.18	Docosanoic acid, Eicosenoic ,methyl ester
45	21.688	0.48	Tricosanoic acid ,methyl ester
46	22.426	3.08	Tetracosanoic acid ,methyl ester
47	23.137	0.88	Pentacosanoic acid ,methyl ester
48	23.195	0.61	Oxirane,hexadecyl
49	23.572	0.55	Tricontane,-1-bromo
50	23.824	2.38	Hexacosanoic acid ,methyl ester

T**able 3.2:** Antimicrobial activity of *Hyphaene thebaica* oil

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

Sa.*: Staphylococcus aureus;*

Ec.*: Escherichia coli;*

Pa.: *Pseudomonas aeruginosa*;

Bs.*: Bacillus subtilis*;

Ca.: *Candida albicans*

3.2-*Acacia mellifera*

3.2.1-Constituents of *Acacia mellifera* oil

The investigation of the constituents of *Acacia mellifera* oil was accomplished by GC-MS analysis using a Shimadzo GC-MS-QP2010 Ultra instrument. Gas chromatogram revealed the presence of 38 components (their abundance is depicted in Table 3.3 and Fig. 3.5). Fatty acids comprised the major constituents (87.40%) followed the steroidal alcohol gamma-ergostenol (7.26%), the aldehyde tridecanedial (3.44%), hydrocarbons (1.81%) and some terpenes and sesquiterpenes (0.09%).

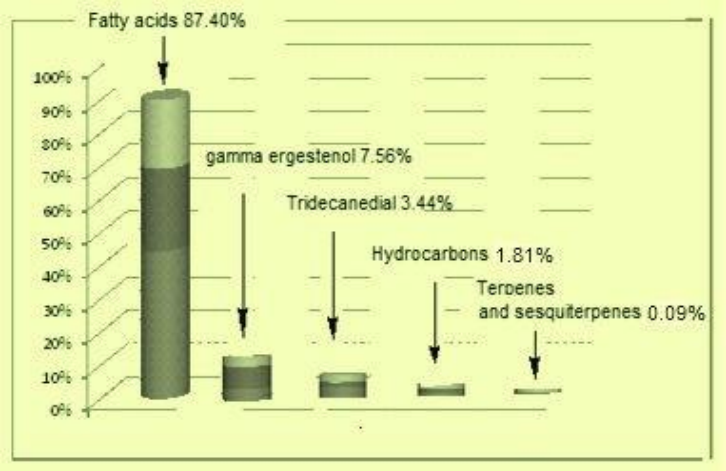


Figure 3.5: Abundance of constituents of the oil

Table 3.3: Constituents of the oil

No.	R. Time	Area%	Name
1	4.583	0.01	6-Heptenoic acid methyl ester
2	7.152	0.04	A-Terpineol
3	7.243	0.01	Cyclohexanol-1-methyl-4-methylethyl
4	11.031	0.01	10,12-Docasadiyndioc acid
5	11.166	0.02	Beta-curcumene
6	11.268	0.01	Alpha-Farnesene
7	11.332	0.01	Isocayophillene
8	11.425	0.03	Dodecanoic acid methyl ester
9	11.538	0.01	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-
10	13.434	0.01	Apiol
11	13.474	0.01	Cis-5-Dodecenoic acid methyl ester
12	13.749	0.31	Methyl Tetradecanoate
13	14.561	0.02	5-Octadecenoic acid methyl ester
14	14.828	0.05	Pentadecanoic acid methyl ester
15	15.562	0.02	n-Propyl-9,12-hexadecadienoate
16	15.621	0.13	7-Hexadecenoic acid methyl ester
17	12.667	1.64	9-Hexadecenoic acid methyl ester
18	15.760	0.08	11-Hexadecenoic acid methyl ester
19	15.882	12.92	Hexadecenoic acid methyl ester
20	16.630	0.28	Cis-10-Heptadecenoic acid methyl ester
21	16.840	0.51	Heptadecanoic acid methyl ester
22	17.544	17.55	9,12-Ocadecadienoic acid methyl ester
23	17.623	12.45	9-Otcadecenoic acid methyl ester
24	17.809	11.23	Methyl stearate
25	18.676	0.10	Nonadecanoic acid methyl ester
26	19.182	3.44	Tridecanedial
27	19.305	3.25	Oxiraneoctanoic acid,3-octyl methyl ester
28	19.340	1.37	11-Eicosenoic acid methyl ester
29	19.549	6.54	Eicosanoic acid methyl ester

30	19.604	0.88	PGH1 methyl ester	
31	19.711	1.13	Methyl 15-hydroxy-9,12- ocadecadienoic	acid methyl ester
32	20.365	0.50	Heneicosanoic acid methyl ester	
33	21.070	5.55	Fumaric acid, 3-heptyl nonyl ester	
34	21.174	6.51	Docosanoic acid methyl ester	
35	21.515	1.51	1-Nonadecene	
36	21.926	0.76	Tricosanoic acid methyl ester	
37	22.669	3.58	Tetracosanoic acid methyl ester	
38	23.811	7.56	ϐ-Ergostenol	
		100%		

The major constituents of the hexane fraction are briefly discussed below:

i)- 9,12-Ocadecadienoic acid methyl ester(17.55%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.3.6. The signal which was observed at m/z294(R.T. 17.544) is due to M+[$C_{19}H_{34}O_2$]+, while the signal at m/z263 corresponds to loss of a methoxyl.

ii)- Hexadecanoic acid methyl ester(12.92%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.3.7. The peak at m/z 270 (R.T. 15.882) accounts for M^+ [C17H34O2] $^+$. The signal at m/z 239 is due to loss of methoxyl.

iii)- 9-Octadecenoic acid methyl ester(12.45%)

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3.8. The signal at m/z296 (RT, 17.623) is due to the molecular ion M+[C19H36O2]+. The peak at m/z266 is due to loss of a methoxyl.

iv)- Methyl stearate(11.23%)

The mass spectrum of methyl stearate is shown in Fig. 3.9. The peak at m/z 298 (R.T.17.809) is due to M^+ $[C19H38O2]^+$, while the signal at m/z 267 correspond to loss of a methoxyl.

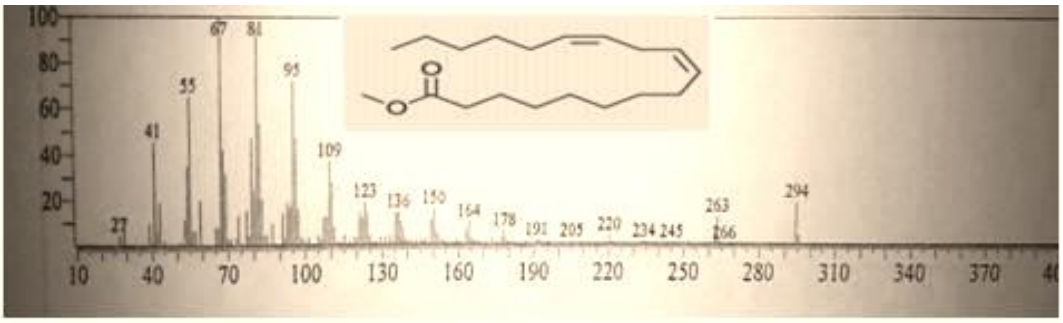


Figure 3.6: mass spectrum of 9,12-octadecadienoic acid methyl ester

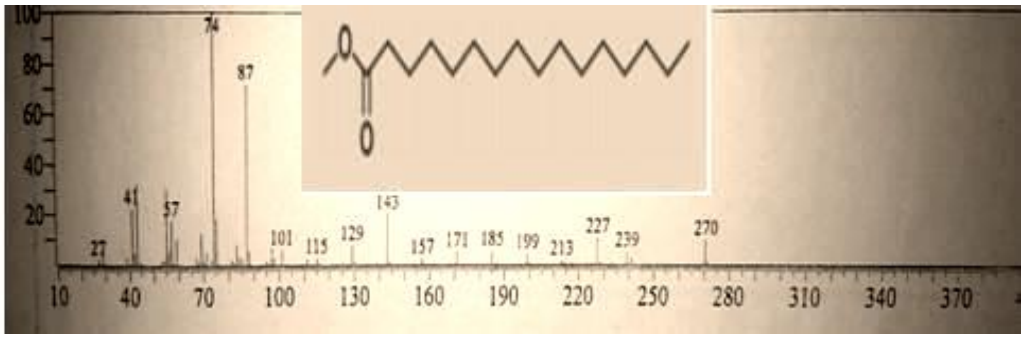


Figure 3.7: mass spectrum of hexadecanoic acid, methyl ester

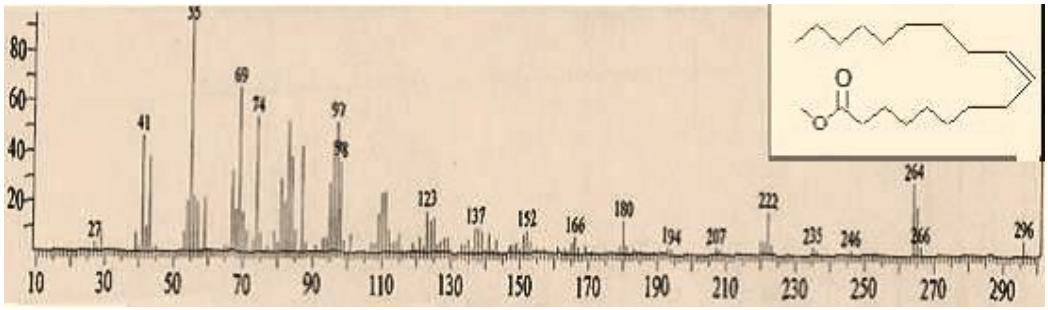


Figure 3.8: Mass spectrum of 9-octadecenoic acid methyl ester

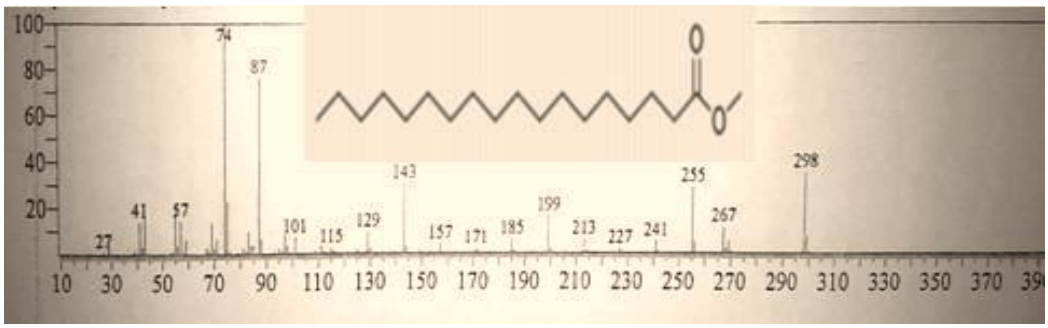


Figure 3.9: mass spectrum of methyl stearate

3.3.3Antimicrobial activity

In the cup plate diffusion assay, *Acacia mellifera* hexane fraction was assessed for antimicrobial potential using five standard human pathogens: *Bacillus subtilis, Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa*, *Escherichia coli* (G-ve) and the fungal species *Candida albicans*. The inhibition zones are shown in Table 3.4.

At a concentration of 100mg/ml, the hexane extract showed moderate activity against all test organisms. It also exhibited moderate activity at 50mg/ml against *Pseudomonas aeroginosa*, *Bacillus subtilis* and the fungal species *Candida albicans*. Moderate activity against *Bacillus subtilis* and *Candida albicans* was also observed at 25mg/ml. Ampicilin, gentamycen and clotrimazole were used as positive controls, while DMSO was used as negative control.

Table 3.4: Antibacterial activity of the oil

Type	Conc. (mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	13	15	15	14	15
	50	12	14	12	13	14
	25	12	13	10	12	14
	12.5	10	12	10	--	--
	6.25	10	13	7	--	--
Ampicilin	40	30	15	--	--	--
Gentamycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

3.3-*Parkinsonia aculeata*

3.3.1-GC-MS analysis of *Parkinsonia aculeata* oil

The total ion chromatograms of *Parkinsonia aculeata* oil is shown in Fig. 3.10 and the constituents of the oil are depicted in Table 3.5. The GC-MS analysis revealed the presence of 33 components dominated by (i) 9,12- octadecadienoic acid methyl ester (40.51%) (ii) of 33 components dominated by (i) 9,12-octadecadienoic acid methyl ester (40.51%) (ii) hexdecanoic acid methyl ester (16.89%) (iii) methyl stearate (10.97%) and (iv) 9,12- octadienoyl chloride (5.05%).

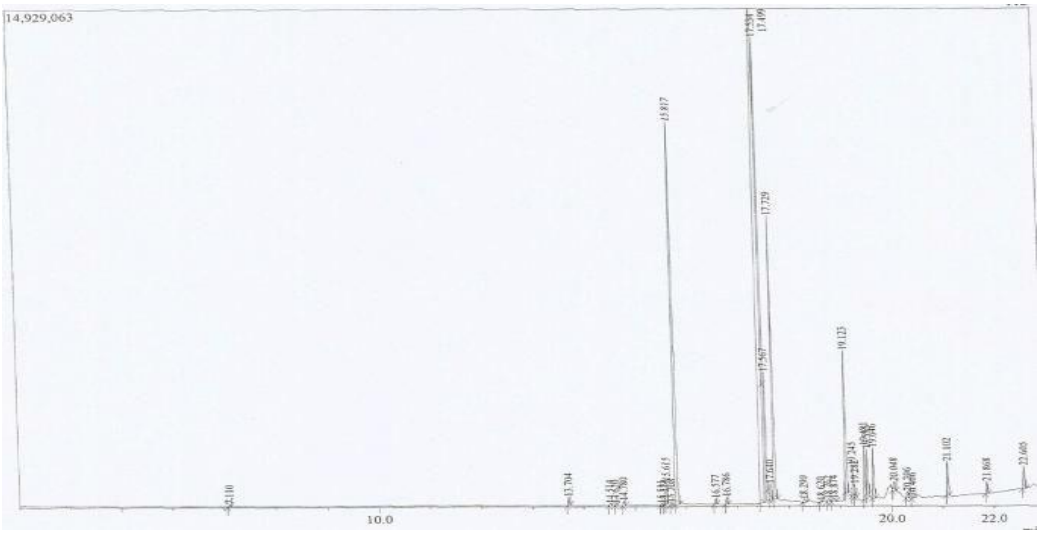


Figure 3.10: Total ions chromatograms

Major components are briefly discussed below:

i)9,12-octadecadienoic acid methyl ester (40.51%)

Fig. 3.11 shows the mass spectrum of the 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z 294 (RT. 17.499) corresponds to the molecular ion: $M^{+}[C_{19}H_{34}O_2]^{+}$, while the signal at m/z 263 accounts for loss of a methoxyl.

ii)Hexadecanoic acid, methyl ester (16.80%)

The mass spectrum of the hexadecanoic acid methyl ester is presented in Fig. 3.12. The peak at m/z 270 (RT. 15.817) is due to the molecular ion: $M^{+}[C_{17}H_{34}O_2]^{+}$. The signal at m/z: 239 is attributed to loss of a methoxyl function.

Table 3.5: Constituents of *Parkinsonia aculeata* oil

No.	RT.	Area%	Name
1	7.110	0.07	Alpha -Terpineol
2	13.704	0.28	Methyl tetradecanoate
3	14.514	0.05	4-Octadecenoic acid methyl ester
4	14.620	0.05	5-Octadecenoic acid methyl ester
5	14.780	0.16	Pentadecanoic acid methyl ester
6	15.511	0.04	7,10-hexadecadienoic acid methyl
7	15.574	0.06	Z-7-Hexadecenoic acid methyl ester
8	15.615	0.73	Cis-10-Nonadecenoic acid methyl
9	15.708	0.08	9-Hexadecenoic acid methyl ester
10	15.817	16.80	Hexadecanoic acid methyl ester
11	16.577	0.21	Cis-10-Heptadecenoic acid methyl
12	16.786	0.23	Heptadecanoic acid methyl ester
13	17.499	40.51	9,12-Octadecadienoic acid methyl
14	17.534	9.98	9-Octadecenoic –(Z)- acid methyl
15	17.567	3.47	9-Octadecenoic acid methyl ester
16	17.640	0.68	Phytol
17	17.729	10.97	Methyl stearate
18	18.290	0.14	Methyl 9-cis-11-trans-
19	18.620	0.05	Nonadecanoic acid methyl ester
20	18.762	0.13	Gamma-Linolenic acid methyl ester
21	18.874	0.17	Methyl 9-cis-11-trans-13-trans-
22	19.123	5.05	9,12-Octadecanoyl chloride
23	19.245	1.04	Oxiraneoctanoic acid , 3-octyl
24	19.282	0.38	Cis-11-Eicosenoic acid methyl ester
25	19.481	1.89	Eicosanoic acid methyl ester
26	19.535	1.51	PGH, methyl ester
27	19.646	2.08	1-Naphthalenol, decahydro-4a-
28	20.048	0.47	Stigmast-7-en-3-ol , (3.beta,
29	20.306	0.23	Heneicosanoic acid methyl ester
30	20.406	0.06	Phenol,2,2`-methylene-bis(6-(1,1-
31	21.102	1.15	Docosanoic acid methyl ester
32	21.868	0.42	Tricosanoic acid methyl ester
33	22.605	0.87	Tetracosanoic acid methyl ester
		100.00	

iii)Methyl stearate (10.97%)

In Fig. 3.13 (mass spectrum of methyl stearate), the signal at m/z 298 (RT. 17.729) accounts for $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 is due to loss of a methoxyl.

9,12-Octadecadienoyl chloride (5.05%)

Fig. 3.14 shows the mass spectrum of the 9,12-octadecadienoyl chloride - (Z,Z). The peak at m/z 298 (RT. 19.123) corresponds $M^+[C_{18}H_{31}ClO]^+$. The signal at m/z 264 accounts for loss of chlorine**.**

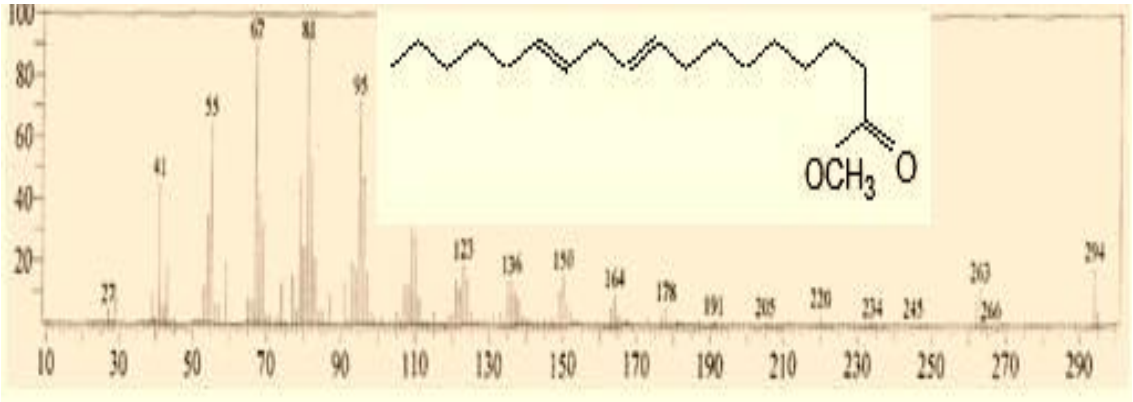


Figure 3.11: Mass spectrum of 9,12-octadecadienoic acid methyl ester

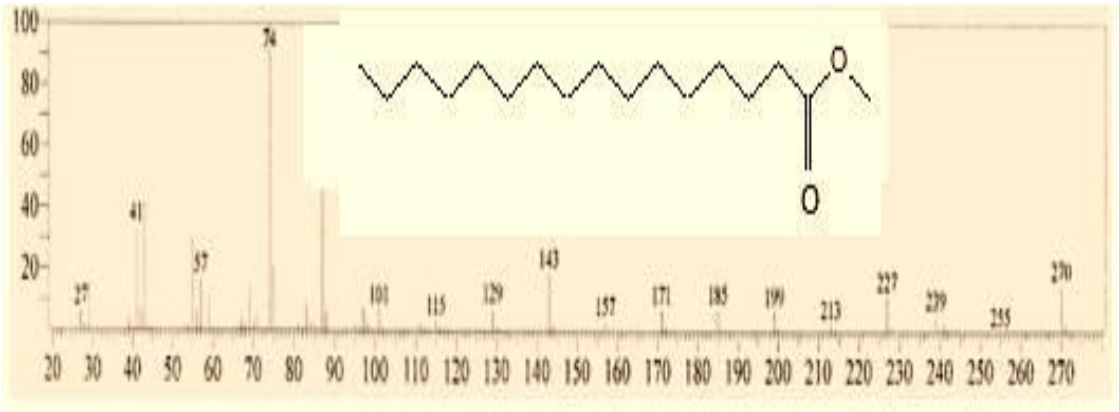


Figure 3.12: Mass spectrum of hexadecanoic acid, methyl ester

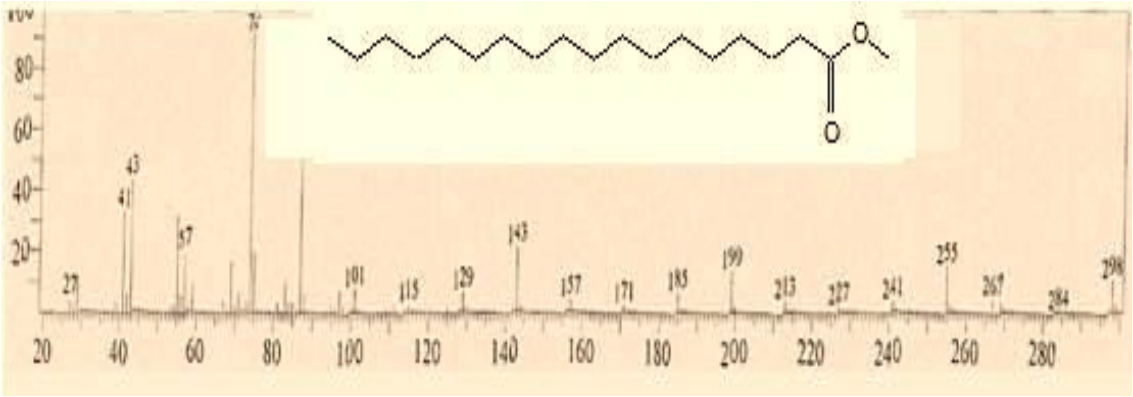


Figure 3.13: Mass spectrum of methyl stearate

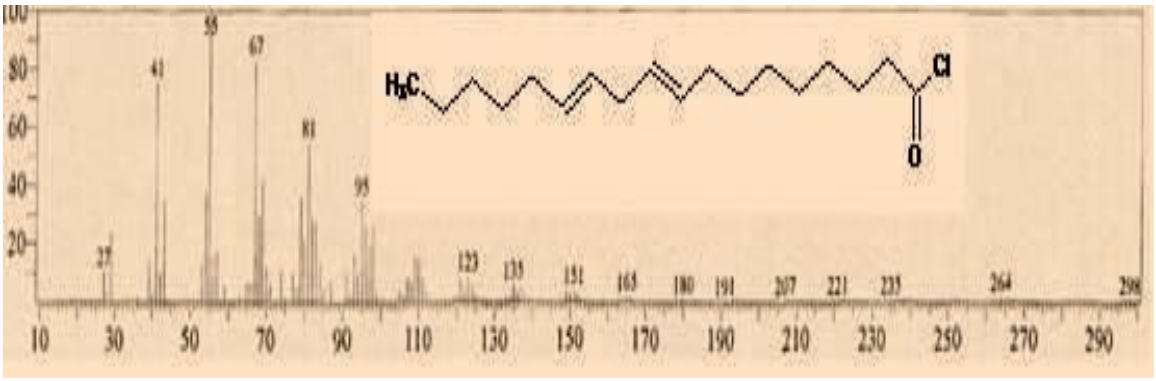


Figure 3. 14: Mass spectrum of 9,12-octadecadienoyl chloride

3.3.2- Antimicrobial Activity

Parkinsonia aculeata oil was evaluated for antimicrobial activity against five standard microorganisms. The results are depicted in Table 3.6. Results were interpreted in the following conventional terms: (>9mm: inactive; 9-12mm: partially active; 13-18mm: active; <18mm: very active). The studied oil showed moderate activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* .

Table 3.6: Inhibition zones of oil and standard drugs

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

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

3.4-*Lucaena leucocephala*

3.4.1- GC-MS analysis of *Lucaena leucocephala* oil

GC-MS analysis of *Lucaena leucocephala* oil was performed. Thirty constituents were detected. The constituents of the oil are presented in Table 3.7. Identification of the constituents was based on retention times and MS library data. Major constituents are briefly discussed below:

The mass spectrum of 9,12-octadecadienoic acid-z,z- methyl ester (37.29%) is shown in Fig. 3.15. The peak at m/z294, which appeared at RT 17,519 in total ions chromatograms corresponds $M^+[C_{19}H_{34}O_2]$. The signal at m/z 263 is due to loss of a methoxyl. Fig**. 3.**16 shows the mass spectrum of hexadecanoic acid methyl ester (18.17%). The molecular ion $M^+[C_{17}H_{34}O_2]$ appeared at m/z 270 (RT,15.830). The signal at m/z 239 accounts for loss of a methoxyl.

The EI mass spectrum of eicosanoic acid methyl ester(6.46%) is shown in Fig. 3.17. The peak at m/z 326, which appeared at R.T. 19.487 accounts for the molecular ion : $M^+[C_{21}H_{42}O_2]^+$. The peak at m/z 295 corresponds to loss of a methoxyl function.

The mass spectrum of 9-octadecenoic acid methyl ester(6.36%) is presented in Fig. 3.18. The signal at m/z 296 (RT,17.555) is due to the molecular ion $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 266 is due to loss of a methoxyl.

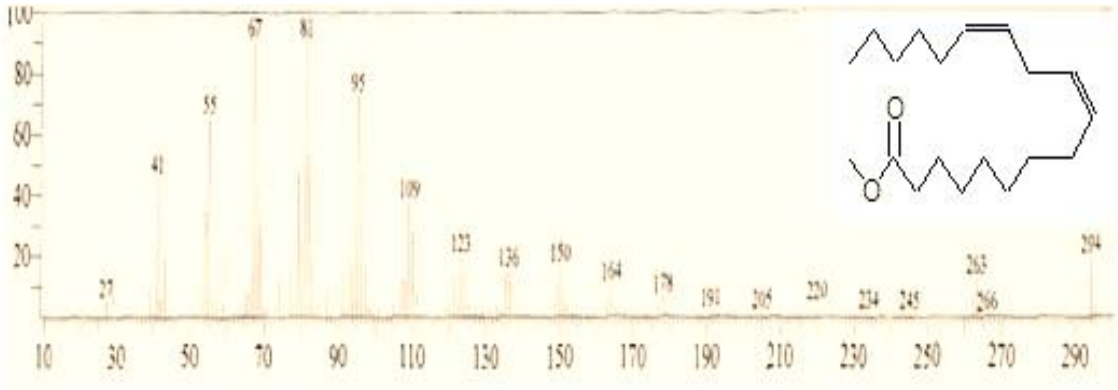


Figure 3.15: Mass spectrum of 9,12-octadecadienoic acid methyl ester

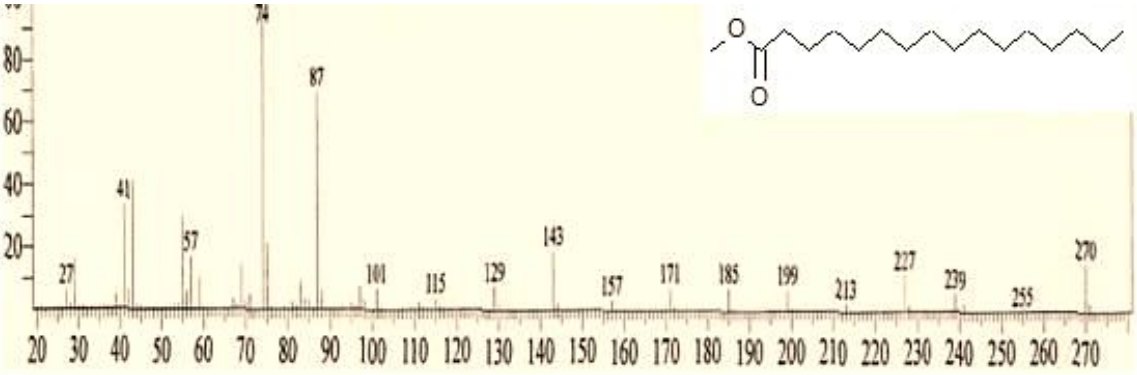


Figure 3.16: Mass spectrum of hexadecanoic acid methyl ester

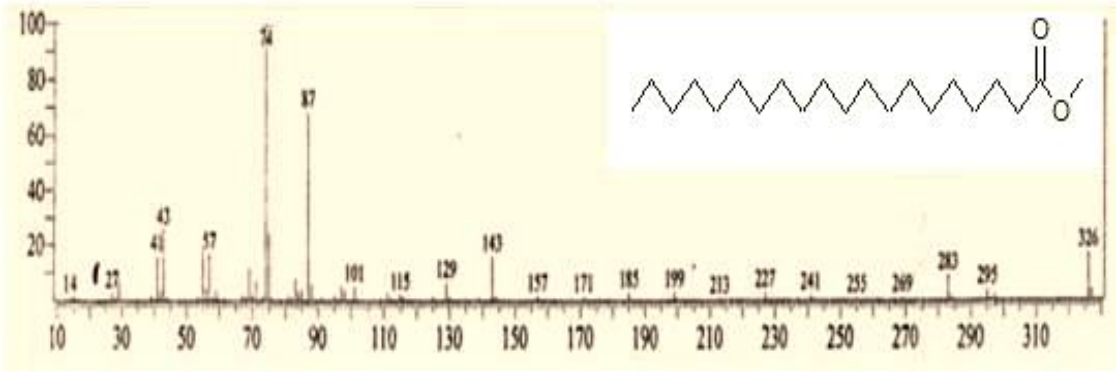


Figure 3.17: Mass spectrum of eicosanoic acid methyl ester

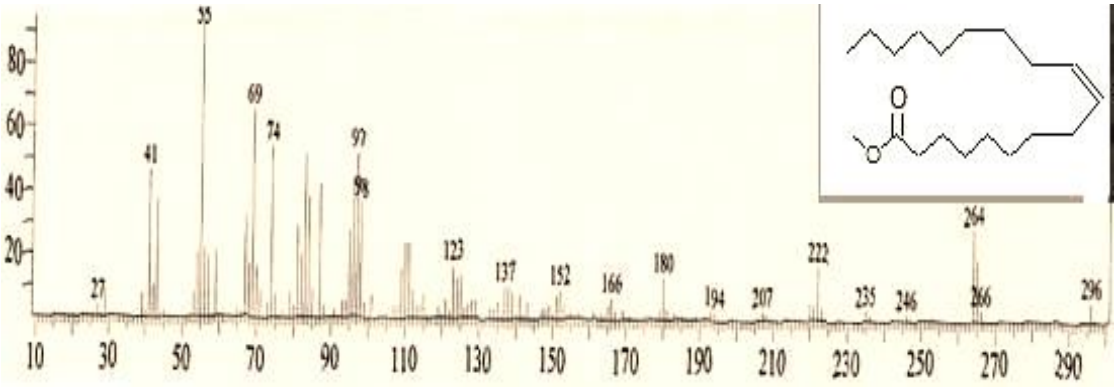


Figure.3. 18: Mass spectrum of 9-octadecenoic acid methyl ester

3.4.2 Antimicrobial assay

The oil was evaluated for antimicrobial activity against five standard microorganisms using the disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (3.8). Results were interpreted as follows: (>9mm: inative;9-12mm:partially active;13-18mm: active;<18mm:very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The studied oil showed moderate activity against *Staphylococcus aureus*. It also exhibited partial activity against *Pseudomonas aeruginosa*.

Table 3.7: Constituents of the oil

No.	Name	Ret.time	Area %
1	Alpha-Terpeneol	7.112	0.06
2	1H-Cyclprop[e]azulene,1a,2,3,4,4a,56,7]	10.047	0.02
3	Methyl tetradecaanoate	13.706	0.10
4	5-Octadecenoic acid ,methyl ester	14.518	0.03
5	Cis-5-Dodecenoic acid methyl ester	14.621	0.2
6	Pentadecanoic acid methyl ester	14.783	1.48
7	9-Hexadecenoic acid methyl ester(Z)	15.618	0.77
8	Hexadecanoic acid methyl ester	15.830	18.17
9	Methyl -9,12-Heptadecadienoate	16.579	0.24
10	Cis-10-Heotaddecenoic acid methvl ester	16.578	0.20
11	Heotadecanoic acid methvl ester	16.788	0.27
12	9.12-Octadecadienoic acid (Z) methvl ester	17.519	37.29
13	9-Octadecenoic acid (Z) methvl ester	17.555	6.36
14	9-Octadecenoic acid(E) methyl ester	17.651	0.36
15	Phytol	17.647	0.41
16	Methyl stearate	18.294	0.41
17	Methyl 9-cis-,11-trans-octadecadienoate	18.622	0.09
18	Trans-Generylgereiol	18.396	0.11
19	Nonadecanoic acid methyl ester	18.604	0.04
20	9,12-Octadecadienoyl chloride (Z,Z)-	19.123	0.74
21	Oxiraneoctanoic acid methyl ester	19.248	0.27
22	Cie-11-Eicosenoic acid methyl ester	19.284	0.61
23	Eicosanoic acid , methyl ester	19.487	6.46
24	PGHI , methyl ester	19.537	1.17
25	Methyl 13,16-docosadienoate	19.649	0.18
26	Heneicosanoic acid methyl ester	20.308	0.38
27	Docosanoic acid methvl ester	21.106	5.78
28	Tricosanoic acid PGHI , methyl ester	21.867	0.59
29	9.19-Cvclolanost-24-en-3-ol-(3-beta)-	22.497	0.43
30	Tetracosanoic acid methyl ester	22.608	3.26

Table 3.8: Antimicrobial activity of oil

Sample	**Sa**	**Bs**	**Ec**	**Ps**	**Ca.**
Oil (100mg/ml)	15	--	--	12	7
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamycin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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

3.5-*Sorghum bicolor*

3.5.1 The GC-MS analysis

Sorghum bicolor oil has been analyzed by GC-MS. Thirty three components were detected (Table 3.9). Major constituents of the oil are:

i)-9,12-Octadecadienoic acid (35.91%),

9,12-octadecadienoic acid

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Figure 3.19. The peak at m/z 294, which appeared at R.T. 19.003 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z263 corresponds to loss of a methoxyl function.

ii)-Pentadecanoic acid (11.46%)

Pentadecanoic acid

The EI mass spectrum of pentadecanoic acid methyl ester is displayed in Figure 3.20. The peak at m/z 256, which appeared at R.T. 17.249 in total ion chromatogram, corresponds

$M^+[C16H32O2]^+$. The peak at m/z225 corresponds to loss of a methoxyl function.

iii)- Tetrapentacontane (11.45%)

Tetrapentacontane

Figure 3.21 shows the mass spectrum of the tetrapentacontane. The peak at m/z 743 (RT., 22.163) is due to $[M - CH3]^+$.

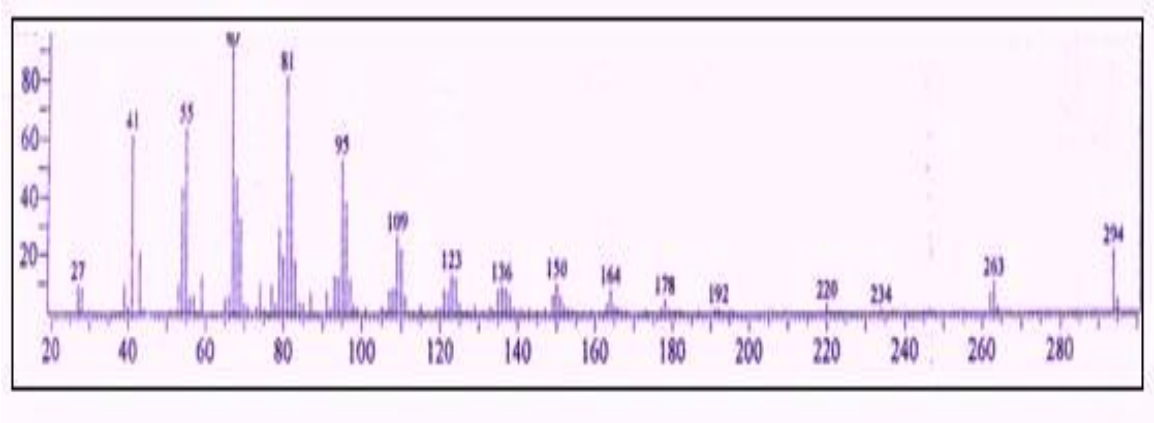


Figure 3.19: Mass spectrum of 9,12-octadecanoic acid methyl ester

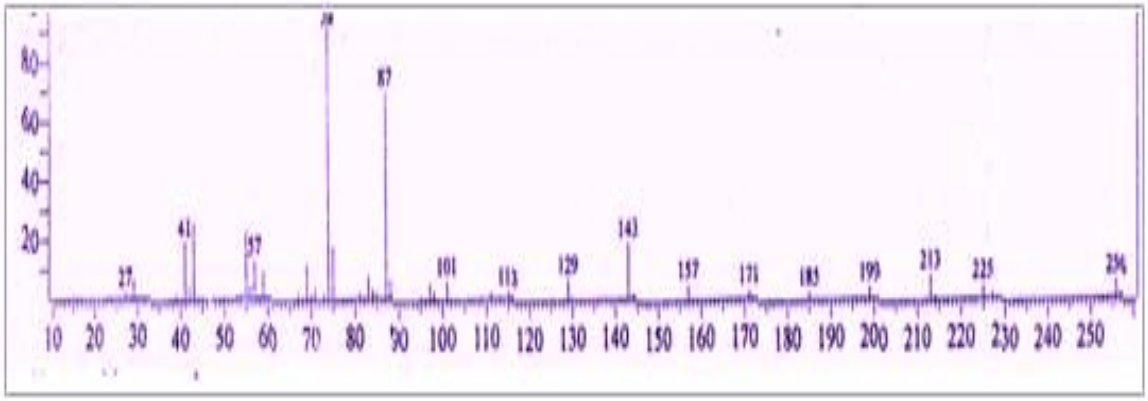


Figure 3.20: Mass spectrum of pentadecanoic acid methyl ester

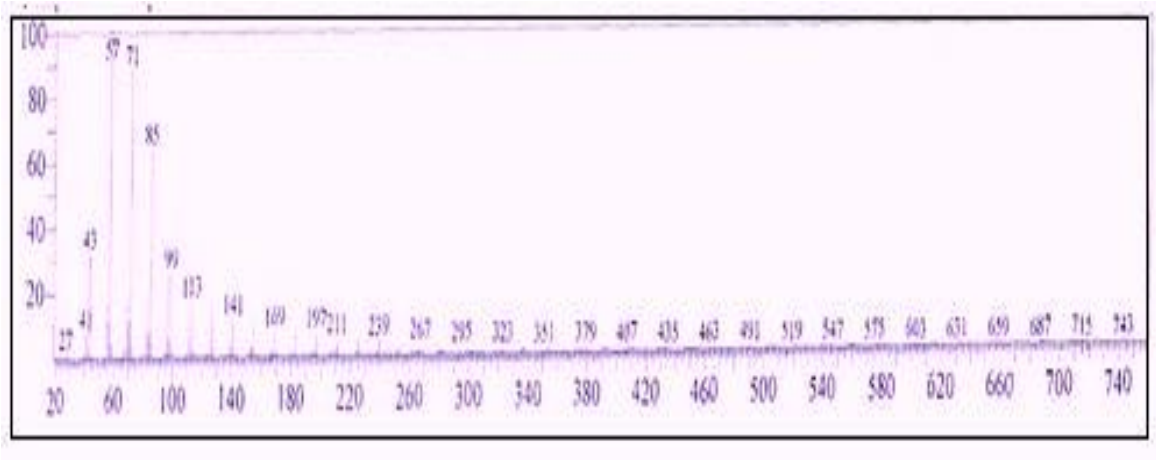


Figure 3.21: Mass spectrum of tetrapentacontane

3.5.2 Antimicrobial activity

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

Table 3.9 :Constituents of the oil

No.	Name	R. Time	Area
1	2-Heptanal	4.583	0.07
2	Butyric acid, 4-pentadecyl ester	6.560	0.07
3	Nonanal	6.700	0.10
4	Octanoic acid methyl ester	6.975	0.16
5	Octanoic acid	7.698	0.22
6	Alpha – Terpineol	8.063	0.06
7	3,4-Nonadienal	8.344	0.03
8	2-Decenal	8.995	0.27
9	2,4-Dodecadienal	9.459	0.64
10	2,4-Dodecadienal , E,E-	9.776	1.31
11	2-Undecenal	10.392	0.19
12	Nonanoic acid , 9-oxo, methyl ester	11.317	0.17
13	2-Pentadecanone, 6,10,14- trimethyl	16.014	0.07
14	9-Hexadecenoic acid , methyl ester -Z-	16.630	0.16
15	Hexadecenoic acid , methyl ester	16.824	6.22
16	Palmitoleic acid	17.026	0.19
17	Pentadecanoic acid	17.249	11.46
18	9,12-Octadecadienoic acid (Z,Z) ,	18.485	8.43
19	9-Octadecenoic acid (Z) , methyl ester	18.533	6.85
20	9-Octadecenoic acid , methyl ester	18.571	0.61
21	Methyl stearate	18.739	1.02
22	9,12-Octadecadienoic acid (Z,Z)	19.003	35.91
23	Oleic acid	19.038	9.24
24	Oxiraneoctanoic acid, 30octyl- , methyl	20.142	0.16
25	Eicosanoic acid, methyl ester	20.500	0.33
26	PGHI, methyl ester	20.557	0.26
27	Methyl 13,16-docosadienoate	20.666	0.28
28	9,12,15-Octadecatrienoic acid methyl	21.005	0.68
29	Phenol , 2,2` - methylenebis[6-(1,1-	21.422	0.19
30	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	21.753	0.61
31	Dotriacontane	21.861	0.34
32	Tetrapentacontane	22.163	11.45
33	Octacosanoic acid , methyl ester	22.913	2.24
			100.00

Table 3.10: Inhibition zones(mm/mg sample)

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

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

Conclusion

Five plants of medicinal attributes(*Hyphane thebaica, Acacia mellifera, Parkinsonia aculeata , Lucaena leucocephala, Sorghum bicolor*) have been investigated. The oils of these plants have been extracted by maceration and then characterized by GC-MS. In addition the antimicrobial activity of the targeted oils has been assessed and different antimicrobial responses have been observed.

Recommendations

It is highly recommended that the isolated oils should be evaluated for a wider range of biological activities including antimalarial, antiviral …etc.

Also other secondary metabolites existing in the studied plant species may be isolated, characterized and their biological activity could be evauated.

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