Sudan University of Science and Technology

College of Post-Graduate Studies

Chemical Characterization of Constituents of *Coffea arabica* Seed Oil and its Biological Activity

التوصيف الكيميائى لزيت بذور البن وفعاليته البيولوجية

A Thesis Submitted in Partial Fulfillment of the Requirements of the Master Degree in Chemistry

By

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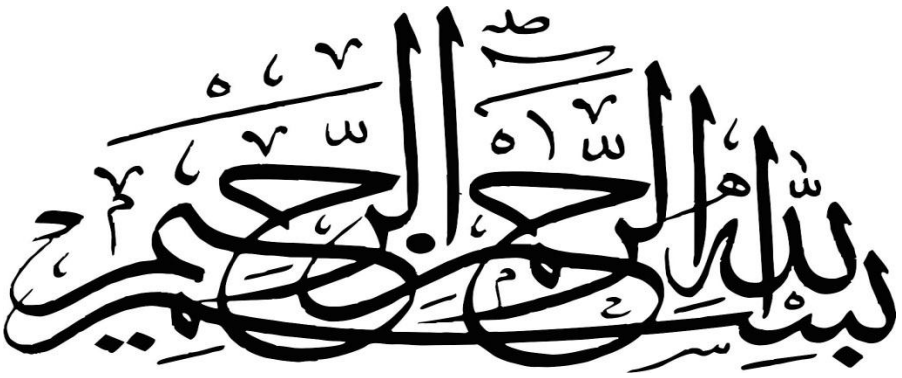
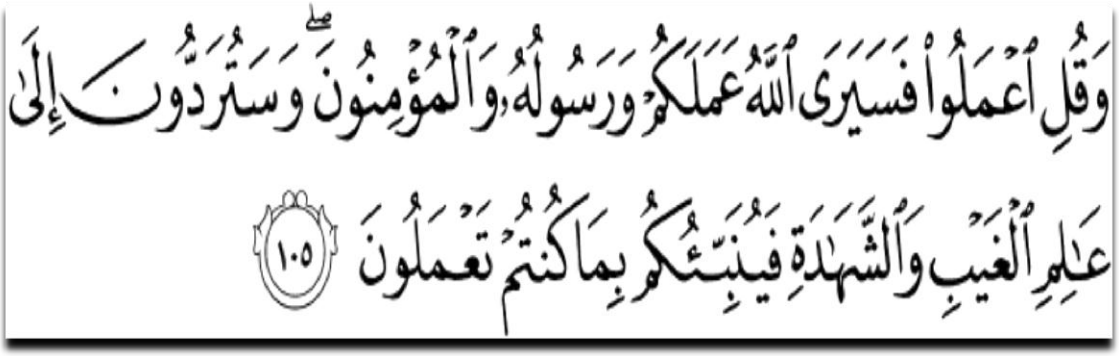
March ,2019





صدق الله العظيم

(التوبة-105)

Dedication

To my mother

To my father

To my sisters

To my brother

Acknowledgement

First of all, I would like to thank **Alla Almighty** who gave me health, strength and patience to complete this work.

I would like to express my deep gratitude and respect to my

supervisor Prof. Mohammed Abdel Karim, for his guidance,

support, valuable advice and kindness . I would like to express

my sincere thanks to my family for their support.

Abstract

The oil from *Coffea Arabica* was analyzed by GC-MS.The analysis showed 22 components. The major components are:

Hexadecanoic acid, methyl ester (31.84%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (%31.46), 9-Octadecanoic acid methyl ester (17.40%), Methyl stearate (10.84%), Eicosanoic acid methyl ester (5.04%).

Antimicrobial activity of Coffea Arabica oil was conducted.

The oil showed significant activity against *Bacillus subtilis*, moderate activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

المستخلص

أستخلصت بذور نبات القهوة العربية بالهكسان حيث تم إستخلاص الزيت الثابت . ثم حلل

الزيت بإستخدام بتقنية كروموتوغرافيا الغاز- مطياف الكتلة والتي أوضحت وجود 22 مكونا

أهمها :

Hexadecanoic acid, methyl ester (31.84%)

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (31.46%)

9-Octadecanoic acid methyl ester (17.40%)

Methyl stearate (10.84%)

Eicosanoic acid methyl ester (5.04%)

وفي إختبارمضادالميكروبات أظهر الزيت فعاليه واضحة ضد:

(Bacillus subtilis)

وفعاليةجزئية ضد:

*(Staphylococcus aureus),(Pseudomonas aeruginosa)*and

(Candida albicans).

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Chapter one

INTRODUCTION

1-Introduction

1.1-Gas chromatography

Chromatography is defined as : process of separating a mixture into individual components. Through the separation process each component in the sample can identified and quantified.

Indeed several kinds of chromatographic techniques exist each is accomplished with its corresponding technique. Gas chromatography (GC) is one of the popular techniques. GC is used for compounds that are thermally stable and volatile (or can be made volatile). Because of its simplicity, sensitivity and effectiveness in separating components, GC is one of the most important tools in chemistry [1].

When a test sample is introduced onto the GC column, the individual molecules continuously transfer between the stationary and mobile phases as the injected solute band moves down the column. Each solute moves at a rate determined by its partition between the gas and liquid phase and the flow rate of carrier gas.Selective partitioning between the mobile and stationary phases is highly dependent on the analyte molecule's solubility in each phase. Thus, solutes that are less soluble in the stationary phase will elute from the column first while solutes that are more soluble

will elute later. Because solubility depends on the physical and chemical properties of a compound, a series of compounds exhibiting a systematic variation in structure and thus, chemical properties, would be expected to partition between the mobile and stationary phases to different extents. Column temperature is an important variable that must be controlled for precise work, so the column is usually housed in a thermostated oven. The optimum temperature depends on the boiling point of the sample and the degree of separation required.

A temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time (2 to 30 min). For samples with a broad boiling point range, it is often desireable to employ temperature programming, whereby the column temperature is increased either continuously or in steps as the separation proceeds [2].

1.1.1- GC Instrumentation

The GC-MS instruments usually consist of the following components:

i. Column

Column is made from metal (stainless steel or copper) or glass tube of varyinglengths.Packed columns contain a granular solid of uniform particle size, evenly coated with a thin layer of the liquid

phase. Capillary columns are fabricated from very narrow-bore tubing with the liquid phase distributed along the column walls.

ii. Carrier Gas

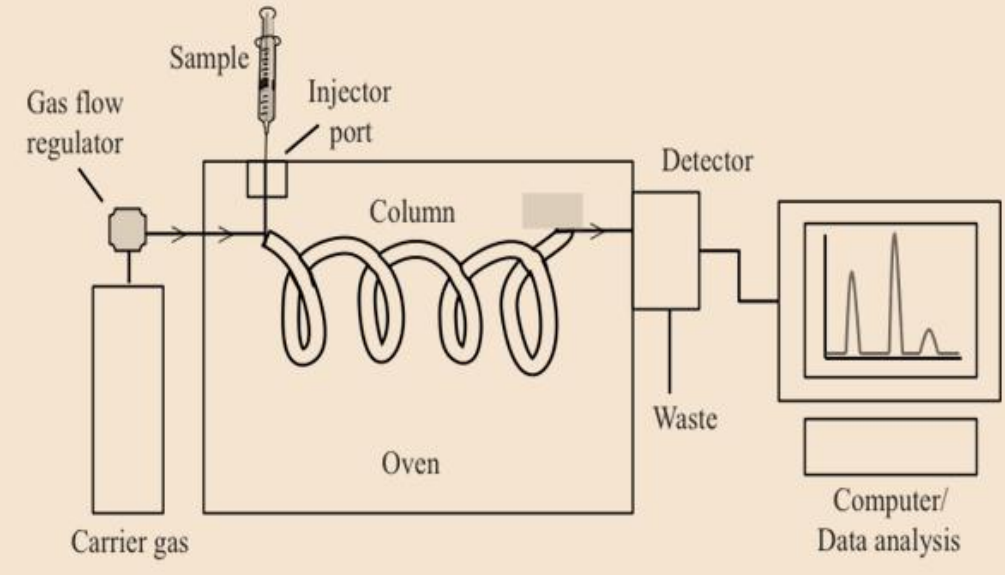
In the GC_MS instrument an inert carrier gas passes through the column and transports the solute from the injection port to the detector. Hydrogen, helium and nitrogen are the most common carrier gases.

iii. Injection System

During a GC-MS experiment , the sample is injected from via a microliter syringe. Usually, a 1, 5 or 10 microliter syringe is used. The entire sample should be vaporized immediately and introduced directly into the column.

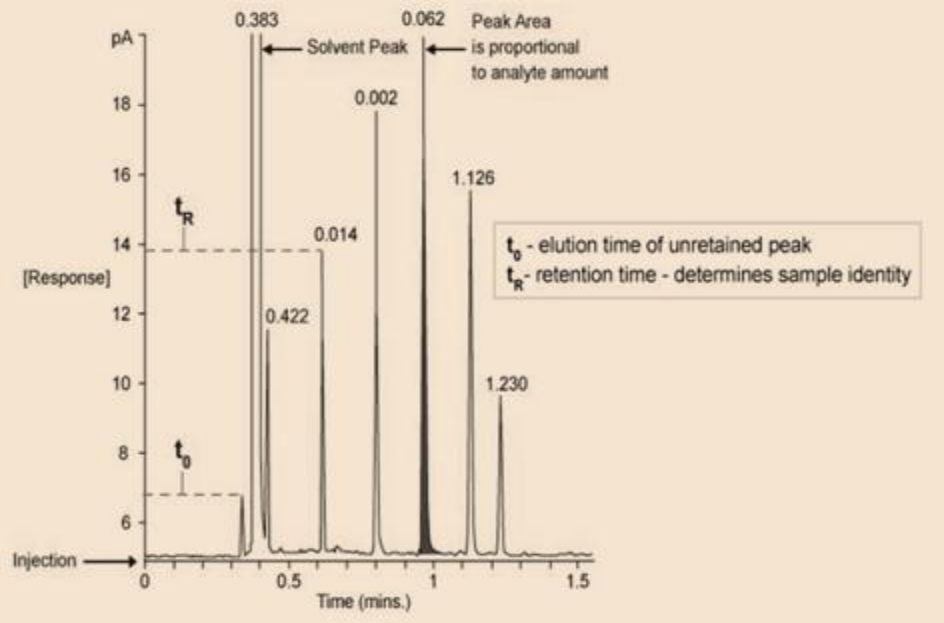
iv. Detection System

The main function of the detector is tomonitor the carrier gas stream as it passes out of the column. The signal is generally proportional to the concentration or the total amount of solute present. The most common types of detectors are :thermal conductivity detector (TCD) and the flame ionization detector (FID) [2].



GC Instrument

When the different constituents elute from the column they pass into a detector – where some physicochemical property of the analyte produces a response from the detector. This response is amplified and plotted against time – giving rise to a 'chromatogram' Components (such as the injection solvent) that are not retained within the column elute at the 'dead time' t0. There are various ways of measuring this parameter using unretained compounds such as methane or hexane.



Chromatograms generated by GC

It should be emphasized that the technique of gas chromatography has several important advantages. GC techniques produce fast analyses because of the highly efficient nature of the separations achieved. It can be argued that modern GC produces the fastest separations of all chromatographic techniques. A column has been produced to separate 970 components within a reasonable analysis time - proving that very complex separations may be carried out using GC. By using a combination of oven temperature and stationary phase polarity very comples separations may achieved – including separations of chiral and other positional isomers. GC is excellent for quantitative analysis with a range of sensitive and linear detectors to choose from.

Gas chromatography is resticted to volatile analytes. Some highly polar analytes can be derivatized to impart a degree of volatility, but this process can be difficult and may incur quantitative errors. A practical upper temperature limit for conventional GC columns is around 350-380°C. Analyte boiling points rarely exceed 400 °C in GC analysis and the upper molecular weight is usually around 500 Da.

Among advantages of GC-MS are:

- High speed analysis.

-High efficiency – leading to high resolution.

-Possess sensitive detectors (ppb).

-It is non-destructive – enabling coupling to Mass Spectrometers (MS) - an instrument that measures the masses of individual molecules that have been converted into ions, i.e. molecules that have been electrically charged.

-High quantitative accuracy (<1% RSD typical).

-Requires small samples (<1 mL).

-reliable techniques.

- endowed with extensive literature and applications.

However this technique has some limitations including:

- restricted to volatile analytes.

-Not used for samples that decompose at high temperatures .

-Not suited to preparative chromatography.

- It requires MS detector for analyte characterization.

- It is known that most MS detectors are destructive [3-5].

1.2-Mass Spectrometry (MS)

MS isdefined as : a technique utilized to specify the molecular composition of an analyte. Mass spectrometry starts by vaporizing and ionizing the sample, most often using heat and a beam of electrons. The sample is then accelerated through a magnetic field, where its path is curved. Small particles bend more along the path. Once they reach the detector, they can be analyzed according to their mass to charge ratio. The computer attached to the detector will print a graph, showing which species were most abundant, along with their mass to charge (m/z) ratio.

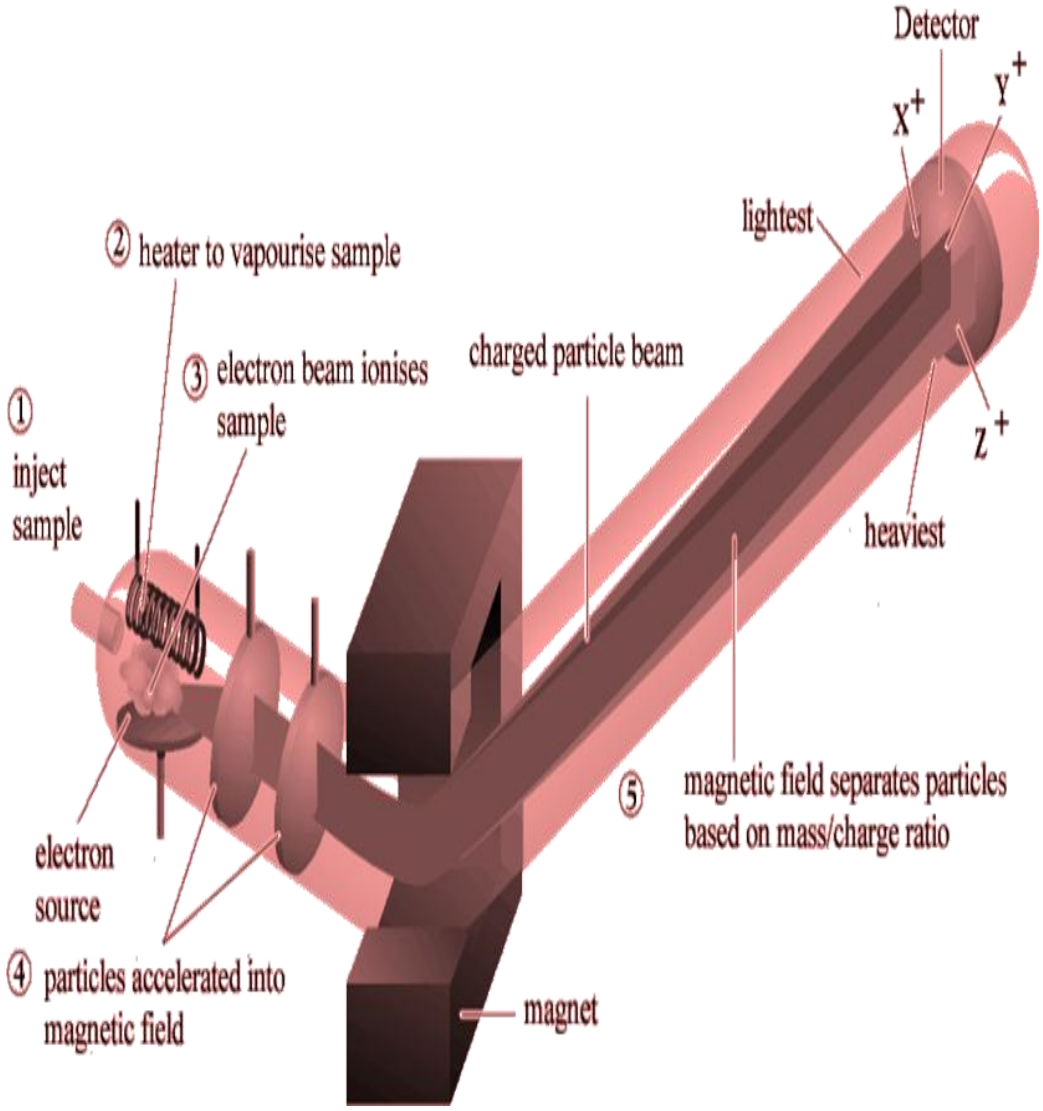
Mass spectrometry is used in many different fields to analyze unknown substances. The data received can be used to determine the formula and structure of many samples. High resolution mass spectrometry, which contains the most advanced ionization and detection equipment, can accurately determine the formula and structure of almost any compound. Mass spectrometry is used not

only in sciences such as chemistry and biology to identify substances, but also in criminal forensics, to identify almost any substance encountered[6].

During the MS experiment the first stage of mass spectrometry is ionization. Molecules tend to be hard to work with, unless they are charged. Charged molecules can easily be manipulated using electric and magnetic fields. To create ions, the substance must first be vaporized. A mass spectrometer will contain a chamber in which the sample is placed. This chamber will be heated, until a vapor is created of the sample. This vapor will move through an electric field, through which electrons are passing. These electrons will knock electrons off of the molecules being analyzed. Alternatively, the sample could be chemically induced to create ions. Sometimes, the removal and redistribution of electrons causes different species of the molecule to form, which are fractions of the original molecule. These will be seen on the final output from mass spectrometry, and give vital clues to the structure of a molecule. This ionic vapor is now ready to be accelerated and analyzed. The ions are accelerated between two negative plates. This means that only positive cations can be detected. The negative species are removed via a vacuum pump. The positive ions travel down a curved tube. To bend the path of the ions, a large electromagnet encircles the tube. The voltage to this magnet can be adjusted,

which will increase or decrease its overall strength. At the end of the tube is a small slit, through which molecules can exit and be detected by a sensitive screen.

Since the magnet can be adjusted, the computer controlling the unit will cycle through different power levels. The slit is small, and the power must be exactly right to get a particle to curve just right and exit the slit. Larger molecules will tend to curve less than small molecules. The computer graphs the output at each level, which corresponds the mass to charge ratio of the molecules at each level. In other words, only molecules of a certain size and charge can get through the slit at any given level of power. As this is adjusted, the computer can sense the total amount of molecules which make it through the slit. This allows the operator to effectively sort and analyze the entirety of any sample by processing it through mass spectroscopy. However, because the molecules must be ionized, there are also fractional species produced. This can aid in identifying the structure of the molecule, but it can also make identification vastly more complicated. The sensitive screen on the other side of the silt is bombarded with molecules. The computer analyzing this screen tracks their relative abundance at each mass to charge ratio [4].



Components of Mass Spectrometry Instrument

MS has a wide range of applicationsincluding the ion and weights separation. The samples are usually introduced through a heated batch inlet, heated direct insertion probe, or a gas chromatography. Ionization mass spectrometry (ESI-MS) which has become an increasingly important technique in the clinical laboratory for structural study or quantitative measurement of metabolites in a

complex biological sample. MS/MS applications are plentiful. Mass spectrometry is an analytical method with high specificity and a growing presence in laboratory medicine. Various types of mass spectrometers are being used in an increasing number of clinical laboratories around the world, and, as a result, significant improvements in assay performance are occurring rapidly in areas such as toxicology, endocrinology, and biochemical markers [5].

1.2.1-Advantages and disadvantages of MS

Mass spectrometry possesses distinct advantages including: hgih sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference.

MS technique has excellent specificity where characteristic fragmentation patterns can identify unknowns or confirm the presence of suspected compounds. It provides information about molecular weight and isotopic abundance of elements [6].

However, disadvantages resides in the fact that often mass spectrometry fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and para positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions [7].

1.3-Gas chromatography- Mass spectrometry (GC-MS)

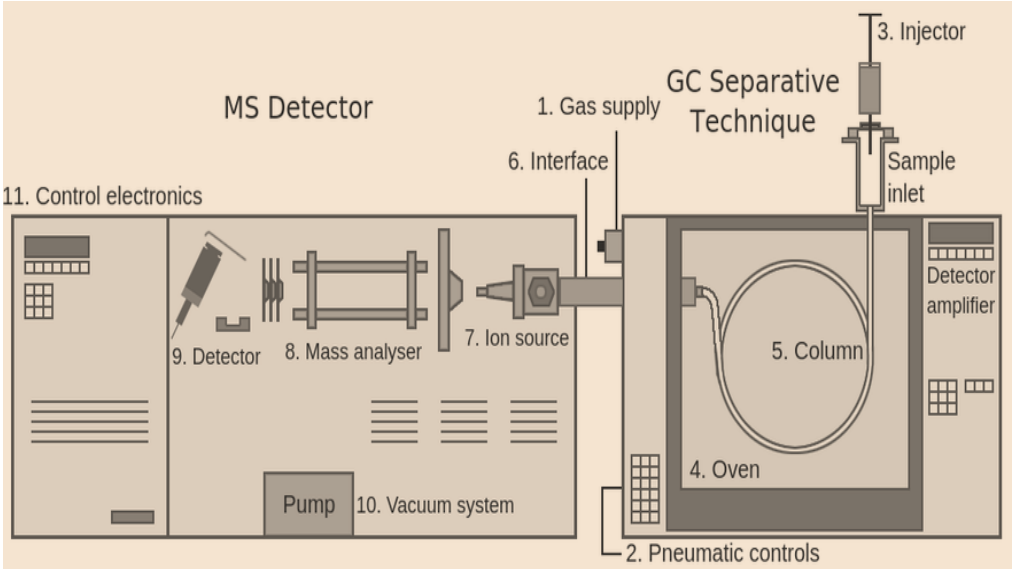
The combination of gas liquid chromatography (GC) for separation and mass spectrometry (MS) for detection and identification of the components of an analyte is rapidly becoming the definitive analytical tool in the research and commercial analytical laboratory. The GC/MS systems come in many varieties and sizes depending on the work they are designed to accomplish.

GLC is a popular, powerful, reasonably inexpensive, and easy-to-use analytical tool. Mixtures to be analyzed are injected into an inert gas stream and swept into a tube packed with a solid support coated with a resolving liquid phase. Absorptive interaction between the components in the gas stream and the coating leads to a differential separation of the components of the mixture, which are then swept in order through a detector flow cell. Gas chromatography suffers from a few weaknesses such as its requirement for volatile compounds, but its major problem is the lack of definitive proof of the nature of the detected compounds as they are separated. For most GC detectors, identification is based solely on retention time on the column. Since many compounds may possess the same retention time, we are left in doubt about the nature and purity of the compound(s) in the separated peak. The mass spectrometer takes injected material, ionizes it in a high vacuum, propels and focuses these ions and their fragmentation

products through a magnetic mass analyzer, and then collects and measures the amounts of each selected ion in a detector. A mass spectrometer is an excellent tool for clearly identifying the structure of a single compound, but is less useful when presented with a mixture.

The combination of the two GC and MS into a single GC/MS system forms an instrument capable of separating mixtures into their individual components(GC), identifying, and then providing quantitative and qualitative(MS) information on the amounts and chemical structure of each compound.

However, GC-MS still possesses the weaknesses of both components. It requires volatile components, and because of this requirement, has some molecular weight limits. The mass spectrometer must be tuned and calibrated before meaningful data can be obtained. The data produced has time, intensity, and spectral components and requires a computer with a large storage system for processing and identifying components [8].



GC-MS Instrument

GC requires the analyte to have significant vapor pressure between 30 and 300°C. GC presents an insufficient proof of the nature of the detected compounds. The identification is based on retention time matching that may be inaccurate or misleading. GC-MS represents the mass of a given particle (Da) to the number (z) of electrostatic charges (e) that the particle carries. The term m/z is measured in DA/e. GC-MS commonly uses electron impact (EI) and chemical ionization (CI) techniques. The main features of enhanced molecular ion, improved confidence in sample identification, significantly increased range of thermally labile and low volatility samples amenable for analysis, much faster analysis,

and improved sensitivity particularly for compounds that are hard to analyze [9].

1.3.1-Applications of GC-MS

As far as pollution is concerned , organic pollutants in the environment can be detected by GC-MS.. The determination of polycyclic aromatic hydrocarbons (PAH), unleaded gasoline, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, sulphur in air chloro-phenols in water and soil, is very convenient to be screened by this GC-MS.

GC-MS could be used to detect the degradation products of lignin in bio-mass research, pesticides in food products. Analysis of some C_{60} degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization.

In foods there are several aromatic compounds existing naturally in native state or formed while processing and such compounds could be screened by GC-MS. The techniqueis also used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. GC-MS is also used to detect and measure contaminants, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should to be controlled and checked as regulated by governmental issues.

GC-MS is used in the analysis of piperine , spearmint oil, lavender oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrances, menthol, allergens, olive oil, lemon oil, peppermint oil, yiang oil, strawberry syrup, butter triglycerides, residual pesticides in food and wine.

In criminal cases GC-MS can analyze the particles from suspect to correlate his involvement in case. Also the analysis of fire debris using GC-MS can be established.

GC-MS is used in sports anti-doping laboratories to test athlete's urine samples for prohibited performance enhancing drugs like anabolic steroids. It is also commonly used in forensic toxicology to find poisons, steroids in biological specimens of suspects, victims, or the deceased.

The gas chromatography – MS technique is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents and drugs like anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, sedative hypnotics, narcotics and food items.

GC-MS could be used for detecting adulterations, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, oregano-chlorinated pesticides in river water, drinking water, soft drinks.

Detection of explosivescould be accomplished via GC-MS. Is an essential part of chemical analysis unit. For enhancing capability in homeland security and public health preparedness, traditional GC-MS units have been modified for field portability.

GC-MS could successfully be employed for analysis of an extended range of low volatilite hydrocarbons that are amenable for analysis including waxes up to $C_{74}H_{150}$.

GC-MS analysis could successfully be used for petrochemicals, fuels and hydrocarbon mixtures, including gasoline, kerosene, naphthenic acids, diesel fuel, various oil types, transformer oil, biodiesel, wax and broad range of geochemical samples.

A Wide spectrum of congenital metabolic diseases called as inborn error of metabolism are now detectable in newborn by screening tests using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering from metabolic disorders. This is easy, effective and efficient way to diagnose the problem like in case of genetic metabolic disorders by a urine test at birth. In combination with isotopic labeling of metabolite, the GCMS is used for determining metabolic activity. Most applications are based on the use of 13C labeling and the measurement of ^{13}C-^{12}C ratios with an isotope

ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few select ions and return values as ratios.

GC-MS is extensively used in pharmaceutical industries forquality control, quality assurance, production, analytical research and development, , pilot plants departments for active pharmaceutical ingredients , bulk drugs and formulations.

GC-MS is used for process and method development, identification of impurities in active pharmaceutical ingredients. It is an integral part of research associated with synthesis and characterization of compounds, pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc.

a-Clinical toxicology

Due to many advantages including :enhanced molecular ions, extended range of compounds amenable for analysis, superior sensitivity for compounds and faster analysis, GC-MS is used in clinical toxicology. Through GC-MS, toxins and venoms are easily identified and quantified.

GC-MS is used in industries for the analysis of aromatic solvents, inorganic gases, amino alcohol in water, impurities in styrene, glycol, diols, xylene, allergens in cosmetics etc. GC-MS is used for the characterization of formic acid in acetic acid for industrial use.

In Industries acetic acid is important intermediate in coal chemical synthesis. It is used in the production of poly ethylene, cellulose acetate and poly vinyl as well as synthesic fiber and fabrics.

b-Energy and fuel applications

GC-MS is used for the analysis of aromatic solvents, sulphur, impurities in polypropylene, sulphur in menthane, natural gases, 1,3 butadiene, ethylene, gas oil, unleaded gasoline, polyethene, diesel oil, unleaded gasoline, polyethylene, diesel, modified biomass, grafted polymers etc. GC-MS has triggered a new arena of research and taken to new heights of impactful presentation and characterization of compounds by its wide range of applications.

c-Academic research

As a unique and powerful technology the GC-MS provides a rare opportunity to perform the analysis of new compounds for characterization and identification of synthesized or derivatized compound. It is widely used in pure and applied sciences like Chemistry, Polymers, Nanotechnology and Biotechnology etc. It yields useful information that can be used in research publication internationally [9].

1.4-Essential Oils

Essential oils are concentrated volatile aromatic compounds produced by plants - the easily evaporated essences that give plants their wonderful scents. Each of these complex precious liquids is extracted from a particular species of plant life. Each plant species originates in certain regions of the world, with particular environmental conditions and neighboring fauna and flora.

Essential oils are frequently referred to as the “life force” of plants. Unlike fatty oils, these "essential" oils are volatile, highly concentrated, substances extracted from flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01 percent to 10 percent of the total. That's why tons of plant material is required for just a few hundred pounds of oil. These oils have potent antimicrobial factors, having wide range of therapeutic constituents. These oils are often used for their flavor and their therapeutic or odoriferous properties, in a wide selection of products such as foods, medicines, and cosmetics [10].

Throughout history, the essential oils of plants were used in many cultures for their medicinal and therapeutic benefits. Egyptian culture used resins, balms and fragrant oils for medical, magical and religious ceremonies, for embalming, and as an offering to

their gods. Other ancient cultures recognized the physical and psychological benefits of scented ointments and oils, including China and India, during the same period as ancient Egypt. Greek and Roman cultures refined and added to this knowledge. Hippocrates, known as the father of modern medicine, maintained 2,500 years ago that "the key to good health rests on having a daily aromatic bath and scented massage'. Dioscorides wrote about aromatics in his Materia Medica about 100 AD. With the fall of the Roman Empire and the expansion of Christianity, Roman physicians fled to Constantinople with the books of Galen, Dioscorides and Hippocrates. These were translated into Persian and Arabic and passed into the Arab world. Most aromatic oils used (prior to about 1600 AD) were not distilled, as we know today, but were produced by macerating plants in hot vegetable oils or more commonly in animal fats.

However, distillation of some sort is an age-old process, which may have begun as early as 2000 BC. This suggests that the Arabs revived or improved upon the process that had been known but perhaps little used for over four thousand years. Between the 7th and the 13th centuries Alchemist Arabic philosophers devoted themselves to the old hermetic art of alchemy, the purification and concentration of spiritual forces. Reviving the use of aromatics in medicine and perfumery, they perfected the techniques. Alchemists

optimistically searching for the "elixir of life" and "the philosophers' stone" made many chemical discoveries [11].

1.4.1-Classifying essential oils

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

i-Consistency

Depending on their consistency, essential oils are classified as:

a-Essences: Fluid essences are liquids which are volatile at room temperature.

b-Balsams

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

c-Resins

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

1. 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.

2. 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, micro biotic and chemical uniformity, and easy to add). They have the aroma of the plant in concentrated form and are highly viscous liquids or semi-solid substances (black pepper, paprika oleoresin, cloves…).

3. Gum-resins: These are natural plant or tree extracts. They are a mix of gums and resins.

ii- Origin

Depending on their origin, essential oils are classified as:

- 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.

- 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 ethanol.

-Synthetic oils: are usually produced by combining their chemically synthesized components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences…).

iii- Chemical nature

The total essential oil content of a plant is generally low (less than 1%). However, by extraction we obtain a highly concentrated form which is used in industrial processes. Most of these are highly complex chemical compounds. The proportion of these substances varies depending on the oil, but also on season, time of day, growing conditions, and genetics [12].

1.4.2- Properties of essential oils

1.4.2.1- Physical properties

Essential oils are volatile and become liquid at room temperature, when distilled they are at first colourless or slightly yellowish, they are less dense than water, they are nearly always rotational and have a high refractory index, they are soluble in alcohol and in the usual organic solvents, such as ether or chloroform, and also in high grade alcohol, they are lipo-soluble and not very soluble in water, but can be dragged using steam [12].

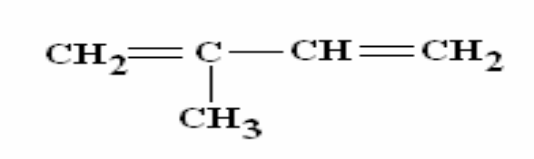
1.4.2.2- Chemical properties

Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

i) Volatile fraction

Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters. The hydrocarbons are the Compounds that have hydrogen and carbon as their building blocks. Basic

Hydrocarbon found in plants is isoprene having the following structure.



(Isoprene Unit)

-Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene (C_5) units under the Hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene (C_{10}), when three join, it's a sesquiterpene (C_{15}) and four linked isoprene units are diterpenes (C_{20}).

-Alcohols are the compounds which contains Hydroxyl compounds. Alcohols exist naturally, either as a free compound, or combined with a terpenes or ester. When terpenes are attached to an oxygen atom, and hydrogen atom, the result is an alcohol. When the terpene is monoterpene, the resulting alcohol is called a monoterpenol. Alcohols have a very low or totally absent toxic reaction in the body or on the skin. Therefore, they are considered safe to use.

- Medicinally, essential oils containing aldehydes are effective in treating Candida and other fungal infections.

-Organic acids in their free state are generally found in very small quantities within Essential oils. Plant acids act as components or buffer systems to control acidity.

-Essential oils containing esters are used for their soothing, balancing effects. Because of the presence of alcohol, they are effective antimicrobial agents. Medicinally, esters are characterized as antifungal and sedative, with a balancing action on the nervous system. They generally are free from precautions with the exception of methyl salicylate found in birch and wintergreen which is toxic within the system.

-ketones are beneficial for promoting wound healing and encouraging the formation of scar tissue. Ketones are usually (not always) very toxic. The most toxic ketone is Thujone found in mugwort, sage, tansy, thuja and wormwood oils.

ii) Nonvolatile residue or fixed oils

That comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids [10].

1.4.3-Biological activities of essential oils

i)Antibacterial and antifungal action

Because of the variability of amounts and profiles of the components of essential oils, it is likely that their antimicrobial activity is not due to a single mechanism, but to several sites of action at the cellular level. Then, different modes of action are involved in the antimicrobial activity of essential oils. One of the possibilities for action is the generation of irreversible damage to the membrane of bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energy substrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore to its death. Another possibility of action is inhibition of production of amylase and protease which stop the toxin production, electron flow and result in coagulation of the cell content [13]. It has been reported that EOs containing mainly aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol were characterized by the highest antibacterial activity, followed by EOs containing terpene alcohols. Other EOs, containing ketones or esters, such as -myrcene, -thujone, or geranyl acetate, had much weaker activity; while volatile oils containing terpene hydrocarbons were usually inactive. Generally, essential oils characterized by a high level of phenolic compounds, such as

carvacrol, eugenol, and thymol, have important antibacterial activities [14].

Antifungal actions are quite similar to those described for bacteria. However, two additional phenomena inhibiting the action of yeast are worth mentioning: the establishment of a pH gradient across the cytoplasmic membrane and the blocking of energy production of yeasts which involve the disruption of the bacterial membrane [13].

ii)Antioxidant Activity

The antioxidant potential of an essential oil depends on its composition. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial ant oxidative properties. 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. 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. EOs have shown their action as hepatoprotective agents in ageing polyunsaturated fatty acids mammals and it has been proved that they possess a beneficial impact upon the PUFAs, in particular the long chain C20 and C22 acids [14].

iii) Antiviral activity

The complex mixture of essential oils usually shows a higher antiviral activity than individual compounds (due probably to synergism phenomena); with exception of β- caryophyllene which is the most famous antiviral compounds found in many different essential oils from different plant families. Different mechanisms of antiviral activity of different essential oils and their constituents seem to be present. The antiviral activity of the essential oil is principally due to direct virucidal effects (by denaturing viral structural proteins or glycoproteins). Proposed mechanisms suggest that essential oils interfere with the virus envelope by inhibiting specific processes in the viral replication cycle or by masking viral components, which are necessary for adsorption or entry into host cells, thus, they prevent the cell-to-cell virus diffusion [13].

iv) 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. The inflammatory response induces an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines. It also stimulates the activity of several enzymes (oxygenase's, nitric oxide synthases, peroxidases, etc.), as well as the arachidonic acid metabolism. Recently, essential oils have been used in clinical settings to treat inflammatory diseases, such as rheumatism, allergies, or arthritis. Melaleuca alternifolia EO was reported to have a considerable anti-inflammatory activity. This activity is correlated with its major compound: -terpineol. The active compounds act by inhibiting the release of histamine or reducing the production of inflammation mediators. 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.

v) Cancer Chemo protective Activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researchers for their potential activity against cancer. They and their volatile constituents of the studies target the discovery of new anticancer natural products. 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. Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer.

vi) Cytotoxicity

Due to their complex chemical composition, essential oils have no specific cellular ligands. As lipophilic mixtures, they are able to cross the cell membrane and degrade the layers of polysaccharides, phospholipids and fatty acids, and permeabilize. This cytotoxicity appears to include such membrane damage. In bacteria, the membrane permeabilization is associated with the loss of ions and the reduction of the membrane potential, the collapse of the proton pump and the depletion of the ATP pool. Essential oils may coagulate the cytoplasm and damage lipids and proteins. Damage to the wall and the cell membrane can lead to the leakage of macromolecules and lysis. Recent work on the yeast

Saccharomyces cerevisiae has shown that the cytotoxicity of some essential oils based on the ability to form colonies differs significantly in relation to their chemical composition. Generally, 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, viruses, fungi, protozoa, parasites, mites, and others.

vii) Repellent and Insecticidal Activity

Essential oils constitute a rich bank of structurally-diverse compounds with a variety of insecticidal and repellent mechanisms. Numerous studies have demonstrated that these compounds, as well as their parent blends, possess biological activity capable of eliciting adverse effects in arthropod pests. Several factors affecting the commercialization of plant essential oil extracts as repellents include regulatory requirements, intellectual property value, biological activity, product performance, and product quality. The toxic effect of essential oils was not only suitable for granary insects but also for flying insects.

Actually, the activities of essential oils on species are manifold. Commonly, essential oils can be inhaled, ingested, or skin-absorbed by insects. The fumigant toxicity of essential oils and their main components, the volatile monoterpenes, has been described [14].

1.5- The target species - *Coffea arabica*

*Coffea arabica*is the older type of coffee and is considered the best. It is more aromatic, less bitter and contains less caffeine. The bean is longish and has a curved cut. Arabica matures within nine to eleven months. Its share on the world market amounts to more than 60%. It is mainly cultivated in Brazil, Columbia and Central America.

Another species of *Coffea* is*Coffea canephora* (Robusta)**.**Robusta contains twice as much caffeine as Arabica, and is less aromatic. It is more resistant against heat, diseases and parasites. Its bean is round-shaped and the cut is straight. It matures within six to eight months. Its share reaches nearly 40 %. Its growing areas are primarily located in Central and West Africa, South East Asia and Brazil [19].

-Common Names

- **USA:** Coffee,Java, Joe.
- **Middle East:** Qahwah, Caova, Cova.
- **Panama:**Cabi.
- **Spanish:** Café [20].
- **Turkish:**kahveh[21].
- **Sudanese:** Bunn.

Taxonomy

- **Kingdom:** Plantae – plants, Planta, Vegetal, plants.
- **Subkingdom:**Viridiplantae – green plants.
- **Infrakingdom:** Streptophyta – land plants.
- **Super division:**Embryophyta.
- **Division:** Tracheophyta – vascular plants, tracheophytes.
- **Subdivision:** Spermatophytina – spermatophytes, seed plants, phanérogames.
- **Class:** Magnoliopsida.
- **Superorder:** Asteranae.
- **Order:** Gentianales.
- **Family:**Rubiaceae – madders, rubiacées.
- **Genus:** Coffea L. – coffee.
- **Species:** Coffea arabica L. – Arabian coffee [22].

*Coffea Arabica*is an upright tropical evergreen shrub or small tree that grows to 10-15’ tall. Although native to Ethiopia, Arabian coffee has been cultivated in Arabia for over 1000 years. It is now commercially grown in subtropical and tropical areas throughout the world. In coffee plantations, plants are kept trimmed to 6’ tall for ease of harvest and best production. Indoor container plants typically grow to about 4-6’ tall.

Plants feature (a) glabrous, glossy, elliptical, dark green leaves with prominent veins and wavy margins, (b) fragrant, star-shaped, white flowers that bloom in axillary clusters of 2-9, (c) green two-seeded fruits (berries) that gradually mature to yellow, light red and finally deep red and (d) fissured bark on older branches. Berries are edible with a pulpy grape-like texture, but it is the seeds (coffee beans) that are coveted. Berries are picked by hand when ripe and depulped, with the extracted seeds/beans then dried in the sun before roasting [23].

Coffee beans contain caffeine, a widely used stimulant that is also used in proprietary painkillers to potentiate the effect of aspirin and paracetamol. It also contains the stimulants theobromine and theophylline, as well as chlorogenic acid, which are stimulant and diuretic as well as a known allergen. The seed is a bitter, aromatic, stimulant herb that has diuretic effects and controls vomiting. It is reported to be analgesic, an aphrodisiac, anorexic, antidotal,

cardiotonic, CNS-stimulant, counterirritant, diuretic, hypnotic, galactagogue and nervine.

Whilst not usually recognised as a medical herb, coffee is a highly effective general stimulant, having a particular effect upon the central nervous system, improving perception and physical performance. It has been found of help in some cases of headache or migraine. An enema made using coffee beans is an effective cleanser for the large bowel. Coffee is a folk remedy for asthma, atropine poisoning, fever, flu, headache, jaundice, malaria, migraine, narcosis, nephrosis, opium poisoning, sores and vertigo. The dried seeds ('beans') are roasted, ground, and brewed to make one of the two most popular beverages in the world. Coffee is widely used as flavoring in ice cream, pastries, candies, and liqueurs.

The seed has been used as a masticatory since ancient times. Cooked in butter, it can be used to make rich flat cakes.An extract from the seeds is used as a flavoring in ice cream etc.

The dried, roasted green seeds have been used as an appetizer, whilst chocolate-covered roasted seeds are used as a gourmet snack.The red fruits and leaves are chewed for their stimulating properties.

The cooked leaves have a strong brown colour, a good texture and a rather neutral flavor with only a hint of bitterness.The leaves contain more caffeine than the fruit and are sometimes used as a tea substitute.

Coffea arabica

Aim of this study

This study was carried out to:

1-Extract oil from *Coffea Arabica*.

2-Conduct GC-MS analysis.

3-Evaluate the potential antimicrobial activity of the extracted oil.

Chapter two

Materials and Method

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

The plant material seeds of *Coffea Arabica* were purchased from the local market – Omdurman (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2-Instruments

The GC-MS analysis was performed on a GC-MS-QP2010 Ultra instrument (a product of Shimaduzu-Japan

2.1.3-Solvents

n-hexane used for extraction and other solvents used in this study are analytical grade solvents.

2.1.4-Test organisms

The oil extracted from Coffea Arabica was assessed for its antimicrobial properties against the following human pathogens : G+ve) *Bacillus subtilis, Staphylococcus aureus*;(G-

ve),*Pseudomonas aeroginosa, Escherichia coli*and the fungus*Candida albicans.*

2.2- Methods

2.2.1-Extraction of oil

Coffea Arabica seeds (250g) werepowdered and percolated with n-hexane. The solvent was evaporated under reduced pressure to yield the oil. For the CG-MS analysis, the oil was esterified using a methanolic solution of sodium hydroxide and a methanolicsulphuric acid.

2.2.2- GC-MS analysis

*Coffea Arabica*oil was investigated by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness).

Chromatographic conditions (oven temperature program and other conditions) are outlined below:

Rate: - **; temperature**: 150.0^{o}C ; **hold time** : 1.00 min.$^{-1}$

Rate: 4.00 ; **temperature**: 300.0^{o}C ; **hold time** : 0.00min^{-1}

Table 2.1: Chromatographic conditions

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

2.2.3-Antimicrobial test

Meida for microbial growth were prepared according manufacturer instructions. A Suspension containing about 10^8-10^9 colony forming units per ml was prepared in Muller Hinton agar (for bacteria) . For fungal growth Sabouraud dextrose agar was 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 dry , and then incubated at 37^{o}C for 24 hours.

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

Plate agar diffusion assay was used to screen 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^{o}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 each of these plates which were divided into two halves, two cups in each half (10 mm in dimeter) 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 micro pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37^{o}C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial

chemotherapeutics. After incubation, the dimeters of the resultant growth inhibition zones were measured in triplicates and averaged.

Chapter three

Results and Discussion

3-Results and Discussion

3.1-GC-MS analysis of *Coffea Arabica* oil

The components of *Coffea Arabica* L. oil were identified by GC-MS analysis , where retention times and the fragmentation patterns were used for identification purposes. Eleven constituents were identified by GC-MS. The typical total ion chromatograms is presented in Fig. (1) . Different constituents of the oil are outlined in Table 1 .

Table 1: Constituents of ***Coffea arabica*** oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	14.183	0.20
2.	Hexadecanoic acid, methyl ester	16.414	31.84
3.	Heptadecanoic acid, methyl ester	17.420	0.14
4.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.168	31.46
5.	9-Octadecenoic acid (Z)-, methyl ester	18.204	17.40
6.	Methyl stearate	18.412	10.84
7.	cis-11-Eicosenoic acid, methyl ester	20.049	0.91
8.	Eicosanoic acid, methyl ester	20.253	5.04
9.	Docosanoic acid, methyl ester	21.956	0.95
10.	Tetracosanoic acid, methyl ester	23.535	0.38
11.	.gamma.-Sitosterol	23.969	0.84

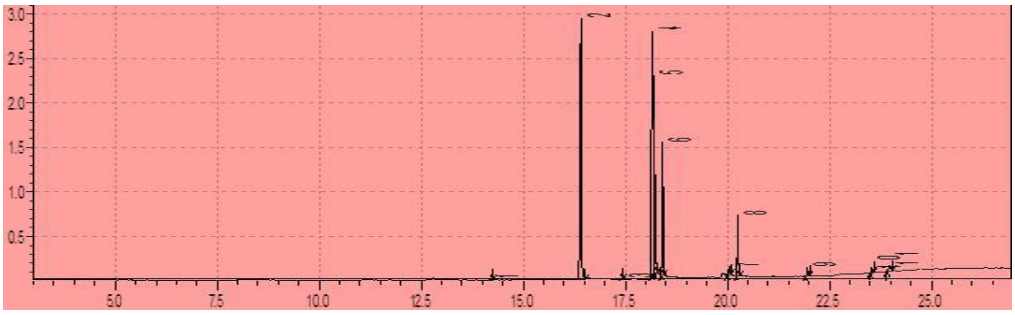


Fig.1: Total ion chromatograms

3.2-Major constituents

The following compounds were detected by GC-MS as major constituents:

3.2.1--Hexadecanoic acid methyl ester(31.84%)

Figure 2 shows the mass spectrum of hexadecanoic acid methyl ester .The molecular ion : $M^{+}[C_{17}H_{34}O_2]^{+}$ appeared at m/z 270 with R.T. 16.414 in total ion chromatograms.The fragment at m/z239 is due to loss of a methoxyl .

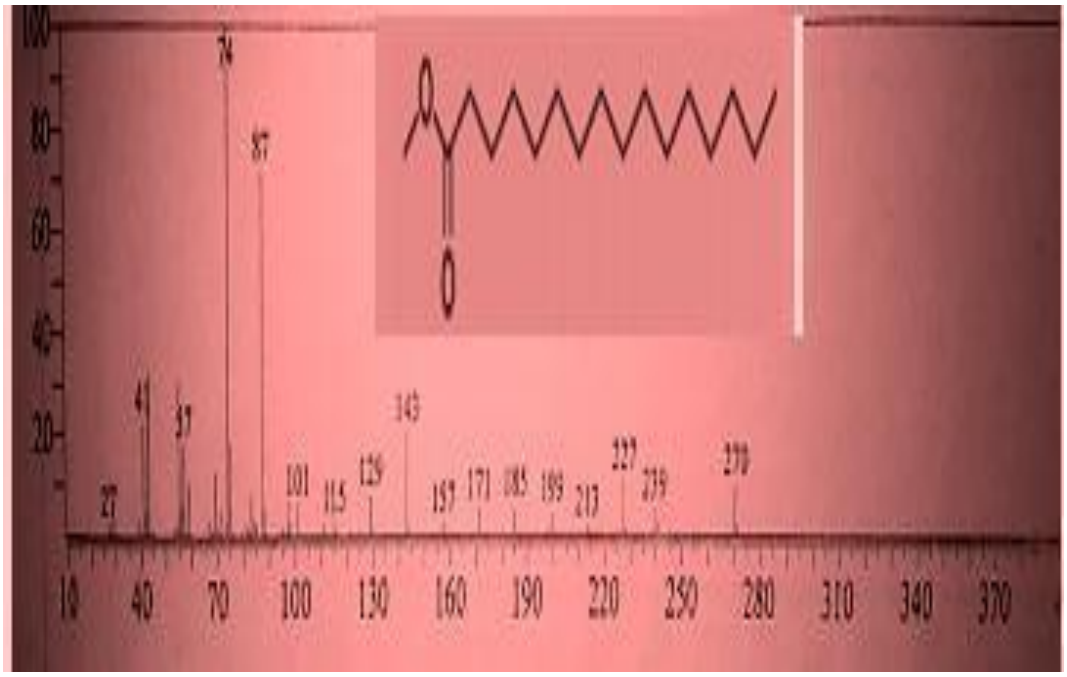


Fig. 2: Mass spectrum of hexadecanoic acid methyl ester

3.2.2- 9,12-Octadecadienoic acid methyl ester(31.46%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig. 3 .The peak at m/z294 (R.T. 18.168) coincides with $M^{+}[C_{19}H_{34}O_2]^{+}$, while the peak at m/z263 is due to loss of a methoxyl .

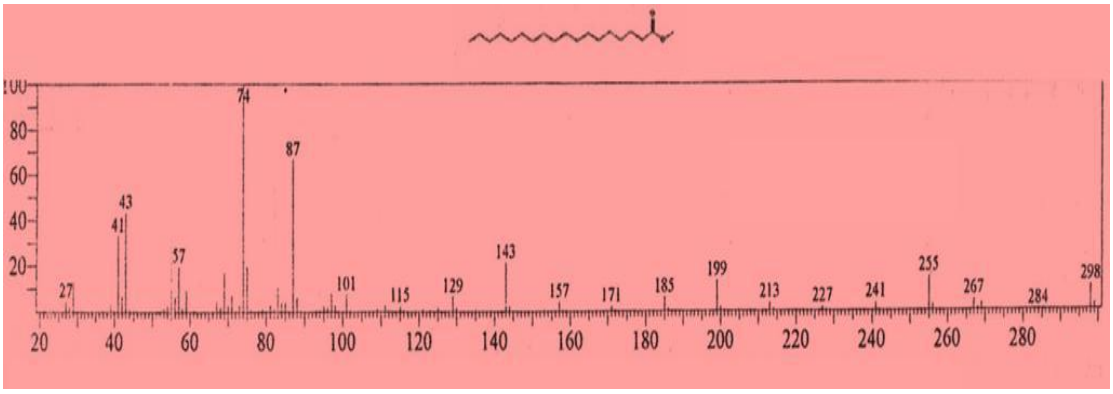


Fig. 3: Mass spectrum of 9,12-octadecenoic acid methyl ester

3.2.3- 9-Octadecanoic acid methyl ester(17.40%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.4.The peak at m/z 296 (R.T. 18.204) corresponds $M^{+}[C_{19}H_{36}O_2]^{+}$, while the signal at m/z266 is attributed to loss of a methoxyl .

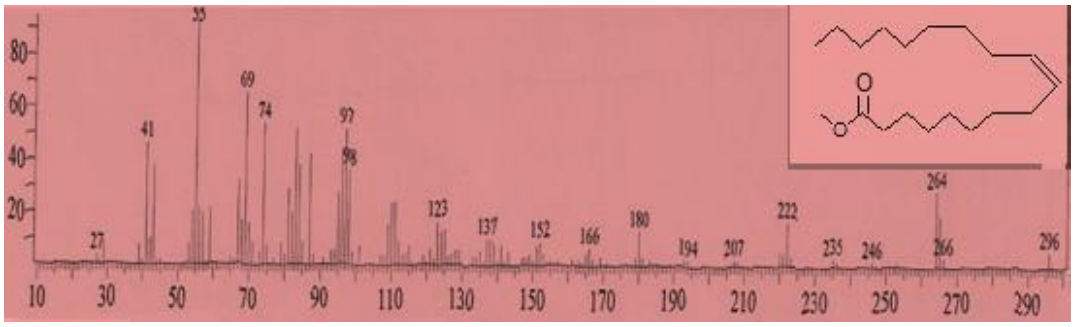


Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester

3.2.4-Methyl stearate(10.84%)

The EI mass spectrum of methyl stearate is depicted in Fig.5.The peak at m/z 298 with R.T. 18.412 is due to $M^{+}[C19H38O2]^{+}$.

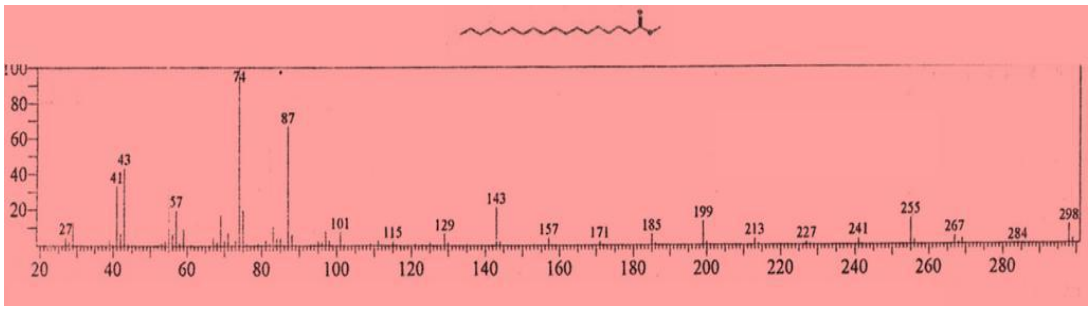


Fig. 5: Mass spectrum of methyl stearate

3.25-Eicosanoic acid methyl ester(5.04%)

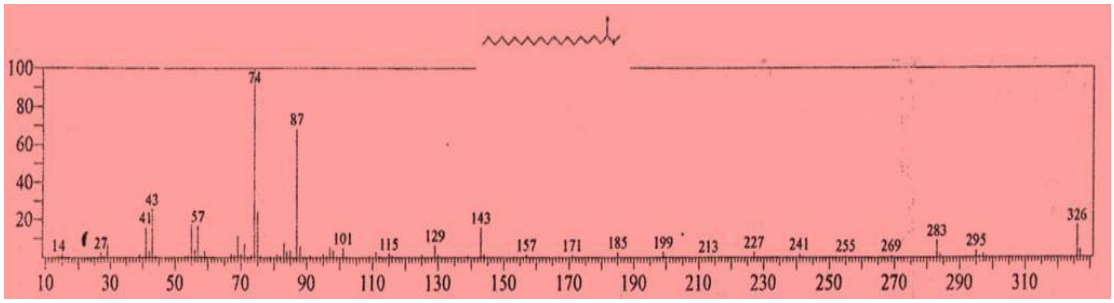


Fig. 6: Mass spectrum of eicosanoic acid methyl ester

Fig. 6 shows the mass spectrum of eicosanoic acid methyl ester.The peak at m/z326, which appeared at R.T. 20.253 accounts for : $M^{+}[C_{21}H_{42}O_2]^{+}$, while the signal at m/z295 corresponds to loss of a methoxyl function.

The phytosterol gamma sitosterol appeared as minor constituent at m/z414 (RT, 23.969)- see Fig.7.

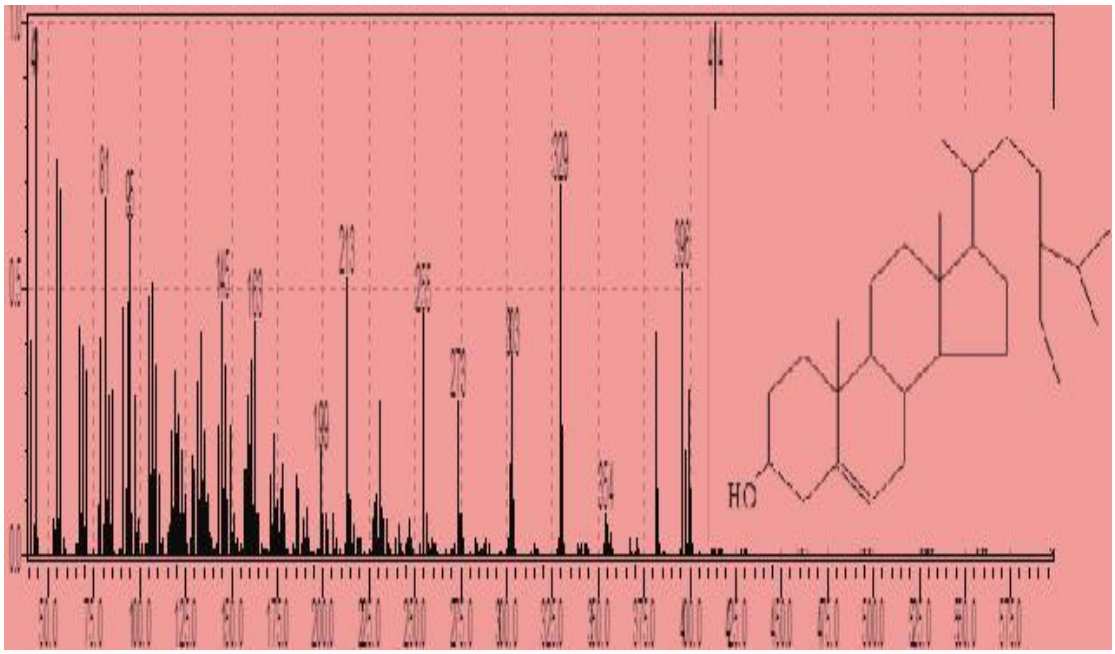


Fig. 7: Mass spectrum of gama-sitosterol

3.3-Antimicrobial activity of the oil

Coffea Arabica oil was examined for antimicrobial activity against five standard microbial isolates. The diameters of the growth of inhibition zones are shown in Table 2. Results were interpreted according to the following data :(< 9mm: inactive; 9-12 mm : partially active ; 13- 18 mm : active ; >18mm : very active). The oil showed significant activity against G+ve *Bacillus subtilis,* moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*It also showed moderate anticandidal activity, but it failed to give inhibitory effect of G-ve: *Escherichia coli*. Ampicilin, gentamycin and clotrimazole were used as positive controls(Tables 3 and 4) .

Table 2: Antimicrobial activity of the oil

Sample						
		Ec	Pa	Sa	Bs	Ca
Solanum						
melogena	oil	--	14	15	17	14
(100mg/ml)						

Table 3 : Antibacterial activity of standard drugs

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

Table 4 : Antifungal activity of standard drug

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

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

Conclusion

The oil from Coffea Arabica was analyzed by GC-MS.The analysis showed 22 components. The major components are :hexadecanoic acid, methyl ester (31.84%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (%31.46), 9-octadecanoic acid methyl ester (17.40%), methyl stearate (10.84%), eicosanoic acid methyl ester (5.04%).

Antimicrobial activity of Coffea Arabica oil was conducted. The oil showed partial activity against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans.

Recommendations

The following is recommended:

-The extracted oil may be assessed for other biological activities such antimalarial ,antiinflammatory….etc.

-Other constituents of the target plant may be isolated , identified and screened for biological activity.

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