Sudan University of Science and Technology

College of Post Graduate Studies

Characterization of Constituents of Jasminum humile Seeds Fixed oil and its Biological Activity

توصيف مكونات الزيت الثابت لبذور نبات الياسمين ونشاطه البيولوجي

A Thesis Submitted in Partial Fulfillment of The Requirement of the Master Degree in Chemistry

By

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Nov., 2017


بسم الله الرحمن الرحيم

الإستهلال

قال تعالي (إقرأ بإسم ربك الذي خلق (1) خلق الانسان من علق (2) إقرأ وربك الاكرم (3) الذي علم بالقلم (4) علم الانسان ما لم يعلم (5)).

سورة العلق (الأيات1-5)

صدق الله العظيم

Dedication

To

My parents

My uncles and aunts

My brothers and sisters

Acknowledgment

First I would like to thank **Almighty Allah**for giving me health, strength to perform this work.

I would like to express my gratitude to my supervisor: prof. Mohammed Abdel Karim Mohammed for his helpful suggestions, continuous encouragement and help during his supervision of this work.

My thanks would also extend to the staff of Chemistry dept. at Sudan University of Science and Technology for their infinite help and support.

I would like to extend my thanks to my colleagues for their valuable assistance.

My very special thanks are due to my aunt, Nafisa Omer and my uncle, Dr.Mohammed Nasor. for their continuous support, encouragement throughout my studies.

Finally my great thanks go to my family for their consist love and understanding and to all those who were not mentioned but had helped me in some way or another.

Abstract

In this study, the fixed oil of *Jasminum humile was* extracted from seeds. The oil was analyzed by gas chromatography-mass spectrometry (GC-MS) and 20 component were identified Major constituent were: 9-Z-Octadecenoic acid methyl ester(30.19%%) , 9,12-Z,Z-Octadecadienoic acid methyl ester (23.96%) , Hexadecanoic acid methyl ester(15.853%) , Methyl stearate(13.22%) .

The oil was screened for antimicrobial activities using five stander bacterial strains. The cup plate agar diffusion technique was used to assess the antimicrobial activity of the oil against (Bacillus subtilis and Staphylococcus aureus- Gram positive ; Escherichia coli and Pseudomonas aeruginosa- Gram negative bacteria and the fungal species Candida albinos. The results indelicate that *Jasminum humile* fixed oil contains bioactive constituents having significant antimicrobial properties.

المستخلص

أستخلص الزيت الثابت لبذور الياسمين ودرس بواسطة الكروماتوغرافيا الغازية- طيف الكتلة حيث إتضح أن الزيت يحتوي على 20 مكونا المكونات الرئيسية هي:

9-Z-Octadecenoic acid methyl ester(30.19%%) , 9,12-Z,Z-Octadecadienoic acid methyl ester (23.96%) , Hexadecanoic acid methyl ester(15.853%) , Methyl stearate(13.22%) .

ثم أخضع زيت الياسمين لإختبارات بيولوجية كمضاد للميكروبات حيث استخدمت خمسة انواع من البكتيريا القياسية وهي :

Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*
Gram negative bacteria *; Escherichia coli* and *Pseudomonas aeruginosa* and the fungal species *Candida albicans*.

وقد أوضحت نتائج الاختبارات البيولوجية أن الزيت الثابت لبذور الياسمين يحتوي علي مواد مثبطة لعمل الميكروبات.

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Chapter one introduction

1-Introduction

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

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples, including that of material samples obtained from planet Mars during probe missions as early as the 1970s. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. Like liquid chromatography–mass spectrometry, it allows analysis and detection even of tiny amounts of a substance[1].

GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a 100% specifictest, which positively identifies the presence of a particular substance. A nonspecific test merely indicates that any of several in a category of substances is present. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification[1].

1.1-1 History

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952. These comparatively sensitive devices were originally limited to laboratory settings.[1]

The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1964, Electronic Associates, Inc. (EAI), a leading U.S. supplier of analog computers, began development of a computer controlled quadrupole mass spectrometerunder the direction of Robert E. Finnigan2-4.

By 1966 Finnigan and collaborator Mike Uthe's EAI division had sold over 500 quadrupole residual gas-analyzer instruments2. In 1967, Finnigan left EAI to form the Finnigan Instrument Corporation along with Roger Sant, T. Z. Chou, Michael Story, and William Fies5. In early 1968, they delivered the first prototype quadrupole GC/MS instruments to Stanford and Purdue University2. When Finnigan Instrument Corporation was acquired by Thermo Instrument Systems (later Thermo Fisher Scientific) in 1990, it was considered "the world's leading manufacturer of mass spectrometers"6.

In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. By the 2000s computerized GC/MS instruments using quadruple technology had become both essential to chemical research and one of the foremost instruments used for organic analysis. Today computerized GC/MS instruments are widely used in environmental monitoring of water, air, and soil; in the regulation of agriculture and food safety; and in the discovery and production of medicine[7].

1.1.2-Instrumentation

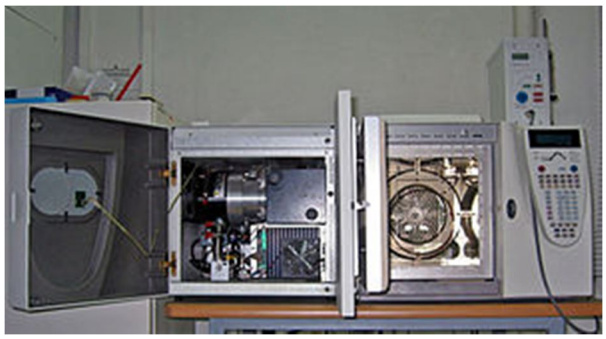

Figure1: The insides of the GC-MS, with the column of the gas chromatograph in the oven on the right.

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g.

5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio [8].

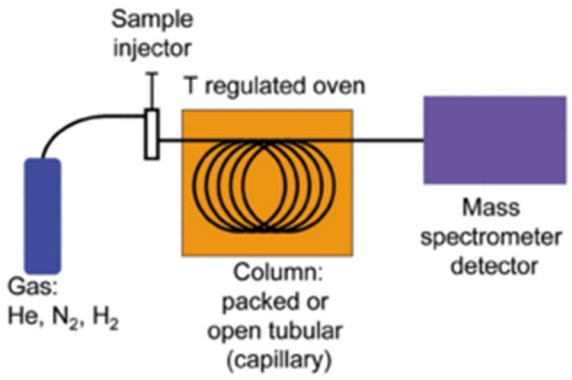

Figure2: Schematic of GC-MS

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography

using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample [8].

1.1.2.1- Types of mass spectrometer detectors

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

(TOF), tandem quadruples (MS-MS) (see below), or in the case of an ion trap MS^n where n indicates the number mass spectrometry stages[9].

1.1.3-Ionization

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.The ionization technique chosen is independent of using full scan or SIM.

1.1.3.1-Electron ionization

By far the most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupled or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectrometrists as the employ of molecular electron

bombardment, whereas "soft ionization" is done by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA). Spectral library searches employ matching algorithms such as Probability Based Matching and dot-product matching that are used with methods of analysis written by many method standardization agencies[9,10].

1.1.3.2-Cold electron ionization

The "hard ionization" process of electron ionization can be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information[11]. In this method named cold electron ionization (Cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic molecular beam (SMB). Collisions with the make up gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process[11]. Cold-EI mass spectra are characterized by an abundant molecular ion while the usual

fragmentation pattern is retained, thus making Cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae[12].

1.1.3.3-Chemical ionization

In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced[13].

In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts[13].

In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply[13].

1.1.4-Analysis

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

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

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the

spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample[14].

A “full spectrum” analysis considers all the “peaks” within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced[14].

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be *m/z* 50 to *m/z* 400. The determination of what range to use is largely

dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as *m/z* 28 due to nitrogen), carbon dioxide (*m/z* 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively

important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard[14].

1.1.5-Applications of GC\MS

1.1.5.1- Environmental monitoring and cleanup

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies[14].

1.1.5.2- Criminal forensics

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate[14].

1.1.5.3- Law enforcement

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased[1].

1.1.5.4-Sports anti-doping analysis

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids[14] .

1.1.5.5-Security

Explosive detecting systems have become a part of all international airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith 's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems[15].

1.1.5.6- Chemical warfare agent detection

Traditional GC-MS units with transmission quadruple mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, so man, and VX.[15] These complex and large GC-MS systems have been modified and configured with resistively heated low thermal mass (LTM) gas chromatographs that reduce analysis time

to less than ten percent of the time required in traditional laboratory systems.[19] Additionally, the systems are smallerand systems that are hand-carried by two-person teams or individuals, much ado to the smaller mass detectors. Depending on the system, the analyses can be introduced via liquid injection, desorbed from sorbent tubes through a thermal desorption process, or with solid-phase micro extraction (SPME) [16].

1.1.5.7- Chemical Engineering

GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass [17].

1.1.5.8-Medicine

Dozens of congenital metabolic diseases also known as inborn error of metabolism are now detectable by newborn screening tests, especially the testing 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 with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a

newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the 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.

1.1.5.9-Astrochemistry

Several GC-MS have left earth. Two were brought to Mars by the Viking program[18]. Venera 11 and 12 and Pioneer Venusanalysed the atmosphere of Venus with GC-MS[19]. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan[20,21]..

1.2-Antimicrobial

An **antimicrobial** is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are

called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis[22].

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones. The term also used to be restricted to antibacterials (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth.

Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection[23]. More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the

merits of controlling these interactions in medicine[28]. In 1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as *Penicilliumrubens*. The substance extracted from the fungus he named penicillin and in 1942 it was successfully used to treat a *Streptococcus* infection[24]. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients until then[24].

Many antimicrobial agents exist, for use against a wide range of infectious diseases[24].

Many essential oils included in herbal pharmacopoeias are claimed to possess antimicrobial activity, with the oils of bay, cinnamon, clove and thyme reported to be the most potent in studies with food borne bacterial pathogens[25,26]. Active constituents include terpenoid chemicals and other secondary metabolites. Despite their prevalent use in alternative medicine, essential oils have seen limited use in mainstream medicine. While 25 to 50% of pharmaceutical compounds are plant-derived, none are used as antimicrobials, though there has been increased research in this direction. Barriers to increased usage in mainstream medicine include poor regulatory oversight and quality control, mislabeled or misidentified products, and limited modes of delivery[27].

1.3-Essential Oils(EOs)

Essential oil is a highly concentrated hydrophobic liquid of complex mixtures containing volatile aroma compounds and can be extracted from several parts of plant , for example ,leaves, peels, barks, flowers , buds, seeds, and so on which serve as the major source of essential oil[28].

1.3.1- History of essential oils

Throughout history, the essential oils of plants were used in many cultures for their medicinal and therapeutic benefits. The Egyptians were renowned for using essential oils extensively in medical practice, beauty treatment, food preparation, and in religious ceremony. Frankincense, sandalwood, myrrh and cinnamon were considered very valuable cargo along the ancient caravan trade routes and were some-times exchanged for gold.

Borrowing from the Egyptians, the Greeks used essential oils in their practices of therapeutic massage and aromatherapy. The Romans also used essential oils to promote health and personal hygiene. Influenced by the Greeks and Romans, as well as Chinese and Indian Ayurvedic use of aromatic herbs, the Persians began to refine distillation methods for extracting essential oils from aromatic plants [29]. Essential oil extracts were used throughout the dark ages in Europe for their antibacterial and fragrant properties.

More recently, the powerful healing properties of essential oils were rediscovered in 1937 by a French chemist, Rene Maurice Gattefosse, who plunged his badly burnt hand into a vat of lavender oil (mistaking it for water) and was surprised to see no injury or scarring. A French contemporary, Dr. Jean Valnet, used therapeutic-grade essential oils to successfully treat injured soldiers during World War II. Dr. Valnet went on to become a world leader in the development of aromatherapy practices[29].

With the invention of synthetic drugs, the use of plants and herbs for their therapeutic properties temporarily declined. That trend is reversing as health scientists and medical practitioners continue to research and validate the benefits of therapeutic-grade essential oils to protect the body, boost the immune system, and revitalize the mind and influence mood, without the nega-tive side effects commonly associated with synthetic drugs [29].

1.3.2- Origin of essential oils in plants

The popularity of spiced food has led in recent years to increase in consumption of spices and essential oils. Black pepper, ginger and cloves for example are grown in many countries.

More and more temperate zone spices are being grown in the United States, such as basilparsley. thyme. mustard seed, tarragon and marjorarn. Most of these are processed for dryspices. Essential oils are isolated from various parts of the plant. such as leaves (patchouli. pines.

cedar). hits (mandarin). Bark(cinnamon), root (ginger). grass (citronella). gum (myrrh and balsam oils). Bemes(pimenta).seed (cmway). tlorvers (rose) twiigs (clove stem), buds (cloves), wood(amyris), heartwood(cedar).and saw dust(cedar oil). These plants are processed to yieldtheir quintessence or essential oils by separation from cellulose. glycerides. starches.sugars. tannins. salts. andminerais in the botanicals. The most widespread physical method for isolating essential oils Erom the botanical is CO-distillation with steam[30].A small group of products are exception to the simple definition of an essential oil.

Garlic oil.mustard oil. sweet birch oil and the like require enzymatic release of the volatile components before they can be freed from the residual biomass by steam distillation. There are other flower oils or resin oils obtained by extraction which contain only a small portion of volatile oil, but nonetheless are called essential oils. There are also several oils coming via dry-distillation which also contain only a limited arnount of volatiles. but which nonetheless fall within the designation of essential oil. e.g. oil Labdanrrm, oiI balsam Peru[30].Getting the odorous principal from the botanical is called ‘expressing' the essential oil.

In the case of the citrus oils, this in fact does involve 'pressing' as implied in the verbitself. Formerly produced by tedious hand-pressing or sponge-pressing, they are nowproduced by modern high speed. multifunctional machines. Many flower oils are extracted with a purified

petroleum solvent. Enfleurage is an old process in which delicateflower petals were physically stuck ont a purified fat, is no longer in common use.

Maceration as a process is used frequently toda[34].The yield of essential oils frombotmicals varies widely. Nutmegs yield (10-12wt %) ofoil. whereas onions yield less than 0.01 % after enzymatic treatment. Thuga wood oil yield istypicaliy between 0.60-1.00% wet material .Essential oils are typically liquid at room temperature.

1.3.3- The role and location of essential oil

The function of essential oil in the plant is not fùlly understood. Microscopicexamination of plant parts that contain the oil sacs readily shows their presence when the sac on the foliage is pricked and the aroma appears.

The odour oraroma of the flowers are said to act as attractants for insects involved in pollination. Andthus aid in preservation of species and natural selection. Essential oils are almost alwaysbacteriostatic and often bactericidal. Many components of essential oils are chemicallyactive. They are sources of plant metabolic energy if present in large quantities although some chemists have labeled them as waste products of plant metabolism. Exudates such as balsam and resins which contain essential oils act as protective seals against disease or parasite. They prevent loss of sap and are formed readily when the tree trunks are damaged[31].

1.3.4- Uses of essential oils

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oil uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments. Essential oil benefits come from their antioxidant, antimicrobial and anti-inflammatory properties.

The amount of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

The Essential oils are good source of several bioactive compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved.

Essential oils are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products and have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims

for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most countries[32-34]

1.3.5- Pharmacological Properties of Essential Oils

Essential oils have antiseptic properties and are active against a wide range of bacteria as well as on antibio-resistant strains. Moreover, they are also known to be active against fungi and yeasts (Candida).The most common sources of essential oils used as antiseptics are: Cinnamon, Thyme; Clover; Eucalyptus; Culin savory; Lavender. Citral, geraniol, linalool and thymol are much more potent than phenol[35]**.**

When used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anaesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains. Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilatation and in consequence bring about a diuretic effect.

Essential oils from the Umbellifereae family, Mentha species and verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia[35]. Essential oils are also glagtogugeand anti-inflammatory[35] .

1.3.6- Chemistry of essential oils

The molecular structure of essential oils are ring like and far more complex than the simpler, linear carbon-hydrogen structure of fatty oils. The essential oil chains are held together by carbon atoms linked with oxygen and hydrogen, along with nitrogen and sulfur atoms.

No two essential oils are alike in their structure and each oil is comprised of a combination of hundreds - even thousands - of different natural chemicals. The average essential oil may contain anywhere from 80 to 400 known chemical constituents, making them ideal for killing and preventing the spread of bacteria.

While synthetic antibiotics often contain only one active chemical allowing bacteria (like MRSA) to mutate to survive the attack, the large and varied number of antiseptic and antibacterial constituents in essential oils make it impossible for bacteria to mutate enough to survive each and every one. This is what makes essential oils such effective as natural antibiotics.

The therapeutic benefits of the oil are often related to the compound(s) at the highest amount in the oil. In general, essential oils can be subdivided into two distinct groups of chemical constituents:

a-Hydrocarbons

Made of terpenes which inhibit the accumulation of toxins and help discharge existing toxins from the liver and kidneys. Key terpenes include:

-**Monoterpenes**– (Found in Grapefruit, Orange, and Balsam Fir) . They inhibit the accumulation of toxins, detoxify kidneys and liver, enhance balance, restore information in DNA.

-**Sesquiterpenes**– (Found in Cedarwood, Sandal-wood and Myrrh) Antiseptic, anti-inflammatory, stimulate liver, increase oxygenation around the pineal and pituitary glands, can surpass the blood-brain barrier and enter the brain tissue[36].

b-Oxbygenated compounds

-**Alcohols** (found in Ravensara, Rosewood, Gera-nium) – Uplifting, high resistance to oxidation, antiseptic, antiviral, revert cells to normal function.

-**Aldehydes** (found in Cinnamon Bark, Lemongrass) - Calming, relieve stress and blood pressure, sedative, antiviral, anti-inflammatory.

-**Esters** (found in Lavender, Bergamot, Valerian and Roman Chamomile) - Balancing, relaxing and calming, antifungal, anti-inflammatory and antispasmodic.

-**Ketones** (found in Hyssop, Patchouli Rosemary, Sage) - Calming, sedative, stimulate cell and tissue regeneration, liquefy mucous.

-**Oxides** – (Found in Ravensara, Rosemary and Eucalyptus) derived from other compounds such as alcohols, terpenes or ketones which have been oxidized. Can be mildly stimulating.

-**Phenols** (found in Oregano, Thyme Clove and Tea Tree) - Stimulating to the nervous and immune systems, highly antibacterial/antimicrobial antioxi-dant, clean cell receptor sites[36].

1.3.7-Methods of extracting essential oils

Early efforts at extraction used alcohol and a fermentation process. New methods of essential oils extraction are entering the mainstream of aromatherapy, offering new choices in oils never before available. With the new labels of CO_2 and Super Critical CO_2, along with the traditional ‘steam’ and ‘hydro’ distillations, 'absolutes', and 'cold pressing', a little education for the aromatherapy enthusiast can go a long way in essential oil selection. Is one process better than another? Does one produce nicer smelling oil, or one with greater aroma therapeutic value? It turns out that essential oil production is an art form as well as a science. The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties. Some plants, and particularly flowers, do not lend themselves to steam distilling. They are too delicate, or their fragrance and therapeutic essences cannot be completely released by water alone. These oils will

be produced as 'absolutes' – and while not technically considered essential oils they can still be of therapeutic value. Jasmine oil and Rose oil in particular are delicate flowers whose oils are often found in 'absolute' form.

The value of the newer processing methods depends greatly on the experience of the distiller, as well as the intended application of the final product. Each method is important, and has its place in the making of aromatherapy-grade essential oils. Some of the few methods available for extractions of essential oils are given below:

1.3.7.1-Maceration

In this process, the whole or coarsely powdered plant material is placed in a stopper container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc(the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing[37].

1.3.7.2Cold Pressing

Cold pressing is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the simppressing of the rind at about 120 degrees F to extract the oil. The rinds are separated from the fruit, are ground or chopped and are then pressed. The result is a watery mixture of essential oil and liquid which will separate given time. Little, if any, alteration from the oil's original

state occurs – these citrus oils retain their bright, fresh, uplifting aromas like that of smelling a wonderfully ripe fruit. It is important to note that oils extracted using this method have a relatively short shelf life, so make or purchase only what you will be using within the next six months[38].

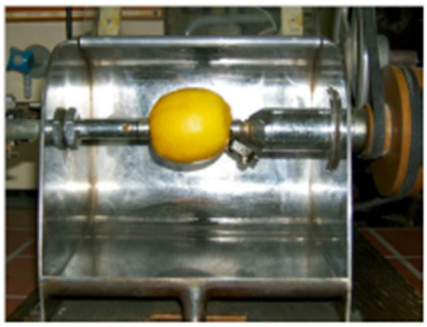

Figure3: Schematic of of cold expression

1.3.7.3-Solvent extraction

A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing resin (resinoid), or a combination of wax and essential oil (known as concrete) remains. From the concentrate, pure alcohol is used to extract the oil. When the alcohol evaporates, the oil is left behind. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system[38].

1.3.7.4-Enfleurage

Enfleurage is an intensive and traditional way of extracting oil from flowers. The process involves layering fat over the flower petals. After the fat has absorbed the essential oils, alcohol is used to separate and extract the oils from the fat. The alcohol is then evaporated and the essential oil collected[37].

1.3.7.5-Hydrodistillation

Some process becomes obsolete to carry out extraction process like Hydro Distillation which often used in primitive countries. The risk is that the still can run dry, or be overheated, burning the aromatics and resulting in an Essential Oil with a burnt smell. Hydro distillation seems to work best for powders (i.e., spice powders, ground wood, etc.) and very tough materials like roots, wood, or nuts[38].

1.3.7.6- CO_2 and Supercritical CO_2 extraction

The most modern technologies, carbon dioxide and supercritical carbon dioxide extraction involve the use of carbon dioxide as the 'solvent' which carries the essential oil away from the raw plant material. The lower pressure CO_2 extraction involves chilling carbon dioxide to between 35 and 55 degrees F, and pumping it through the plant material at about 1000 psi. The carbon dioxide in this condition is condensed to be a liquid. Supercritical CO_2 extraction (SCO_2) involves carbon dioxide heated to 87 degrees F and pumped through the plant material at around 8,000 psi – under these conditions; the carbon dioxide is likened to a

'dense fog' or vapor. With release of the pressure in either process, the carbon dioxide escapes in its gaseous form, leaving the essential oil behind. The usual method of extraction is through steam distillation. After extraction, the properties of a good quality essential oil should be as close as possible to the "essence" of the original plant. The key to a 'good' essential oil is through low pressure and low temperature processing. High temperatures, rapid processing and the use of solvents alter the molecular structure and will destroy the therapeutic value and alter the fragrance[38].

1.3.7.7-Turbo distillation extraction

Turbo distillation is suitable for hard-to-extract or coarse plant material, such as bark, roots, and seeds. In this process, the plants soak in water and steam is circulated through this plant and water mixture. Throughout the entire process, the same water is continually recycled through the plant material. This method allows faster extraction of essential oils from hard-to-extract plant materials[37].

1.3.7.8-Steam Distillation

Most commonly, the essence is extracted from the plant using a technique called distillation. One type of distillation places the plants or flowers on a screen. Steam is passed through the area and becomes "charged" with the essence. The steam then passes through an area where it cools and condenses. This mixture of water and essential oil is separated and bottled. Since plants contain such a small amount of this

precious oil, several hundred pounds may need to produce a single ounce.

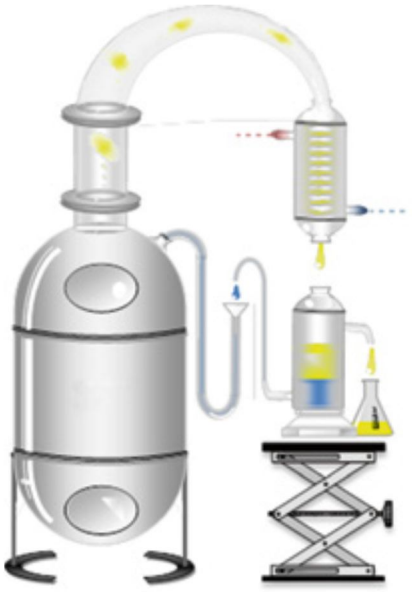

Figure4: A schematic representation of conventional recovery of essential oils

1.4-The target plant *Jasminum humile*

seeds

Taxonomy

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Family: Oleaceae

Genus: *Jasminum*

Species: *Jhumile*

Flower

Jasminum humile the Italian jasmine[39] or yellow jasmine, is a species of flowering plant in the Oleaceae family, native to Afghanistan, Tajikistan, Pakistan, Nepal, Burma (Myanmar), the Himalayas and south China. The species is widely cultivated and reportedly naturalized in Greece, Sicily and the former Yugoslavia[40.]

Jasminum humile is a roundish semi-evergreenshrub with thick stems reaching up to 4m in height. It has stout, dark green leaves, 5 cm long, with 5-7 leaflets. In protected areas it retains its leaves over winter, though in cold winters its foliage and buds may freeze. It blooms in spring and summer with clusters of usually six yellow, scented flowers[40-41]. Numerous cultivars have been developed for garden use**.** The plant is astringent, cardio tonic, decongestant and tonic**.** The flowers are astringent and a tonic for the heart and bowels[42].A paste made from the flowers is considered effective in the treatment of intestinal problems[43].The juice of the root is used in the treatment of ringworm,[43]. The milky juice of the plant is used for destroying the unhealthy lining walls of chronic sinuses and fistulas[42].

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Aim of this study

This study was undertaken to:

-Extract oil from *Jasminum humile* seeds.

-Conduct a GC-MS analysis for oil.

-Evaluate the oil for antimicrobial activity.

chapter two
Materials and Methods

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Seeds of *Jasminum humile* were collected from Nyala western Sudan and authenticated by direct comparison with a herbarium sample.

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

Jasminum humile oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table(2.1).

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
5	*Candida albicans*	fungi

2.2- Methods

2.2.1- Preperations of reagents for phytochemical screening.

a)-Flavonoid test reagents

- Aluminium chloride solution

(1 g) of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

(1 g) of potassium hydroxide was dissolved in 100 ml water.

-Ferric chloride solution

(1 g) of ferric chloride was dissolved in 100 ml methanol.

b)- Alkaloid test reagents

Maeyer reagent

- **Mercuric chloride solution**: 1.36 g in 60 ml. water.

- **Potassium iodide solution** : 5 g in 10 ml. water

The two solutions were combined and then diluted with water up to 100 ml.

-Wagner reagent

(1.27 g) iodine and(2 g) of potassium iodide in (100 ml) water.

2.2.2- Preparation of plant extract for phytochemical screening

(100 g) Of powdered air- dried seeds of *Jasminum humile* were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract(PE) was used for photochemical screening.

2.2.3- Phytochemical screening

i) Test for unsaturated sterols and for triterpenes

(10 ml)of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over anhydrous sodium sulphite . (5 ml) portion of the solution was mixed with(0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

ii)- Test for flavonoids

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.

- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

iii)- Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on a water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into two portions:

To one portion a few drops of Maeyer reagent were added., to the other portion few drops of Wagner reagent were added.

iv)- Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled , filtrated and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

v) -Test for saponins

(1g) of dried powdered plant material was placed in a clean test tube. (10 ml) of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand.

2.2.4-Extraction of oil

Powdered seeds of *Jasminum humile*(500g) were exhaustively extracted with n-hexane (soxhlet).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.

2.2.5- GC-MS analysis

The oil of *Jasminum humile* was 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 given 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

2.2.6-Antimicrobial test

i)-Preparation of 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)-Preparation of 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 antibacterial 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 micrometer 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.

Chapter three
Result and Discussion

3-Results and Discussion

The oil from a key species in Sudanese ethnomedicine-*Jasminum humile*- was analyzed by GC-MS and assessed for antimicrobial activity via cup plate agar diffusion bioassay against five standard human pathogens.

3.1-GC-MS analysis of oil

GC-MS analysis of *Jasminum humile* fixed oil was carried out. The MS library (NIST) was checked for identification of the constituents (a 90-95% match was observed) . Furthermore, the resulting fragmentation pattern was discussed. 20 components were detected by GC-MS anslysis(Table 3.1).The typical total ion chromatogram (TIC) is depicted in Fig.(1).

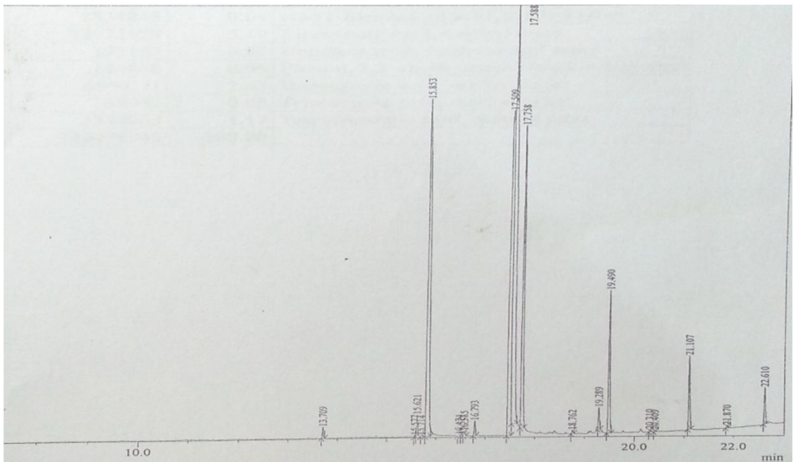

Fig.5: Total ion chromatograms

Table 4: Contituents of *Jasminum humile* oil

k#	R.Time	Area	Area%	Name
1	13.709	986126	0.34	Methyl tetradecanoate
2	15.577	132544	0.05	cis-10-Nonadecenoic acid, methyl ester
3	15.621	1809506	0.63	7-Hexadecenoic acid, methyl ester, (Z)-
4	15.714	102784	0.04	9-Hexadecenoic acid, methyl ester, (Z)-
5	15.853	61082758	21.17	Hexadecanoic acid, methyl ester
6	16.474	90130	0.03	Hexadecanoic acid, ethyl ester
7	16.521	124010	0.04	Hexadecanoic acid, 14-methyl-, methyl est
8	16.585	423979	0.15	cis-10-Heptadecenoic acid, methyl ester
9	16.793	1305296	0.45	Heptadecanoic acid, methyl ester
10	17.509	69111788	23.96	9,12-Octadecadienoic acid (Z,Z)-, methyl e
11	17.588	87080137	30.19	9-Octadecenoic acid,,methyl ester, (E)-
12	17.758	38131437	13.22	Methyl stearate
13	18.762	335711	0.12	.gamma.-Linolenic acid, methyl ester
14	19.289	1834443	0.64	cis-11-Eicosenoic acid, methyl ester
15	19.490	14975929	5.19	Eicosanoic acid, methyl ester
16	20.310	193157	0.07	Heneicosanoic acid, methyl ester
17	20.409	144048	0.05	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
18	21.107	6990111	2.42	Docosanoic acid, methyl ester
19	21.870	238989	0.08	Tricosanoic acid, methyl ester
20	22.610	3378063	1.17	Tetracosanoic acid, methyl ester
		288470946	100.00	

Main constituents of the oil are discussed below:

9-Z-Octadecenoic acid methyl ester(30.19%%)

Fig. 2 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.588 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl .

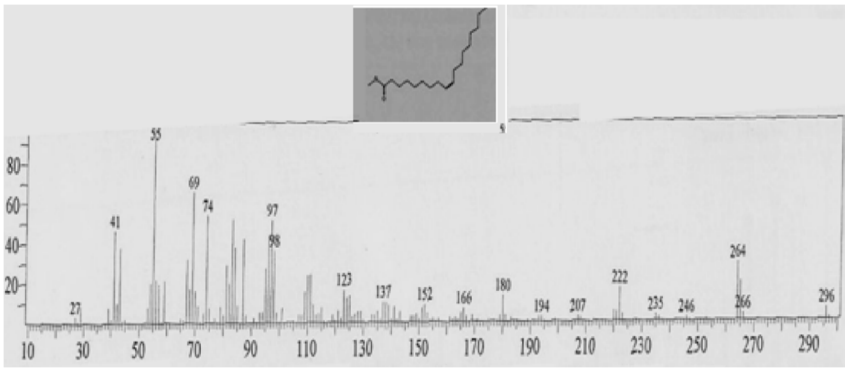

Fig. 6: Mass spectrum of 9-octadecenoic acid methyl ester

9,12-Z,Z-Octadecadienoic acid methyl ester (23.96%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.3.The peak at m/z294(R.T. 17.509 -in total ion chromatogram) corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$ .The signal at m/z263 corresponds methoxyl function.

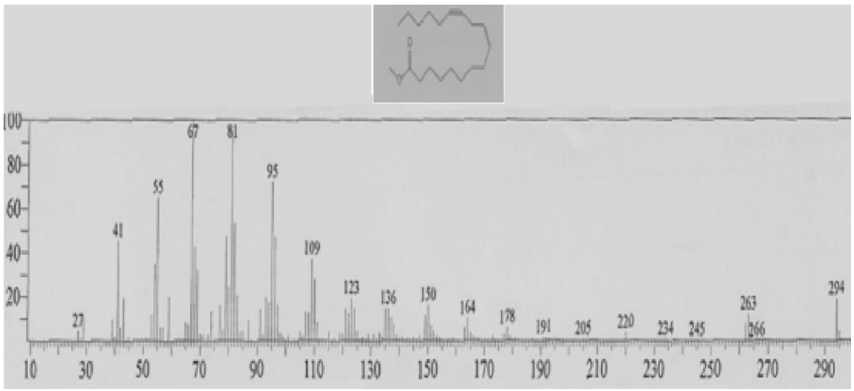

Fig. 7: Mass spectrum of 9,12-octadecadienoic acid methyl ester

Hexadecanoic acid methyl ester(15.853%)

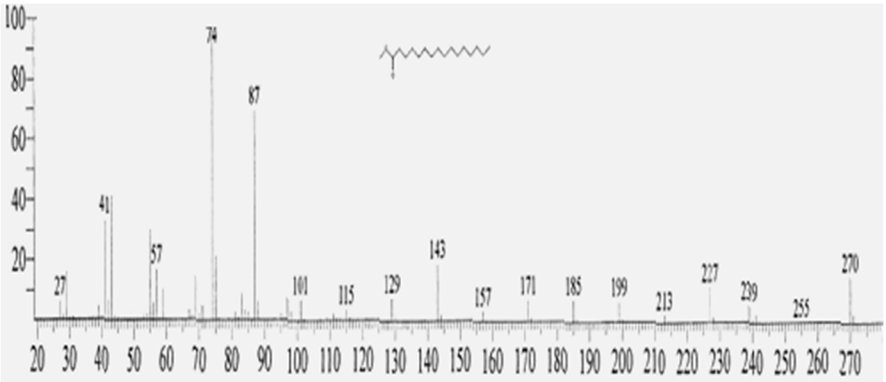

Fig. 8: Mass spectrum of hexadecanoic acid methyl ester

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270 (R.T.15.853) corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$ .The signal at m/z239 corresponds to loss of a methoxyl**.**

Methyl stearate(13.22%)

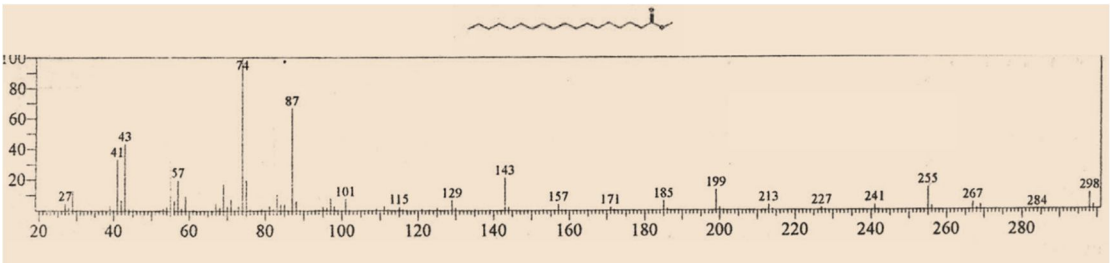

Fig. 9: Mass spectrum of methyl stearate

Fig. 5 shows the mass spectrum of methyl stearate .The signal at m/z 298(R.T.17.758) corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$, while the peak at m/z267 corresponds to loss of a methoxyl group.

Antibacterial activity

Jasminum humile oil was screened for antimicrobial activity against five standard bacterial strains . The diameters of the growth of inhibition zones are shown in Table (3.2) . Conventional terms were used for interpretation of the results : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Tables (3.3) and 3.4) display the antibacterial and antifungal activities of standard chemotherapeutic agents respectively.

Table 5 : Antibacterial activity of *Jasminum humile* oil

Type	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Oil	100	19	7	7	18	7

Table 6 : Antibacterial activity of standard chemotherapeutic agents

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 7 : Antifungal activity of standard chemotherapeutic agent

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

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

Though the oil failed to show anticandidal activity , it exhibited excellent activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at test concentration.

Conclusion

The fixed oil of *Jasminum humile was* extracted by maceration. The oil was analyzed by gas chromatography-mass spectrometry (GC-MS) and thoctadecenoic acid methyl ester(30.19%%) , 9,12-Z,Z-octadecadienoic acid methyl ester (23.96%) , hexadecanoic acid methyl ester(15.853%) , methyl stearate(13.22%) . The oil was screened for antimicrobial activity. The results indelicated that *Jasminum humile* fixed oil contains bioactive constituents having significant antimicrobial properties.

Recommendations

-Other phytochemicals (steroids, alkaloids ..etc) of *Jasminum humile* may be isolated and investigated.

- *Jasminum humile* oil may be screened for other biological effects like antimalarial , anti-inflammatory ..etc.

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