Sudan University of Science and Technology

College of Graduate Studies

Constituents of Lemon Grass (*Cymbopogon citratus*) Oil

مكونات زيت حشيشة الليمون

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

by

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August, 2020

استهلال

قال تعالى:

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ{1} خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ{2} اقْرَأْ وَرَبُّكَ الْأَكْرَمُ{3} الَّذِي عَلَّمَ بِالْقَلَمِ{4} عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ {5}﴾

صدق الله العظيم – سورة العلق (1-5)

Dedication

To

My parents, my brothers and sisters

Acknowledgment

I would like to thank **Allah Almighty** for giving me health and strength to accomplish this work.

I would like to express my thanks and respect to my supervisor Prof. Mohamed Abdel Kareem, for his infinite support, close supervision and valuable advice.

Thanks for staff, Department of Chemistry, Sudan University of Science and Technology for all facilities.

Also, thanks are extended to my family for their unlimited support.

Abstract

Cymbopogen ctratus oil was extracted by maceration using n-hexane. The oil was then analyzed by GC-MS. The GC-MS analysis showed the presence of 57constituents. Major components are:

1. 2,6 octadienal, 3,7-dimethyl, (E) (43.20%).
2. 2,6 octadienal, 3,7-dimethyl, (Z) (31.36%).
3. 5, hepten-2-one, 6methyl, (3.87%).

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مستخلص

إستخلص زيت حشيشة الليمون بواسطة الهكسان العادي باستخدام النقع، ثم أجري تحليل الكروموتوغرافيا الغازية – طيف الكتلة حيث اتضح أن الزيت يحوي 57 مكوناً أهمها:

1. 2,6 octadienal, 3,7-dimethyl, (E) (43.20%).
2. 2,6 octadienal, 3,7-dimethyl, (Z) (31.36%).
3. 5, hepten-2-one, 6methyl, (3.87%).

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Introduction

1. Introduction

1-1 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 are 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. Beware of imitations. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate[1].

1.2 Role of essential oils in plants

Essential oils are extracted from oil 'sacs' in flowers, leaves, stems, roots, seeds, wood and bark. They differ significantly from the well-known vegetable, nut and seed oils which are made up of various fatty acids (essential oils are not). Essential oils are used by the plants in somewhat the same way they are by humans - they fight infection, contain hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal,

viral, and animal foes. Despite their foliar origins however, essential oils have a similar structure to some compounds found in blood and tissues, allowing them to be compatible with our own physiology[1].

1.3 Uses of essential oils

The most effective way to use most essential oils is by external application or inhalation, though some can be very beneficial when taken internally. The use of essential oils include body oils, compresses, cosmetic lotions, baths, hair rinses, inhalation by steam, perfumes and room sprays. Essential oils are *very* potent - some will cause skin irritation or have other harmful effects if not used properly. Unless specifically noted, it is best to dilute all essential oils in a carrier of *base* oil like Almond, Jojoba or Apricot Kernel before applying to the skin - appropriate dilution is usually only 1 - 10% essential oil in carrier. For inhalation, a diffuser or oil lamp is effective for releasing essential oils into your environment - a very pleasant way of creating a particular atmosphere[2].

Table-(1) **<u>Major Raw Material Use In Extraction Of Essential Oils</u>**

Essential Oils are derived from various parts of Plants

Leaves	Flowers	Peel	Seeds	Wood
Basil	Chamomile	Bergamot	Almond	Camphor
Bay leaf	Clary Sage	Grape fruit	Anise	Cedar
Cinnamon	Clove	Lemon	Celery	Rosewood
Eucalyptus	Geranium	Lime	Cumin	Sandalwood
Lemon Grass	Hyssop	Orange	Nutmeg Oil	
Melaleuca	Jasmine	Tangerine		
Oregano	Lavender			
Patchouli	Manuka			
Peppermint	Marjoram			
Pine Rosemary	Orange Rose			
Spearmint Tea	Ylang-Ylang			
Tree				
Wintergreen				
Thyme				
Berries	**Bark**	**Resins**	**Rhizome**	**Root**
Allspice Juniper	Cassia Cinnamon	Frankincense Myrrh	Ginger	Valerian

1-4 Pharmacological Properties of Essential Oil

1.4.1 Antiseptics 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[2]**.**

1.4.2 Expectorants and diuretics

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 vasodilation and in consequence bring about a diuretic effect[3].

1.4.3 Spasmolytic and sedative

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[3].

Others uses of essential oils include: anti-inflammatory; cicatrizing.

1.5 Chemical constituents of essential oils

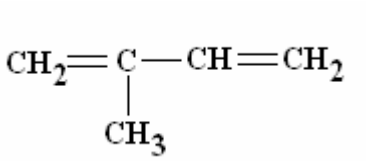
Pure essential oils are mixtures of more than 200 components, normally mixtures of terpenes or phenylpropanic derivatives, in which the chemical and structural differences between compounds are minimal. They can be essentially classified into two groups:

Volatile fraction: these are monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

Nonvolatile residue this comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids[4].

1.5.1. Hydrocarbon

Essential Oils consist of Chemical Compounds that have hydrogen and carbon as their building blocks. Basic Hydrocarbon found in plants are isoprene having the following structure[4].



(Isoprene)

1.5.2. Terpenes

Terpenes generally have names ending in " **ene**."

For examples: Limonene, Pinene, Piperene, Camphene, etc. Terpenes are anti- inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the Hydrocarbon heading, when two of these isoprene units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes[3].

1.5.3. Alcohols

Alcohols are anti-septic, anti-viral, bactericidal and germicidal.

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. Most alcohols have a very low or totally absent toxic reaction in the body or on the skin[4]. Therefore, they are considered safe to use. Examples include:

- ¾ linalool found in ylang-ylang and lavender.
- ¾ Geraniol in geranium and rose.
- ¾ Nerol in neroli.

1.5.4. Aldehydes

Aldehydes possess anti-fungal, anti-inflammatory, anti-septic, anti-viral, bactericidal, disinfectant, sedative.

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

- Citral in lemon.
- Lemongrass and lemon balm.
- Citronellal in lemongrass, lemon balm and citrus eucalyptus[4].

1.5.5. Acids

Essential oils may contain acids which possess anti-inflammatory activity. 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. Examples of such acids include:

- Cinnamic and benzoic acid in benzoin.
- Citric and lactic[4].

1.5.6. Esters

Essential oils may contain esters. Esters are formed through the reaction of alcohols with acids. 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. Examples of essential oils esters include:

- linlyl acetate in bergamot and lavender.
- Geranyl formate in geranium[4].

1.5.7. Ketones

Ketones which may exist in some essential oils possess many interesting biological activities including: anti-catarrhal, cell proliferant, expectorant, vulnery.

Ketones often are found in plants that are used for upper respiratory complaints. They assist the flow of mucus and ease congestion. Essential oils containing 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. Other toxic ketones found in essential oils are pulegone in pennyroyal, and pinocamphone in hyssops. Some non-toxic ketones are jasmone in jasmine oil, fenchone in fennel oil, carvone in spearmint and dill oil and menthone in peppermint oil[4]. Examples fenchone in fennel, carvone in spearmint and Menthone in peppermint[4].

1.5.8. Lactones

Some lactones exhibit anti-inflammatory, antiphlogistic, expectorant, febrifuge. Lactones are known to be particularly effective for their anti-inflammatory action, possibly by their role in the reduction of prostaglandin synthesis and expectorant actions. Lactones have an even stronger expectorant action then ketones[4].

1.6. Methods of extraction of 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, like winemaking, 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[4].

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 are available for extractions of essential oils are given below. [5]

1.6.1. Maceration

Maceration actually creates more of an ”infused oil" rather than an "essential oil". The plant matter is soaked in vegetable oil, heated and strained at which point it can be used for massage.

1.6.2. Cold Pressing

Cold pressing is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. This method involves the simple pressing 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[5].

1.6.3. 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[5].

1.6.4. Enfleurage

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[5].

1.6.5. Hydro distillation

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[5].

1.6.6 CO_2 & Super Critical 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 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, will destroy the therapeutic value and alter the fragrance[5].

1.6.7. 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[5].

1.6.8. Steam Distillation

Most commonly, the essence is extracted from the plant using an 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[5].

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Nowadays, a reputable distiller will try to preserve the original qualities of the plant, but the final therapeutic result is often not formed until after the extraction process. During extraction, the qualities of the oil change to give it more value, for example, chamazulene (characteristic of the pure blue colour of German Chamomile) is formed during the steam distillation process. Currently, the most popular method for extraction is steam distillation[5].

Many old-time distillers favor this method for most oils, and say that none of the newer methods produces better quality oils. Steam distillation is a special type of distillation or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are insoluble in water and may decompose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituent(s) [5].

Essential oils contain substances with boiling points up to 200°C or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to 100°C at atmospheric pressure[5].

Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allows to pass through the herb material under pressure which softens the cells and allows the essential oil to escape in vapor form. The temperature of the steam must be high enough to vaporize the oil present, yet not so high that it destroys the plants or burns the essential oils. As

they are released, the tiny droplets of essential oil evaporate and, together with the steam molecules, travel through a tube into the still condensation chamber. As the steam cools, it condenses into water. The essential oil forms a film on the surface of the water. To separate the essential oil from the water, the film is then decanted or skimmed off the top. The remaining water, a byproduct of distillation, is called floral water, distillate, or hydrosol. It retains many of the therapeutic properties of the plant, making it valuable in skin care for facial mists and toners. In certain situations, floral water may be preferable to be pure essential oil, such as when treating a sensitive individual or a child, or when a more diluted treatment is required. Rose hydrosol, for example, is commonly used for its mild antiseptic and soothing properties, as well as it's pleasing floral aroma[5].

Essential oil isolated by steam distillation are different in composition to those naturally occurring in the oil bearing glands of plants, since the steam distillation conditions cause chemical reactions to occur which result in the formation of certain artificial chemicals, called artifacts. Some of these are considered beneficial e.g. the formation of chamazulene during the steam distillation of Chamomile oil; whilst others may not be e.g. the hydrolysis of linalyl acetate during the distillation of clary sage. Few, if any, essential oils are unscathed by the thermal conditions of steam distillation, but some distillation techniques can, in certain instances, be a measure less damaging than others (e.g. hydro diffusion – a sort of inverted steam distillation where steam is introduced at the top of the vegetable material-packed container, and oil and condensate issue from the bottom – can produce oils with higher ester contents i.e. less thermally induced hydrolysis) [5].

A number of factors determine the final quality of a steam distilled essential oil. Aside from the plant material itself, most important are time, temperature

and pressure, and the quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. So, much like a fine meal is more flavorful when made with patience, most oils benefit from a long, slow 'cooking' process. It is possible that longer distillation times may give more complete oil. It is also possible however, that longer distillation time may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improving the odour, as sometimes when materials that have a larger number of components are sniffed, the perception is often of slightly increased sophistication, added fullness and character, and possibly, and extra pleasantness[5].

The advantage of steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are well known. Newer methodology, such as sub critical water extraction, may well eventually replace steam distillation, but so far even contenders such as carbon dioxide extraction - although establishing a firm market niche - have not really threatened to take over as the major preparative technique[5].

1.7 Medicinal and Pharmacological Uses of Essential Oils.

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides [6]. Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure or mitigate or prevent diseases, infection and indisposition by means of inhalation[7]. Inhalation of essential oils or their individual volatile terpenes has a significant role in controlling the central nervous system. For instance, aroma inhibit of storax pill essential oil and pre

inhalation of *Aconus gramineus* rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy[8]. The fragrance compounds, cisjasmonate, which characterized the aroma of *Jasminum grandiflorum* have a tranquilizing effect on the brain upon inhalation[9]. They significantly increased the sleeping time of mice induced by pentobarbital. Cendrol, which is a major component of cardwood essential oil, shows a sedative effect and prolonged pentobarbitalinduced sleeping time on rats upon inhalation[10]. The vapour of lavender essential oil or one of its main component linalool may also be applicable to the treatment of menopausal disorder through inhalation[11]. Lavender essential oil demonstrated an analgesic activity, mainly relevant after inhalation at the doses devoid of sedative side effects[12]. Medical professionals are more interested in the medicinal properties of essential oils. Many oils show antibacterial, fungicidal, relaxant, stimulating, antidepressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both by pathogenic and non-pathogenic diseases. Pathogenic diseases caused by bacterial, virus, and the fungi can be treated with essential oils.

Essential oils are reported to have insecticidal properties essentially as ovicidal, larvicidal, growth inhibitor, repellence and antifeedant[13]. The influence of certain oils and their constituents on the reproduction of some insect species and on morphological changes in other has also been discussed[14]. According to Laurent (1997), 63 essential oils isolated from Bolivia plants were tested on *Triatomal infestants* for ovicidal and larvicidal properties. This insect is responsible for transmission of Chaga's disease to humans in the region extending from the arid Perivian highlands to the very dry north eastern Brazilian regions, and the plains of Argentina. Three types of test were used; topical application on insects; nymphs on impregnated paper and eggs on

impregnated paper. In all tests, the essential oils were used as ethanol solution with concentration of 2% and 20% (v: v)

1.8 Gas chromatography

It is a unique and versatile technique. In its initial stages of development it was applied to the analysis of gases and vapors from very volatile components. Gas chromatography is the analytical technique used for product identification (under very controlled conditions) and must be directly coupled to a mass spectrometer when information other than a comparative fingerprint (program) is required, such as positive identification of peaks on the chromatogram[15].

1.8.1 Basic principal of gas chromatography

The basic principal of gas chromatography is that greater the affinity of the compound for the stationary phase, more the compound will be retained by the column and longer it will be before it is eluted and detected. Thus the heart of the gas chromatograph is the column in which separation of the component takes place, and to this must be added the source and control of the carrier gas flow through the column, a mean of sample introduction and a means of detection of the components as they elute from the end of the column. Since temperature will influence the volatility of the analytes, the column is placed in a thermostatically controlled oven[16].

The basis of the separation is a retardation of the individual components as they are moved through a long column by a carrier gas, usually helium or nitrogen. The column consists of a steel or glass tube filled with an inert packing material such as glass or ceramic beads (see Figure 1). In gas-liquid chromatography (GLC), these are coated with an in volatile liquid, so that the surface area of the liquid in contact with the gas is large. For some applications, the packing may be a solid without any liquid coating; it is then called gas-solid chromatography (GSC), but this is less widely used than GLC[16].

The sample is injected into the carrier gas stream. As it moves through the column with the carrier gas, the molecules of each substance present in the sample will distribute between the gas and the liquid. Individual molecules will constantly move between the gas and the liquid in a dynamic equilibrium. While a molecule is in the gas phase it will pass along the column, while it remains dissolved in the liquid it will be stationary. The more volatile a substance, the greater proportion of time its molecules will be moving in the carrier gas, and so the sooner it will emerge from the column. In this way each substance will become separated within the column and emerge separated by time at the end.

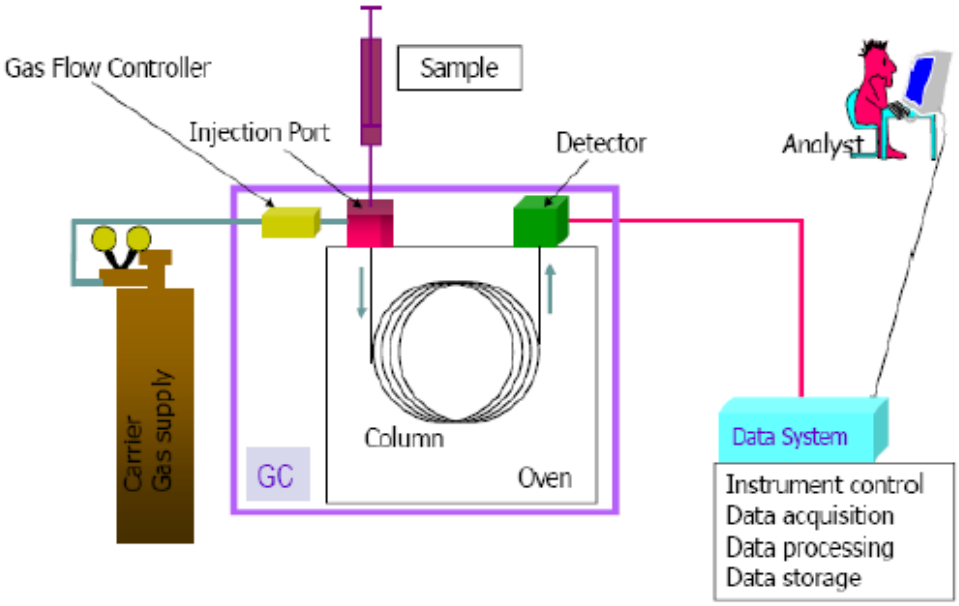


Fig. 1. Schematic Diagram of Gas Chromatography

1.8.2 Applications of GC

Gas chromatography (GC) is used widely in applications involving food analysis. Typical applications pertain to the quantitative and/or qualitative analysis of food composition, natural products, food additives, flavor and aroma components, a variety of transformation products, and contaminants, such as pesticides, fumigants, environmental pollutants, natural toxins, veterinary drugs, and packaging materials[17]. And particular food applications involving

GC, such as carbohydrates and amino acids[18]. Lipids and accompanying lipophilic compounds[19], flavors and aroma[20].

GC can be used for the direct separation and analysis of gaseous samples, liquid solutions, and volatile solids. If the sample to be analyzed is non-volatile, the techniques of derivatization or pyrolysis GC can be utilized. Gas chromatography (GC) has been an indispensable analytical technique in the application of fatty acid determinations in oilseed plant breeding, biosynthesis and human metabolism, as well as the characterization of complex mixtures of geometric isomers when combined with other chromatographic separations and spectroscopic identification[21]. Plant breeders uses GC as a more precise and rapid technique for studying the variation and inheritance of fatty acids in oilseed crops such as rapeseed[22]. flaxseed, and safflower[23].

1.8.3 Advantages of GC

Optimum qualitative and quantitative GC analysis of complex mixtures presupposes:

(i) Good resolution, as shown by sharp and symmetric peaks;

(ii) High repeatability and reproducibility of retention times;

(iii) High precision and accuracy in quantitation based on peak area measurements, i.e. no discrimination of components through volatility, polarity or concentration;

(iv) Minimum thermal and catalytic decomposition of sensitive sample components[24]. The use of fused-silica capillary columns with improved surface inertness, thermal stability and resolution[25]. Best fulfills most of these requirements. In capillary GC the peak resolution, expressed in terms of column efficiency, separation and retention factors[26]. Is primarily affected by the polarity of the stationary phase, column length, internal diameter and film thickness. A variety of columns with different properties are available. In

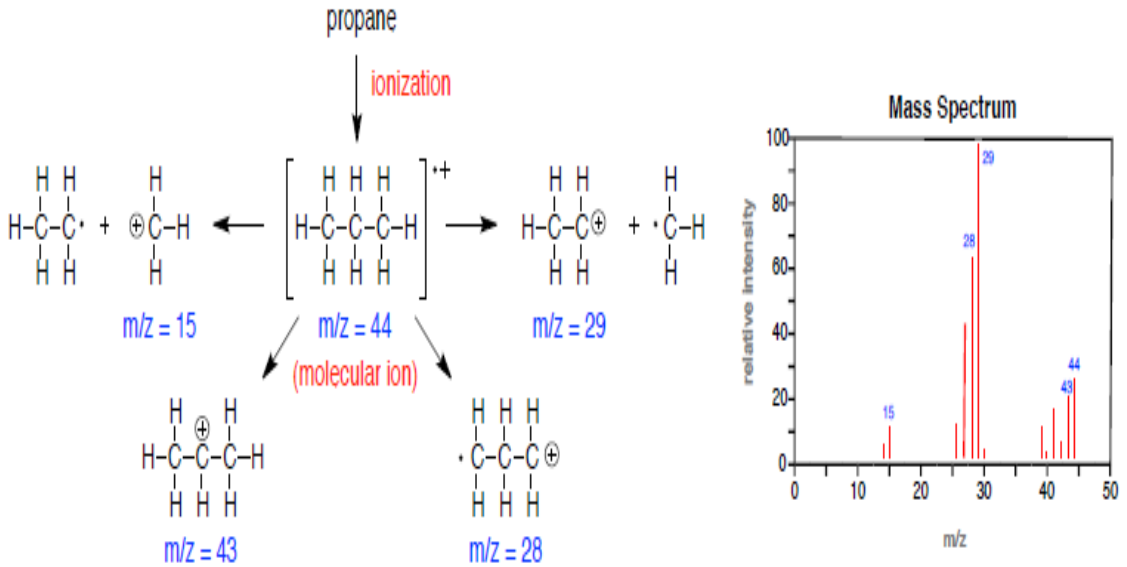
addition, fused-silica columns are highly applicable in practical work due to their flexibility and simplicity in handling and easy connection to GC and mass spectrometers[27]. In order to improve the sensitivity of the GC analyses it is also important to test the effect of carrier gas flow rate, as well as gas flows in the FID, in order to reduce the noise from the hydrogen flame[28]. The carrier gas, usually hydrogen or helium, and its purity can also affect the resolution[29].

1.9 Mass Spectrometry

1.9.1 General Principles

- Converts molecules to ions
- Separates ions (usually positively charged) on the basis of their mass/charge (m/z) ratio
- Quantifies how many units of each ion are formed over a given period

Mass spectrometry provides both molecular weight and fragmentation pattern.



Many different methods for ionization and detection are known:

- Useful for different types of molecules

- Vary in amount of energy delivered à impacts ionization / fragmentation[30]

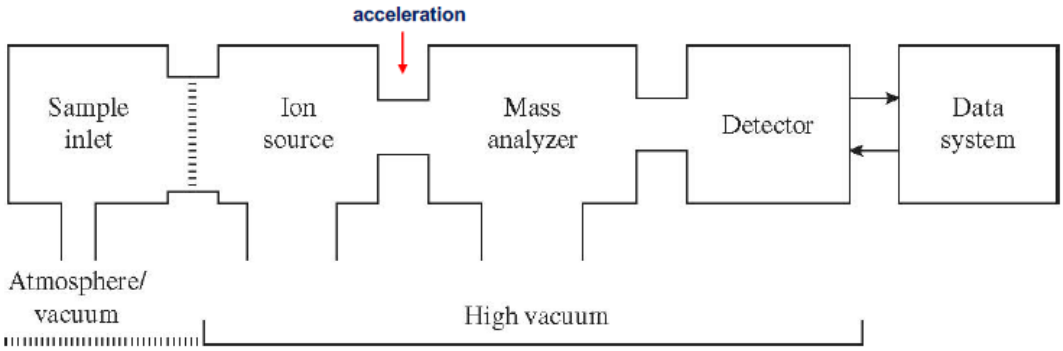


Fig. (2): Scheme of basic mass spectrometer

Table (1): Different methods of ionization are depicted below[30]:

Ionization Method	Typical Analytes	Sample Introduction	Mass Range	Ionization [mass]
Electron Impact (EI)	relatively small, volatile	GC or liquid/solid probe	up to 1000 Daltons	hard, $[M]^{+\cdot}$ if observed
Chemical Ionization (CI)	relatively small, volatile	GC or liquid/solid probe	up to 1000 Daltons	hard to soft; varies with carrier $[M+H]^+$
Fast Atom Bombardment (FAB)	carbohydrates, organometallics, peptides, nonvolatile	sample mixed in viscous matrix	up to 6000 Daltons	soft, but harder than MALDI, ESI $[M+Na]^+$, $[M+H]^+$
Matrix Assisted Laser Desporption (MALDI)	peptides, proteins, nucleotides	sample mixed in solid matrix	up to 500,000 Daltons	soft $[M+H]^+$
Electrospray (ESI)	peptides. proteins, nonvolatile	HPLC or syringe	up to 6000 Daltons	soft $[M+Na]^+$, $[M+H]^+$

1.9.2 Applications

Mass spectrometers are used in industry and academia for both routine and research purposes. The following list is just a brief summary of the major mass spectrometric applications[30]:

- **Biotechnology:** *the analysis of proteins, peptides, oligonucleotides*
- **Pharmaceutical:** *drug discovery, combinatorial chemistry, pharmacokinetics,drug metabolism*
- **Clinical:** *neonatal screening, haemoglobin analysis, drug testing*
- **Environmental:** *PAHs, PCBs, water quality, food contamination*
- **Geological:** *oil composition*

1.10 Gas Chromatography–Mass Spectrometry (GC-MS):

is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to

identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass. The further addition of mass spectrometer in it leads to GC-MS/MS. Superior performance is achieved by single and triple quadrupole modes[30].

1.11 Lemongrass

Lemongrass is a tropical perennial plant which yields aromatic oil. The name lemongrass is derived from the typical lemon-like odor of the essential oil present in the shoot. The herb originated in Asia and Australia. Lemongrass was one of the herbs to travel along the spice route from Asia to Europe. Lemongrass oil of commerce is popularly known as Cochin oil in the world trade, since 90% of it is shipped from Cochin port. The state of Kerala in India had the monopoly in the production and export of lemongrass oil. The annual world production of lemongrass oil is around 1000 t from an area of 16000 ha. In India, it is cultivated in an area of 4000 ha and the annual production is around 250 t. The crop is extensively cultivated in the poor, marginal and waste lands and also along the bunds as live mulch. The well ramified root system of the plant helps in soil and water conservation [31].

Fig. (3): Lemongrass

Below is the Botanical classification of lemon grass, presented in (Table 2).

Table (2): Botanical classification of lemon grass

Kingdom:	Plantae
(Unranked):	Angiosperms
(Unranked):	Monocots
(Unranked):	Commelinids
Order:	Poales
Family:	Poaceae
Subfamily:	Panicoideae
Tribe:	Andropogoneae
Subtribe:	Andropogoninae
Genus:	Cymbopogon

Lemongrass oil is derived from three different species: Cymbopogan flexuosus, C. citratus, and C. pendulus. Its uses as a pesticide active ingredient are primarily as an anti-fungal agent in post-harvest handling and as an insect repellent. Lemongrass oil also has some herbicidal properties. The essential oil is high in citral, which is considered the principal biologically active agent. A common food ingredient in many cuisines, lemongrass oil is non-toxic to humans and most non-target species. Lemongrass has a non-toxic mode of action and is believed to pose a minimal risk to human health and the environment[32].

Lemongrass essential oil is analgesic properties have been found to relieve muscle and joint pains caused by overexertion of muscles through exercise. It is known to boost energy and to reduce fever as well as headaches caused by viral infections such as the flu. It acts as an antiseptic, making it a beneficial ingredient in lotions and creams that prevent wounds from becoming infected. By alleviating abdominal pain, ¡t can relieve stomach aches and ease spasms ¡n the digestive tract. It works as a detoxifying agent by increasing perspiration, thus promoting the expulsion of bodily toxins through sweating[32].

When used cosmetically or topically, lemongrass oil can eliminate or inhibit the growth of harmful bacteria due to its Citral content, which ¡s known to have antimicrobial properties. Lemongrass Oil can reduce inflammation due to its Limonene content, and it can slow down the flow of blood by contracting blood vessels. When used ¡n shampoos, it ¡s believed to prevent hair loss. Lemongrass makes an effective, inexpensive, eco-friendly and long lasting deodorant[33].

Aim of this study:

This study was designed to:

- Extract oil from the medicinal plant lemongrass.
- Analyze the oil via GC-MS technique to identify oil constituents.

Chapter Two

Materials and Methods

2-MateriaIs and Methods

2.1-Materials

2.1.I-Plant material

Seeds of *Cymbopogan citratus* were collected from a forest reserve around Damazin plant was authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2- Instruments

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

2.2- Methods

2.2.1 -Extraction of oil

Powdered seeds of *Cymbopogan citratus* (350g) were exhaustively extracted with n-hexane (maceration). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4ºC for further manipulation,

For GC-MS analysis the studied was esterified as follows: the oil(2mI) 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. (5gl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate (l PI) was injected in the GC-MS vial.

2.3 GC-MS analysis

The oil of *Cymbopogan citratus* was studied by gas chromatography - mass spectrometry using 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 Tale (2), while other chromatographic conditions are depicted in Table(3).

Table (2): Oven temperature program

Rate	Temperature	Hold time (min)
-	150.0	1.00
4.00	300.0	0.00

Table (3): Chromatographic conditions

Column over temperature`	150.0ºC
Injection temperature	300.0ºC
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3kPa
Total flow	50.0nl/min
Column flow	1.54nl/sec
Linear velocity	47.2cm/sec
Purge flow	3.0ml/min
Spill ratio	-1.0

Chapter Three
Results and Discussion

3. Results and Discussion

3.1- *Cymbopogon citratus*

Cymbopogon citrates essential oil was investigated by gas chromatography-mass spectrometry. This analysis revealed the presence of 57 constituents.

3.1.1-GC-MS analysis

When analyzed by GC-MS , the volatile oil of Cymbopogon citratus showed fifty seven components. The total ion chromatogram is shown in (Fig. l). Different components of the oil were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in Table (l).

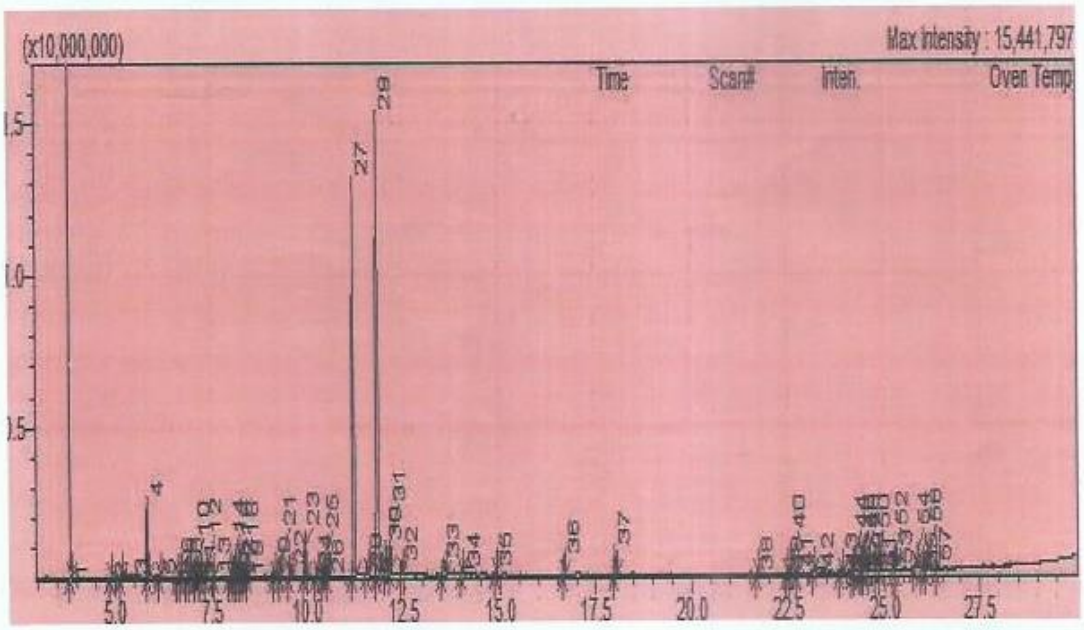


Fig.3.1: Total ion Cromatograms of *Cymbopogon citratus* oil

Table 3.1 Constituents of *Cymbopogon citratus* essential oil

Peak#	R. Time	Area	Area %	Name
1.	3.847	43153	0.04	4-Heplatone
2.	4.886	45854	0.04	Alpha-Pinene
3.	5.173	95965	0.08	Camphene
4.	5.843	4662825	3.87	5-Hepten-2-ne-6methyl
5.	6.155	12056	0.11	Octanal
6.	6.618	65421	0.05	6-octen-1yn-3-ol 3,7-dimenthyl
7.	6.710	113342	0.09	D-Limonene
8.	6.850	38681	0.03	trans—beta-Ocimene
9.	6.933	31921	0.03	Acetic acid cvclohexvl ester
10.	7.069	41165	0.03	1,3 6-Octatriene, 3 7-dimethul0(Z)-
11.	7.203	117239	0.10	Cvci0J>rOJ>ane, 2-(1 1-dimethyl-2-propenyl
12.	7.362	131234	0.11	Cvcelohexane (1 1-dymethv1tjroj>v1)-
13.	7.582	378664	0.31	4-Nonanonc
14.	7.963	54361	0.05	.aij>ha-Methyl-a1J>ha-14-methyi-3-J>enten
15.	8.105	89793	0.07	Ascaridole epoxide
16.	8.174	988876	0.82	1 6-Octatriene-37-dimenthvl-
17.	8.252	95764	0.08	Nonanal
18.	8.338	233438	0.19	Carane 4 5eJJOXY-trans
19.	8.435	216588	0.18	di-t-Butvlacetvlene
20.	9.132	419064	0.35	1-5-Heptadicne-3,3-dimethyle- (E)
21.	9.295	566455	0.47	CycoiOJ>entane, 1-methyl-1(2-methyi-2-2J>ro
22.	9.547	689909	0.57	cis-Verbenol
23.	9.930	1932095	1.60	3-Cyclohexcnc-1-carboxaldehyde, 2,4,6-tri
24.	10.237	1010247	0.84	CyclOJ>entanol 1 2-dimethyl-3-(1-methylet
25.	10.404	355914	0.30	Deeanal
26.	10.529	219237	0.18	Carveol
27.	11.230	37801662	31.36	2 6-Octadienal 3 7-dimethyl-(Z)-
28.	11.550	484671	0.40	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl
29.	11.850	52069929	43.20	2 6-Octadienal 3,7-dimethyl- (E)-
30.	12.042	2095496	1.74	2-Furanmethanol 5-thenyltetrahydro-all
31.	12.153	1982494	1.64	1,3-Propanediol, 2,2-duethyl-
32.	12.455	744966	0.62	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-
33.	13.556	2131724	1.77	Geranic acid
34.	14.017	456007	0.38	Geranyl acetate
35.	14.907	551763	0.46	carvoJ>hyllene
36.	16.665	1517211	1.26	.1!amma-Muurolene
37.	17.989	2148279	1.78	caryophylUene oxide
38.	21.710	95384	0.08	2-Pentadecanone, 6, 10,14-trimethyl-
39.	22.458	57564	0.05	Ethyl I!Cranatc
40.	22.538	111400	0.09	CvclOI>rODanecarboxaldehyde 2 mthyl-2
41.	22.750	278479	0.00	Formic acid 3 7 1 1-trimethyl-1 6 10-dodec
42.	23.179	387004	0.32	(1 R 2R 3S 5R)-(-)-2 3-Pinanediol
43.	23.874	307220	0.25	2-Bornanol 2-methyl-
44.	24.163	422956	0.35	(1 R 4R)-J>Mentba-2,8-diene 1-
45.	24.228	95060	0.08	2-Methylisoborneol
46.	24.362	174988	0.15	Endo-Borneol
47.	24.409	159556	0.13	Bicycloi5.2.0Inonsne 4 8 8-trimethyl-2-mc
48.	24.455	645362	0.54	Bicycloi2.2- 2Joct-2-ene 1,2 3 6-tetramethyl
49.	24.582	204673	0.17	7-Octv;idcncbicvclo 4.1.01 heotane
50.	24.762	225163	0.19	1-Cyclobutanol 1-mcthyl-2-(2,2-dimethyl-
51.	24.896	262622	0.22	Carveol phenylcarbamimate(ester)
52.	25.224	630283	0.52	Neric acid
53.	25.306	4240R6	0.35	2-Ruten-!-onl’ 1-(2.2 “-trimethylnerhwlr
54.	25.793	561911	0.47	Ph vtol
55.	25.973	181991	0.15	2 10-Dodeecadien- 1-ol 3 7 1 1-trimethyl- (E
56.	26.076	186026	015	5-Hydroxymethyl-1, 1 4a-trimethyl-6-meth
57.	26.34	363208	0.30	Myrtanol, 2-mercaJ>to-

3.1.2 Major constituents of the oil

The following major constituents were detected by GC-MS:

2,6-octadienal, 3,7-dimethyl(E) (43.20%)

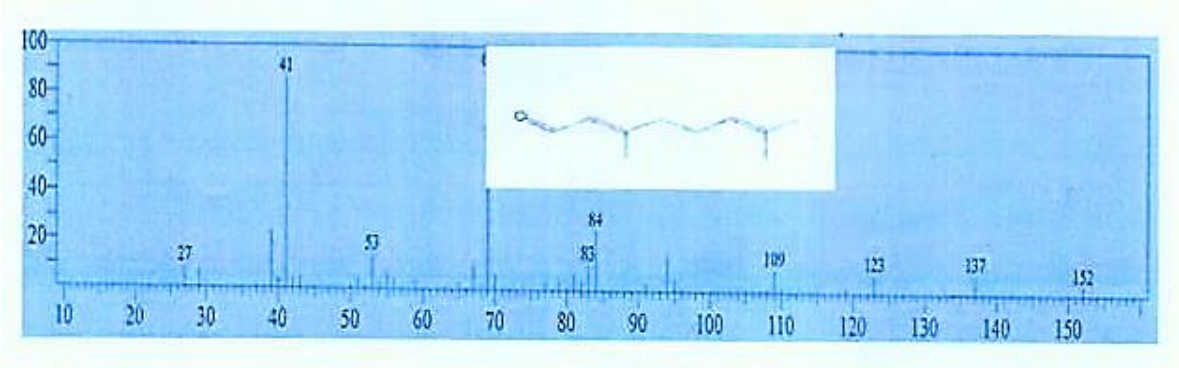


Fig. (2) Mass Spectrum of 2,6-octadienal, 3,7-dimethyl(E)

The EI mass spectrum of 2,6-octadienal, 3,7-dimethyl(E) is shown in Fig. 2. The peak at M/z 152, which appeared at R.T. 11.850 in total ion chromatogram, corresponds to $M^{+}[C_{10}H_{16}O]^{+}$. The peak at m/z137 corresponds to loss of methoxyl function.

2,6-octadienal, 3,7-dimethyl(Z) (31.36%)

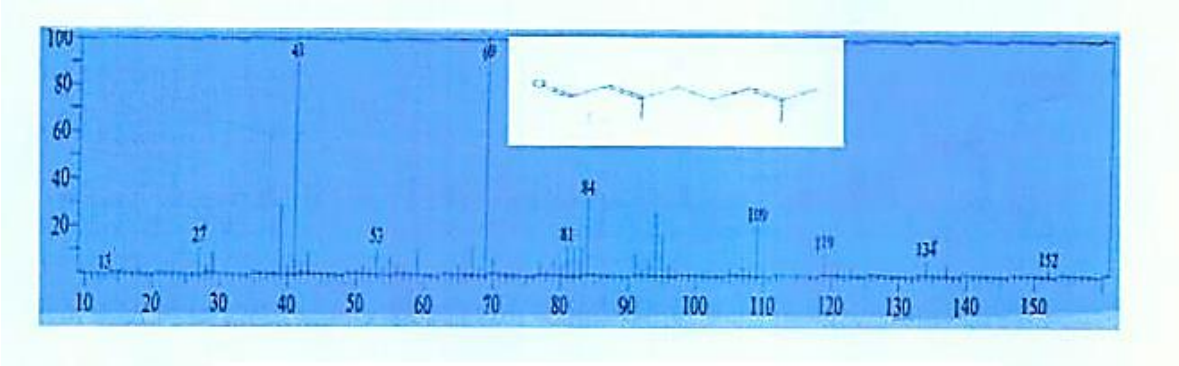


Fig. (3) Mass Spectrum of 2,6-octadienal, 3,7-dimethyl(Z)

The El mass spectrum of 2,6-octadienaI,3,7-dimethyl,(Z) is shown in Fig. 3. The peak at m/z 152, which appeared at R.T. 11.230 in total ion chromatogram, corresponds to $M^{+}[C_{10}H_{16}O]^{+}$. The peak at m/z134 corresponds to loss of a methoxyl function.

5, hepten-2-one, 6-methyl (3.87%)

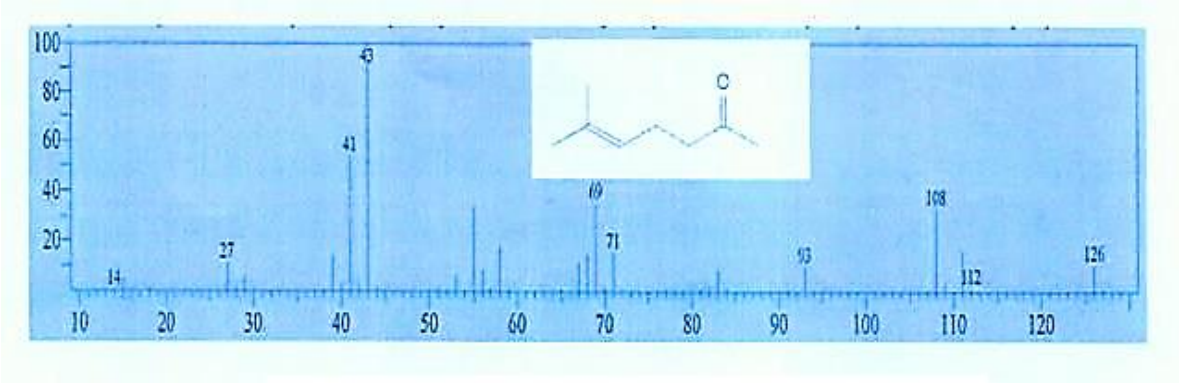


Fig. .4: Mass spectrum of S, hepten-2-one, 6-methyl

The El mass spectrum of 5, hepten-2-one, 6-methyl is shown in Fig.4. The peak at m/z 126, which appeared at R.T. 5.843 in total ion chromatogram, corresponds to $M^+[C_8H_{14}O]^+$. The peak at m/zl12 corresponds to loss of a methoxyl function.

3.2 Conclusion

Cymbopogon citratus oil was investigated by GC-MS analysis. The analysis showed the presence of 57 constituents. Major components are:

4. 2,6 octadienal, 3,7-dimethyl, (E) (43.20%).
5. 2,6 octadienal, 3,7-dimethyl, (Z) (31.36%).
6. 5, hepten-2-one, 6methyl, (3.87%).

3.3 Recommendations

Recommendations include the following:

1. The extracted oil may be evaluated for other pharmacological properties such as antimalarial and antiviral activity.

Other biologically active constituents of the targeted plant may be isolated and their bioactivity could be assessed.

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