Sudan University of Science and Technology

College of Post Graduate Studies

Chemical Constituents of *Elettaria cardamomum* Seed Oil and its Biological Activity

المكونات الكيميائية لزيت بذور الهبهان وفعاليتة البيولوجية

A Thesis Submitted in Partial Fulfillment of the Requirements of the M.Sc. Degree in Chemistry

By

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May,2019

وقل أعملوا فسيرى الله عملكم ورسوله والمؤمنون وستردون إلى عالم الغيب والشهادة فينبئكم بما كنتم تعملون

صدق الله العظيم

(**التوبة**_ 105)

Dedication

Dedicated to

My Parents

Brother and Sister

Abstract

This study was carried out to investigate the phyto constituents of Elttaria cardamomum fruit oil. The oil was study by GC-MS analysis. The analysis showed 39 constituents Major are: cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate (27.64%), Hexadecanoic acid, methyl ester (14.86%), 9-octadecenoic acid (Z)-, methyl ester (8.78%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.35%), L-.alpha.-terpineol (6.98%). The oil showed significant anticandidal activity. It also exhibited moderate activity against Gram positive Staphylococcus aureus, Bacillus subtilis and Gram Negative Escherichia coli, However it failed to give inhibitory effect against Gram negative Paeudomonas aeruginosa.

المستخلص

استخلصت ثمار نبات الهبهان بالهكسان العادي ثم اخدع الزيت الناتج للتحليل بتقنية كروموتوغرافيا الغاز- طيف الكتلة والتي أوضحت وجود 39 مركباً أهمها من حيث التواجد:

cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate (27.64%), Hexadecanoic acid, methyl ester (14.86%), 9-octadecenoic acid (Z)-, methyl ester (8.78%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.35%), L-.alpha.-terpineol (6.98%).

في اختبار مضاد البكتريا ادى الزيت فعالية ضد الفطريات وكذلك ادى الزيت فعالية عالية ضد:

Escherichia coliوStaphylococcus aureus, Bacillus subtilis

لكن لم يعطي الزيت أي فعالية ضد بكتريا:

Paeudomonas aeruginosa.

Acknowledgment

My praise and thanks to **Almighty Allah** for giving me the will and strength to finish this work.

I would like to express my deepest indebtedness and gratitude to my supervisor ***:*** Prof. Mohammed Abdel Karim for his helpful suggestions , guidance , encouragements and useful criticisms throughout the period of the study .

Thanks to my mother for her help and big support

Thank are due to the staff of Chemistry Department – Sudan University of Science and Technology for all facilities . I would like to extend my thanks to my colleagues for their valuable assistance .

Thanks are extended to my teachers specially A.Amin eldeen Essa Mohammed for his help and support.

I would like to express my sincere thanks to my big family and my best friends Balsam Abd ulhadi Fadlallah Ali.

Thanks are also due to all those who helped and encouragement me to do this work.

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Introduction

1-Introduction

1.1-General approach

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. [1,2]

1.2-Rule 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. [3,4]

1.3- 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:

i)Volatile fraction

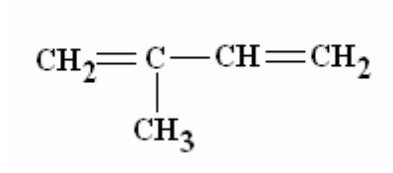
Essential oil constituting of 90–95% of the oil in weight, containing the monoterpene and sesquiterpene hydrocarbons, as well as their oxygenated derivatives along with aliphatic aldehydes, alcohols, and esters.

ii)Nonvolatile residue

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

1.3.1-Hydrocarbons

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.



Isoprene

1.3.2-Terpenes

Generally have names ending in " **ene**."

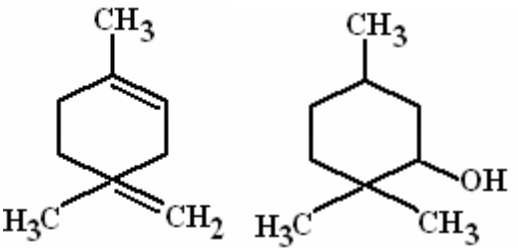
For examples: limonene, pinene, piperene, camphene, etc. Terpenes are anti-inflammatory, antiseptic, antiviral, and bactericidal. Terpenes can be further categorized in monoterpenes, sesquiterpenes and diterpenes. Referring back to isoprene units under the Hydrocarbon heading, when two of these isoprene

units join head to tail, the result is a monoterpene, when three join, it's a sesquiterpene and four linked isoprene units are diterpenes.

1.3.3.-Monoterpenes [$C_{10}H_{16}$]

Properties: analgesic, bactericidal, expectorant, and stimulant.

Monoterpenes are naturally occurring compounds, the majority being unsaturated hydrocarbons (C_{10}).But some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as monoterpenoids.



Limonene Menthol

The branched-chain C_{10} hydrocarbons comprises of two isoprene units and is widely distributed in nature with more than 400 naturally occurring monoterpenes identified. Moreover, besides being linear derivatives (geraniol, citronellol), the monoterpenes can be cyclic molecules (menthol – monocyclic; camphor – bicyclic; pinenes (**α** and **β**) – pine genera as well. Thujone (a monoterpene) is the toxic agent found in *Artemisia absinthium* (wormwood) from which the liqueur, absinthe, is made. Borneol and camphor are two common monoterpenes. Borneol, derived from pine oil, is used as a disinfectant and deodorant. Camphor is used as a counterirritant, anesthetic, expectorant, and antipruritic, among many other uses.

Example: camphene and pinene in cypress oil ; camphene, pinene and thujhene in black pepper.

1.3.4. Sesquiterpenes

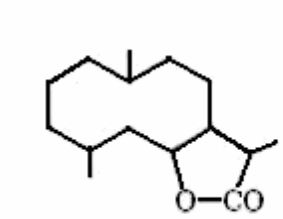
Properties: anti-inflammatory, anti-septic, analgesic, anti-allergic.

Sesquiterpenes are biogenetically derived from farensyl pyrophosphate and in structure may be linear, monocyclic or bicyclic. They constitute a very large group of secondary metabolites, some having been shown to be stress compounds formed as a result of disease or injury.

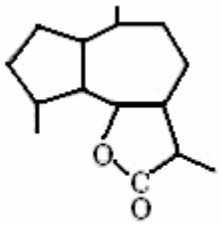
1.3.5-Sesquiterpene lactones

Over 500 compounds of this group are known; they are particularly characteristics of the Compositae but do occur sporadically in other families. Not only have they proved to be of interest from chemical and chemotaxonomic viewpoints, but also possess many antitumor, anti-leukemia, cytotoxic and antimicrobial activities. They can be responsible for skin allergies in humans and they can also act as insect feeding deterrents.

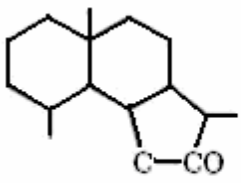
Chemically the compounds can be classified according to their carboxylic skeletons; thus, from the germacranolides can be derived the guaianolides, pseudoguaianolides, eudesmanolides, eremophilanolides, xanthanolides, etc.



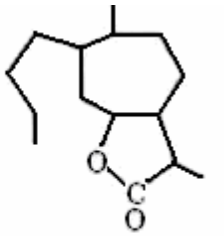
Germacranolides



Guaianlides



Eudesmanolides



Xantnanolides

A structural feature of all these compounds, which appears to be associated with much of the biological activity, is the α, β -unsaturated- γ- lactones.

Example: farnesene in chamomile and lavender ; beta-caryophyllene in basil and black pepper.

1.3.6. Diterpenes

Properties: anti-fungal, expectorant, hormonal balancers, hypotensive .Diterpenes are made of up four isoprene units. This molecule is too heavy to allow for evaporation with steam in the distillation process, so is rarely found in distilled essential oils. Diterpenes occur in all plant families and consist of compounds having a C_{20} skeleton.

skeleton. There are about 2500 known diterpenes that belong to 20 major structural types. Plant hormones gibberellins and phytol occurring as a side chain on chlorophyll are diterpenic derivatives. The biosynthesis occurs in plastids and interestingly mixtures of monoterpenes and diterpenes are the major constituents of plant resins. In a similar manner to monoterpenes, diterpenes arise from metabolism of geranyl geranyl pyrophosphate (GGPP).

Diterpenes have limited therapeutical importance and are used in certain sedatives (coughs) as well as in antispasmodics and antoxiolytics.

Example: sclareol in clary sage is an example of a diterpene alcohol.

1.3.7-Alcohols

Properties: anti-septic, anti-viral, bactericidal and germicidal.

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

Example: linalool found in ylang-ylang and lavender , geraniol in geranium and rose , nerol in neroli.

1.3.8. Aldehydes

Properties: 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.

Example: citral in lemon , lemongrass and lemon balm, citronellal in lemongrass, lemon balm and citrus eucalyptus.

1.3.9- Acids

Properties: anti-inflammatory.

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.

Example: cinnamic and benzoic acid in benzoin , citric and lactic.

1.3.10-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.

Example: linlyl acetate in bergamot and lavender, geranyl formate in geranium.

1.3.11- Ketones

Properties: 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.

Example: fenchone in fennel, carvone in spearmint and dill , menthone in peppermint.

1.3.12- Lactones

Properties: 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.[5,6,7]

1.4- Description of essential oils

Essential oils (EO) contain unpredictable substances that are extracted by physical techniques from plants of a specific plant species. The oils ordinarily bear the name of the plant species from which they are extracted. Essential oils are so termed as they are accepted to speak to the very substance of smell and flavor. Essential oil plants and culinary herbs

incorporate a wide scope of plant species that are utilized for their sweet-smelling quality as flavorings in drinks and as aromas in pharmaceutical and consumer care products.

Essential oils are utilized as a part of the protecting procedures, in prescription and in purification ceremonies. There are likewise more than 200 references to aromatics, incense and balms in the Old and New Testaments. Research has affirmed of the pragmatic utilization of Essential Oils hundreds of years ago; most of these are available in herbal shops. There are around three hundred known fundamental oils on the planet and these key oils are well utilize today by professionals to treat viral diseases caused by, bacterial, parasitic and contagious damages which attack our bodies.[8]

Plants have been used for treatment or prevention of various human diseases throughout history. From the most recent century, enthusiasm for experimental phytotherapy has expanded in a few therapeutic fields, for example, immunology,

oncology, hematology and the utilization of plants in medicine has influenced the distinguishing proof of phytomolecules.[9]

Home grown cures have turned out to be more prevalent in the treatment of minor illnesses these days, because of increasing hospital bills and success stories of the home grown cures. Individuals have utilized a few plants constituents for quite a long time, e.g., to get ready noxious points for fighting and chasing. Plant inferred substances have generally assumed vital parts in the treatment of human ailments. Today, around 80% of the

world populace who lives in underdeveloped nations still depends completely on plant items for their essential human services.[10]

1.5- Medicinal plants extracts

Extraction is the process of selectively removing a compound of interest from a mixture. The extracts so gotten from plants are moderately polluted fluids, semisolids or powders expected just for oral or outer use. These incorporate classes of arrangements known as decoctions, restoratively dynamic parts of plant or creature tissues from the latent or idle segments mixtures, liquid concentrates, arrangements, pilular (semisolid) removes and powdered concentrates. Such arrangements famously have been called galenicals, named after Galen, the second century Greek doctor.[11]

The reasons of institutionalized extraction systems for unrefined medications are to separate the restoratively wanted segment and to dispose of the inactive material by treatment with a specific dissolvable known as menstruum. The concentrate consequently obtained might be prepared for use as a medicinal agent in the form of solution and fluid extracts. It might further be consolidated into various shapes, for example, tablets or capsules, or it might be fractionated to segregate singular synthetic elements, for example, ajmalicine, hyoscine and vincristine, which are modern drugs. Consequently, institutionalization of extraction techniques contributes fundamentally to the last nature of the home grown medication. [11]

1.6- The distinctiveness of essential oils

Essential oils are active substances extracted from various parts of plants, containing many substances, but typically with the prevalence of one, two or three of them that really characterize fragrance. [12]

In early work, the term “fundamental oils” was characterized as the unpredictable oils acquired by the steam refining of plants. This definition was obviously expected to have many kind of effect between “greasy” and essential oils which are easily volatile. With the development of science came enhancements in the techniques for extracting the oils, and parallel with this advancement, a superior knowledge of the constituents of the oils was acquired. It was found that, the oils contain numerous classes of natural substances with different volatilities. In spite of the fact that, a rundown of all the known oil categories, which incorporate an assortment of artificially irrelevant mixtures will entail a long list, it is conceivable to characterize these into four principal gatherings of crucial oils. [13]

These are terpenes, identified with isoprene; straight-chain mixtures, not containing any side branches; benzene subsidiaries; and miscellaneous. [9].

1.7-Extraction of essential oils

Essential oils have high liquor segments. Thus, it has a higher instability and a quick vanishing rate. Keeping in mind the end goal to get the best quality and amount of essential oils, extraction methodology appears to hold the key controlling step. Elements worth considering in the extraction of essential oils are sorts of plant, compound constituents of oils, area of oils inside of the plant i.e. root, bark, wood, branch, leaf, blossom, foods grown from the ground and picking the right extraction strategy. [14]

Some plants like rose and jasmine contain minute essential oil. Their significant sweet-smelling properties are separated utilizing a compound dissolvable. The deciding item, known as a flat out, contains essential oil alongside other plant constituents. (14)

The estimation of the fresher handling strategies depends significantly on the experience of the distiller, and also the expected utilization of the last item. Every strategy is vital, and has its place really taking shape of aromatherapy grade of essential oils(15)

1.7.1- Solvent extraction

A hydrocarbon dissolvable is added to the plant material to disintegrate the fundamental oil. At the point when the mixture is sieved and thought by refining, a substance contains gum (resinoid), or a blend of wax and key oil known as solid remains. From the concentrate, immaculate liquor is utilized to remove the oil. At the point when the liquor vanishes, the oil is deserted. This is not viewed as the best strategy for extraction as the solvents can leave a little measure of buildup behind which could cause allergies and affect the immune system.(16)

1.7.2- Maceration method

Maceration really makes a greater amount of imbued oil instead of an essential oil. This straight forward broadly utilized method includes leaving the pounded plant to absorb a suitable dissolvable in a shut compartment. Basic maceration is performed at room temperature by blending the ground plant with the dissolvable and leaving the blend for a few days with periodic shaking or mixing. The procedure is rehashed for more than one occasion with new dissolvable. Ultimately the last deposit of concentrate is

squeezed out of the plant particles utilizing a mechanical press or an axis. The technique is suitable for both introductory and mass extraction. The fundamental hindrance of maceration is that the procedure can be very tedious, taking from a couple of hours up to a few weeks and some of the time the likelihood of changing the structure of the oil. [8]

1.7.3 -Cold pressing

This strategy is utilized to remove the essential oils from citrus peels, for example, orange, lemon, grapefruit and bergamot. This strategy includes the straightforward squeezing of the peels at around 49 ℃ to extricate the oil. The peels are isolated from the organic product, ground or hacked and after that squeezed. The outcome is a watery blend of crucial oil. The outcome will separate given time by virtue of differences in densities. Little adjustment from the oil's unique state happens and these citrus oils hold their brilliant, crisp, inspiring fragrances like that of noticing a magnificently ready natural product. The downside of this technique is that, the oils removed have a moderately short shelf-life. [17]

1.7.4- Effleurage method

This is one of the conventional methods for separating oil from blooms. The procedure includes layering fat over the blossom petals. After the fat has assimilated the key oils, liquor is utilized to discrete and removes the oils from the fat. The liquor is then vanished and the essential oil is gathered. [18]

1.7.5- Super critical CO_2 extraction

Supercritical CO_2 extraction includes carbon dioxide warmed to 30.6 ℃ and pumped through the plant material at around 551.58 bars, under these

conditions the carbon dioxide is contrasted with a “thick haze” or vapor. With the arrival of the weight in either prepare, the carbon dioxide escapes in its vaporous structure, deserting the essential oil. The typical strategy for extraction is through steam refining. After extraction, the properties of decent quality crucial oil ought to be as close as could be allowed to the pith of the first plant. The way to decent fundamental oil is through low weight and low temperature handling. High temperatures, quick handling and the utilization of solvents change the sub-atomic structure, will annihilate the helpful esteem and modify the scent. [8]

1.7. 6-Water distillation

In this strategy, the material is totally submerged in water, which is boiled by applying heat by direct fire, steam coat, shut steam coat, shut steam loop or open steam curl as shown in figure 2.1 below.

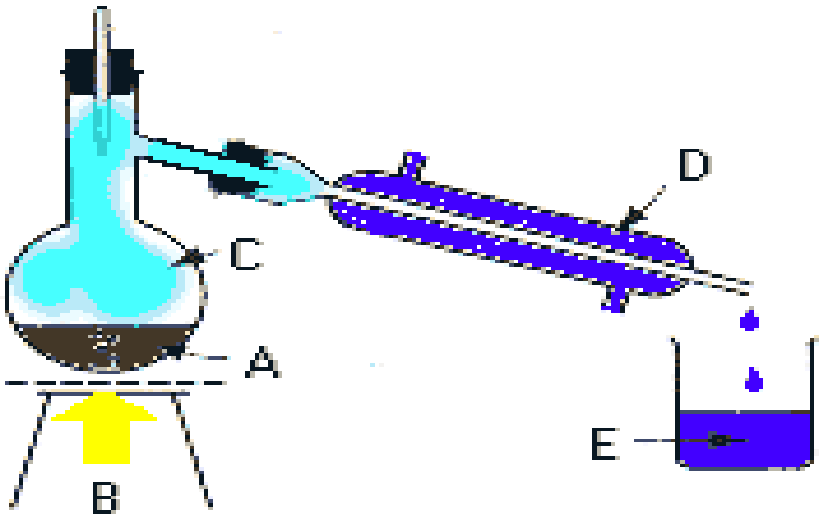


Figure 2.1: Flow process of water distillation method

The procedure is that, there is immediate contact between boiling water and plant material. When the still is warmed by direct fire, sufficient safety

measures are important to keep the charge from overheating. When a steam coat or shut steam loop is utilized, there is less peril of overheating. In any case, with open steam, care must be taken to counteract gathering of dense water inside of the still. In this way, the still ought to be all around protected. The plant material in the still should be upset as the water boils, generally collections of thick material will settle on the base and turn out to be thermally debased. Certain plant materials like cinnamon bark, which are rich in adhesive, must be powdered so that the charge can promptly scatter in the water; as the temperature of the water increases, the separation occurs and the rest settles at the bottom of the still. This enormously builds the consistency of the water charge blend, permitting it to boil. Before any field refining is done, small- scale water refining in a dish is performed to find out whether any advances happen amid the refining process. From this trial, the yield of oil from a known weight of the plant material can be resolved.

Amid water refining, all parts of the plant charge must be kept in movement by boiling water; this is feasible when the refining material is charged freely and stays free in the boiling water. Thus, just water refining has one particular point of interest, i.e. it permits processing of finely powdered material or plant parts that, by contact with live steam, would otherwise form lumps through which the steam cannot penetrate. [19]

1.7.7- Turbo distillation extraction

Turbo refining is suitable for difficult to separate or coarse plant material, for example, bark, roots, and seeds of plants. In this procedure, the plants absorb water and steam is coursed through this plant and water blend. All

through the whole process, the same water is consistently reused through the plant material. This technique permits quicker extraction of fundamental oils from difficult to concentrate plant materials. [19]

1.7. 8- Steam distillations method

As the name implies, direct steam refining is the procedure of refining plant material with steam produced outside the still in a satellite steam generator . With the direct steam refining technique, the plant material is bolstered on a punctured framework over the steam gulf. A genuine reason for preference of satellite steam generator is that the quantity of steam can be promptly controlled. Since steam is produced in a satellite heater, the plant material is warmed to around 100°C and, thus, it ought not to experience warm corruption. The steam which then contains the fundamental oil is passed through a cooling system to condense the steam, which form a fluid from which the essential oil and water is then separated. Direct steam refining (DSD) is the most broadly acknowledged procedure for the generation of crucial oils on substantial scale. In the flavor and fragrance supply business, the direct steam distillation method is a standard practice since this method does not change the composition of the oil.

A conspicuous disadvantage to steam distillation method is the much higher capital cost expected to construct such a facility. The cost of essential oils such as rosemary, Chinese cedar wood, lemongrass, litsea cubeba, spike lavender, Eucalyptus, citronella, cornmint, across the world are sufficiently high to legitimize their generation by steam distillation method without amortizing the capital use required to fabricate the facility over a period of 10 years or more.[19]

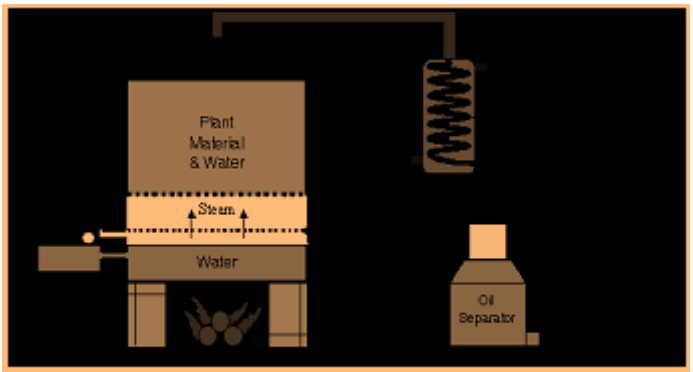


Figure 2.2 Flow process of steam distillation method

1.8- Gas chromatography- mass spectrometry

In its simplest form, the mass spectrometer has five components , and each will be discussed separately .The first component of the mass spectrometer is the sample inlet which brings the sample from the laboratory environment (1 atm) to the lower pressure of the mass spectrometer. Pressures inside the mass spectrometer range from a few millimeters of mercury in a chemical ionization source to a few micrometers of mercury in the mass analyzer and detector regions of the instrument. The sample inlet leads to the ion source where the sample molecules are transformed into gas phase ions. The ions are then accelerated by an electromagnetic field. Next, the mass analyzer separates the sample ions based on their mass-to-charge (*m*/*z*) ratio. The ions then are counted by the detector and the signal is recorded and processed by the data system, typically a personal computer (PC). The output from the data system is the mass spectrum—a graph of the number of ions detected as a function of their *m/z* ratio.[20-24]

Gas Chromatography (GC) is a kind of chromatography in which the versatile stage is a transporter gas, for example, nitrogen, and the stationary stage is an infinitesimal layer of fluid or polymer on an idle strong backing,

inside glass or metal tubing, called a segment. The fine section contains a stationary stage, a fine strong backing covered with a nonvolatile fluid. The strong can itself be the stationary stage. The gas is cleared through the section by a flood of helium gas. The segments are isolated from each other due to the fact that some take more time to go through the section than others. As the sample leaves the end of the GC section it is divided by ionization and the pieces are sorted by mass to frame a discontinuity design. It is specific to the point that; it is regularly alluded to as the atomic unique finger impression. Gas chromatography-mass spectrometry (GC-MS) is a systematic technique that joins the components of gas-fluid chromatography and mass spectrometry to distinguish distinctive substances inside the sample. GC can isolate unpredictable and semi unstable mixes with extraordinary accuracy, yet it can't distinguish the compounds. MS can give point by point auxiliary data on most mixes such that they can be precisely distinguished, yet it can't promptly isolate them. The GC-MS joins two diverse investigative strategies, Gas Chromatography (GC) and Mass Spectrometry (MS), used to dissect complex natural and biochemical blends. The GC-MS instrument comprises of two primary parts. The gas chromatography parcel that isolates distinctive mixes in the example into beats of immaculate chemicals in light of their instability by streaming an idle gas which conveys the specimen, through a stationary stage settled in the section .Spectra of mixes are gathered as they leave a chromatographic segment by the mass spectrometer, which distinguishes and evaluates the chemicals

according to their mass to charge proportion (m/z). These spectra can then be put away on the PC and examined. [24]

1.9-Pharmacological properties of essential oils

i-Antiseptics

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**.**

ii-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.

iii-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 [25,26].

iv) Antibacterial Activity

The antimicrobial properties of essential oils and of their constituents have been considered [27,28] and the mechanism of action has been studied in detail[29]. An important feature of essential oils are their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria,disrupting the structure, and making it more permeable[30]. This can then cause leakage of ions and other cellular molecules [31,32]. Although a certain amount of leakage of bacterial cells can be tolerated without loss of viability, greater loss of cell contents or critical output of molecules and ions can lead to cell death . [33]

EOs and/or their constituents can have a single target or multiple targets of their activity. For instance, trans-cinnamaldehyde can inhibit the growth of Escherichia coli and Salmonella typhimirium without disintegrating the OM or depleting intracellular ATP. Similar to thymol and carvacrol,trans-cinnamaldehyde likely gains access to the periplasm and deeper portions of the cell .[34]

Carvone is also ineffective against the OM and does not affect the cellular ATP pool[35].It has been reported that EOs containing mainly aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol were characterized by the highest antibacterial activity, followed by EOs containing terpene alcohols. Other EOs, containing ketones or esters, such as _-myrcene,-thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene

hydrocarbons were usually inactive .[36,37]

Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, have important antibacterial activities [36,38].These compounds are responsible for the disruption of the cytoplasmic membrane, the driving force of protons, electron flow, active transport, and also coagulation of cell contents [28,33,39]. The chemical structure of essential oils affects their mode of action concerning their antibacterialactivity [38]. The importance of the presence of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, was confirmed.[32,38,40] However, the relative position of the phenolic hydroxyl group on the ring does not appear to influence the intensity of the antibacterial activity.

The action of thymol against Bacillus cereus, Staphylococcus aureus, and Pseudomonas aeruginosa appears to be comparable to that of carvacrol, for example . However, carvacrol and thymol act differently against Gram-positive and Gram-negative species . Thymol, eugenol, and carvacrol have an antimicrobial effect against a broad spectrum of bacteria: Escherichia coli, Bacillus cereus,Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus, and Helicobacter pyroli[41,42]. Other families of compounds also have valuable antibacterial properties: certain alcohols, aldehydes, and ketones, monoterpene (geraniol, linalol, menthol, terpineol, thujanol,myrcenol, citronelîaî, neral, thujone, camphor, carvone, etc.), phenylpropanes (cinnamaldehyde), and monoterpenes (-terpinene, p-cymene). Among these compounds, carvacrol is the most active. Known to be non-toxic, it is used

as a preservative and food flavoring in drinks, sweets, and other preparations.

It is important to mention that essential oils are more active against Gram-positive than Gram-negative bacteria[43] . The latter are less susceptible to the action of essential oils with the outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide film[46]. Furthermore, the antibacterial activity of essential oils related to their chemical composition, the proportions of volatile molecules, and their interactions [38,33,47] . An additive effect is observed when the combination is equal to the sum of the individual effects.Antagonism is observed when the effect of one or both compounds is less important when they are tested together than when used individually [48] .

A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects [49]. Some studies have shown that the use of the whole essential oil provides an effect which is greater than that of the major components used together [50]. This suggests that minor components are essential for activity and may have a synergistic effect. It has been reported additive and synergistic effects of the combinations of 1,8-cineole and aromadendrene against methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) and Enterococcus faecalis by using checkerboard and time-kill assays, respectively [51].The combined effects of plant volatile oils and benzoic acid derivatives against L. monocytogenes and S. enteritidis are considered as synergistic since the combined components allowed

_log10 higher inhibition than the sum of the inhibitory effects of the components used separately [52]. Increased antifungal effects were caused by combinations (1:5, 1:7, and 1:9) of essential oils of S. aromaticum (clove) and *Rosmarinus officinalis* against C. albicans [53]. Moreover, Lambert et. al. [27] reported that, combined, carvacrol and thymol showed additive effects against S. aureus and P. aeruginosa by using half-fold dilutions within the Bioscreen plat. Two hypotheses have been proposed to explain synergistic effects of cinnamaldehyde/thymol or cinnamaldehyde/carvacrol against S. typhimurium: proving, on one hand, that thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell, and, on the other hand, that thymol or carvacrol could increase the number, size, or duration of the existence of the pores created by the binding of cinnamaldehyde to proteins in the cell membrane [54]. These facts justify a synergistic effect achieved when these two components are used in combination. Mechanisms of interaction that produced antagonistic effects were less studied [55].

In addition, essential oils have also revealed to be effective on the inhibition of growth and reduction in numbers of the more serious foodborne pathogens, such as Salmonella spp., E. coli O157:H7, and Listeria monocytogenes [52].

v) Antioxidant activity

Numerous studies have demonstrated the antioxidant properties of essential oils. The antioxidant potential of an essential oil depends on its composition. It is well established that phenolics and secondary metabolites

with conjugated double bonds usually show substantial antioxidative properties [56]. Most of the essential oils are dominated by oxygenated monoterpenes such as alcohols (*Achillea filipendulina*), aldehydes (Ga*lagania fragrantissima*), ketones (*Anethum graveolens,Artemisia rutifolia, Hyssopus seravschanicus, Mentha longifolia*, and *Ziziphora clinopodioides*), and esters (*Salvia sclarea*). *Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons, whereas phenolic terpenoids, such as thymol or carvacrol, characterize *Origanum tyttanthum* and Mentha longifolia EOs, which would explain why both plants exhibited generally the strongest antioxidant activity. Thymol and carvacrol, which are predominant in *Origanum tyttanthum*, are also responsible for the antioxidant activity of several other essential oils, such as Mentha longifolia and Thymus serpyllus [57].

The essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties [53]. Thymol and carvacrol are the most active compounds. Their activity is related to their phenolic structure. These phenolic compounds have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition [50]. The antioxidant activity of essential oils is also due to certain alcohols, ethers,

ketones, aldehydes, and monoterpenes: linalool, 1,8-CineoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: terpinene, terpinene and terpinolene [53].Essential oils with important scavenging capacity of free radicals may play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and immune

system decline. In fact, these diseases may result from cellular damage caused by free radicals [53,54].

EOs have shown their action as hepatoprotective agents in ageing polyunsaturated fatty acids mammals and it has been proved that they possess a beneficial impact upon the PUFAs, in particular the long chain C20 and C22 acids [58]. Moreover, essential oils being able to scavenge free radicals may also play an important role in some disease prevention, such as brain dysfunction, cancer, heart disease, and immune system decline [59]. Sharififar et al. (2011) [60] evaluated the antioxidant activity of *Zataria multiflora* Boiss. (Lamiaceae) essential oil in rats. Antioxidant activity was measured by the test of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition and inhibition of lipid peroxidation by measuring the index of thiobarbituric acid reactive substances (TBARs). Three doses of 100, 200, and 400 _L/kg were administered to animals by intra gastric intubation (i.g) routh for 10 days. The blood was collected in eleventh day through direct puncture and the liver was rapidly excised. The histopathology studies of the animals were compared to animals in butylated hydroxyl toluene (BHT) group. The authors reported that all Zataria multiflora oils ZMO tested doses were able to scavenge DPPH radical ($p < 0.05$). Moreover, ZMO decreased TBARs in a dose-dependent manner. No alteration in liver function test LFT enzymes or changes in histopathology of the liver was considered in ZMO treated groups. The results indicated that ZMO might be used in human healthy and food industry.

According to Manjamalai and Grace [61], essential oil of Wedelia chinensis (Osbeck) increases both the level of catalase and glutathione peroxidase in

the lung and liver tissues, whereas in the serum the level of catalase decreased on the 22nd day (2.32 _ 0.016 Lung tissue 6.47 _ 0.060 liver tissue,0.94 _ 0.007 serum). Furthermore, the level of Glutathione Peroxidase GPx in the liver (the range) was

found to be decreased in the EO-treated group compared to the cancer-induced group and control group, whereas the level of GPx in the lung tissue was found to be low (76.2 _ 1.66).

vi)Anti-Inflammatory Activity

Inflammation is a normal protective response induced by tissue injury or infection and functions to combat invaders in the body (microorganisms and non-self cells) and to remove dead or damaged host cells. The inflammatory response induces an increase of permeability of endothelial lining cells and influxes of blood leukocytes into the interstitium, oxidative burst, and release of cytokines, such as interleukins and tumor necrosis factor(TNF). It also stimulates the activity of several enzymes (oxygenases, nitric oxide synthases, peroxidases, etc.), as well as the arachidonic acid metabolism. Recently, essential oils have been used in clinical settings to treat inflammatory diseases, such as rheumatism, allergies, or arthritis [(55)]. *Melaleuca alternifolia* EO was reported to have a considerable antiinflammatory activity [(56,58)].This activity is correlated with its major compound:alpha_-terpineol [(59)]. The active compounds act by inhibiting the release of histamine or reducing the production of inflammation mediators. Geranium essential oil is another example [(55)]. Linalool and linalyl acetate showed anti-inflammatory activity on oedema of paw-induced mouse carrageenan [(60)].

Yoon et al. [62] reported that the oils of Torreya nucifera Siebold et Zucc. oil, mainly constituted by limonene, 3-carene, and alpha-pinene, have an inhibitory effect on COX-2, thus inducing a significant inhibitory effect on prostaglandin (PGE2) production. Furthermore, 1,8-cineole, present in many essential oils, was reported as an inhibitor of leukotrienes (LTB4) and PGE2, biogenerated both from pathways of arachidonic acid metabolism [62].The anti-inflammatory activity of essential oils may be attributed not only to their antioxidant activities but also to their interactions with signaling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. Essential oils, therefore, represent a new option in the treatment of inflammatory diseases.

vii) Cancer chemoprotective activity

The varied therapeutic potential of essential oils attracted, in recent years, the attention of researchers for their potential activity against cancer. They and their volatile constituents of the studies target the discovery of new anticancer natural products [51]. Essential oils would act in the prevention of cancer, as well as at its removal. It is well known that certain foods, such as garlic and turmeric, are good sources of anticancer agents[63]. Garlic essential oil is a source of sulfur compounds recognized for their preventive effect against cancer [64,65]. Diallylsulfide, diallyldisulfide, and diallyltrisulfide are examples. According to Wu et al. [66], these compounds activate, in rats, the enzymes involved in the detoxification process of hepatic phase 1 (disintegration of chemical bonds that link carcinogenic toxins to each other) and phase 2 (bonds to toxins released detoxifying enzymes, such as glutathioneS-transferase).

Metabolism happens mainly in the liver, the body's largest internal organ. The portal vein carries blood from the small intestine directly to the liver. Sixty percent of liver tissue is made up of hepatic cells. More chemical processes happen in these than in any other group of cells in the body. Phase 1 metabolism involves chemical reactions, such as oxidation (most common), reduction, and hydrolysis. There are three possible results of phase 1 metabolism. The drug becomes completely inactive. In other words, the metabolites are pharmacologically inactive. One or more of the metabolites are pharmacologically active, but less so than the original drug. The original substance is not pharmacologically active, but one of its metabolites is. The original substance is called a prodrug. Phase 2 metabolism involves reactions that chemically change the drug or phase 1 metabolites into compounds that are soluble enough to be excreted in urine. In these reactions, the molecule (drug or metabolite) is attached to an ionisable grouping. This is called conjugation and the product is called a conjugate. Metabolites formed in phase 2 are unlikely to be pharmacologically active. Some drugs undergo either phase 1 or phase 2 metabolism, but most undergo phase 1 metabolism followed by phase 2 metabolism.

Another example is myristicin, an allylbenzene present on a certain essential oil, especially that of nutmeg (Myristica fragrans). This molecule is known to activate glutathione S-transferase in mice [(67)] and inhibit carcinogenesis induced by benzo(a)pyrene in the lungs of mice . Recently, it has been discovered that myristicin induces apoptosis in neuroblastoma (SK-N-SH) in humans [(68)].

There are other volatile compounds that showed a cytotoxic activity against various cancer cell lines [53].Geraniol decreases the resistance of colon cancer cells (TC118) to 5-fluorouracil, an anticancer agent.Therefore, geraniol enhances this inhibitory effect of tumour growth 5-fluorouracil [69,70]. The essential oil of balsam fir and _-Humulene, showed significant anticancer activity in several cell lines and low toxicity to healthy cells [71].
In addition, anticancer activity of D-limonene, the main component of Citrus essential oil has been proven, especially at the level of stomach cancer and liver [72]. The _-Bisabolol, an abundant sesquiterpene alcohol in chamomile essential oil (Matricaria), has an antigliomale activity [73].
Many essential oils have a cytotoxic activity namely Melissa officinalis [74], Melaleuca alternifolia [75], Artemisia annua [76], and Comptonia peregrina [77].

viii)Cytotoxicity

Due to their complex chemical composition, essential oils have no specific cellular ligands [31]. As lipophilic mixtures, they are able to cross the cell membrane and degrade the layers of polysaccharides, phospholipids and fatty acids, and permeabilize. This cytotoxicity appears to include such membrane damage. In bacteria, the membrane permeabilization is associated with the loss of ions and the reduction of the membrane potential, the collapse of the proton pump and the depletion of the ATP pool [32,78,80]. Essential oils may coagulate the cytoplasm [27] and damage lipids and proteins [32,50]. Damage to the wall and the cell membrane can lead to the leakage of macromolecules and lysis [27,30,81].

In addition, essential oils change membrane fluidity, which becomes abnormally permeable, resulting in a leakage of radicals, cytochrome C, the Ca2+ ions, and proteins, like in the case of oxidative stress. This permeabilization of the outer and inner membranes causes cell death by apoptosis and necrosis [82,83]. Ultrastructural alteration of the cell can be observed at a plurality of compartments [62,84,85]. The interruption of the viral envelope herpes simplex virus HSV by essential oils can also be observed by electron microscopy [86]. The induction of membrane damage was also confirmed by an analysis showing that microtubule Saccharomyces cerevisiae genes involved in the biosynthesis of ergosterol, the absorption of sterols, lipid metabolism, the structure and function of cell wall cellular detoxification, and transport are affected by treatment with _-terpinene [87]. Recent work on the yeast Saccharomyces cerevisiae, has shown that the cytotoxicity of some essential oils based on the ability to form colonies differs significantly in relation to their chemical composition. Generally, essential oil cytotoxicity mainly correlates to the presence of phenols, alcohols, and monoterpene aldehydes [88,89]. The cytotoxic properties of essential oils are of great importance because they assume their use not only against certain human pathogens and animal parasites, but also in the preservation of agricultural and marine products against microbial attack. Indeed, some components of essential oils are effective against a variety of microorganisms as bacteria [90], viruses [91], fungi [87,92,94], protozoa [95], parasites [96,98], mites, and others.

In addition, _-humulene shows cytotoxicity against breast cancer cells in vitro. _-humulene was reported to be responsible for cytotoxicity (CI50 55

mM) [99]. It induced a dose- and time-dependent decrease in cellular glutathione (GSH) content and an increase in reactive oxygen species (ROS) production.

Furthermore, Zeytinoglu et al. [100], focusing on the effects of carvacrol, one of the main compounds in the EO of oregano, on the DNA synthesis of N-ras transformed mouse myoblast CO25 cells, finding that this monoterpenic phenol was able to inhibit the DNA synthesis in the growth medium and ras-activating medium, which contained dexamethasone. They proposed that it may be valuable in cancer therapy because of its growth inhibition of myoblast cells, even after activation of mutated N-ras-oncogene.

The EO of the Anonaceae Xylopia aethiopica (Ethiopian pepper), a plant grown in Nigeria, showed, at a concentration of 5 mg/mL, a cytotoxic effect in the carcinoma cell line (Hep-2) [101]. Moreover, Yu et al. [102] tested the essential oil of the rhizome of the Aristolochiaceae Aristolochia mollissima for its cytotoxicity on four human cancer cell lines (ACHN, Bel-7402, Hep G2, HeLa).

The rhizome oil possessed a significantly greater cytotoxic effect on these cell lines than the oil extracted from the aerial plant.

Linalool inhibited only moderate cell proliferation; however, in subtoxic concentrations potentiates doxorubicin-induced cytotoxicity and proapoptotic effects in both cell lines, MCF7 WT and MCF7 AdrR. This monoterpene improves the therapeutic index in the management of breast cancer, especially multidrug resistance (MDR) tumors [103].

An in vitro cytotoxicity assay indicated that the EO of Cyperus rotundus (Cyperaceae) characterized by the predominance of cyperene, _-Cyperone,

isolongifolen-5-one, rotundene, and cyperorotundene, was very effective against L1210 leukemia cells, which correlates with significantly increased apoptotic DNA fragmentation [104].

1.10- *Elettaria cardamomum*

Elettaria cardamomum is a large, perennial, herbaceous, rhizomatous monocot. The rhizome bears many leafy shoots,4–5 m tall. Leaves are alternate, sessile, linear-lanceolate, and 30–90 cm long. The flowers are borne on long racemose panicles that originate from the rhizome. The most conspicuous part of the flower is the whitish lip/labellum located at the tip of the corolla tube. It has violet nectar guides leading to the corolla tube. The single fertile stamen bears bi-lobed anthers. The pistil is trilocular and each locule bears over 20 ovules with axile placentation. The style is filiform and terminates with a laterally compressed, cup-shaped stigma located slightly above the anther. The inner surface of the stigmatic cup is receptive and is lined with a viscous exudate. Several varieties of cardamom, developed largely through clonal selection, have been released commercially.[130]

Elettaria cardamomum

Aim of this study

The aim of this study was:

-To extract the fixed oil from *Elettaria cardamomum*.

-To study the constituents of the oil by GC-MS.

-To evaluate the oil for antimicrobial potential.

Chapter Two

Material and Methods

2-Materials and Methods

2.1-Materials

2.1.1- 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)**.**

2.1.2-Test organisms

Elttaria cardamomum oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Candida albicans*.

2.1.3-Plant material

Seeds of Elttaria cardamomum _were purchased from the local market and identified by direct comparison with a herbarium sample.

2.2- Methods

2.2.1-Extraction of oil

Powdered seeds of Elttaria cardamomum (300g) were exhaustively macerated with n-hexane.The solvent was removed under reduced pressure to afford the oil.

2.2.2- GC-MS analysis

The oil of Elttaria cardamomum was analyzed by GC-MS. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)was used.. Oven temperature program and other chromatographic conditions are shown below:

Table 2: Oven temperature program

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

Table 3: Chromatographic conditions

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

2.2.3-Antimicrobial assay

(i)Bacterial suspensions

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

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

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

ii)-Fungal suspensions

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

iii)-Antibacterial test

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

3.1.- GC-MS analysis of *Elettaria cardamomum* oil

In this study the essential oil of the medicinally important plant *Elettaria cardamomum* was investigated**.** GC-MS analysis showed 39 constituents. Major are : 3-cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate(27.66%),hexadecanoic acid, methyl ester(14.86%) ,9-octadecenoic acid (Z)-, methyl ester (8.78%),9,12-octadecadienoic acid (Z,Z), methyl ester (8.35%)**,** L-.alpha.-terpineol (6.98%).The total ion chromatogram is presented in Fig.1 , while the oil components are displayed in Table 1.

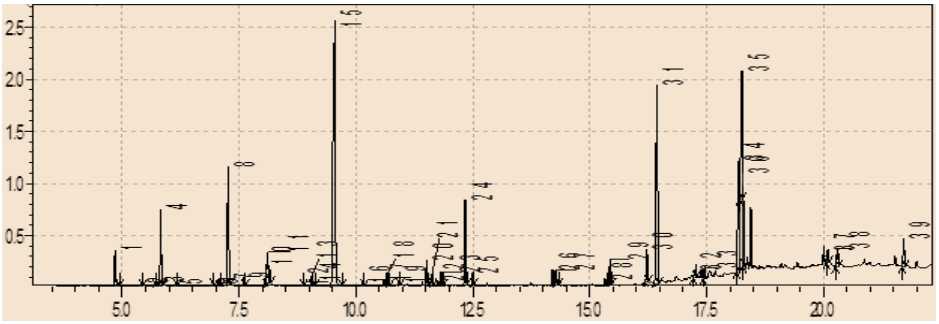


Fig.1: Total ions chromatograms

Major components are discussed below:

a- **3-cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate (27.64%)**

Fig. 2 presents the mass spectrum of 3-cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate.The peak at m/z181 which appeared at retention time 9.547 is due to M^+ $[C_{12}H_{20}O_2]^+$-3H.

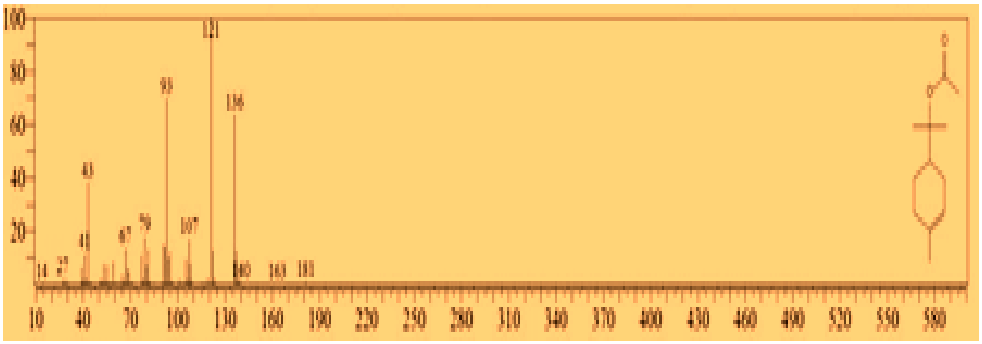


Fig. 2: Mass spectrum of 3-cyclohexene-1-methanol, -alpha-,-alpha-,4-trimethyl-, acetate (27.66%)

b- Hexadecanoic acid, methyl ester(14.86%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.The signal at m/z 270 (R.T. 16.439) corresponds $M^{+}[C_{17}H_{34}O_{2}]^{+}$ .The signal at m/z239 is due to loss of a methoxyl .

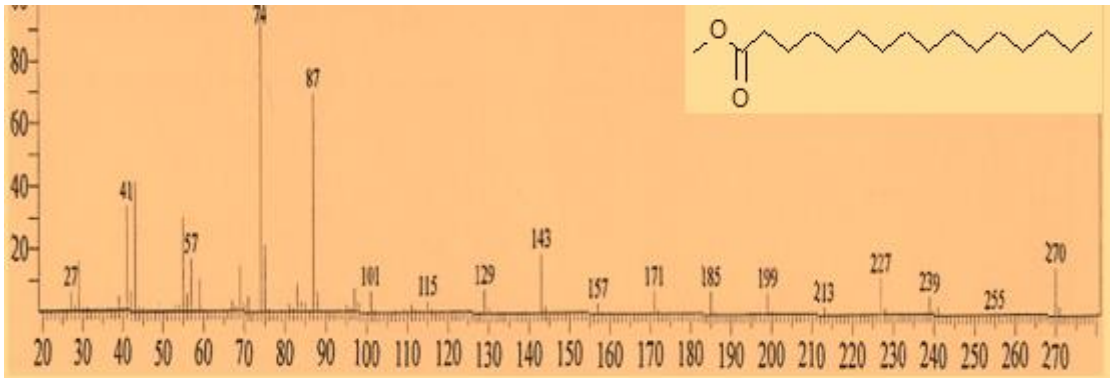


Fig. 3: Mass spectrum of hexadecanoic methyl ester

c-9-octadecenoic acid (Z)-, methyl ester (8.78%)

The mass spectrum of 9-octadecanoic acid methyl ester is displayed in Fig.4.The peak at m/z 296, which appeared at R.T. 18.253 accounts for the molecular ion: $M^{+}[C_{19}H_{36}O_{2}]^{+}$ .The signal at m/z265 accounts for loss of a methoxyl function

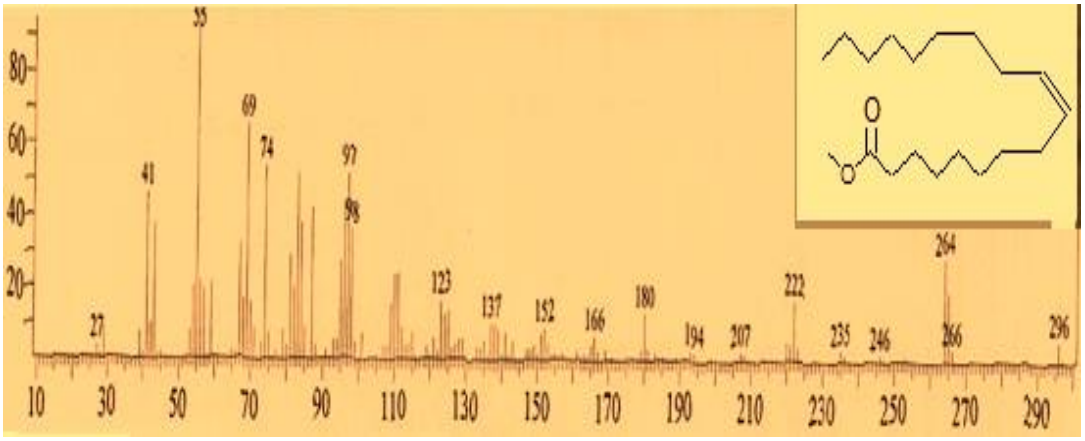


Fig. 4: Mass spectrum of 9-octadecanoic acid methyl ester

d-9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.35%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 5.The peak at m/z 294, which appeared at R.T. 18.185 in total ion chromatogram, corresponds the molecular ion : $M^{+}[C_{19}H_{34}O_2]^{+}$ .The signal at m/z263 corresponds to loss of a methoxyl function.

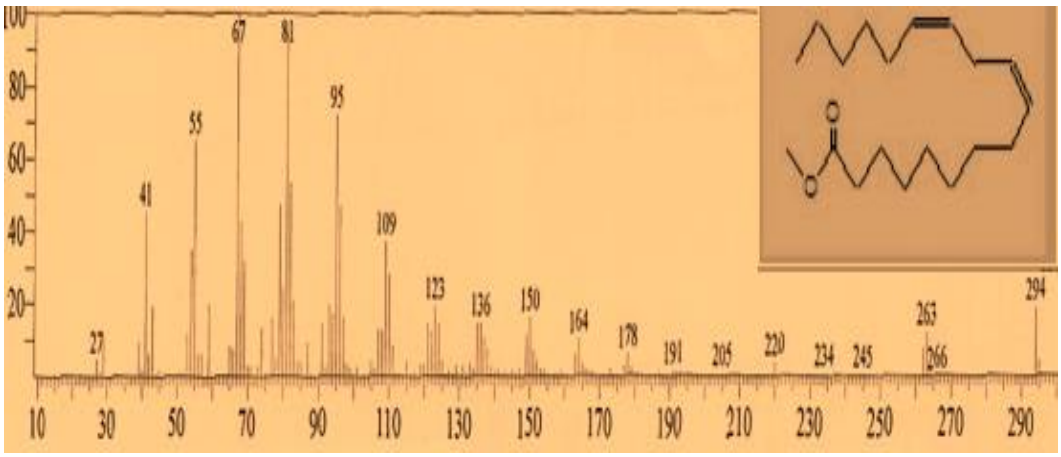


Fig. 5: Mass spectrum of 9,12-octadecanoic acid methyl ester

e- L-.alpha.-terpineol (6.98%)

The mass specrum of L-.alpha.-terpineol is shown in Fig.6. The molecular ion $M^+[C_{10}H_{18}O]^+$ appeared at m/z154 with retention time 7.265.

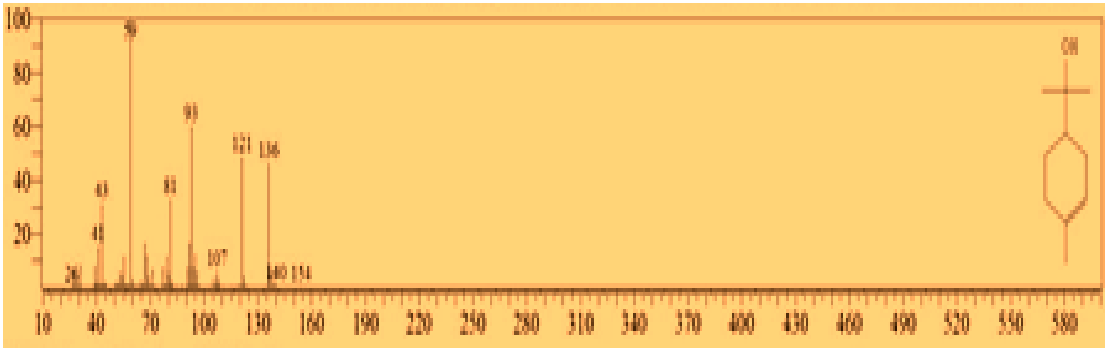


Fig. 6 : Mass spectrum of L-.alpha.-terpineol

Table 1: Constituents of the oil

No.	Name	Ret.Time	Area%
1.	D-Limonene	4.847	1.75
2.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	5.412	0.26
3.	(+)-2-Carene	5.699	0.21
4.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.825	3.95
5.	Octanoic acid, methyl ester	6.138	0.15
6.	.alpha.-Terpineol	6.911	0.11
7.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	7.066	0.37
8.	L-.alpha.-Terpineol	7.265	6.98
9.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	7.606	0.41
10.	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	8.075	1.07
11.	Geraniol	8.108	1.91
12.	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-,	8.859	0.08

	[1R-(1.alpha.,3.beta.,4.alpha.,6.alpha.)]-		
13.	6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-	9.050	0.49
14.	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	9.087	0.26
15.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	9.547	27.64
16.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	10.148	0.18

Table 1 Contd.

17	Caryophyllene	10.602	0.29
18	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, propanoate	10.690	0.74
19	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	10.920	0.13
20	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	11.512	1.34
21	Guaia-1(10),11-diene	11.617	0.51
22	Dodecanoic acid, methyl ester	11.755	0.26
23	.gamma.-Muurolene	11.835	0.73
24	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	12.335	4.66
25	Supraene	12.491	0.59
26	Methyl tetradecanoate	14.203	0.86
27	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	14.270	1.05
28	Pentadecanoic acid, methyl ester	15.338	0.30
29	4-Hexen-1-ol, 6-(2,6,6-trimethyl-1-cyclohexenyl)-4-methyl-, (E)-	15.433	1.50

30	9-Hexadecenoic acid, methyl ester, (Z)-	16.221	1.68
31	Hexadecanoic acid, methyl ester	16.439	14.86
32	cis-10-Heptadecenoic acid, methyl ester	17.240	0.41
33	Heptadecanoic acid, methyl ester	17.454	0.54
34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.185	8.35
35	9-Octadecenoic acid (Z)-, methyl ester	18.253	8.78
36	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.275	3.16
37	cis-13-Eicosenoic acid, methyl ester	20.088	0.59
38	Eicosanoic acid, methyl ester	20.291	1.17
39	Tetratriacontane	21.714	1.68

3.2-Antimicrobial assay

Elettaria cardamomum essential oil was investigated for antimicrobial activity via the cup plate agar diffusion bioassay using five standard pathogenic bacteria. The average of the diameters of the growth inhibition zones are displayed in Table (2) . Results were interpreted as follows:>9 considered inactive; 9-12:weak activity ; 13-18: active and <18 : very active.

Ampicilin,gentamycin and clotrimazole have been used as positive controls .

Table 2: Antimicrobial activity of the oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	16	15	--	17
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

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

At a concentration of 100mg/ml , the oil showed significant anticandidal activity. It also exhibited moderate activity against Gram positive *Staphylococcus aureus* , *Bacillus subtilis* and Gram negative *Escherichia coli.* However it failed to give inhibitory effect against Gram negative *Pseudomonas aeruginosa.*

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