Sudan University of Science and Technology

College of Graduate Studies

Characterization and Antimicrobial Activity of Oils from Five Medicinal Herbs

توصيف المكونات والنشاط المضاد للميكروبات لزيوت خمسة نباتات طبية

A Thesis Submitted in Fulfillment for the Requirements of the Ph.D. Degree in Chemistry

by

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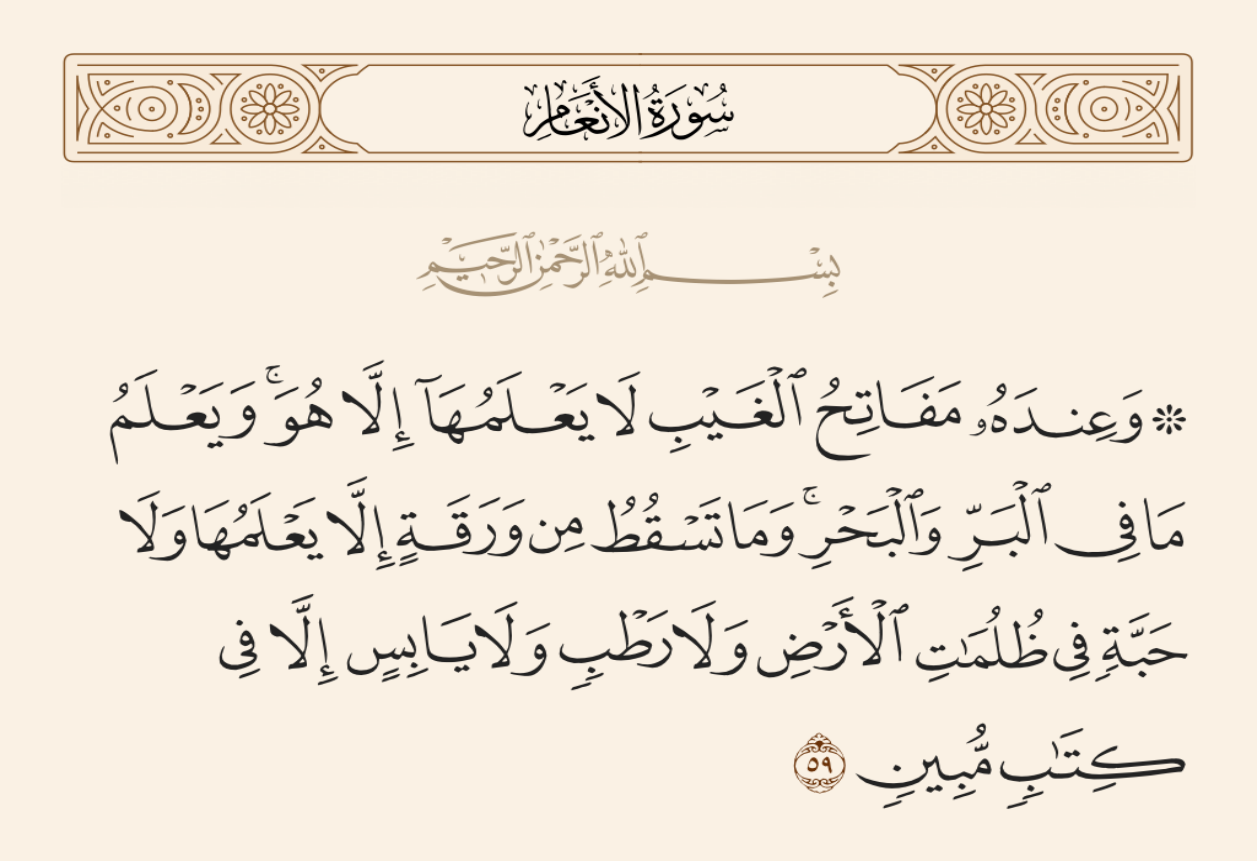
الإستهلال

قال تعالى:



صدق الله العظيم

Dedication

To.....

The soul of my father

My mother

My husband

Brothers and sister

Acknowledgement

First and foremost, I would like to thank the most **Merciful, Gracious and Omniscient Almighty Allah** for the great help and blessing in my whole life.

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Abstract

This research was designed to study the seed oils of five potential medicinal plants. (*Carum carvi*, *Coriandrum sativum*, *Hibiscus sabdariffa* (grown in Egypt); *Nerium oleander*, *Lagenaria siceraria*(grown in Sudan). The constituents of the target oils have been studied by GC-MS and the antimicrobial activity has been evaluated.

9-Octadecanoic acid methyl ester appeared in different ratios for all samples except for *Legenaria siceraria* which contained a component (linoleic acid ethyl ester) which is absent in the rest of oil samples. 9,12-Octadecadienoic acid methyl ester appeared in the five oils at different ratios, also all the studied oils contained different ratios of hexadecanoic acid methyl ester. Methyl stearate appeared at different ratios in all oil samples with the exception of *carum carvi* oil which contained a constituent – 10-nonadecanone- which is absent in other studied oils. All oil samples have been evaluated for antimicrobial activity and they showed different antimicrobial responses.

المستخلص

يشتمل البحث على دراسة لزيوت خمسة نبات ات طبية وهي الكراويا، الكسبرة، الكركدي (والتى جمعت من اسوان – مصر) بالاضافة الى إثنان من النباتات السودانية وهى الورد الكاذب والقرع الحلو. تمت دراسة المكونات الكيميائية للزيوت بواسطة الكروماتوغرافيا الغازية- طيف الكتلة. كما وأُجرى إختبار مضاد الميكروبات لهذه العينات. ظهر مركب استر حمض 9- اوكتاديكنويك فى جميع العينات بنسب متفاوته ما عدا عينة القرع الحلو والتى إحتوت على استر اثيل لحمض لينالويك وهو مركب لم يتوفر فى بقية العينات. أما استر حمض 12,9- اوكتاديكاداينويك فقد كان متوفرا فى جميع العينات بنسب متفاوه. إحتوت جميع العينات , ما عدا الكراويا, على مثيل استيرات بنسب متفاوته .أيضا إحتوت عينة الكراويا على مكونا لم يتوفر فى بقية العينات وهو المركب الكيتونى : 10- نونـاديكنون. جميع الزيوت قيد الدراسة إحتوت على استر حمض هكسانويك. أيضا أُجرى إختبار مضاد الميكروبات لجميع العينات والتى أبدت نشاطا متفاوتا.

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1 Introduction

1.1 Natural products

Natural products have historically been an extremely productive source for new medicines in all cultures and continue to deliver a great variety of structural templates for drug discovery and development. Although products derived from natural sources may not necessarily represent active ingredients in their final form, the majority of all drugs in the market have their origin in nature[1,2].

Generally, the term “natural product” is regarded as being synonymous with “secondary metabolite”[3]. Secondary metabolites are organic compounds in the correct chiral configuration to exert biological activity, but have no “primary” function directly involved in the normal growth, development or reproduction of an organism[4]. Natural products are usually relatively small molecules with a molecular weight below 3,000 Daltons and exhibit considerable structural diversity[3].

Natural substances have evolved over a very long selection process to form optimal interactions with biological macromolecules[5] which have activity on a biological system that is relevant to the target disease. They have historically been the most productive source of active compounds and chemical lead structures for the discovery and development of new medicines[6].

Historically, the most important sources for biologically active natural products have been terrestrial plants and microorganisms such as fungi and bacteria. Terrestrial and aquatic species of plants and microorganisms, especially those of marine origin, produce unique bioactive substances yielding a large variety of valuable therapeutics and lead structures for potential new drugs. Even though natural products may not have coevolved with human proteins, they have emerged in nature to interact with biomolecules[7]. Natural products interact with a wide variety of proteins and other biological targets, acting also as modulators of cellular processes when they inhibit the difficult to target protein-protein interactions[8].

Natural products can be defined as the products of natural backgrounds. Natural products include: (1) a complete organism (e.g., a microorganism, a plant or an animal) that has not been exposed to any type of treatment other than a simple course of preservation (e.g., drying), (2) part of an organism (e.g., an isolated animal organ, flowers or leaves of a plant), (3) part of an organism, exudates and an organism extract and (4) pure compounds (e.g., terpenoids, coumarins, alkaloids, glycosides, flavonoids, steroids, sugars, lignans, etc.) isolated from microorganisms, animals or plants[9].

1.2Fixed oils

Fixed oils and fats (lipids) are present in virtually all the food we eat[10]. It is the only material present in vegetable oils and animal fats, it is an

important component of milk and milk–based products such as cream and cheese, eggs and meat, and is even present in leaves and green vegetables[11].

Fixed oil and fat are present in many plants such as castor seed, olive, peanut, soybean, sesame, almond, cotton seed, corn, safflower, cocoa butter, linseed, sunflower, oil palm and shea butter. It is also a component of animal fats especially milk, meat and egg[12]. The presence of these substances in human and in its food has been speculated to confer some physiological functions as well assist in the prevention and or treatment of some diseases and infections.

Fixed oil and fats are involved in cancer in many ways. Dietary lipids, including micronutrients such as fat–soluble antioxidants, may play a role in predisposing to, or protecting from cancer. The development of cancer involves cellular changes that include alterations to lipid components of the cell (and this may be relevant to the design of new treatments). Finally, there may be a role for certain dietary lipids in the control or treatment of certain cancers or aspects of cancer[13].

Fixed oil and fats are believed to act in diverse ways, all of which could be seen as targeting cells with a high rate of cell division. They may act by disrupting signal transduction and signaling pathways, e.g. inhibition of the phosphoinositide–specific phospholipase C with suppression of the diacylglycerol–protein kinase C pathway, or inhibition of phosphocholine cytidylyl transferase leading to inhibition of

phosphatidyl choline biosynthesis de novo[14]. These compounds have shown great promise in cellular systems, but their clinical application has been limited by problems of toxicity to the intestinal tract (whose cells are also characterized by a high rate of division)[15].

Though fixed oil and fats serve as one of the main components of human diets, however some of them portend danger to man as a result of their toxicity. Toxic fixed oil and fats include ones containing cyclopropenes, long–chain monoenes, trans–unsaturated fatty acids and lipid peroxides. Cyclopropenes of the fatty acids, those containing a cyclopropene ring have been considered toxic as a result of their ability to inhibit the Ä9–desaturase.

One result of this is to alter membrane permeability as seen in 'pink–white disease'. If cyclopropene fatty acids are present in the diet of laying hens, the permeability of the membrane surrounding the yolk is increased, allowing release of pigments into the yolk[16]. Cottonseed oil is the only important oil in the human diet that contains cyclopropene fatty acids. However, their concentration in the natural oil is low (0.6–1.2%) and is reduced still further to harmless levels (0.1–0.5%) by processing. There has been no evidence that consumption of cottonseed oil in manufactured products has had any adverse nutritional effects[17].

1.3 Essential oils

Essential oils are highly volatile, aromatic yields obtained from plants. Due to their volatility, they can easily be extracted by the method of steam distillation from different natural sources[18].

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. They are concentrated hydrophobic liquid containing volatile (easily evaporated at room temperatures) chemical compounds from plants. Because of their antidepressant, stimulating, detoxifying, antibacterial, antiviral and calming properties, they are recently gaining popularity as a natural, safe and cost-effective therapy for a number of health concerns. Essential oils are aromatic compounds found in great quantities in oil sacs or oil glands present at different depths in the fruit peel, mainly flavedo part and cuticles[19]. In addition, essential oils are aromatic oily liquids extracted from different parts of plants for instance, leaves, barks, seeds, flowers and peels[20]. They can be obtained by expression, fermentation, effleurage or extraction but among all the methods, steam distillation and hydro distillation are widely used for commercial production of essential oils[21,22].

The essential oils have been used for centuries for different purposes and regarded with great intrigue, albeit many their uses have been lost with time, it is by and large acknowledged that people have been extracting them from fragrant plants since the very beginning of

humankind. Essential oils have been used by ancient Egyptians in medication, perfumery, and in the craft of planning bodies for entombment through preservation. In Asian region, the Vedas classified the employments of these aromatic essences for remedial and worship purposes. Indeed, through the ages, humans have utilized essential oils for different purposes, including religious uses, production of scents or for curing purposes against deadly ailments[23].

Nearly 3000 diverse essential oils have been depicted. Of these, around 300 are utilized monetarily in the seasoning and scents advertise. In any case, the high diversity in the chemical composition of aromatic plants shows a possibly significant problem for the scent producing industry[21].

1.3.1 Extraction methods of essential oils

Different methods are used in extraction, but the most common and prevalent methods are Steam Distillation, Cold Pressing and Solvent Extraction.

Thus, the chemical composition of the oil, both quantitative and qualitative, differs according to the extraction technique. For example, hydro-distillation and steam-distillation methods yield oils rich in terpene hydrocarbons. In contrast, the super-critical extracted oils contained a higher percentage of oxygenated compounds[24].

Some of the extraction methods are given below:

1.3.1.1 Hydro-distillation

The technique involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into a set up known as Clevenger apparatus. Plant material is heated in two to three times its weight of water with direct steam.

The distillation vessel is heated over heating mantle and the water vapour and oil are removed through a water cool condenser.

1.3.1.2 Hydro-diffusion

Hydro-diffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma of the plants[25].

1.3.1.3 Steam-Distillation

Steam distillation is the oldest and the traditional method of oil extraction[26]. In this method, pure aromatherapy oils extracted yield pristine oil, free from impurities. The process works by placing plant material in a container while steam is passed through it. Heat from the steam opens pockets of plant containing aromatic molecules and oils. When released, these molecules rise with the steam and pass through a closed system. The aromatic steam is then passed through a cooling process and distilled with cold water. During this process, the essential

oils condense and transform into liquid state[27]. The liquid mixture is separated later into two-essential oils and aromatic water or hydrosol[28]. Steam distillation takes into account a variety of things, including the pressure of steam passed through plant material, the coolant used and the temperature of the closed system during production of oil etc.,[29].

An oil's quality and purity are based on all these factors and the skill of the distiller. Reputed distillers' oils are rated high owing to the quality and purity of their extracts[30].

1.3.1.4 Cold-pressing

This method is used to extract oils from the citrus family of fruits where oils are produced from the rind of fruits like tangerines, grapefruits, lemons, oranges and others[31]. Though they are only known as expressed oils, they are classified under essential oils due to their high therapeutic value. Using mechanical pressure, oils are forced out of the fruits in juice form. Since the juicy form of oils contain a lot of water, a separation process is carried out to separate oils from water. One downside to this method is that cold-pressed oils spoil quickly than other oils. Therefore, it is recommended that these oils are bought in small quantities and refilled whenever required[32].

1.3.1.5 Solvent Extraction

Some plant material cannot tolerate heat (in steam form) or be subjected to cold-pressing. When they are subjected to any such method, the oil thus produced may be contaminated or impure in quality[33]. To avoid

this, some plants like Jasmine, Rose, Orange Blossom (Neroli), Tuberose and Oak are extracted through solvents. Solvents such as ethanol, ether, methanol, hexane, alcohol, and petroleum are used to extract essential oils[34,35]. This process works by passing plant materials through hydrocarbon solvents. The solvent mixture is then filtered and distilled in low pressure to produce essential oils[35]. A downside to this method is that, sometimes, solvent residues remain in the oils, which can cause allergic reactions in certain individuals.

1.3.1.6 Microwave assisted process (MAP)

The MAP process uses microwave to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants[36].

This technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction[37].

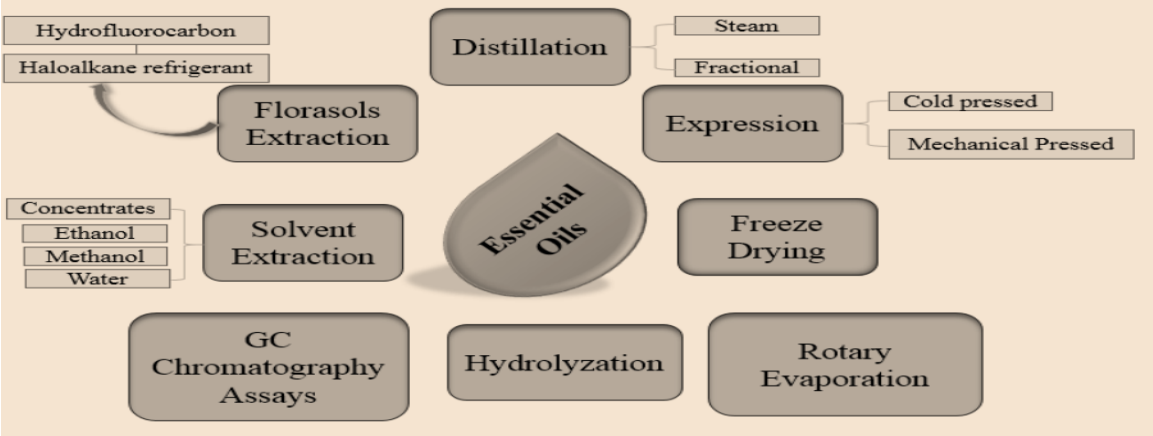


Figure 1.1: Several methods for extracting essential oils form different plants

Other forms for acquiring essential oils incorporate effleurage and maceration with the recent systems employing extraction with supercritical liquids or solvents. Maceration can be utilized when the yield from distillation is low, while effleurage and solvent extraction is reasonable for sensitive, costly and thermally unstable materials[38]. Generally, essential oils are acquired through water distillation or steam distillation from various organs of the plant, including the entire plant or simply the fruits, wood, leaves, roots, bark, or seeds[18].

1.3.2Components of Essential Oils

Every single oil normally has more than a hundred components, but the number of component changes depending on the oil in question. However, the most important active compounds are included in two chemical groups: terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids. These two groups originate from different precursors of the primary metabolism and are synthesized through separate metabolic pathways. Like all organic compounds, essential oils, are made up of hydrocarbon molecules and can further be classified as terpenes, alcohols, esters, aldehydes, ketones and phenols etc.

Other components of essential oils which include Oxygenated compounds, phenols, alcohols, monoterpene alcohols, sesquiterpene alcohols, aldehydes, Ketones, esters, lactones, coumarins, ethers[39].

1.3.2.1 Terpenoids

Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers[40]. Within terpenoids, the most important components of essential oils of the majority of plants are found in the monoterpenoid and sesquiterpenoid families[41].

Monoterpene/monoterpenoid compounds are found in nearly all essential oils and have a structure of 10 carbon atoms with at least one double bond. Examples of monoterpenes and monoterpenoids include geraniol, terpineol (present in lilacs), limonene (present in citrus fruits), myrcene (present in hops), linalool (present in lavender) or pinene (present in pine trees)[42]. They react readily to air and heat sources and for this reason, citrus oils do not last long, since they are high in monoterpene hydrocarbons and have a quick reaction to air, and are readily oxidized[43].

sesquiterpenes consist of 15 carbon atoms with the molecular formula $C_{15}H_{24}$ and have complex pharmacological actions such as chamazulene, which is found in German chamomile[44]. Oxygenated groups are the most common type of functional group found in essential oils. As with terpenes, it is important to understand the different classes of oxygenated compounds that exist, as each class contributes its own unique potential health benefits[43].

1.3.2.2 Esters

Esters are compounds that result from the reaction of an alcohol with an acid (esterification) and are very common and are found in a large number of essential oils. They are calming and relaxing and tend to be fruity with therapeutic effects, which include being sedative and antispasmodic. Linalyl acetate, a well-known ester which is found in bergamot, clary sage, lavender as well as petit grain with geraniol acetate found in sweet marjoram are one of the beneficial compounds in essential oils[45]. Some esters also have anti-fungal and anti-microbial properties like the anti-fungal properties in geranium oil[46].

1.3.2.3 Ketones

Ketones are sometimes mucolytic and neuro-toxic when isolated from other constituents. They stimulate cell regeneration, promote the formation of tissue, and liquefy mucous. They are helpful with conditions such as dry asthma, colds, flu, and dry cough and are largely found in oils used for the upper respiratory system Essential oils that contain Ketones include Clary sage, Hyssop, Idaho, Tansy, Rosemary and Western red cedar[47].

1.3.3 Classification of essential oils Based on Aroma

Essential Oils can also be classified based on aroma/smell of the oil. This classification of oils can be categorized into Citrus, Herbaceous, Medicinal/Camphorous, Floral, Resinous oils and Woody, Earthy, Minty and Spicy oils[48].

1.3.3.1 Citrus Oils

Essential oils that have a distinct citrus flavor fall into this category. Bergamot, Grapefruit, Lemon, Lime, Orange and Tangerine are some of the plants that produce Citrus oils[49].

1.3.3.2 Herbaceous Oils

Oils that are extracted from plants, which are otherwise most useful herbs. These oils can be extracted from plants such as Basil, Chamomile, Melissa, Clary Sage, Hyssop, Marjoram, Peppermint and Rosemary are some of this kind[50].

1.3.3.3 Camphoraceous Oils

These are essential oils with a particular healing property. Some of these essential oils are obtained from Cajeput, Tea Tree, borneol-like, earthy and mugwort-like and rosemary- like, with a fruity, dried plum-like background[48].

1.3.3.4 Floral Oils

Oils made from floral parts or which carry the floral essence of plants fall under this group. Geranium, Jasmine, Lavender, Rose, Neroli, Chamomile, Ylang-Ylang etc. are some of the plants that produce these oils[51].

1.3.3.5 Woody Oils

Essential oils that are woody in aromas or extracted from the barks and other woody parts of plants. Cedar wood, Cinnamon, Cypress, Juniper Berry, Pine and Sandalwood etc. produce such oils[52].

1.3.3.6 Earthy Oils

Essential oils that have a distinct earthy aroma or are extracted from plants' roots and other earthy parts. Angelica, Patchouli, Vetiver and Valerian produce some of these oils[53].

1.3.3.7 Spicy Oils

Oils extracted from spices or spicy plants such as thyme, cloves, Aniseed, Black Pepper, Cardamom, Cinnamon, Coriander, Cumin, Ginger and Nutmeg[54].

1.3.4 Applications of essential oils

The utilization of essential oils is to a great degree assorted relying upon the source, quality, extraction strategy, and so on. Essential oils have demonstrated modern applications in the fabricate of fragrances, beautifying agents, soaps, shampoos, or cleaning gels. Another fascinating part of these oils is their potential as medicines in aroma based therapies or as carriers for drug delivery. Another major utilization of essential oils is in the agro food business, both for creating refreshments and for enhancing sensorial properties of food items.

1.3.4.1 Beauty care products

The utilization of essential oils in the beauty care products, detergent, soap and scent industry is of great concern from a financial point of view. The generation of essential oils for preparation of perfumes and scents has expanded enormously on a global level and simultaneously collection of these aromatic plants. Salvia, lavender and thyme species

are highly consumed to produce these aromatic yields. Sufficient determination of the crude source material and method employed for the extraction are fundamental components for enhancing the nature of the volatile yields.

1.3.4.2 Pharmaceutic and therapeutics

Essential oils are utilized as a part of pharmaceutics for their potential as therapeutic agents[55]. This is particularly the instance of the essential oils from peppermint (*Mentha piperita*), sage (*Salvia officinalis*), anise (*P. anisum*), eucalyptus (*E. globulus*), clove (*S. aromaticum*), and tea tree (*M. alternifolia*). These oils are utilized as an expectorant for treating bronchitis and cough (eucalyptus essential oil), as antibacterial agents (sage, clove and tea tree oil), as a decongestant of the respiratory tract (peppermint oil), and as a carminative (anise oil). Moreover, clove oil is utilized as a part of dentistry for its antimicrobial and pain-relieving properties while tea tree oil is utilized in the field of dermatology (anti-acne drug) as it possesses antimicrobial properties against Gram-positive microbes[56]. In pharmaceutics, essential oils are used to enhance sensory attributes of pharmaceutical drugs. The prime application of essential oils in pharmaceutics is aromatherapy. Different strategies can be utilized to administer essential oils isolated from different plant sources. The term "aromatherapy" was coined by Gattefossé in the 1920s and was restored by Maury in the 1960s. Since the 1980s, its prominence has expanded relentlessly. In current times, it is genuinely entrenched in

Germany, New Zealand, Australia, Canada, France, Switzerland, the United States, and United Kingdom[57]. The most well-known application strategy for essential oils is local application of these oils along with some carrier oils after being diluted to a concentration. They can likewise be breathed in the steaming water after addition of few drops or by methods for a humidifier or atomizer. Moreover, they can be used as balms, compresses and creams. In any case, oral utilization of essential oils through encapsulation or other customized discharge techniques has been presented as a successful strategy for getting the helpful impacts of these essences[58].

1.3.4.3 Agro food

Essential oils are utilized as a part of a wide range of food products, for example, confectionery sodas, and alcoholic drinks. Apart from being consumed as a seasoning material, they are also utilized as a part of agriculture and food industry for their antimicrobial, antiviral, antifungal, insecticidal, nematocidal, and anticancer attributes[59,60]. Due to these reasons, their use as preservatives in food and as an agent has been indicated[61]. Numerous essential oils have antibacterial as well as anti-oxidative properties[62]. yet their application as additives in food items requires a detailed learning of their properties, including the inhibition of the microorganisms on target, the particular method of activity, their antibacterial effectiveness, and the possible interaction impact on their antibacterial attributes with food components[18].

1.3.5 Biological Activity of Essential Oils

1.3.5.1 Antibacterial Activity

An important feature of essential oils is their hydrophobicity, which allows them to partition into lipids of the cell membrane of bacteria, disrupting the structure, and making it more permeable[63]. This can then cause leakage of ions and other cellular molecules[64]. 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[65].

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[66]. Carvone is also ineffective against the OM and does not affect the cellular ATP pool[67].

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 β-myrcene, α-thujone, or geranyl acetate, had much weaker activity, while volatile oils containing terpene hydrocarbons were usually inactive[68,69].

Generally, essential oils with a high level of phenolic compounds, such as carvacrol, eugenoland thymol have important antibacterial activities[68]. 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[63,65]. Evidently, the structure of essential oils affects their mode of action concerning their antibacterial activity[70].

The presence of hydroxyl group in the phenolic compounds, such as carvacrol and thymol, is extremely vital for the antimicrobial activity of essential oils[64,70].

The relative position of the phenolic hydroxyl group on the aromatic ring of phenols does not appear to influence the intensity of the antibacterial activity. For example, the activity of thymol against *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* appears to be comparable to that of carvacrol[64,71].

It has been shown that carvacrol and thymol act differently against Gram-positive and Gram-negative species[70]. Thymol, eugenol, and carvacrol have exhibit antimicrobial activity against a broad spectrum of bacteria including *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus*, and *Helicobacter pyroli*[72].

Many other compounds also have valuable antibacterial properties including certain alcohols, aldehydes, and ketones, monoterpene

(geraniol, linalol, menthol, terpineol, thujanol, myrcenol, citronelîaî, neral, thujone, camphor, carvone, etc.), phenylpropanes (cinnamaldehyde), and monoterpenes (γ-terpinene, *p*-cymene). Among these compounds, carvacrol is the most active. Since is non-toxic, it is used as a preservative and food flavoring in drinks, sweets, and other preparations.

Noteworthy that essential oils are more active against Gram-positive than Gram-negative bacteria[73,74]. 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[73].

The antibacterial activity of essential oils related to their chemical composition, the proportions of volatile molecules, and their interactions[70,74]. Antagonism is observed when the effect of one or both compounds is less important when they are tested together than when used individually[75]. A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects[76].

Literature data suggests that the use of the whole essential oil provides an effect which is greater than that of the major components used together[21]. This suggests that minor components are essential for activity and may have a synergistic effect.

A synergistic effect has been observed when a combination of 1,8-cineole and aromadendrene is used against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococcus *faecalis*[55]. 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[77].

Enhanced antifungal activity was observed when using combinations (1:5, 1:7, and 1:9) of essential oils of *S. aromaticum* (clove) and *Rosmarinus officinalis* against *C. albicans*[78]. Lambert et al. (2001) reported that a combined, carvacrol and thymol showed additive effects against *S. aureus and P. aeruginosa*.

Thesynergistic effects of cinnamaldehyde/thymol orcinnamaldehyde/carvacrol against *S. typhimurium* has been explained as follows : 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[79]. 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[80].

Moreover, essential oils have also revealed to be effective on the inhibition of growth and reduction in numbers of the more serious food-borne pathogens, such as *Salmonella* spp., *E. coli*, and *Listeria monocytogenes*[77].

1.3.5.2 Antioxidant Activity

It has been shown that the antioxidant properties of essential oils depend on its composition. Those phenolics and secondary metabolites with conjugated double bonds usually exhibit substantial antioxidative effect[81].

Generally essential oils are dominated by oxygenated monoterpenes such as alcohols (*Achillea filipendulina*), aldehydes (*Galaganiafragrantissima*), ketones (*Anethumgraveolens, Artemisia rutifolia, Hyssopusseravschanicus, Mentha longifolia,* and *Ziziphoraclinopodioides*), andesters (*Salvia sclarea*). *Artemisia absinthium* and *Artemisia scoparia* predominantly contain monoterpene hydrocarbons, whereas phenolic terpenoids, such as thymol or carvacrol, characterize *Origanumtyttanthum* and *Mentha longifolia*essential oils, which would explain why both plants exhibited generally the strongest antioxidant activity. Thymol and carvacrol are major components in *Origanumtyttanthum*, these components are responsible for the antioxidant activity of several other essential oils, such as *Mentha longifolia* and *Thymus serpyllus*[82].

It has been reported that the essential oils of cinnamon, nutmeg, clove, basil, parsley, oregano, and thyme are characterized by the most important antioxidant properties[78]. Thymol and carvacrol are the most active compounds. Their activity is related to their phenolic structure.

Phenolics of essential oils have redox properties and, thus, play an important role in neutralizing free radicals and also in peroxide decomposition[21]. The antioxidant potential of essential oils is also attributed to certain alcohols, ethers, ketones, aldehydes and monoterpenes like linalool, 1,8-cineoIe, geranial/neral, citronellal, isomenthone, menthone, and some monoterpenes: α-terpinene, β-terpinene and α-terpinolene[78].

Those essential oils which possess significant 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. Such diseases may result from cellular damage caused by free radicals[78]. Several essential oils exhibited hepatoprotective effect in ageing mammals[83].

Wedeliachinensis essential oil seems to increase 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)[84].

1.3.5.3 Anti-inflammatory Activity

The inflammatory response is a normal protective response induced by tissue injury or infection and is initiated 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-α (TNF-α). It also stimulates the activity of several enzymes (oxygenases, nitric oxide synthases, peroxidases, etc.), as well as the arachidonic acid metabolism.

In recent years essential oils have been used in clinical settings to treat inflammatory diseases, such as rheumatism, allergies, or arthritis[80].

It has been shown that *Melaleuca alternifolia* essential oil has a considerable anti-inflammatory activity[81,82,83]. This activity is correlated with its major compound: α-terpineol[85]. The active compounds act by inhibiting the release of histamine or reducing the production of inflammation mediators. The essential oil from Geranium is another example[80]. Linalool and linalyl acetate showed anti-inflammatory activity on oedema of paw-induced mouse carrageenan[86].

Essential oil from *Torreya nucifera* which mainly contain limonene, δ-3-carene, and α-pinene, has 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, bio generated both from pathways of arachidonic acid metabolism[87].

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.

1.4 Gas Chromatography–Mass Spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical tool that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample.

Gas-liquid chromatography separates 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[88].

The presence of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952.

1.4.1 Principle of GC-MS

The gas chromatography-mass spectrometry machine consists of two major components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility[89], by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column[90]. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed[89].

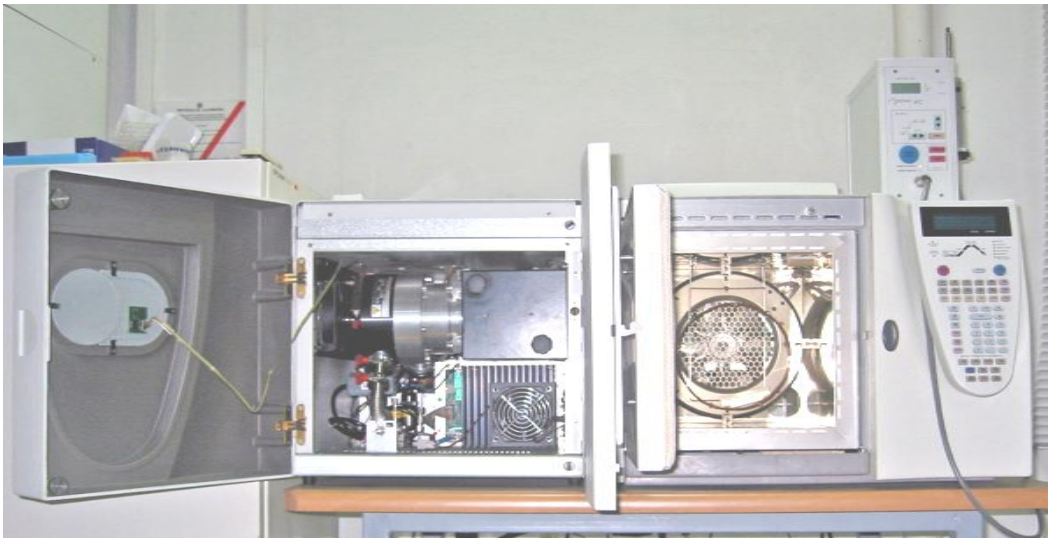


Figure 1.2 :The insides of the GC-MS, with the column of the gas chromatography on the right

The gas chromatography-mass spectrometry instrument is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute from the column at different times (the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detect the fragments[91].

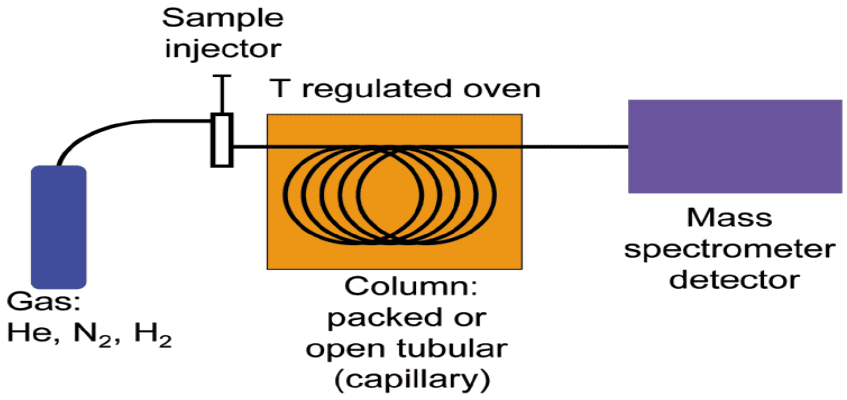


Figure 1- 3: TheGC-MS schematic

When GC and MS are used together, GC-MS allows a much finer degree of substance identification than used separately. It is not possible to

make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute[92]. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error.

1.5 The target plant species

1.5.1 Caraway (*Carumcarvi*L.)

-Scientific classification:

Kingdom: Plantae

Clade: Tracheophytes

Order: Apiales

Family: Apiaceae

Genus: *Carum*

Species: ***C. carvi***

*Carumcarvi*L. is a plant in the family Apiaceae. It is one of the earliest cultivated herbs in Asia, Africa and Europe. *Carumcarvi* is used traditionally as a carminative for stomach disorders, diarrhea, and colic, it

is also used in veterinary medicine[93]. This plant has a long history of use as a household remedy especially in the treatment of digestive complaints where its antispasmodic action soothes the digestive tract and its carminative action relieves bloating caused by wind and improves the appetite[94,95].

Seeds of *Carumcarvi* can be chewed raw for the almost immediate relief of indigestion and can also be made into infusions. The seed is also used in the treatment of bronchitis and are an ingredient of cough remedies. This plant is especially useful for children and for mothers for increasing breast milk. A tea made from the seeds is a pleasant stomachic and carminative, it has been used to treat flatulent colic[96,97]. The seed is used in Tibetan medicine where it is considered to have an acrid taste and a heating potency. It is used to treat failing vision and loss of appetite[98].

Two components isolated from *Carumcarvi*-Carvone and limonene - were found to induce the detoxifying enzyme glutathione-*S*-transferase in several mouse target tissues[99]. Carvone, specifically, was found to be responsible for the high enzyme-inducing and related anticarcinogenic activities.*Carumcarvi*showed a dose-dependent antiulcerogenic activity, associated with a reduced acid output and an increased mucin secretion as well as an increase in prostaglandin E_2 release and a decrease in leucotrienes[100]. Moreover, antibacterial and potent antioxidant activities (through hydroxyl and lipid peroxide superoxide radicals inhibition) have been demonstrated for *Carum carvi*[101].

In clinical studies, phytotherapeutic combinations containing caraway oil exhibited beneficial effects on gastro-intestinal symptoms, such as dyspepsia and functional dyspeptic syndrome[102,103]. None of these studies clearly defined the effects of caraway on gastro-intestinal motor activities, as the clinical dyspeptic syndrome is a complex pathophysiological process in which alteration of motility, alteration of sensation as well as psychosocial factors are involved in the generation of dyspepsia[104]. Caraway oil inhibited the motor activities of SMC of the gallbladder, stomach, trachea and ileum[103,105]. Recently, *in vitro* and *in vivo* experiments demonstrated antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities for *C. carvi* seeds[106].

Figure 1.4:*Carumcarvi*L. Seeds.

1.5.2 Coriandrum sativum L. (Coriander)

-Scientific classification:

Kingdom: Plantae

Order: Apiales

Family: Apiaceae

Genus: *Coriandrum*

Species: ***C. sativum***

Coriander (*Coriandrum sativum* L.) is an annual herb in the family Apiaceae. Coriander is native to south-western parts of Asia and North Africa. This plant grows up to 50 cm tall. The leaves are variable in shape, broadly lobed at the base of the plant and slender and feathery higher on the flowering stems. The flowers are borne in small umbels, white or very pale pink. The fruit is a globular dry schizocarp which commonly called seed[107]. Being known as aromatic, medicinal and condimental plant. The whole aerial parts of Coriander, specially the leaves, present essential oil with an unpleasant odour, while the dry fruits are rich in essential oil and have both odor and taste very pleasant. So, they have been widely used in food industry to prepare liqueur[108].

Coriander has a long history of traditional uses including: antioxidant, hypoglycemic, hypolipidemic, antibacterial, anti-mutagenic potential[109], stimulant, diuretic and diaphoretic activity, so it is used in disorders of respiratory, digestive and urinary systems in the Indian traditional medicine. It also has been indicated for a number of medical problems in Iranian traditional medicine such as convulsion, dyspeptic complaints, insomnia, loss of appetite[110], to cure ulcer, inflammation, spasm and acts as an expectorant, protects and soothes liver. Coriander fruits have a

health-supporting reputation that is high on the list of the healing spices. They are used in medicine as a carminative, diuretic and also used in the preparation of many house hold medicines to cure bed cold, seasonal fever, nausea, and stomach disorders[109].

The use of coriander dated back to around 1550 BC, and it was one of the oldest spice crops in the world. The powdered fruit, fluid extract and oil are chiefly used medicinally as flavouring to disguise the taste of active purgatives and correct their griping tendencies. The whole or ground seed (fruit) was an ingredient of pickling spices, also used to flavor various commercial foods, particularly, to prepare some instant soups and dishes, in many cakes, breads and other pastries, alcoholic beverages, frozen dairy desserts, candy, and puddings. The fruit essential oil was a common ingredient in creams, detergents, surfactants, emulsifiers, lotions, and perfumes[111]. However, seeds were applied locally to alleviate swelling and pains. Externally, powdered green coriander was used to alleviate burning sensation and pain in diseases like inflammation caused by erysipelas and lymphadenopathy. Decoction of green coriander was used in stomatitis. Nasal drops of green coriander act as a hemostat and thus stop bleeding in epistaxis. Juice or decoction of green coriander was used in conjunctivitis. The seeds were included in many prescriptions as carminative and for the treatment of fever, diarrhea, vomiting and indigestion. Coriander was used internally as tonics. It was also used for syncope and memory loss.

Fresh juice of leaves was used as gargle in sore throat and stomatitis. Paste of leaves were locally applied for swellings and boils and were applied over forehead and temples for headache[112].

Figure1.5: *Coriandrum sativum*L. Seeds.

1.5.3 *Hibiscus sabdariffa* L. (Roselle)

-Scientific classification:

Kingdom: Plantae
Order: Malvales
Family: Malvaceae
Genus: *Hibiscus*
Species: ***H. sabdariffa***

Hibiscus (*H.*) *sabdariffa* Linn. is a plant in the family Malvaceae. It is commonly termed as "*Roselle*" or "*Red sorrel*" in English. This plant is widely grown in Central and West Africa, South-East Asia, and in parts of West India, Jamaica and Central America[113]. Its native distribution is uncertain, some believe that is from India or Saudi Arabia[114], while Murdock[115] showed evidence that *Hibiscus sabdariffa* L. was domesticated by the black populations of western Sudan (Africa) before 4000 BC. It has been traditionally used as antiseptic, astringent, cholagogue, aphrodisiac, demulcent, diuretic, emollient, purgative,

digestive, stomachic, sedative and tonic. The plant was also reported to be used for high blood pressure, liver diseases, fever, ulcers, abscesses and anemia[116]. In Egypt, preparations from the calyces have been used to treat cardiac andnerve diseases and also to increase the production of urine (diuresis). In Egypt and Sudan, an infusion of ‘‘Karkade’’ calyces are also used to help lower body temperature[117]. In Guatemala it is used for treating drunkenness[118]. In North Africa, calyces’ preparations are used to treat sore throats and coughs, as well as genital problems, while the emollient leaf pulp is used for treating external wounds and abscesses[119]. In India, a decoction from the seeds is used to relieve pain in urination and indigestion. In Brazil, the roots are believed to have stomachic and emollient properties. In Chinese folk medicine, it is used to treat liver disorders and high blood pressure[118]. In Iran, sour hibiscus tea is reportedly a traditional treatment for hypertension[120], while in Nigeria the decoction of the seeds is traditionally used to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality[121].

The seed oil of *H. sabdariffa* revealed the presence of cholesterol, campasterol, stigmasterol, ß-sitosterol, α-spinasterol, and ergosterol[113] (Ali et al., 2005). Besides, the seed oil contains steroids, tocopherols, unsaturated fatty acids (70%) such as linoleic acid, cellulose, pentosans, and starch[116]. Seed is an excellent source of proteins (25.2%) they also contain protease inhibitors, phytic acid and gossypol[122]. The infusion

prepared from the calyx of *Hibiscus sabdariffa* has been shown to decrease both systolic and diastolic blood pressure in patients from 30 to 80 years of age with diagnosed hypertension[123].

Figure 1.6: *Hibiscus sabdariffa* L. Seeds.

1.5.4*Lagenaria siceraria*L.

-Scientific classification:

Kingdom:	Plantae
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	*Lagenaria*
Species:	***L. siceraria***

Lagenaria siceraria **Standley** is a common fruit vegetable in the family Cucurbitaceae. The fruit has many uses in ethnomedicine, it is also valued as a nutrient. Fruit is considered as a good source of carotene, vitamin C and B complex [124,125].

Preliminary phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, and steroids[126,127]. The plant also contains polyphenols, cucurbitacins and fibre[128].Fruit is used as diuretic, immunosuppressant, cardio-protective and cardio-tonic[129,130].

It has been reported that *Lagenaria siceraria* possesses antioxidant[131,132], antidepressant[133] and hepatoprotective properties[134]. Seeds which contain leganin and a ribosome - inactivating protein has anti- HIV, antiproliferative and antitumor potential[135]. Seeds are also used traditionally against dropsy and intestinal worms Seeds are also valuable nutrient containing amino acids, minerals and vitamins[136,137].

Figure 1.7: *Lagenaria siceraria*L. (bottle gourd) seeds

1.5.5 *Nerium oleander*L.

-Scientific classification:

Kingdom:	Plantae
Order:	Gentianales
Family:	Apocynaceae
Genus:	*Nerium*
Species:	***N. oleander***

Nerium oleander is an evergreen small tree in the family Apocynaceae. The centre of origin of this species is the Mediterranean region and the

Indo-Pakistan subcontinent[138]. This draught-tolerant plant is widely grown in tropics, subtropics and temperate regions as an ornamental plant[139].

For many years *Nerium oleander* has been mentioned in ancient texts and folklore medicine. All parts of the plant have been used in ethnomedicne. The leave juice, in small doses, is used against eye diseases and snake bite. Bark is expectorant, diuretic, emetic and heart tonic.[138] Root is used for leprosy, ulcer, hemorrhoids and cancer[140,141]. Roots showed digoxin-like cardiac activity beside antimicrobial effect[142].Leaves are applied externally for scabies. The flowers are diuretic, cardiotonic, expectorant and emetic[143].Leaves are also used for baldness and diabtes[144]. The stem extracts showed antimicrobial potency[145].The antiviral[146], anti-inflammatory[146-148], anticancer[146,149], antimicrobial[150], larvicidal[150-154], immunomodulating[148], antidiabetic[155-157] and diuretic[148] activities of *Nerium oleander* have been reported.

Figure 1.8: *Nerium oleander* L.(Oleandrin) seeds

Aim of study

This study was aimed to:

-Extract oils from five medicinal plants (*Carum carvi*L., *Coriandrum sativum* L., *Hibiscus sabdariffa* L., *Lagenaria siceraria* and *Nerium oleander* L.).

-Analyze the extracted oils by GC-MS.

-Evaluate the antimicrobial activitغ of the target oils.

Chapter Two
Materials and Methods

2 Materials and Methods

2.1 Materials

2.1.1 Plant materials

*Carum carvi*L. *Coriandrum sativum* L. and *Hibiscus sabdariffa* L. seeds were purchased from Dakahlia Market in Egypt. The seeds of *Lagenaria siceraria*L. were collected from Nyala-western Sudan while *Nerium oleander* L. seeds were collected from Kordofan-western Sudan. The plants were identified and authenticated by direct comparison with reference herbarium samples. The plant material was shade - dried at room temperature and finally powdered.

2.1.2 Instruments

The GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness).

2.1.3 Test organisms

The targeted oils were screened for antimicrobial activity using five standardmicroorganisms,Gram +ve:*Bacillus subtilis* and *Staphylococcus aureus*;Gram -ve**:***Pseudomonas aeruginosa* and *Escherichia coli*; fungal strain**:***Candida albicans.*

2.2 Methods

2.2.1 Extraction of oil

Powdered seeds of target plants (400g) were exhaustively macerated with n-hexane. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

2.2.2 Gas Chromatography–Mass Spectrometry analysis

The target oils were 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. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program and other chromatographic conditions are shown below:

Table 2.1: Oven temperature program

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

Table 2.2: Chromatographic conditions

Spilt ratio	- 1.0
Column oven temperature	150.0°C
Injection temperature	300.0°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.

2.2.3 Antimicrobial assay

a) Preparation of bacterial suspensions

One mL aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 mL sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/mL. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms 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 solution and 0.02 mL volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 mL) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colonies forming units per ml suspension.

b) Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100mL of sterile normal saline, and the suspension were stored in the refrigerator until used.

c) Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ mL (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on

surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µL of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and recorded as average of two replicates.

Chapter Three
Results and Discussion

3Results and Discussion

This research was designed to study the oils of five potential medicinal plants (*Carumcarvi*L., *Coriandrum sativum* L., *Hibiscus sabdariffa* L., *Lagenariasicerariа*L. and *Nerium oleander* L.). The constituents of the oils have been characterized by GC-MS and the antimicrobial potential of the oils has been evaluated.

3.1 *Carumcarvi*L.

3.1.1 The GC-MS analysis of *Carumcarvi*L.

*Carumcarvi*L. oil was studied by GC-MS. The analysis showed thirteen constituents dominated by,9-octadecenoic acid (Z)- methyl ester (69.67%); 9,12-octadecadienoic acid (Z,Z)-methyl ester (12.07%) and 10-nonadecanone (5.84%)-Table 3.1.

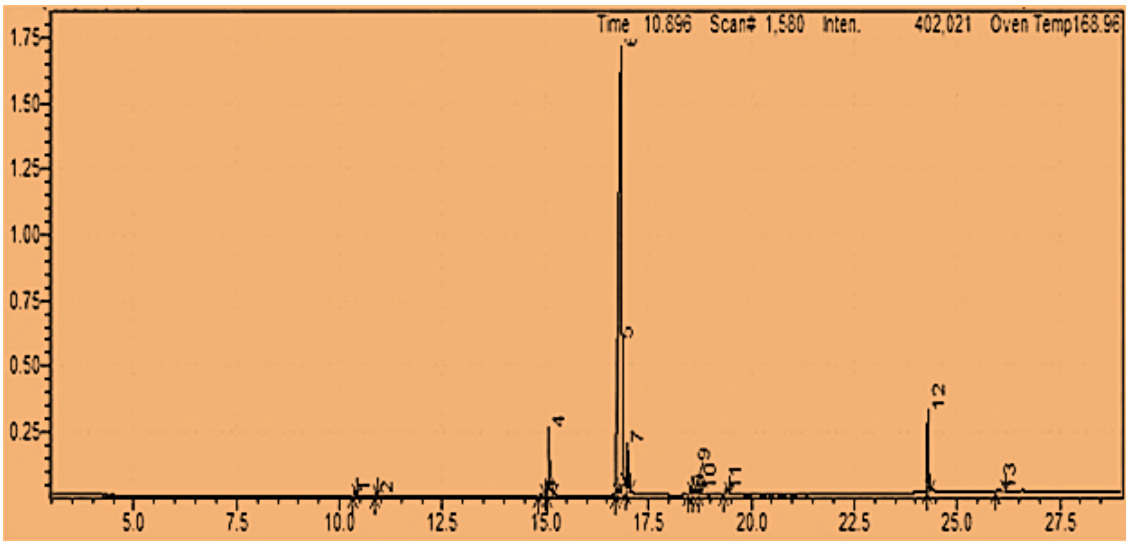


Fig 3.1: Total ions chromatograms of *Carumcarvi*L.oil

Table 3.1: Constituents of *Carumcarvi*L. oil

No.	Name	Ret.Time	Area%
1.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	10.366	0.23
2.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	10.884	0.12
3.	9-Hexadecenoic acid, methyl ester, (Z)-	14.898	0.30
4.	Hexadecanoic acid, methyl ester	15.083	5.39
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.736	12.07
6.	9-Octadecenoic acid (Z)-, methyl ester	16.846	69.67
7.	Methyl stearate	17.001	3.38
8.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	18.515	0.78
9.	8,11,14-Eicosatrienoic acid, methyl ester	18.618	0.62
10.	Eicosanoic acid, methyl ester	18.745	0.33
11.	Hexadecanoic acid, 2-hydroxy-, methyl ester	19.377	0.71
12.	10-Nonadecanone	24.294	5.84
13.	Stigmasterol	26.023	0.56

Major components of the oil are :

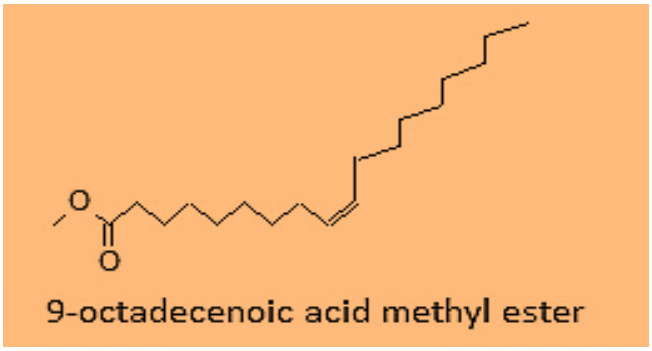
i) 9-Octadecenoic acid (Z)-, methyl ester (69.67%)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (12.07%)

iii) 10-Nonadecanone (5.84%)

iv) Hexadecanoic acid, methyl ester (5.39%)

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is presented in Fig 3.2. The peak at m/z 296, which appeared at R.T. 16.846, corresponds to $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z 265 corresponds to loss of a methoxyl function. The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is shown in Fig3.3. The peak at m/z 294(R.T. 16.736) corresponds to $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 corresponds to loss of a methoxyl group. The mass spectrum of 10-nonadecanone is displayed in Fig3.4. The signal at m/z 282(R.T. 24.294) is due to $M^+[C_{19}H_{38}O]^+$. The peak at m/z 251 accounts for loss of a methoxyl group. The mass spectrum of hexadecanoic acid, methyl ester is presented in Fig 3.5. The peak at m/z 270, which appeared at R.T. 15.083, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of a methoxyl function.



9-octadecenoic acid methyl ester

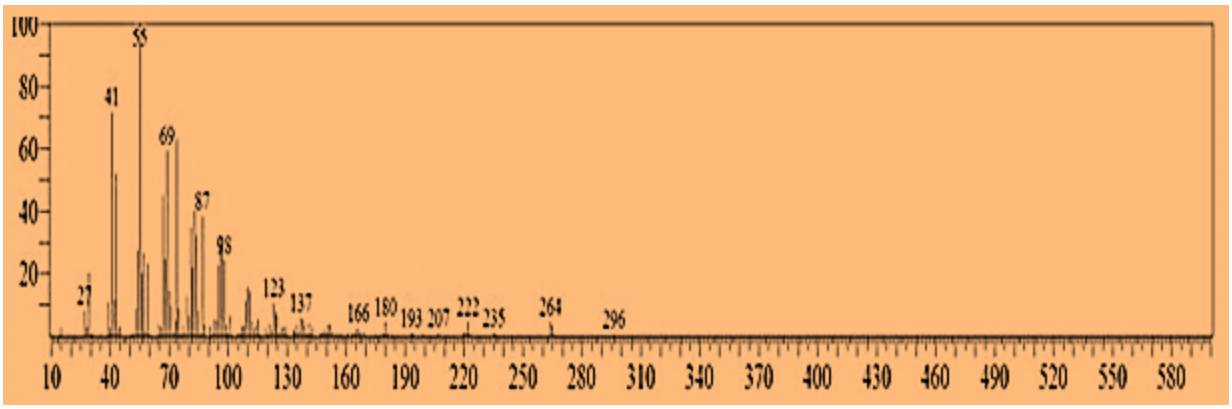
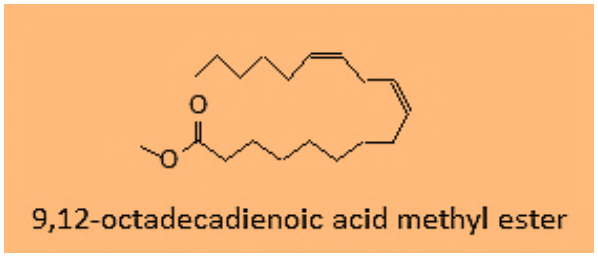


Fig3.2: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester



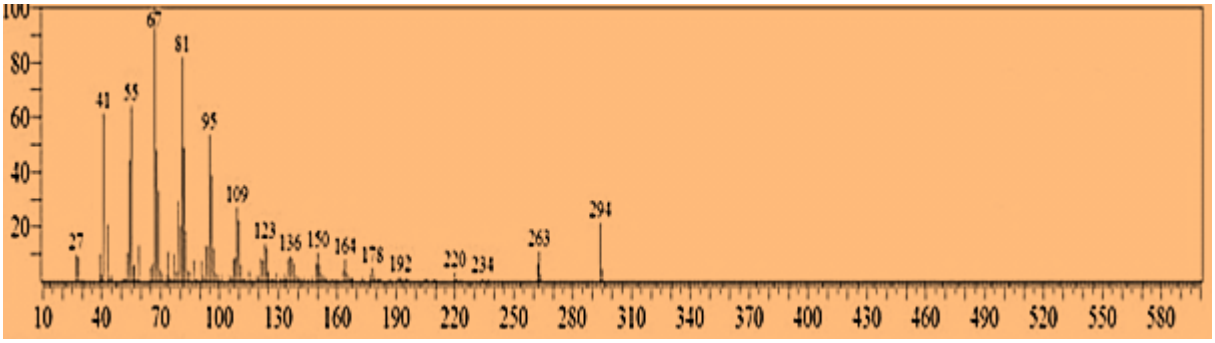
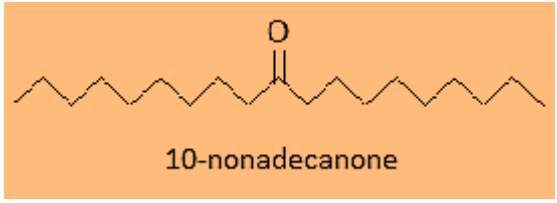


Fig. 3.3: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester



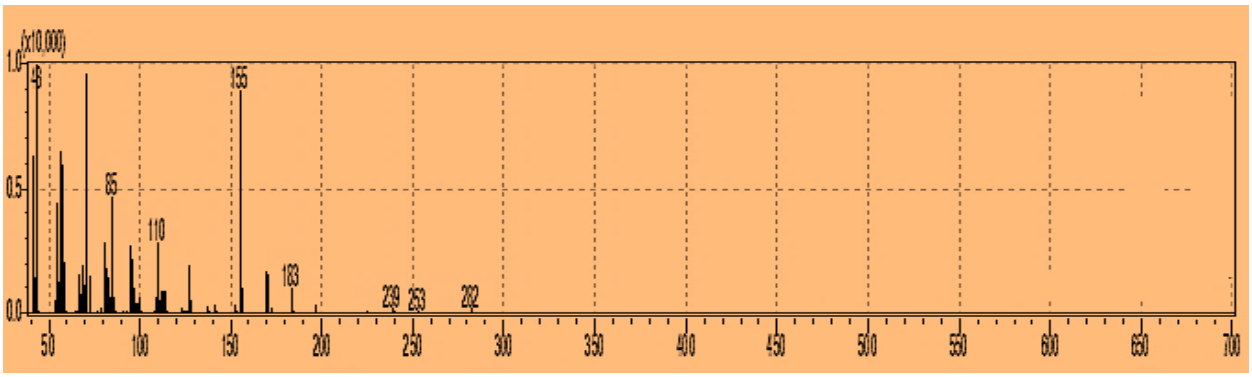
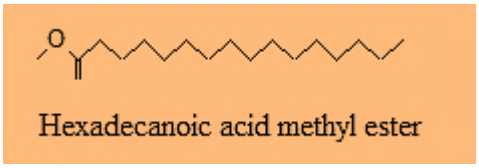


Fig3.4: Mass spectrum of 10-nonadecanone



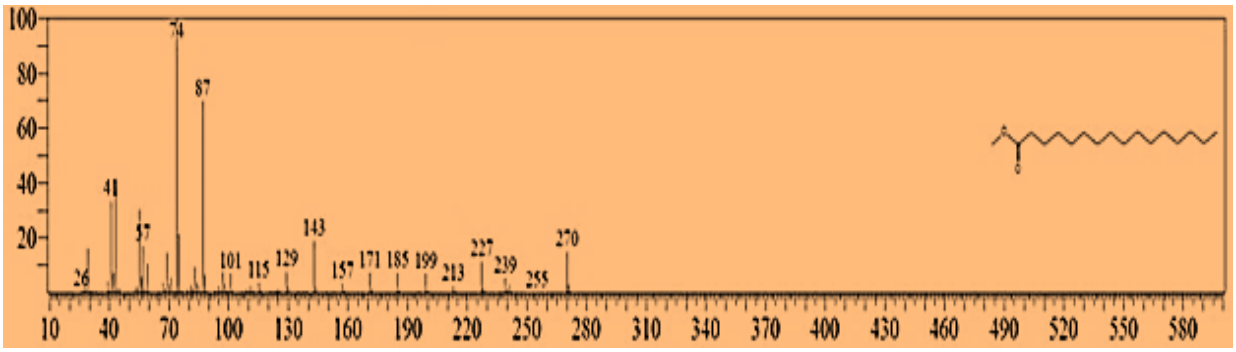


Fig 3.5: Mass spectrum of hexadecanoic acid, methyl ester

3.1.2 Antimicrobial activity

The oil from seeds of *Carumcarvi*was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are depicted in Table (3.2).

Results were interpreted as follows:(<9mm: inactive;9-12mm: partially active;13-18mm: active;>18mm: very active). Ampicilin, gentamicin

and clotrimazole were used as positive controls. The oil showed moderate activity against *Escherichia coli* and *Bacillus subtilis*, it also shown moderate anticandidal activity. However, it exhibited partial activity against *Staphylococcus aureus*.

Table 3.2: Antimicrobial activity of *Carumcarvi L.* oil

Sample	Sa	Bs	Ec	Pa	Ca
Oil(100 mg/mL)	12	15	15	--	15

Table 3.3: Antibacterial activity of standard antibacterial agents

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

Table 3.4: Antifungal activity of standard antifungal agent

Drug	Conc.(mg/mL)	Ca
Clotramizole	30	38
	15	31
	7.5	29

Sa.: *Staphylococcus aureus*

Bs.: *Bacillus subtilis*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Ca.: *Candida albicans*

3.2 *Coriandrum sativum* L.

3.2.1 The GC-MS analysis of *Coriandrum sativum* L.

The GC-MS analysis of *Coriandrum sativum* L. oil showed twenty constituents. The total ion chromatogram (TIC) is given in Fig. (3.6) while the constituents of the oil are outlined in Table 3.5.

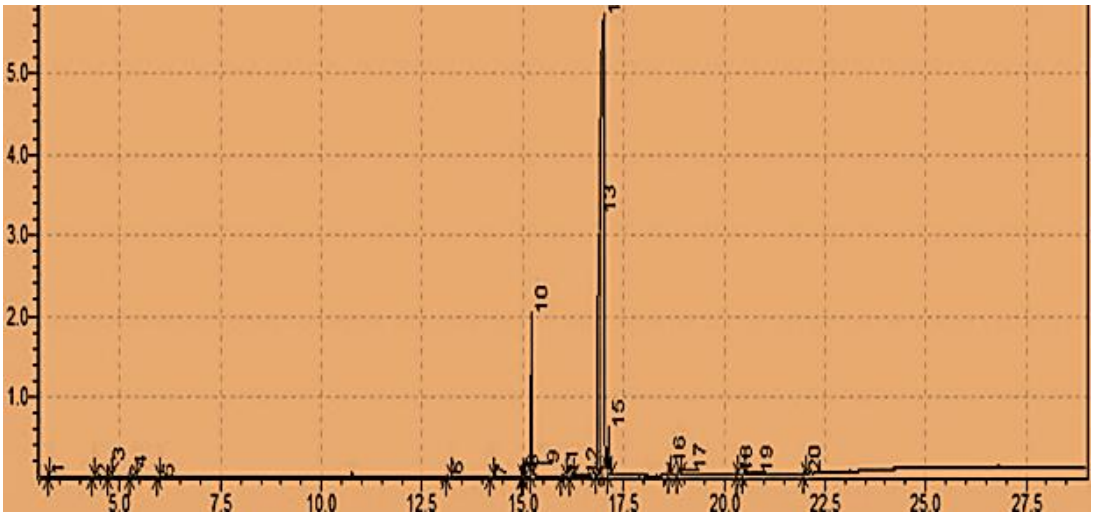


Fig3.6: Total ions chromatograms of *Coriandrum sativum* L. oil

Major components of the oil are discussed below:

i) 9-Octadecenoic acid (Z)-, methyl ester (72.22%)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (16.20%)

iii)Hexadecanoic acid, methyl ester (6.52%).

Table 3.5: Constituents of *Coriandrum sativum* L.oil

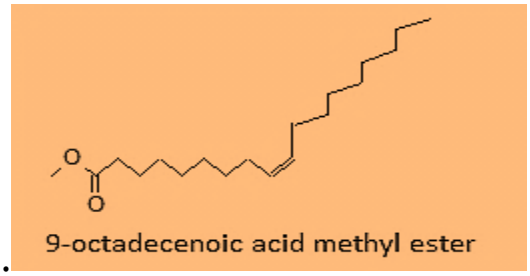
No.	Name	Ret.Time	Area%
1.	.alpha.-Pinene	3.225	0.02
2.	D-Limonene	4.339	0.02
3.	.gamma.-Terpinene	4.721	0.09
4.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.261	0.60
5.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	5.957	0.03
6.	Methyl tetradecanoate	13.129	0.09
7.	Pentadecanoic acid, methyl ester	14.200	0.05
8.	7-Hexadecenoic acid, methyl ester, (Z)-	14.991	0.30
9.	9-Hexadecenoic acid, methyl ester, (Z)-	15.014	0.66
10.	Hexadecanoic acid, methyl ester	15.214	6.52
11.	cis-10-Heptadecenoic acid, methyl ester	15.981	0.12
12.	Heptadecanoic acid, methyl ester	16.190	0.05
13.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.900	16.20
14.	9-Octadecenoic acid (Z)-, methyl ester	17.015	72.22
15.	Methyl stearate	17.134	1.93
16.	cis-11-Eicosenoic acid, methyl ester	18.645	0.65
17.	Eicosanoic acid, methyl ester	18.868	0.22
18.	13-Docosenoic acid, methyl ester, (Z)-	20.305	0.07
19.	Docosanoic acid, methyl ester	20.484	0.09

20.	Tetracosanoic acid, methyl ester	21.983	0.07

The EI mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is presented in Fig. 3.7. The peak at m/z 296, which appeared at R.T. 17.015, in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$. The signal at m/z 265 corresponds to loss of a methoxyl function

Fig (3.8) demonstrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester**.** The signal at m/z 294(R.T. 16.900) is due to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 accounts for loss a methoxyl group.

The mass spectrum of hexadecanoic acid, methyl ester is presented in Fig 3.9. The signal at m/z 270(R.T. 15.214) corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 is due to loss of a methoxyl .



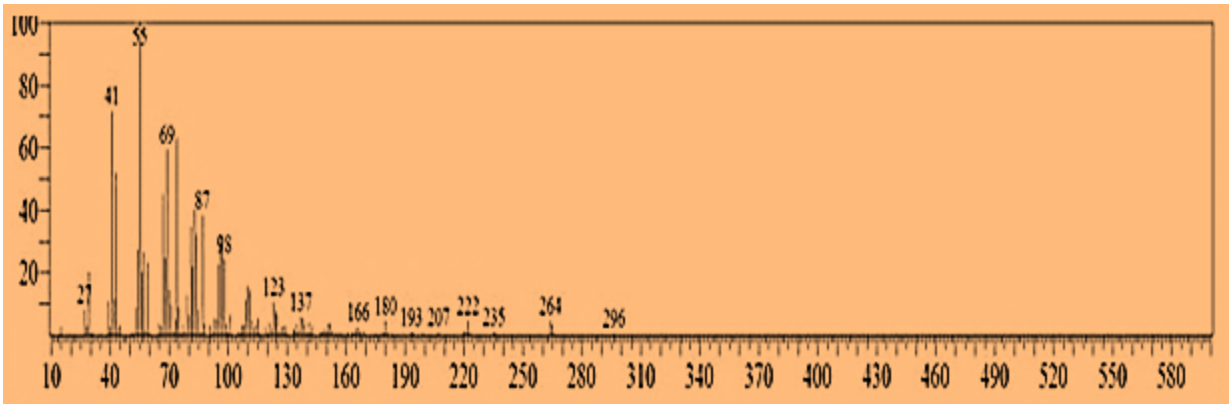
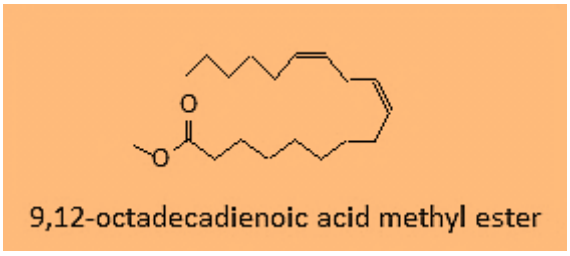


Fig. 3.7: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester



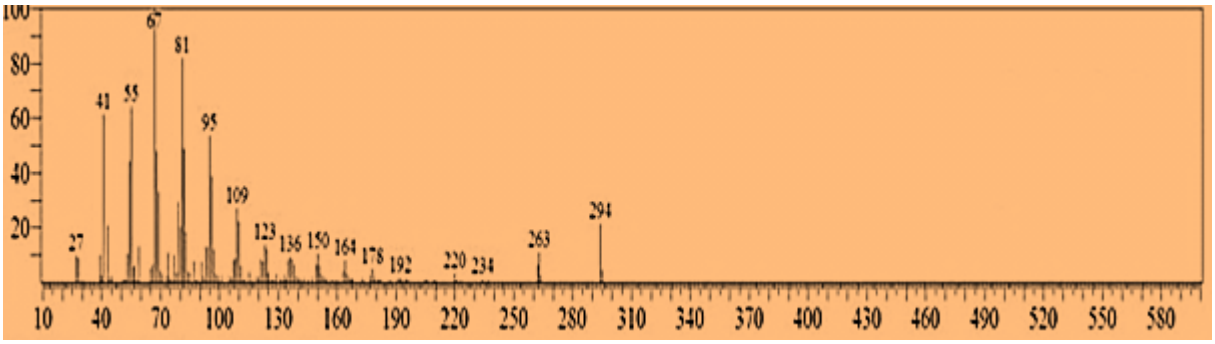


Fig3.8: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

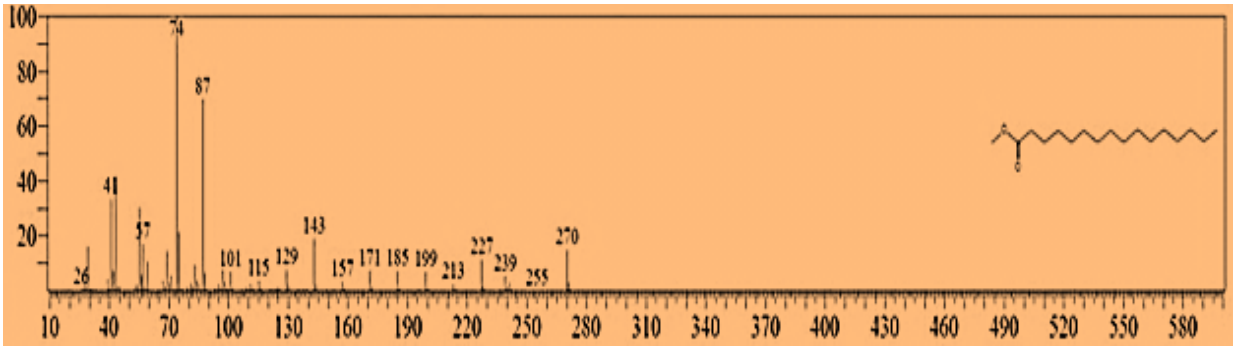


Fig 3.9: Mass spectrum of hexadecanoic acid, methyl ester

3.2.2 Antimicrobial activity

The oil was evaluated for antimicrobial activity. the averages of diameters of the growth inhibition zones are shown in Table 3.6.

Table 3.6: Inhibition zones(mm/mg sample)

Type	Sa	Bs	Ec	Pa	Ca
Oil(100mg/mL)	11	14	14	11	14
Ampicilin(40mg/mL)	30	15	--	--	--
Gentamicin (40mg/mL)	19	25	22	21	--
Clotrimazole (30mg/mL)	--	--	--	--	38

The oil showed moderate antibacterial activity against *Escherichia coli* and *Bacillus subtilis*, it also shown moderate anticandidal activity. However, it exhibited partial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

3.3 *Hibiscus sabdariffa* L.

3.3.1 The GC-MS analysis of *Hibiscus sabdariffa* L.

GC-MS analysis of *Hibiscus sabdariffa* L. oil revealed twenty four constituents (Table 3.7). The total ion chromatogram (TIC) is given in Fig. (3.10).

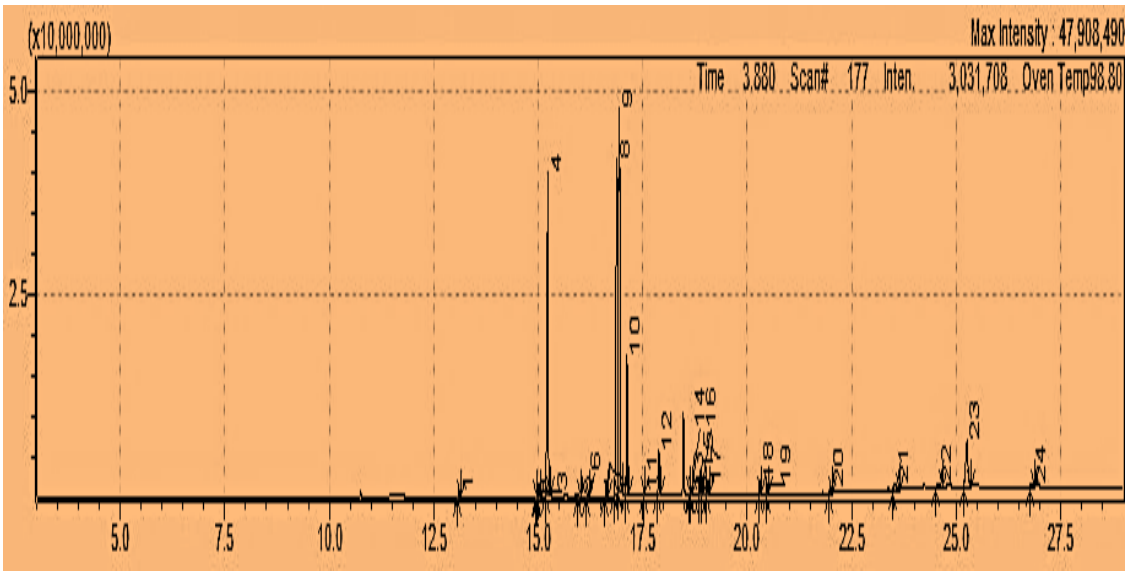


Fig 3.10: Total ions chromatogramsof *Hibiscus sabdariffa* L. oil

Major components of the oil are:

i)9-Octadecenoic acid (Z)-, methyl ester (30.23%)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (28.33%)

iii) Hexadecanoic acid, methyl ester (19.37%)

iv)Methyl stearate (6.66%)

The EI mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is presented in Fig 3.11. The peak at m/z 296, which appeared at R.T. 16.944, in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_2]^{+}$. The peak at m/z 265 corresponds to loss of a methoxyl function

Fig (3.12) demonstrates mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester**.** The peak at m/z 294, which appeared at R.T.

16.888, corresponds to $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 corresponds to loss of a methoxyl group.

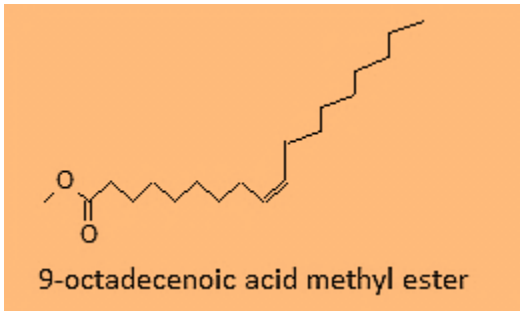
Mass spectrum of hexadecanoic acid, methyl ester methyl ester is shown in Fig. 3.13. The peak at m/z 270, which appeared at R.T. 15.228, corresponds to $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 corresponds to loss of a methoxyl function.

Mass spectrum of methyl stearate is presented in Fig 3.14. The peak at m/z 298, which appeared at R.T. 17.124, in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267 corresponds to loss of a methoxyl group.

Table 3.7: Constituents of *Hibiscus sabdariffa* L. oil

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.109	0.27
2.	7-Hexadecenoic acid, methyl ester, (Z)-	14.970	0.03
3.	9-Hexadecenoic acid, methyl ester, (Z)-	15.009	0.27
4.	Hexadecanoic acid, methyl ester	15.228	19.37
5.	cis-10-Heptadecenoic acid, methyl ester	15.956	0.18
6.	Heptadecanoic acid, methyl ester	16.180	0.13
7.	8,11-Octadecadienoic acid, methyl ester	16.626	0.69
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.888	28.33
9.	9-Octadecenoic acid (Z)-, methyl ester	16.944	30.23
10.	Methyl stearate	17.124	6.66

11.	Methyl 2-octylcyclopropene-1-octanoate	17.533	0.23
12.	10-Nonadecenoic acid, methyl ester	17.886	2.08
13.	11-Octadecenoic acid, methyl ester	18.645	0.17
14.	cis-11-Eicosenoic acid, methyl ester	18.662	0.25
15.	Eicosanoic acid, methyl ester	18.864	1.13
16.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.942	0.70
17.	8,11,14-Docosatrienoic acid, methyl ester	19.055	0.62
18.	13-Docosenoic acid, methyl ester, (Z)-	20.301	0.70
19.	Docosanoic acid, methyl ester	20.479	0.45
20.	Tetracosanoic acid, methyl ester	21.977	0.36
21.	.gamma.-Sitosterol	23.545	0.78
22.	Stigmasterol	24.568	0.52
23.	.beta.-Sitosterol	25.254	5.50
24.	Campesterol	26.813	0.35



9-octadecenoic acid methyl ester

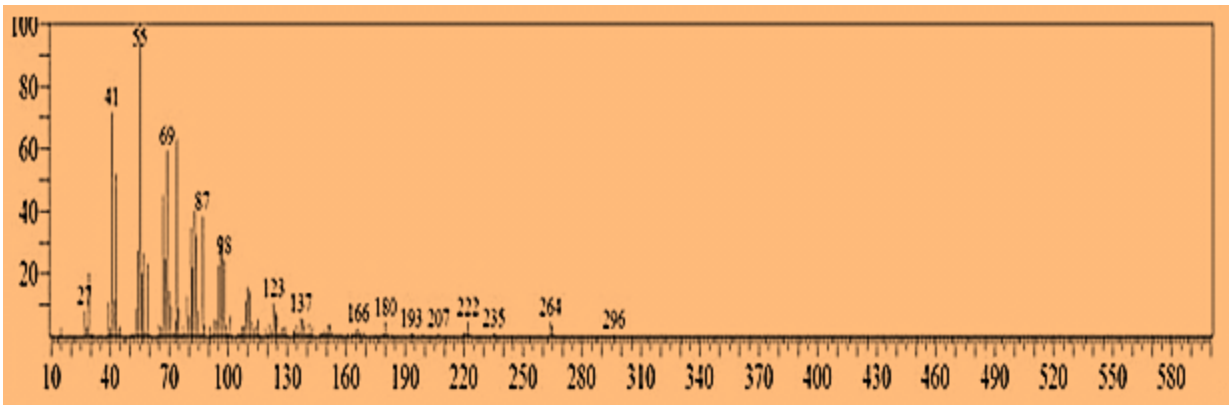
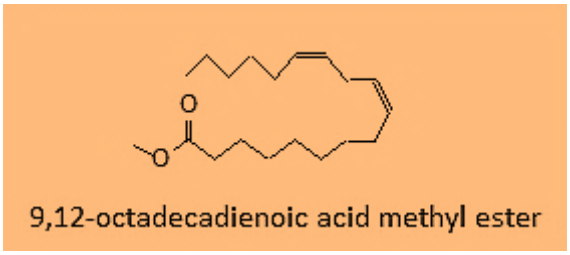


Fig 3.11: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester



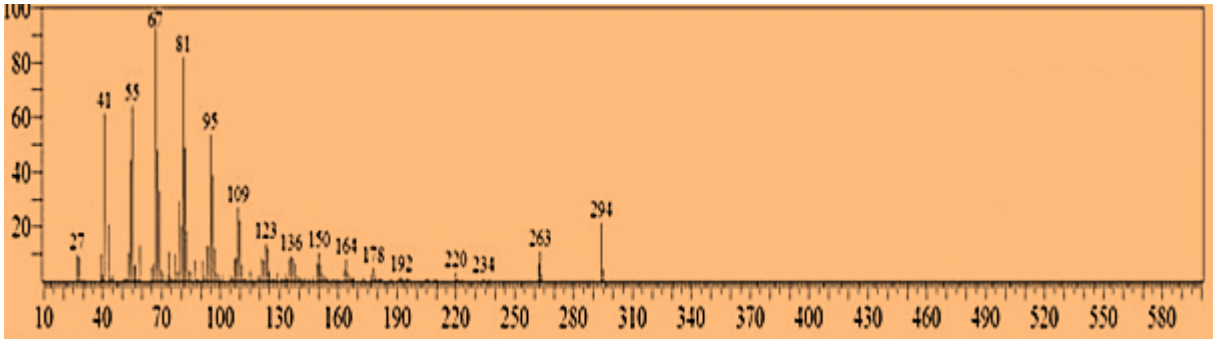


Fig 3.12: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester

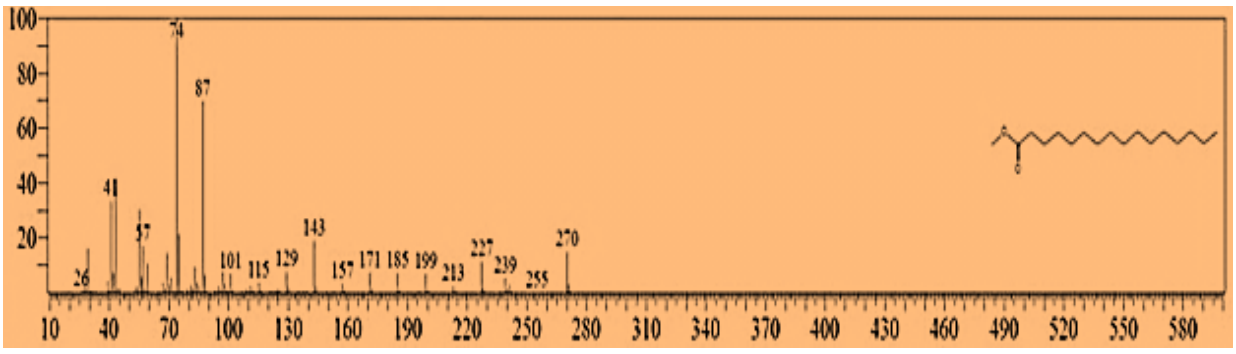


Fig 3.13: Mass spectrum of hexadecanoic acid, methyl ester

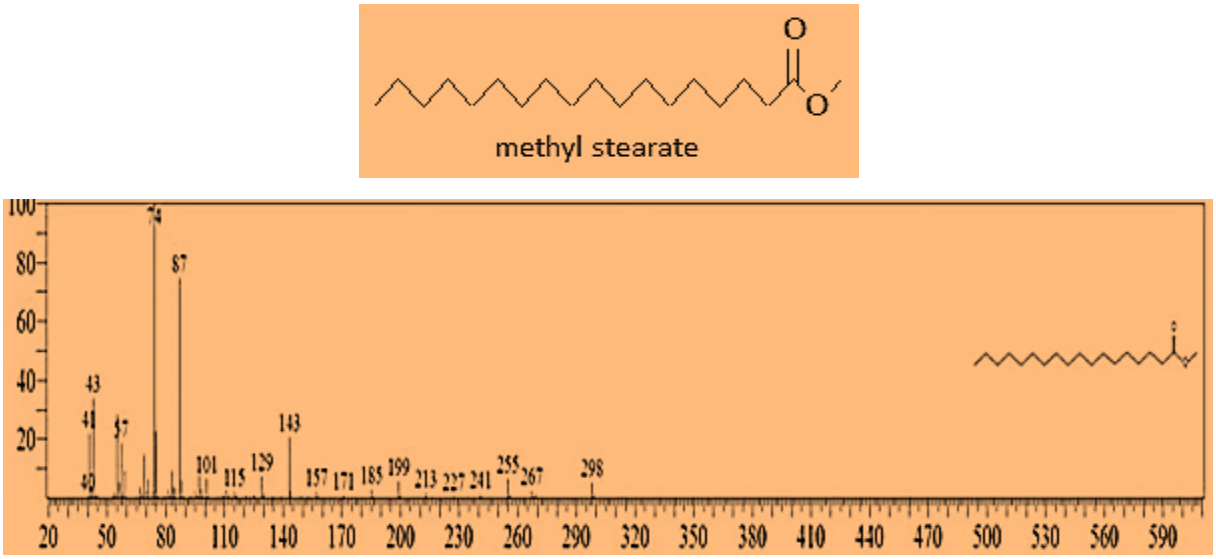


Fig 3.14: Mass spectrum of methyl stearate

3.3.2 Antibacterial activity

The oil was screened for antimicrobial activity against five standard organisms. The averages of diameters of the growth inhibition zones are shown in Table 3.8.

Table 3.8: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Pa	Ca
Oil(100mg/mL)	12	13	12	14	14
Ampicilin (40mg/mL)	30	15	--	--	--
Gentamicin (40mg/mL)	19	25	22	21	--
Clotrimazole(30mg/mL)	--	--	--	--	38

The oil showed moderate antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*, it also shown moderate anticandidal activity. However, it exhibited partial activity against *Escherichia coli* and *Staphylococcus aureus*.

3.4 *Lagenariasiceraria* L.

3.4.1 The GC-MS analysis of *Lagenariasiceraria* L.

The GC-MS analysis of *Lagenariasiceraria*L.oil showed 9 components dominated by fatty acids (Table 3.9). The total ions chromatogram is shown in Fig.3.15.

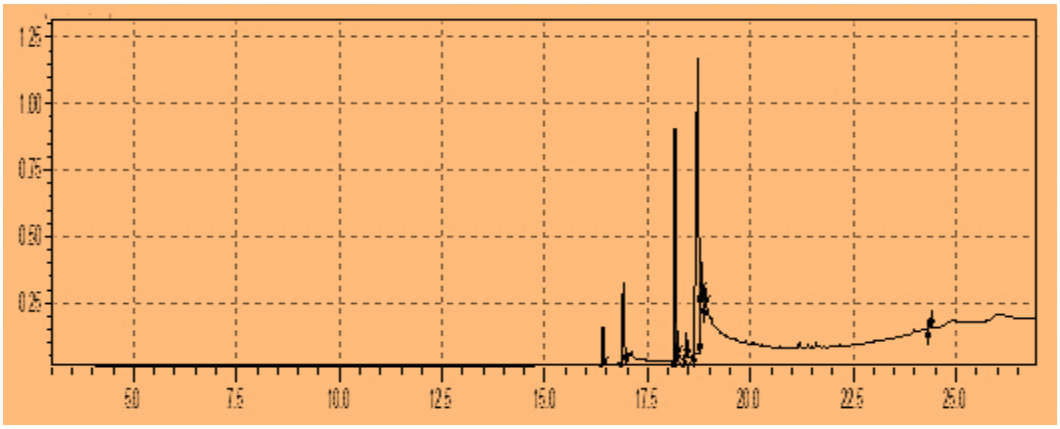


Fig.3.15: Total ions chromatogramsof *Lagenariasiceraria*L. oil

Table 3.9: Constituents of *Lagenariasiceraria*L. oil

No.	Name	Ret.Time	Area%
1.	Hexadecanoic acid, methyl ester	16.411	3.39
2.	n-Hexadecanoic acid	16.908	10.15
3.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.164	19.56
4.	9-Octadecenoic acid (Z)-, methyl ester	18.205	1.43
5.	Methyl stearate	18.428	2.02
6.	Linoleic acid ethyl ester	18.716	57.96
7.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	18.797	3.15
8.	Octadecanoic acid	18.891	1.41
9.	Squalene	24.354	0.93

The oil was dominated by:

i)- Linoleic acid ethyl ester (57.96%).

ii)- 9,12-Octadecadienoic acid methyl ester (19.56%).

iii) n-Hexadecanoic acid (10.15%).

The mass spectrum of linoleic acid ethyl ester is illustrated in Fig. 3.16. The signal at m/z308(RT.18.716) is due to the molecular ion :[$C_{20}H_{36}O_2$]. Fig.3.17 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 18.164) corresponds : M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of n-hexadecanoic acid is presented in Fig.3.18. The peak at m/z 256 (RT.16.908) is due to M^+ $[C_{16}H_{32}O_2]^+$.

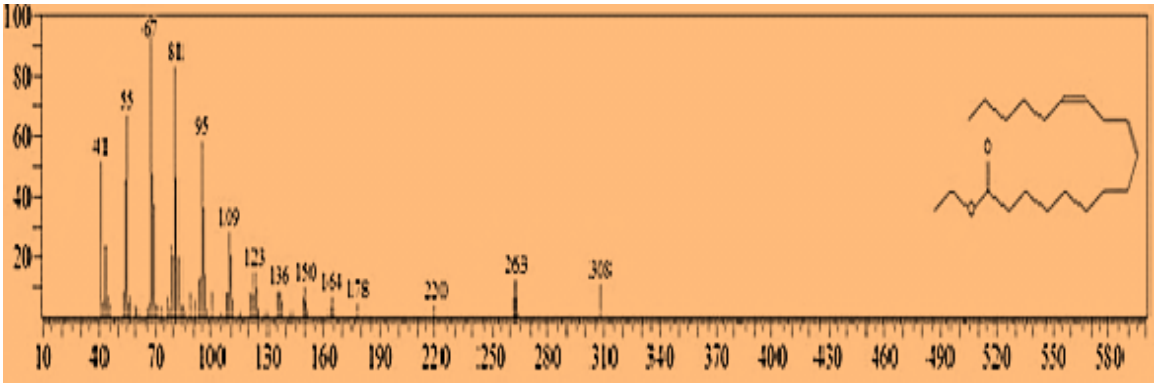


Fig. 3.16 :Mass spectrum of linoleic acid ethyl ester

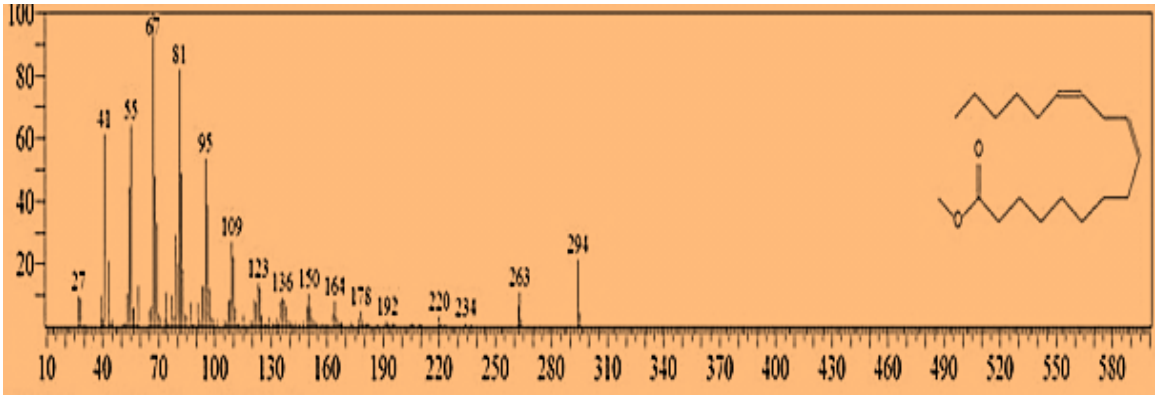


Fig. 3.17 : Mass spectrum of 9,12-octadecadienoic acid methyl ester

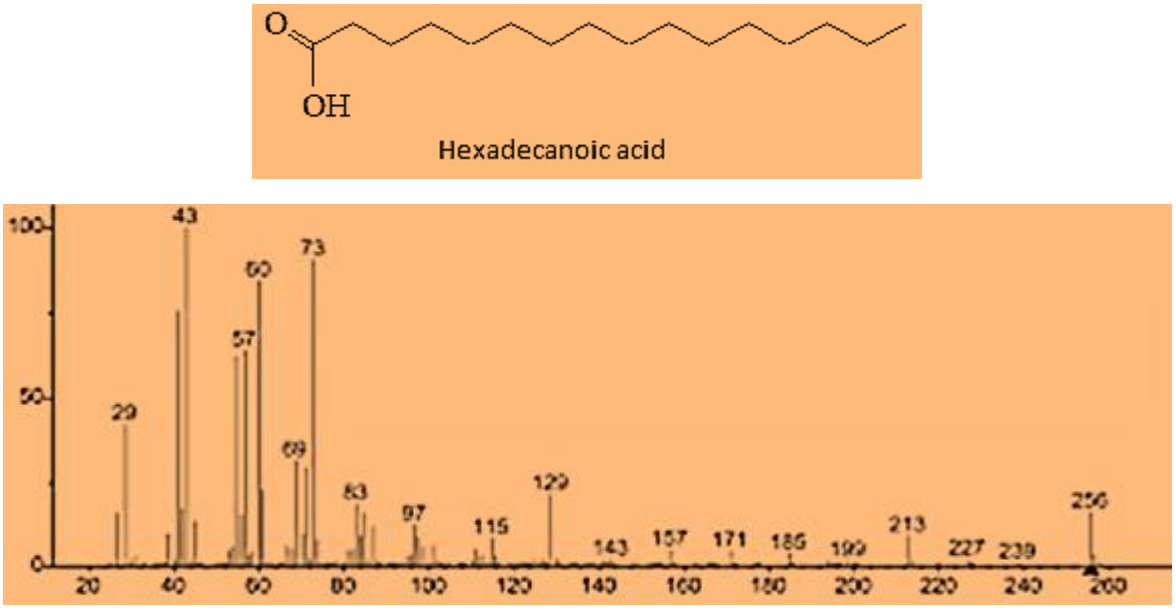


Fig.3.18 :Mass spectrum of hexadecanoic acid

3.4.2 Antimicrobial activity

The oil from seeds of *Lagenariasiceraria*was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are depicted in Table (3.10). Results were interpreted as follows: (<9mm: inative;9-12mm: partially active;13-18mm: active;>18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed moderate activity against *Pseudomonas aeruginosa* beside weak activity against *Staphylococcus aureus* and *Bacillus subtilis.*

Table 3.10 : Inhibition zones(mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/mL)	12	10	--	15	--
Ampicilin(40mg/mL)	30	15	--	--	--
Gentamicin(40mg/mL)	19	25	22	21	--
Clotrimazole(30mg/mL)	--	--	--	--	38

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

3.5 *Nerium oleander* L.

3.5.1 The GC-MS analysis of *Nerium oleander* L.

*Nerium oleander*L.oil was studied by GC-MS which revealed 23 components (Table 3.11). Fig. 3.19 shows the total ions chromatograms.

Fig.3.19: Total ions chromatogramsof *Nerium oleander* L. oil

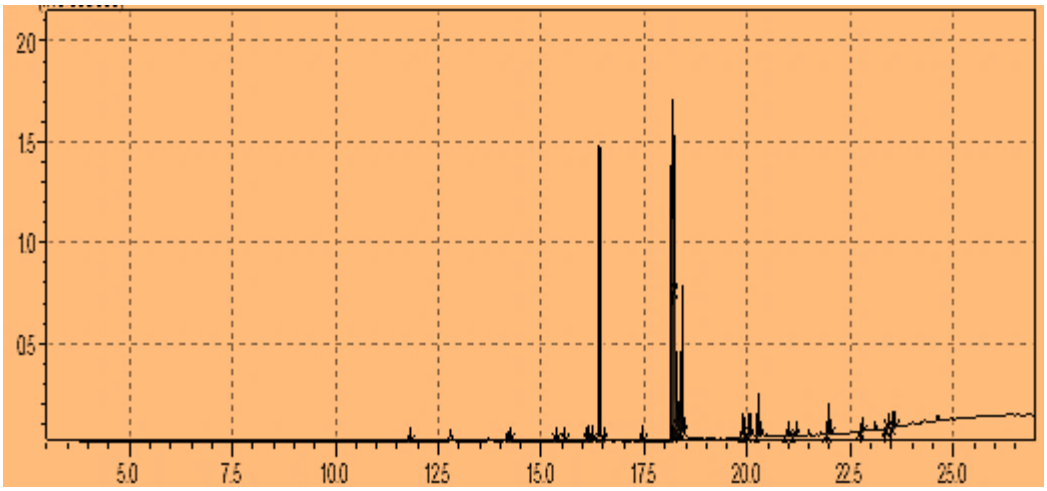


Table 3.11: Constituents of *Nerium oleander* L. oil

ID#	Name	Ret.Time	Area%
1	Dodecanoic acid, methyl ester	11.715	0.30
2	Methyl tetradecanoate	14.135	0.77
3	Pentadecanoic acid, methyl ester	15.285	0.17
4	2-Pentadecanone, 6,10,14-trimethyl-	15.525	0.05
5	7,10-Hexadecadienoic acid, methyl ester	16.065	0.03
6	7-Hexadecenoic acid, methyl ester, (Z)-	16.130	0.05
7	9-Hexadecenoic acid, methyl ester, (Z)-	16.175	0.53
8	Hexadecanoic acid, methyl ester	16.345	24.85
9	Heptadecanoic acid, methyl ester	17.380	0.20
10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.100	34.95
11	9-Octadecenoic acid (Z)-, methyl ester	18.195	7.69
12	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.235	6.36
13	Phytol	18.305	1.93
14	Methyl stearate	18.380	9.66
15	8,11,14-Docosatrienoic acid, methyl ester	19.830	2.71

16	cis-11-Eicosenoic acid, methyl ester	19.995	1.97
17	Eicosanoic acid, methyl ester	20.220	2.82
18	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	20.915	0.33
19	Heneicosanoic acid, methyl ester	21.085	0.31
20	Docosanoic acid, methyl ester	21.920	2.18
21	Tricosanoic acid, methyl ester	22.735	0.44
22	15-Tetracosenoic acid, methyl ester, (Z)-	23.345	0.36
23	Tetracosanoic acid, methyl ester	23.500	1.34

Dominant constituents of the oil are:

i- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester(34.05%).

ii- Hexadecanoic acid, methyl ester(24.85%)

iii- Methyl stearate(9.66%)

iv- 9-Octadecenoic acid (Z)-, methyl ester(7.69%)

v- 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(6.36%).

Fig.3.20 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.100)corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.21. The peak at m/z 270 (RT.16.345) is due to M^+ $[C_{17}H_{34}O_2]^+$. Fig.3.22 showsthemassspectrumofmethylstearate.Thesignalatm/z298(R.T.18.380) correspondsM$^+$[$C_{19}H_{38}O_2$]$^+$,whilethepeakat

m/z267accountsforlossofamethoxyl.The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig.3.23. The signal at m/z296 (RT.18.195) corresponds M^+ $[C_{19}H_{36}O_2]^+$. Fig.3.24 illustrates the mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester. The molecular ion $[C_{19}H_{32}O_2]^+$ appeared at m/z292(RT. 18.235).

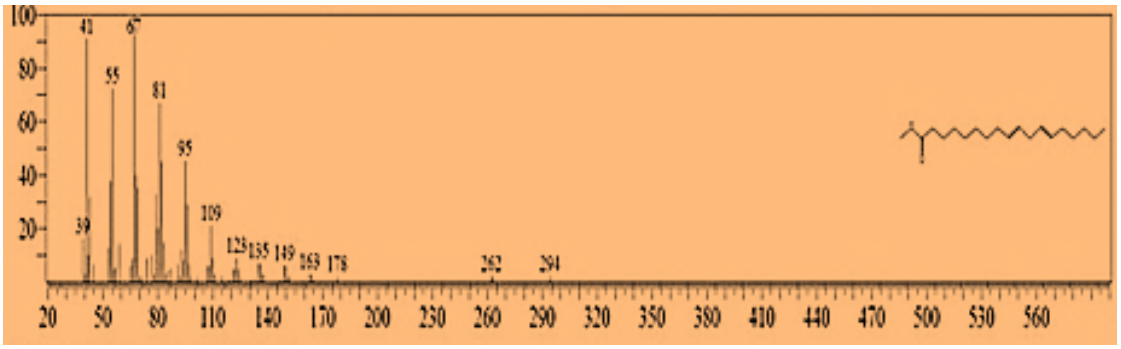


Fig.3.20: Mass spectrum of 9,12-octadecadienoic acid methyl ester

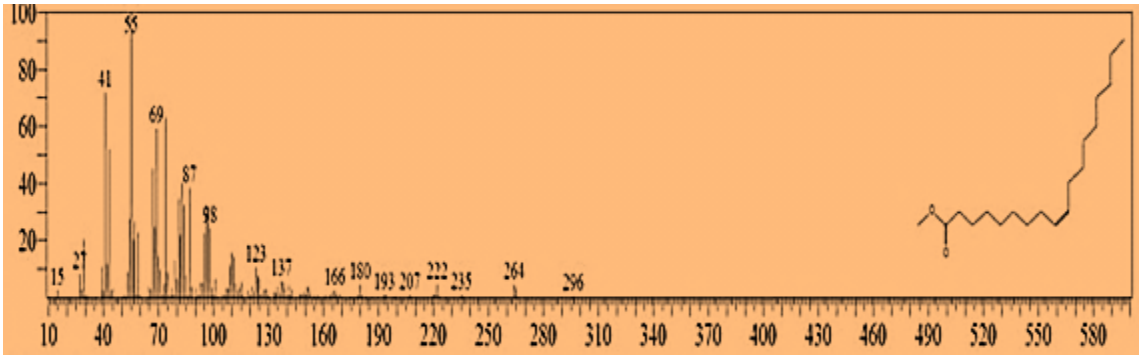


Fig.3.21: Mass spectrum of hexadecanoic acid methyl ester

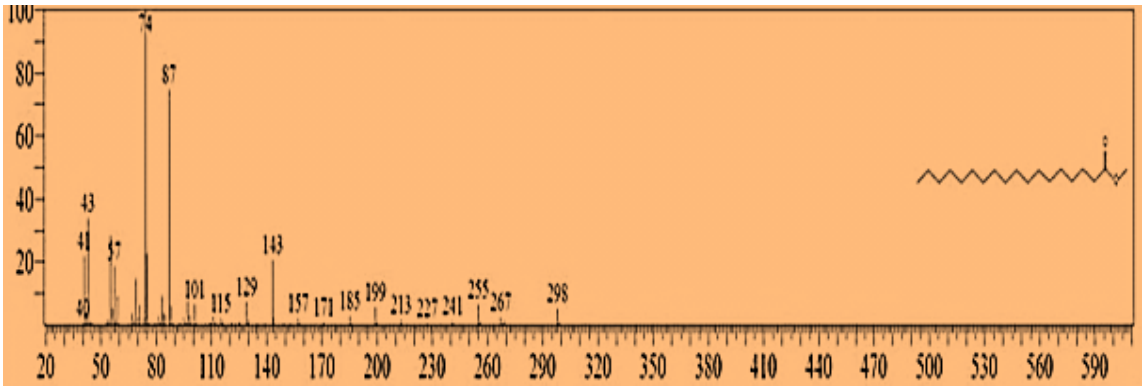


Fig.3.22: Mass spectrum of methyl stearate

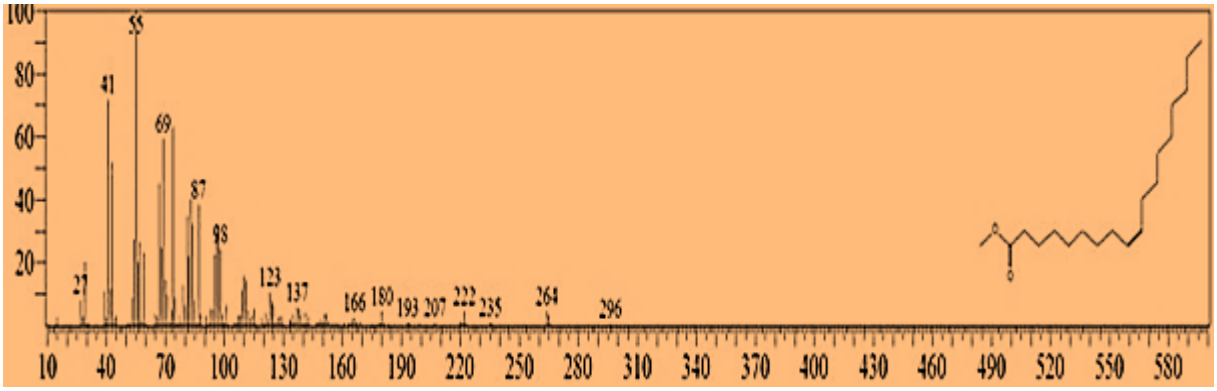
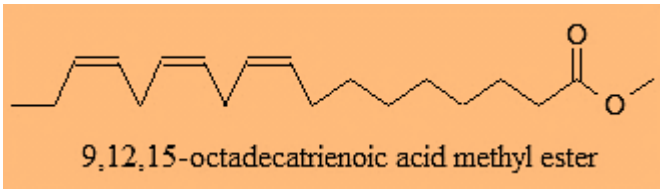


Fig. 3.23: Mass spectrum of 9-octadecenoic acid methyl ester



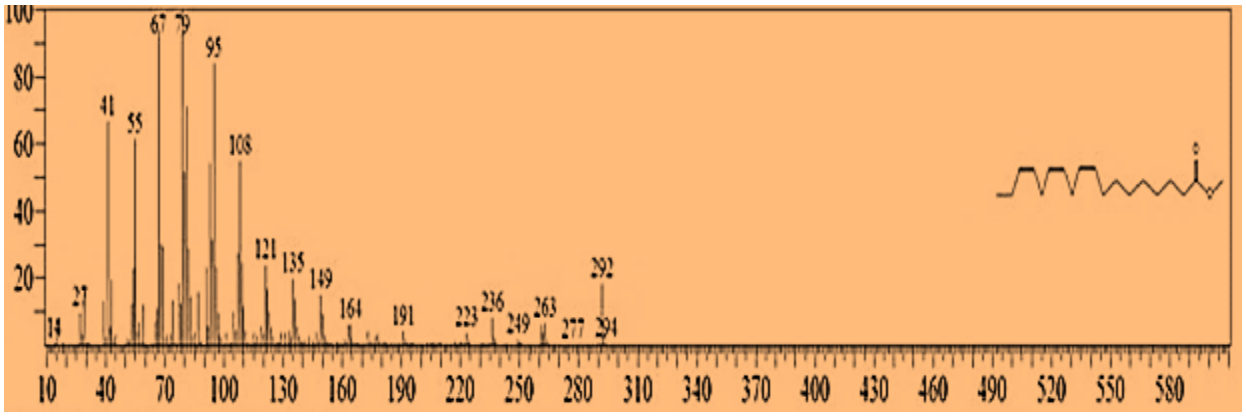


Fig.3.24: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester

3.5.2 Antimicrobial activity

Nerium oleander oil was assessed for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are presented in Table (3.12). Results were interpreted in the following terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed moderate activity against all test organisms with the exception of the fungal species *Candida albicans*.

Table 3.12 : Inhibition zones(mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/mL)	14	13	14	15	--
Ampicilin(40mg/mL)	30	15	--	--	--
Gentamicin(40mg/mL)	19	25	22	21	--
Clotrimazole(30mg/mL)	--	--	--	--	38

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

Conclusion

Five medicinal plants oils: *Carumcarvi*L., *Coriandrum sativum* L., *Hibiscus sabdariffa* L., *Lagenariasiceraria* L. and *Nerium oleander* L. have been studied. GC-MS analysis of the target oils was conducted and the identification of the constituents was accomplished. In the antimicrobial assay the oils exhibited different antimicrobial responses.

Recommendations

- Other phytochemicals (steroids, alkaloids ...etc) of the studied plants may be isolated and their structures may be elucidated and the biological activity could be screened.

- In vivo antimicrobial potency of the isolated oil may be conducted.

- The isolated oils may be assessed for their antiviral, antimalarial and other biological activities.

References

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products. 2012 Mar 23;75(3):311-35.

2. Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. The AAPS journal. 2006;8(2): E239-53.

3. Kinghorn AD, Chin YW, Swanson SM. Discovery of natural product anticancer agents from biodiverse organisms. Current opinion in drug discovery & development. 2009 Mar;12(2):189-96.

4. Zähner H. What are secondary metabolites? Folia Microbiol. 1979 1979/10/01;24(5): 435-43.

5. Ertl P, Schuffenhauer A. Cheminformatics analysis of natural products: lessons from nature inspiring the design of new drugs. Progress in drug research Fortschritte der Arzneimittelforschung Progres des recherches pharmaceutiques. 2008;66:217, 9-35.

6. Harvey AL, Clark RL, Mackay SP, Johnston BF. Current strategies for drug discovery through natural products. Expert opinion on drug discovery. 2010 Jun;5(6):559-68.

7. Piggott AM, Karuso P. Quality, not quantity: the role of natural products and chemical proteomics in modern drug discovery. Combinatorial chemistry & high throughput screening. 2004 Nov;7(7):607-30.

8. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nature reviews Drug discovery. 2005 Mar;4(3):206-20.

9. Samuelsson G. Drugs of Natural Origin: A Textbook of Pharmacognosy. 4th revised ed. Swedish Pharmaceutical Press, Stockholm, Sweden, 1999.

10. Hands, E.S. Lipid composition of selected foods. In: Bailey's Industrial Oil and Fat Products, 5th edition, (Ed. Hui, Y.H.) Wiley, New York. 1996, 441–505.

11. Kritchevsky, D. Fats and oils in human health. In: Food Lipids chemistry, nutrition, and biotechnology, 2nd edition, Akoh, C.C. and Min, D.B. (Eds.), Marcel Dekker, New York. 2002, 543–558.

12. Chow, C.K. (Ed.). Fatty acids in foods and their health implications. Marcel Dekker, New York, 1992.

13. Ching, K.C. (Ed.). Fatty acids in foods and their health implications, 2nd edition, Marcel Dekker, New York, 2000.

14. Hakomori, S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. Cancer Research. 1996, 56: 5309–5318.

15. Guthrie, N. and Carroll, K.K. Specific versus non–specific effects of dietary fat on carcinogenesis. Progress in Lipid Research. 1999, 38: 261–271.

16. Hunt, S.M. and Groff, J.L. Advanced nutrition and human metabolism. West Publishing, St Paul, MN, 1990.

17. Knapp, H.K., Salem, Jr.N. and Cunnane, C. Dietary fats and health (ISSFAL Congress), Lipids. 2003, 38: 299–496.

18. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol. 2012; 3:12.

19. Mahato N, Sharma K, Koteswararao R, Sinha M, Baral E, *et al.* Citrus essential oils: Extraction, authentication and application in food preservation. Crit Rev Food Sci Nutr. 2019, 59: 611-625.

20. Tongnuanchan P and S Benjakul. Essential oils: extraction, bioactivities, and their uses for food preservation. Journal of food science. 2014, 79: R1231-R1249.
21. Burt SA. Essential oils: their antibacterial properties and potential applications in foods: a review. Inter J Food Microbiol. 2004; 94:223-253.
22. Tajkarimi M, SA Ibrahim and D Cliver. Antimicrobial herb and spice compounds in food. Food control. 2010, 21: 1199-1218.

23. Mekem Sonwa M. Isolation and structure elucidation of essential oil constituents: comparative study of the oils of Cyperus alopecuroides, Cyperus papyrus, and Cyperus rotundus. 2000.

24. Donelian A, Carlson L, Lopes T, *et al.* Comparison of extraction of patchouli (Pogostemon cablin) essential oil with supercritical CO 2 and by steam distillation. *The Journal of Supercritical Fluids*. 2009; 48:15-20.

25. Buchbauer G. The detailed analysis of essential oils leads to the understanding of their properties. *Perfumer & flavorist*. 2000; 25:64-67.

26. Ozel MZ and H Kaymaz. Superheated water extraction, steam distillation and Soxhlet extraction of essential oils of Origanum onites. Analytical and bioanalytical chemistry. 2004, 379: 1127-1133.

27. Guan W, Li S, Yan R, *et al.* Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food Chemistry* 2007; 101:1558-1564.

28. Rao BR. Hydrosols and water-soluble essential oils of aromatic plants: Future economic products. Indian Perfum, 2012, 56: 29-33.

29. Casselm E, RMF Vargas, N Martinez, D Lorenzo, E Dellacassa. Steam distillation modeling for essential oil extraction process. Industrial crops and products. 2009, 29: 171-176.

30. Battaglia S. The complete guide to aromatherapy. International Centre of Holistic Aromatherapy. 2003.

31. Shaw PE. Review of quantitative analyses of citrus essential oils. Journal of Agricultural and Food Chemistry. 1979, 27: 246-257.

32. Ryman D. The Aromatherapy Handbook: The Secret Healing Power of Essential Oils. Random House. 2012.

33. Handa S. An overview of extraction techniques for medicinal and aromatic plants. Extraction technologies for medicinal and aromatic plants 1. 2008.

34. Babita Singh, Sellam P, Jayoti Majumder and Puja Rai. Flo Ral Es Sen Tial Oils: Im Portance And Uses For Man Kind. HortFlora Research Spectrum. 2014, 3: 7-13.

35. Aizpurua-Olaizola O, Ormazabal M, Vallejo A, Olivares M, Navarro P, *et al.* Optimization of supercritical fluid consecutive extractions of fatty acids and polyphenols from Vitis vinifera grape wastes. Journal of food science. 2015, 80: E101-E107.

36. Bélanger A, Landry B, Dextraze L, *et al*. Extraction et détermination de composés volatils de l'ail (Allium sativum). *Rivista Italiana EPPOS*. 1991; 2:455.

37. Chiasson H, Bélanger A, Bostanian N, *et al*. Acaricidal properties of Artemisia absinthium and Tanacetum vulgare (Asteraceae) essential oils obtained by three methods of extraction. *Journal of Economic Entomology* 2001; 94:167-171.

38. Chamorro ER, Zambón SN, Morales WG, Sequeira AF, Velasco GA. Study of the chemical composition of essential oils by gas chromatography. Gas chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications. 2012.

39. Başer KHC and F Demirci. Chemistry of essential oils. Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability, edited by Berger RG. New York: Springer 2007, 43-86.

40. Thimmappa R, Geisler K, Louveau T, O Maille P, Osbourn A. Triterpene biosynthesis in plants. Annual Review of Plant Biology. 2014, 65: 225-257.

41. Ludwiczuk A, K Skalicka-Woźniak and M Georgiev. Terpenoids. In: Badal S, Delgoda R (eds) Pharmacognosy: Fundamentals, applications and Strategied. Elsevier. London. 2017, 233-266.

42. Breitmaier E. Terpenes: flavors, fragrances, pharmaca, pheromones. John Wiley and Sons. 2006.

43. Swamy MK, Sudipta Kumar Mohanty, Uma Rani Sinniah, Anuradha Maniyam. Evaluation of patchouli (Pogostemon cablin Benth.) cultivars for growth, yield and quality parameters. Journal of Essential Oil Bearing Plants. 2015,18: 826-832.

44. Safayhi H, Sabieraj J, Sailer ER, Ammon HP. Chamazulene: an antioxidant-type inhibitor of leukotriene B4 formation. Planta medica. 1994, 60: 410-413.

45. Arumugam G, M Swamy and U Sinniah. Plectranthus amboinicus (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance Molecules. 2016, 21: 369.

46. Lang G, Buchbaue G. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. Flavour and Fragrance Journal. 2012; 27(1):13-39.

47. Filomena Nazzaro, Florinda Fratianni, Laura De Martino, Raffaele Coppola and Vincenzo De Feo. Effect of essential oils on pathogenic bacteria Pharmaceuticals. 2013, 6: 1451-1474.

48. Peter Weyerstahl, Sabina Schneider, Helga Marschall, Abdolhossein Rustaiyan. The essential oil of Artemisia sieberi Bess. Flavour and fragrance journal. 1993, 8: 139-145.

49. Viuda-Martos M, Y Ruiz-Navajas, J Fernández-López, J Pérez-Álvarez. Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils. Food control. 2008, 19: 1130-1138.

50. Yepez B, M Espinosa, S López, G Bolaños. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction. Fluid Phase Equilibria. 2002, 194: 879-884.

51. Simpson BB, JL Neff and G Dieringer. The production of floral oils byMonttea (Scrophulariaceae) and the function of tarsal pads inCentris bees. Plant Systematics and Evolution. 1990, 173: 209-222.

52. Xu Junming, Jiang Jianchun, Chen Jie, Sun Yunjuan. Biofuel production from catalytic cracking of woody oils. Bioresource technology. 2010, 101: 5586-5591.

53. Priestap H, G Rücker, M Neugebauer, AL Bandoni. Investigation of the essential oils from Aristolochia triangularis. Journal of Essential Oil Research. 1990, 2: 95-98.

54. López-Cortés I, DC Salazar-García, B Velázquez-Martí, DM Salazar. Chemical characterization of traditional varietal olive oils in East of Spain. Food research international. 2013, 54: 1934-1940.

55. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res. 2007; 21(4):308-23.

56. Buchbauer G. Biological activities of essential oils. Handbook of essential oils: Science, technology, and applications. 2009; 235-280.

57. Koroch AR, Juliani HR, Zygadlo JA. Bioactivity of essential oils and their components. Flavours and fragrances. 2007; 87-115.

58. Boehm K, Büssing A, Ostermann T. Aromatherapy as an adjuvant treatment in cancer care–a descriptive systematic review. Afr J Tradit Complement Altern Med. 2012; 9(4):503-18.

59. Adorjan B, Buchbauer G. Biological properties of essential oils: an updated review. Flavour and Fragrance Journal. 2010; 25(6):407-26.

60. Adlard ERK. Hüsnü Can Başer and Gerhard Buchbauer (Eds.): Handbook of Essential Oils. Science, Technology and Applications. Chromatographia. 2016; 79(11-12):791.

61. Lopez-Reyes JG, Spadaro D, Prelle A, Garibaldi A, Gullino ML. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. J Food prot. 2013; 76(4):631-9.

62. Lang G, Buchbaue G. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review. Flavour and Fragrance Journal. 2012; 27(1):13-39.

63. Sikkema, J.; de Bont, J.A.M.; Poolman, B. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. 1994, 269, 8022–8028.

64. Ultee, A.; Bennink, M.H.J.; Moezelaar, R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. Appl. Environ. Microbiol. 2002, 68, 1561–1568.

65. Denyer, S.P.; Hugo, W.B. Biocide-induced damage to the bacterial cytoplasmic membrane. In Mechanisms of Action of

Chemical Biocides, the Society for Applied Bacteriology, Technical Series No 27; Denyer, S.P., Hugo, W.B., Eds.; Oxford Blackwell Scientific Publication: Oxford, UK, 1991; 171–188.

66. Farag, R.S.; Daw, Z.Y.; Hewedi, F.M.; El-Baroty, G.S.A. Antimicrobial activity of some Egyptian spice essential oils. J. Food Prot. 1989, 52, 665–667.

67. Cosentino, S.; Tuberoso, C.I.G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; Palmas, F. In vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Lett. Appl. Microbiol. 2002, 29, 130–135.

68. Dorman, H.J.D.; Deans, S.G. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 2000, 88, 308–316.

69. Davidson, P.M. Chemical preservatives and natural antimicrobial compounds. In Food Microbiology: Fundamentals and Frontiers; Doyle, M.P., Beuchat, L.R., Montville, T.J., Eds.; ASM Press: Washington, DC, USA, 1997; 520–556.

70. Knobloch, K.; Weigand, H.; Weis, N.; Schwarm, H.M.; Vigenschow, H. Action of terpenoids on energy metabolism. In Progress in Essential Oil Research: 16th International Symposium on Essential Oils; Brunke, E.J., Ed.; De Walter de Gruyter: Berlin, Germany, 1986, 429–445.

71. Lambert, R.J.W.; Skandamis, P.N.; Coote, P.; Nychas, G.J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 2001, 91, 453–462.

72. Marino, M.; Bersani, C.; Comi, G. Antimicrobial activity of the essential oils of Thymus vulgaris L. measured using a bioimpedometric method. J. Food Prot. 1999, 62, 1017–1023.

73. Ratledge, C.; Wilkinson, S.G. An overview of microbial lipids. In Microbial Lipids; Ratledge, C., Wilkinson, S.G., Eds.; Academic Press Limited: London, UK. 1988, 1, 3–22.

74. Davidson, P.M.; Parish, M.E. Methods for testing the efficacy of food antimicrobials. Food Technol. 1989, 43, 148–155.

75. Gill, A.O.; Delaquis, P.; Russo, P.; Holley, R.A. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int. J. Food Microbiol. 2002, 73, 83–92.

76. Reichling, J.; Schnitzler, P.; Suschke, U.; Saller, R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotocic properties-an overview. Forsch. Komplement. 2009, 16, 79–90.

77. Braga, P.C.; dal Sasso, M.; Culici, M.; Gasastri, L.; Marceca, M.X.; Guffanti, E.E. Antioxidant potential of thymol determined by chemiluminescence inhibition in human neutrophils and cell-free systems. Pharmacology. 2006, 76, 61–68.

78. Aruoma, O.I. Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil Chem. Soc. 1998, 75, 199–212.

79. Kamatou, G.P.P.; Viljoen, A.M. A review of the application and pharmacological properties of α-Bisabolol and α-Bisabolol-rich oils. J. Am. Oil Chem. Soc. 2010, 87, 1–7.

80. Maruyama, N.; Sekimoto, N.; Ishibashi, H. Suppression of neutrophil accumulation in mice by cutaneous application of geranium essential oil. J. Inflamm. 2005, 2, 1–11.

81. Koh, K.J.; Pearce, A.L.; Marshman, G.; Finlay-Jones, J.J.; Hart, P.H. Tea tree oil reduces histamine-induced skin inflammation. Br. J. Dermatol. 2002, 147, 1212–1217.

82. Caldefie-Chézet, F.; Guerry, M.; Chalchat, J.C.; Fusillier, C.; Vasson, M.P.; Guillot, J. Antiinflammatory effects of Malaleuca alternifolia essential oil on human polymorphonuciear neutrophils and monocytes. Free Radic. Res. 2004, 38, 805–811.

83. Caldefie-Chézet, F.; Fusillier, C.; Jarde, T.; Laroye, H.; Damez, M.; Vasson, M.P. Potential antiinflammatory effects of Malaleuca alternifolia essential oil on human peripheral blood leukocytes. Phytother. Res. 2006, 20, 364–370.

84. Manjamalai, A.; Jiflin, G.J.; Grace, V.M. Study on the effect of essential oil of Wedelia chinensis (Osbeck) against microbes and inflammation. Asian J. Pharm. Clin. Res. 2012, 5, 155–163.

85. Hart, P.H.; Brand, C.; Carson, C.F.; Riley, T.V.; Prager, R.H.; Finlay-Jones, J.J. Terpinen-4-ol, the main component of the essential oil of Malaleuca altemifolia (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. Inflamm. Res. 2000, 49, 619–626.

86. Sharififar, F.; Mirtajadini, M.; Azampour, M.J.; Zamani, E. Essential Oil and Methanolic Extract of Zataria multiflora Boiss with Anticholinesterase Effect. Pak. J. Biol. Sci. 2012, 15, 49–53.

87. Yoon, H.S.; Moon, S.C.; Kim, N.D.; Park, B.S.; Jeong, M.H.; Yoo, Y.H. Genistein induces apoptosis of RPE-J cells by opening mitochondrial PTP. Biochem. Biophys. Res. Commun. 2000, 276, 151–156.

88. Sahil K, Prashant B, Akanksha M, Premjeet S, Devashish R. GC-MS: Applications. International Journal Pharma & Biological Archives. 2011, 2: 1544-1560.

89. Oregon State University (2012). GC-MS: How does it Work? Environmental Health Sciences Center Corvallis OR 97331 http://www.unsolvedmysteries.oregonstate.edu/MS_05

90. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th Edition. Brooks/Cole Cengage Learning.2007, Chapters 11, 20, 26, 27.

91. Alon T, Amirav A. Isotope abundance analysis methods and software for improved sample identification with supersonic gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2006, 20: 2579-2588.

92. Robert P, Dr. Adams. Identification of Essential Oil Components by Gas Chromatography/Mass spectrometry. 4th edition, Allured Pub Corp. 2007.

93. Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines, 3rd Edition. Medical Economics Company. 2004; 245- 246.

94. Grieve M. *A Modern Herbal.* Penguin Books, Harmondsworth, London. 1984.

95. Bown D. *Encyclopaedia of Herbs and Their Uses.* Dorling Kindersley, London. 1995.

96. Foster S. and Duke JA. *A. Field Guide to Medicinal Plants: Eastern and Central North America.* HoughtonMifflin Co, Boston. 1990.

97. Coffey T. *The History and Folklore of North American Wild Flowers.* Facts on File, New York. 1993.

98. Tsarong TJ. *Tibetan Medicinal Plants*, 1st edn. Tibetan Medical Publications, Darjeeling. 1994.

99. Zheng GQ, Kenney PM, Lam LK: Anethofuran, carvone and limonene: Potential cancer chemopreventive agents from dill weed oil and caraway oil. Planta Med. 1992; 58: 338–341.

100. Khayyal MT, El-Ghazzaly MA, Kanawy SA, Seif-El-Naser M, Mahran GL, Kafafi AY, Okpanyi SN: Antiulcerogenic effect of some gastrointestinally acting plant extracts and their combination. Arzneimittelforschung. 2001; 51: 545–553.

101. Iacobellis NS, Lo Contore P, Capasso F, Senatore F: Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Agri Food Chem. 2005; 53: 57–61.

102. Coon JT, Ernst E: Herbal medicinal products for non-ulcer dyspepsia. Aliment Pharmacol Ther. 2002; 16: 1689–1699.

103. Micklefield G, Jung O, Greving I, May B: Effects of intraduodenal application of peppermint oil (WS(R)1340) and caraway oil (WS(R)1520) on gastroduodenal motility in healthy volunteers. Phytother Res. 2003; 17: 135–140.
104. Camilleri M: Dyspepsia, irritable bowel syndrome, and constipation: Review and what's new. Reviews Gastroenterol Disorders. 2001; 1: 2–17.
105. Boskabady MH, Ramazani M, Tabei T (2003): Relaxant effects of different fractions of essential oil from *Carum copticum* on guinea pig tracheal chains. Phytother Res. 2003; 17: 1145–1149.
106. Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS: Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J Ethnopharmacol. 2005; 98: 127–135.
107. Omidbaigi R. Approaches to Production and Processing of Medicinal Plants. Vol. Tarrahan-e-Nashr; Tehran, Iran. 1997; 1–424.
108. Figueiredo RO, Nakagawa J, Ming LC, Marques MOM. Composition of Coriander Essential Oil from Brazil. Can. Int. Dev. Agency. 2004; 1(4):135-137.
109. Rajeshwari U, Andallu B. Medicinal benefits of coriander (*Coriandrum sativum* L). Spatula DD. 2011; 1(1):51-58.
110. Priyadarshi S, Borse BB. Effect of the Environment on Content and Composition of Essential oil in Coriander. International Journal of Scientific & Engineering Research. 2014; 5(2):57-65.
111. Coskuner Y and Karababa E. Physical properties of coriander seeds (*Coriandrum sativum* L.). J Food Engin 2007; 80(2):408-416.
112. Craker LE and Simon JE. Herb spices and medicinal plant, vol.3, CBS Publishers and Distributors, New Delhi 2002.
113. Ali BH, Wabel NA, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. Phytotherapy Research. 2005; 19: 369-375.

114. Ismail, A., Ikram, E. H. K., and Nazri, H. S. M. Roselle (Hibiscus sabdariffa L.) seeds nutritional composition protein quality and health benefits. Food. 2008; 2(1), 1–16.
115. Murdock, G. P. Africa, its peoples and their culture history. New York: McGraw-Hill. 1959.
116. Mahadevan N, Shivali, Kamboj P. *Hibiscus sabdariffa* Linn. – an overview. Natural Product Radiance. 2009; 8: 77-83.
117. Leung, A. Y., and Foster, S. Encyclopedia of common natural ingredients used in food, drugs, and cosmetics (2nd ed.). New York: John Wiley and Sons. 1996.
118. Morton, J. F. Fruits of warm climates. Florida Flair Books. 1987.
119. Neuwinger, H. African traditional medicine. Stuttgart: Medpharm Scientific Publication. 2000.
120. Burnham, T., Wickersham, R., and Novak, K. The review of natural products (3rd ed.). St. Louis, MO: Facts and Comparisons. 2002.
121. Gaya, I. B., Mohammad, O. M. A., Suleiman, A. M., Maje, M. I., and Adekunle, A. B. toxicological and lactogenic studies on the seeds of Hibiscus Sabdariffa Linn (Malvaceae) extract on serum prolactin levels of albino wistar rats. The Internet Journal of Endocrinology. 2009; 5(2).
122. Abu-Tarboush, H. M. and Ahmed, S. B. Studies on karkade (*Hibiscus sabdariffa*) protease inhibitors, phytate, in vitro protein digestibility and gossypol content. Journal. Agric. Food Chem. 1996; 56, 15 – 19.
123. Herrera AA, Flores RS, Chavez-Soto MA, Tortoriello J: Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertention: A controlled and randomized clinical trial. *Phytomedicine*. 2004; 11: 375–382.
124. Rahman AHMM, Anisuzzaman M, Ahmed F, Rafiul Islam AKM, Naderuzzaman ATM. J of Applied Sci Res 2008; 4(5):555- 558.
125. Kirtikar KR, Basu BD. Indian Medicinal Plants. Ed 2nd, Oriental Enterprises, Dehradun, 2001.

126. The Wealth of India. A Dictionary of Indian raw materials and industrial products, CSIR, New Delhi III, 2004, 16- 19.
127. Shirwaikar A, Sreenivasan KK. Chemical investigation and antihepatotoxic activity of the fruits of Lagenaria siceraria, Indian Journal of Pharmaceutical Sciences, 1996; 58(5):197- 202.
128. Nadkarni AK. Indian Material Medica. Ed 3rd, Vol. 1, Bombay Popular Book Depot, India, 1954, 181.
129. Sankari M, Chitra V, Jubilee R, Janaki PS, Raju D. Der Pharmacia Lettre, 2010, 2(1), 291- 296.
130. Ghule BV, Ghante MH, Yeole PG, Sauji AN. Ind J Pharm Sci, 2007; 69(6):817- 819
131. Shirwaikar A, Sreenivasan KK. Indian J Pharm Sci, 1996; 58:197- 202.
132. Jiwjinda S, Santisopasin V, Murakam A, Kim OK, Kim HW, Ohigashi H. Suppressive effect of edible thai plants on superoxide and NO generation. Asian pacific J Cancer Prev, 2002; 3:215- 223.
133. Gangwal A, Parmar SK, Gupta GL, Rana AC, Sheth NR. Immunomodulatory effects of Lagenaria siceraria fruits in rats. Pharmacogn Mag 2008; 4: 240–244.
134. Nagata S, Tsuchiya M, Asano S, Kaziroya Y, Yamzaki T, Yamamoto O. Nature, 1986, 31,(9): 415- 418.
135. Wang HX, Ng TB. Lagenin, a noble ribosomeinactivating protein with ribonucleolytic activity from bottle gourd Lagenaria siceraria seeds. Life Sci, 2000; 67(21):2631- 2638.
136. Rahman ASH. Bottle gourd (Lagenaria siceraria) a vegetable for good health, natural product radiance, 2003, 2(5):249- 256.
137. Lagenaria siceraria (Mol.) Standley In: Indian Medicinal Plants, Warrier PK, VPK Nambiar, C Ramankutty (Ed), Vol. 3rd, Orient Longman Limited, Madras, 1995, 292- 296.
138. Patel G., Physiological evaluation and qualitative chemical examination of Methanolic extract of Nerium indicum. International Journal of Biomedical Research, 2020, 1(5): 209–213.

139. Kingsbury JM, Poisonous plants of the United States and Canada. Soil Science, 1964, 98(5): 349.
140. Ahmed US, Ali MS, Begum F and Alimuzzaman M, Analgesic Activity of Methanolic Extract of Nerium indicum Mill. Dhaka University Journal of Pharmaceutical Sciences, 2006, (1–2): 85–87.
141. Chauhan S, Singh M, Thakur M and Dogra MS, Antibacterial activity of Nerium indicum against some Gram positive bacterial species. International Journal of Drug Research and Technology, 2013, 3(1): 8–11.
142. Huq MM, Jabbar A, Rashid MA and Hasan CM, A novel antibacterial and cardiac steroid from the roots of *Nerium oleander*. Fitoterapia, 1999, 70(1): 5–9.
143. Hseini S and Kahouadji A, Ethnobotanical study of medicinal flora in the region of Rabat (Morocco Western). Lazaroa, 2007, 28: 79–92.
144. Lahsissene H, Kahouadji A and Hseini S, Catalog of medicinal plants used in the region of Zaer (Morocco Occidental). Lejeunia, 2009, 186: 0457–4184.
145. Jude CA, Extraction, Characterization and industrial applications of Tobacco Seed Oil (Nicotiana tabacum). Chemistry and Materials Research, 2013, 3(2): 19–21.
146. Dey, P. Chaudhuri, T.K., Pharmacological aspects of Nerium indicum Mill: A comprehensive: review. Pharmacognosy Reviews, 2014, 8(16): 156-162.
147. Farooqui, S. Tyagi, T., Nerium oleander: it's application in basic and applied science: a review. International Journal of Pharmacy and Pharmaceutical Sciences, 2018, 10(3): 1-4.
148. Zibbu, G. Batra, A.: A review on chemistry and pharmacological activity of *Nerium oleander* L. Journal of Chemical and Pharmaceutical Research, 2010, 2(6): 351-358.
149. Ali HFM, El-Ella FMA and Nasr NF, Screening of chemical analysis, antioxidant antimicrobial and antitumor activities of essential oil of oleander (Nerium oleander) flower. International journal of biological Chemistry, 2010, 4(4): 190–202.

150. Sinha, S.N. Biswas, K., A concise review on Nerium oleander L. - An important medicinal plant. Tropical Plant Research, 2016, 3(2): 408-412.
151. Gupta PD and Thorsteinson AJ, Food plant relationships of the diamondback moth (Plutella maculipennis (Curt)). I. Gustation and olfaction in relation to botanical specificity of the larva. Entomologia Experimentalis et Applicata, 1960, 3: 241–250.
152. Jacobson M (1975) Insecticides from plants a review of the literature, 1954–1971. Agriculture Handbook 461, USDA, Washington, DC, P. 138.
153. Grainge M (1985) Plant species reportedly possessing pest-control properties: an EWC/UH database. Resource Systems Institute, East-West Center (EWC).
154. Kumar G, Karthik L, Rao KVB, Kirthi AV and Rahuman AA., Phytochemical composition and mosquito controlling property of Nerium oleander leaves (Apocynaceae) against Culex tritaeniorhynchus and Culex gelidus (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, 2012, 2: 1–6.
155. Bas, A.L. Demirci, S. Yazihan, N. Uney, K. Kaya, E.E., Nerium oleander distillate improves fat and glucose metabolism in high-fat diet-fed streptozotocin-induced diabetic rats. International Journal of Endocrinology. 2012: Article ID 947187.
156. Sikarwar, M.S. Patil, M.B. Kokate, C.K. Sharma, S. Bhat, V. ; Antidiabetic activity of Nerium indicum leaf extract in alloxan-induced diabetic rats. Journal of Young Pharmacists, 2009, 1(4): 333-335.
157. Yanardag, S.B. Akkoca, A. Çiçek, F. Ayaz, M.; Effect of distillated Nerium oleander etract on diabetic neuropathy: Animal model study. Journal of Advanced Neuroscience Research., 2015, 2: 16-21.