An-Najah National University

Faculty of Graduate Studies

ORIGANUM SYRIACUM AND *ALOYSIA CITRODORA* ESSENTIAL OIL: CHEMICAL PROFILE, ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY

By

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Dedication

This thesis is wholeheartedly dedicated to my sons Kinan and Elleen, who have been my source of strength and passion, and to my dear husband for his trust and support.

To my mom and sisters who gave me moral, spiritual, and emotional support.

To my husband's family for their encouragement.

I dedicate this work

Acknowledgements

First and foremost, I want to express my gratitude to God Almighty for providing me the chance and direction I needed to accomplish my goal and succeed in this area of my life.

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Last but not least, I want to thank my family, especially my sister lama, my husband, and my kids. Without their wonderful support and understanding throughout the previous few years, I would not have been able to finish my education.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

***ORIGANUM SYRIACUM* AND *ALOYSIA CITRODORA* ESSENTIAL OIL: CHEMICAL PROFILE, ANTIBACTERIAL, ANTIOXIDANT AND ANTICANCER ACTIVITY**

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: _____

Signature: _____

Date: _____

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Origanum syriacum and *Aloysia Citrodora* Essential Oil: Chemical Profile, Antibacterial, Antioxidant and Anticancer Activity

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Abstract

Objectives: The belief that some plants have the power to treat many illnesses without side effects is one of the reasons why interest in medicinal plants is increasing day by day. In our area, two common plants *Origanum syriacum* "Za'atar" and *Aloysia citrodora* have long been employed in numerous traditional treatments. This study aims to explore the chemical profiles of the essential oils (EO) of *Origanum syriacum* collected in Jerusalem and *Aloysia citrodora* gathered in Jericho and Qalailya, as well as their antioxidant, anti-microbial, and anticancer properties. Studying the synergistic impact of their combination on cell proliferation of various cancer cell lines is another goal.

Materials and methods: Using hydro distillation extraction, *Origanum syriacum* EO and *Aloysia citrodora* EO were produced, and the chemical components of the EO were identified qualitatively and quantitatively using GC-MS. By inhibiting DPPH free radicals, the antioxidant activity of the EOs was evaluated. The MTS assay was used to examine the anticancer activity. The micro-dillution method was used to test the antibacterial activity.

Results: 11 compounds were identified in *O. syriacum* EO, Carvacrol(79.46%), thymol (15.87%) and cuminol(3.25 %) were the major components. 37 compounds found in *A. citrodora* EO from Jericho, the most abundant compounds were α-curcumene(26.94%), spathulenol(13.69%), geranial(10.79%), caryophellene oxide(8.66%), neral(7.59%) and ß-caryophyllene(6.14%); whearas 31 compounds identified in *A. citrodora* EO from Qalqilya, geranial(37.00%), neral(29.00%), α-curcumene(7.76%), ß-caryophyllene (6.00%), and bicyclogermacrene(2.79%) were the main constituents. The IC_{50} value of *O. syriacum* was 9.29±0.52 µg/mL while *A. citrodora* IC_{50} was 31.35±0.33 µg/mL. IC_{50} values of *A. citrodora* EO against the cancer cell lines were between 13.5±1.41 and

87.6±3.17 µg/mL, while *O. syriacum* EO ranging from 32.5±1.20−84.9±3.41 µg/mL. The MIC values for *O. syriacum* EO ranged from 48.7−25000 µg/mL, whereas those for *A. citrodora* EO were between 3125 and 10000 µg/mL.

Conclusions: The antioxidant and antibacterial activity of *O. syriacum* exceeded that of *A. citrodora* EO. *A. citriodora* EO had more growth-inhibitory effects on all cell lines than *O. syriacum* EO. Their mixture (1:1 w: w) doesn't show an enhancement on the anticancer activity.

Keywords: *Aloysia citrodora* essential oil Antibacterial; Anticancer; Antioxidant; Chemical profile; *Origanum syriacum* essential oil; Synergistic effect.

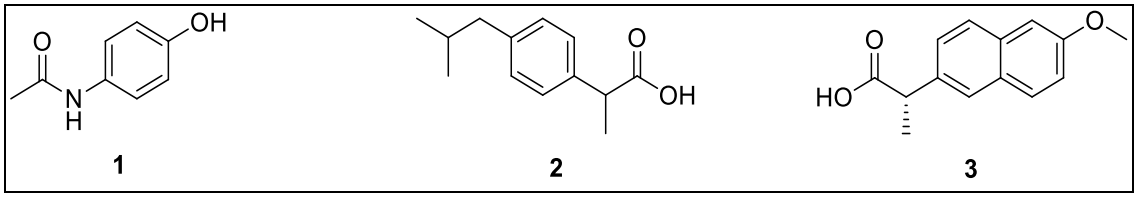
Chapter One

Introduction

1.1 Medicinal plant

Across the past few decades, there has been a significant rise in the prevalence of infectious diseases all over the world, making people's health an increasingly important topic of concern. In 2005, chronic diseases such as cancer and diabetes accounted for more than 60% of all fatalities worldwide. Furthermore, a number of infectious diseases, such as Zika virus and coronavirus, have recently spread considerably beyond their previously understood geographic limits (1, 2). In light of the increasing prevalence of diseases, supporting and maintaining health and well-being has become a necessity for all humans. Due to this health risk, the search for alternative treatments and pharmaceuticals that are both effective and safe is crucial.

Medicines are divided into two categories: conventional modern medicine and herbal or alternative medicine. The origins of modern pharmaceuticals can be traced back to the nineteenth century. New medications were being created to treat complex disorders, but these drugs came with a variety of side effects ranging from minor to fatal. For example, Paracetamol **1** and Ibuprofen **2** are common antipyretics but they have serious adverse effects including liver poisoning. Also Naproxen **3** can induce gastrointestinal problems (3).



1 **2** **3**

Despite the rapid progress of contemporary medicine, its technological advancements have reached an impasse (4). In the United States, toxins resulting from adverse reactions or side effects of drugs claim the lives of at least three times as many individuals as intoxicated drivers. Unlike herbal-related deaths which are quite uncommon. Therefore, individuals flock to herbal medications because they believe it is devoid of harmful side effects (5).

Medicinal plants and herbs are called traditional medicine also referred to as indigenous or folk medicine, it was utilized in early civilizations dating back to 5000 years ago. Indigenous cultures created traditional medical systems such as Siddha and Ayurveda medicines in India, Kampo Medicine in Japan, traditional Chinese medicine (TCM), and Unani medicine in the Middle East and South Asia (6, 7).

Patients seek herbal remedies for a variety of reasons, not only because they are less expensive and herbs are wildly available, but often citing it gives them a "sense of control, a mental comfort from taking action," In addition, many people suffering from chronic diseases such as cancer and diabetes, where they frequently think that traditional medicine has let them down in such circumstances will seek out herbs as a cure. Patients who have acute, self-limiting conditions like colds or sore throats tend to use alternative medicines because expert care is unavailable right away, is uncomfortable, or time-consuming (6). Moreover, cultural variables can also promote the usage of herbs “man earth relationship.” There is a belief that if an illness appears in some region, the plants there will be responded to this deviation and be supported naturally to treat this condition. As per the World Health Organization, traditional medicine is used by around 80% of the world's population (7, 8).

Taking all the above into account, a high need for plant-based medications, health goods, pharmaceuticals, food supplements, and cosmetics in both developing and developed nations has appeared. Therefore, chemists are shifting their fields from synthetic to natural medicines to meet these demands and to learn more about nature (3). Compounds derived from medicinal plants have been shown in several studies to have an antioxidant impact in addition to inhibitory effects on cancer cells, bacteria, and a variety of viruses, including hepatitis B, HIV, herpes, and influenza. Approximately 60% of commonly used anticancer medications are derived from natural products, according to recent statistics (8).

The main difference between conventional and herbal medicine is that the first one has a single active compound that chemically was synthesized or isolated from fungi, bacteria, or plant, while the last one is not a single substance, it contains several compounds which works synergistically. They could be extracted from flowers, leaves, stems, bark, or roots of plants (9). As a byproduct of their regular metabolic processes,

all plants produce chemical compounds. These include primary metabolites, such as sugars and lipids, which are found in all plants, as well as secondary metabolites, which are found in a restricted number of plant species. These secondary metabolites are the medicinal plant's foundation, and they may have therapeutic effects on humans (10).

1.2 Secondary metabolites

Secondary metabolites (SMs) are organic substances produced by plants that are frequently produced at a period after growth and are not directly associated in the growth, development, or reproduction of the plants. They are not essential for short term survival of plants, but their absence may influence their ability to reproduce, look well, or survive over the long term. Occasionally they will not have any apparent change at all. In addition to their use in medicines, they are distinctive sources for flavors, colorant, spices, and aromatics (11).

SMs are classified into three major classes, terpenes, phenols, and alkaloids. Their classification is based on their composition, chemical make-up, solubility in different solvents, or method of synthesis. Also, essential oils are one of the secondary plant metabolites that are lipophilic and very volatile (12, 13).

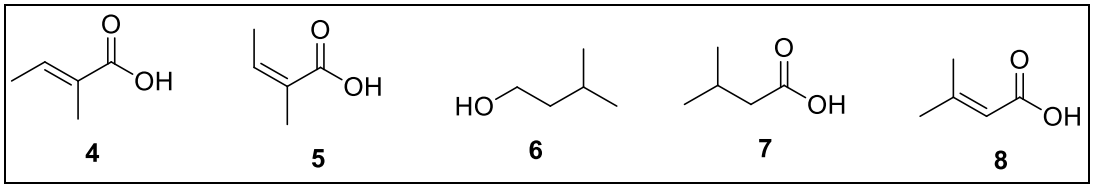
1.2.1 Terpenes

They are also known as isoprenoids or terpenoids and are generated from C5 isoprene units that are connected head-to-tail followed by cyclization and reorganizations of the carbon skeleton. Hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40) which known as carotenoids are some of the several types of terpenes (14). They serve a variety of purposes in plants, including thermoprotection, signaling, taste, and solvents (15). They also have several medical applications, it has been found that cannabis plant which is the most common source of terpene has anticancer, antimicrobial, antifungal, antiviral, antihyperglycemic, analgesic, anti-inflammatory, and antiparasitic activity [15].

1.2.1.1 Hemiterpenes(C5)

Their general formula is C_5H_8 and they represent the simplest terpenes. The leaves of several trees and herbs are the main source of them. Tiglic acid **4** and angelic acid **5** are examples of compounds belonging to hemiterpenes that can interact with other natural

substances to form esters, also isoamyl alcohol **6**, isovaleric acid **7** and senecioic acid **8** are hemiterpenoids (16).

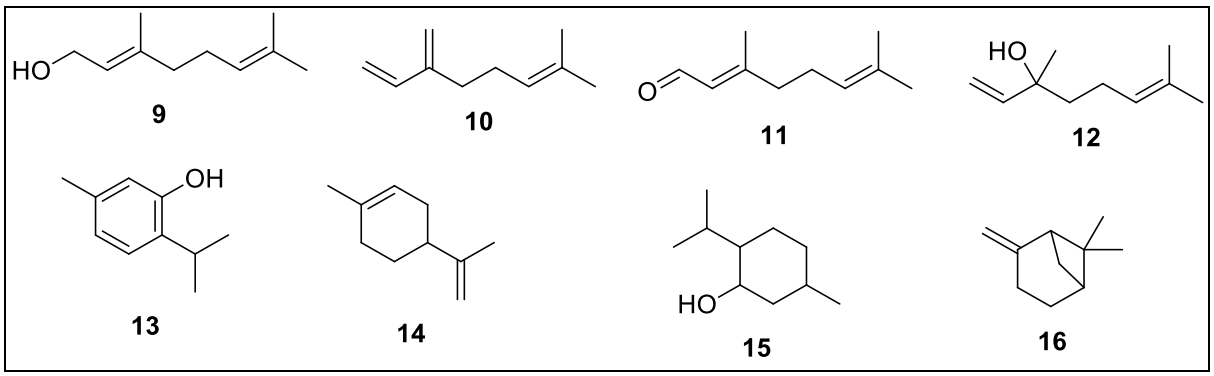


1.2.1.2 Monoterpenes (C10)

The typical formula for monoterpenes is $C_{10}H_{16}$. They are formed by two isoprene units and can be cyclized or oxidized to produce a variety of chemicals. These compounds have a distinct scent. They are also the most prevalent terpene in essential oils, so they are responsible for the aroma of plants. This special odor is due to the different functional groups that are attached to them, in addition to their responsibility in their biological activity. They are categorized into acyclic, monocyclic, and bicyclic monoterpenes (16).

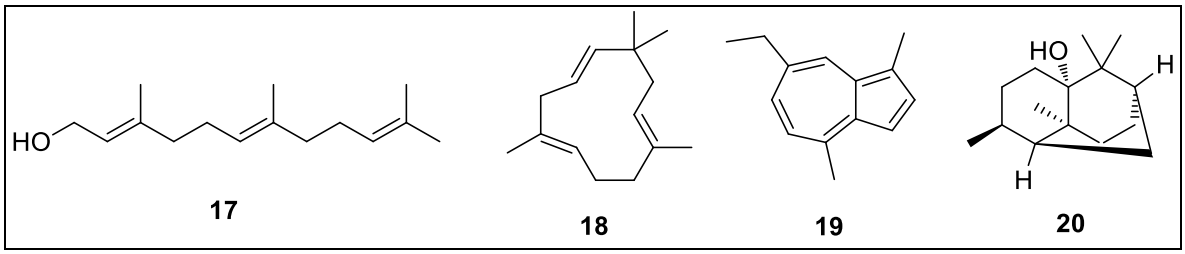
Geraniol ($C_{10}H_{18}O$) **9** is an example of acyclic monoterpene. Its scent is like that of flowers and roses. Geraniol has many advantageous medical qualities, including antioxidant, anti-inflammatory, antibacterial, anticancer, hepatoprotective, cardioprotective, and neuroprotective effects. Myrecene ($C_{10}H_{16}$) **10** which is an intermediate to manufacture perfumes, Citral ($C_{10}H_{16}O$) **11** which is a starting material of vitamin A, and Linalool ($C_{10}H_{18}O$) **12** which is an important synthetic intermediate are other acyclic monoterpenes (16, 17). Thymol ($C_{10}H_{14}O$) **13** is a monocyclic monoterpene, most of the monoterpenes in essential oils are made up of it. Lamiaceae family plants are the source of thymol such as Thymus, Ocimum, Origanum, Satureja, Thymbra, and Monarda. It contains antimicrobial and antioxidant properties; therefore, it has been used as a flavoring and preservative agent in the food industry. Furthermore, it is used in the pharmacological industries due to their positive antioxidant, anti-inflammatory, local anesthetic, antinociceptive, cicatrizing, antiseptic, antibacterial, antifungal properties as well as its beneficial effects on the cardiovascular system (18). Limonene ($C_{10}H_{16}$) **14** and Menthol ($C_{10}H_{20}O$) **15** are other monocyclic monoterpenes. Pinene ($C_{10}H_{16}$) **16** a bicyclic monoterpene (6+4 membered ring). Two isomers of pinene namely α- and β-pinene were isolated from plants. Their main source is the wood of coniferous trees. Each isomer has two enantiomers (+) and (-), resulting in four

active isomers. Pinenes find a wide range of applications, due to their different biological actions. It has been discovered that α- and β-pinene have inhibitory effects on leukemia and breast cancer, furthermore, they are used as antibacterial agents due to their damaging effects on membranes, in addition to their usage as fungicidal agents, tastes, perfumes, antiviral and antimicrobial agents (19).



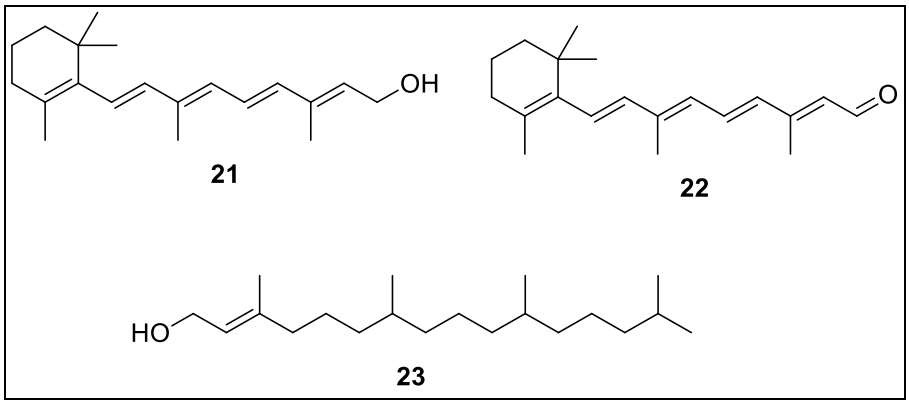
1.2.1.3 Sesquiterpenes (C15)

Their general formula is $C_{15}H_{24}$. They consist of three isoprene units. Sesquiterpenes can be acyclic, monocyclic, bicyclic, or tricyclic. Their properties are similar to monoterpenes, where they share the same ring classification as monoterpenes except for a few tricyclic terpenes. The stacking of the functional groups, the positioning of the 15-carbon skeletons, and the backbone substituents all contribute to the diversity of these terpenes. Farnesol ($C_{15}H_{26}O$) **17**, Humulene ($C_{15}H_{24}$) **18**, Chamazulene ($C_{15}H_{16}$) **19**, Patchouli alcohol ($C_{15}H_{26}O$) **20** are an example of acyclic, monocyclic, bicyclic, and tricyclic sesquiterpene, respectively. It has been reported that they have antibacterial, antifungal, antitumor, and anti-inflammatory activity. They also play an active role as ingredients in the fragrance industry and have numerous applications in plant defense against herbivores (16, 20).

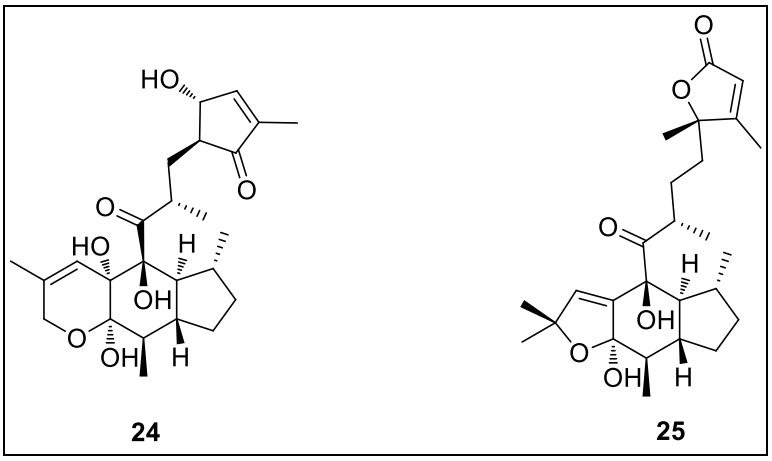


1.2.1.4 Diterpenes (C20) and Sesterterpenes (C25)

Diterpenes consist of four isoprene unites. They are classified based on the number and the cyclization patterns into acyclic, bicyclic, tricyclic, tetracyclic, and macrocyclic diterpenes, as well as mixed compounds that were isolated and identified from nature. Diterpenes serve as the building blocks for several biologically significant chemicals, including retinol **21**, retinal **22**, and phytol **23**. They have antioxidant, anti-microbial, anti-cancer, anti-inflammatory, wound-healing, anti-hypertensive, analgesic, and neuropharmacological properties. They can also be used as sweeteners, hallucinogens, and uterine contraction stimulants (21, 22).



Sesterterpenes (C25) are the least frequent class of terpenoids. This group of substances is descended from geranyl farnesyl diphosphate, which can be cyclized to produce several skeletal types with varied amounts of oxidation and biological activity. Even though there are numerous examples of these natural terpenoids. They are mainly found in marine and fungi-based organisms. Despite that, they can be extracted from plants, where Leucosesterterpenone **24** and leucosterlactone **25** are two tetracyclic sesterterpenoids isolated from Himalayan plant *Leusceptrum canum.* additionally, twenty-four tricyclic sesterterpenoids were extracted from *Salvia dominica* where they are all connected by a substituted pentane side chain and have a decalin with differently functionalized furan-2-one rings (12, 23).



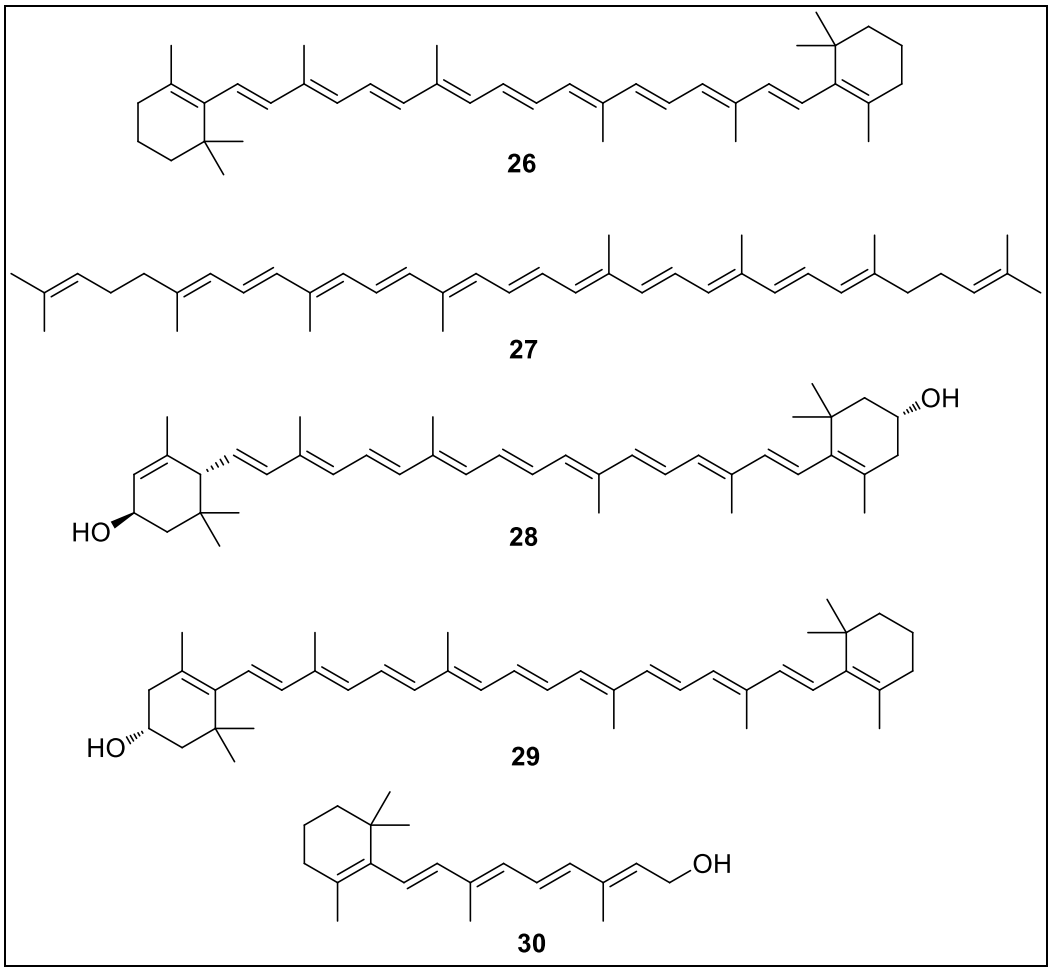
24 **25**

1.2.1.5 Triterpenes (C30) and Tetraterpenes (C40)

Triterpenes general formula is $C_{30}H_{48}$. Squalene, polyunsaturated hydrocarbon triterpene. and related acyclic 30-carbon precursors are created by combining two C15 units. Triterpenes are a sizable collection of naturally occurring chemicals with a wide range of structural characteristics.

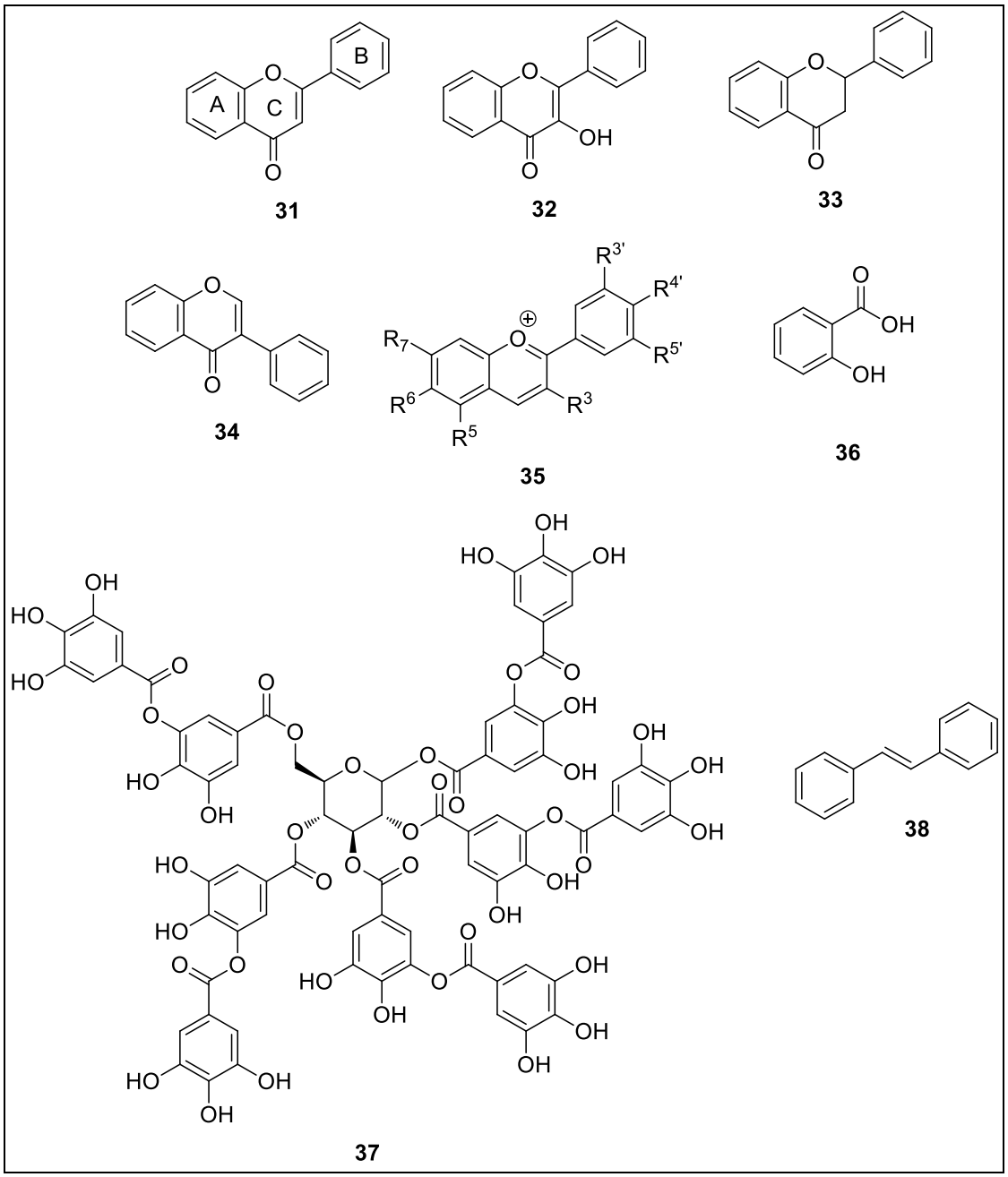
Different structures are created because of their cyclization and oxidation. There are two different ways that transformations take place: one results in tetra- and pentacyclic triterpenes, while the other passes through cycloartenole and cucurbitacines or cholesterol before continuing to phytosterols, cardiac glycosides, and steroid saponins (24).

Tetraterpenes, which have the chemical formula $C_{40}H_{56}$ and are also referred to as carotenoids. They are categorized into carotenes and xanthophylls. The first type occurs when the chain's terminus is converted into a cyclic ionone ring, while the second type is formed via addition of oxygen-containing functional groups such as hydroxyl-, oxo-, epoxy-, methoxy-, or carboxyl groups. β-carotene **26** and lycopene **27** are examples of carotenes while lutein **28** and cryptoxanthin **29** are xanthophylls. They can be found in many kinds of fungi, bacteria, and plants. and they are primarily in charge of producing the red, yellow, or orange fat-soluble pigments present in both plants and animals. They represent the greatest class of natural dyes. The yellow pigment in carrots is a result of beta-carotene, one of the most important and prevalent tetraterpenes. It is essential for mammals as it is a precursor to the production of vitamin A-30 and other essential terpenoids for vision (15, 25).



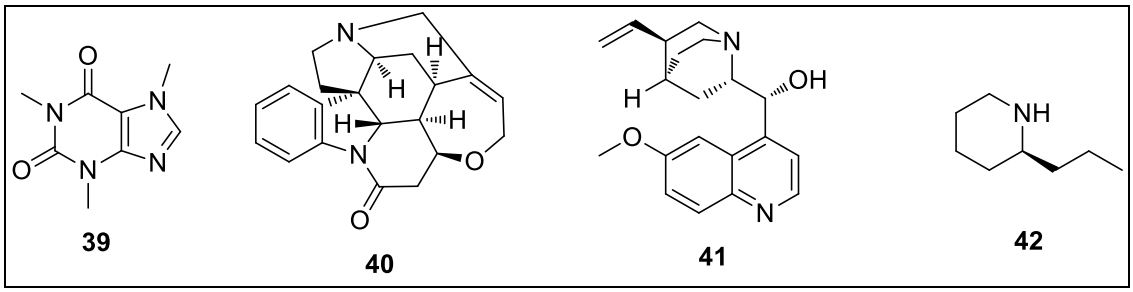
1.2.2 Poly Phenols

Polyphenols are the most abundant subgroups of secondary metabolites with significant physiological and morphological significance in plants. They contain at least one aromatic ring, attached to it one or more hydroxyl and/or methoxy groups. One of the most common polyphenols is flavonoid. Its general structure consists of two phenyl rings (A and B) and a heterocyclic ring (C). Depending on the oxidation state of the C ring, they are further split into six subgroups: flavones **31**, flavanols **32**, flavanones **33**, isoflavones **34**, and anthocyanins **35**. Phenolic acids **36** and tannins **37** are the other two major groups of polyphenols, whereas the less frequent categories are stilbenes **38** and lignans. Polyphenols have strong antioxidant properties. Epidemiological research as well as in vitro and in vivo experiments have suggested that these second plant metabolites may aid in the prevention of cancer, cardiovascular disease, and neurological disorders (26, 27).



1.2.3 Alkaloids

The primary requirement for classification as an alkaloid is the presence of a basic nitrogen atom anywhere in the scaffold of the molecule, with the exception of amide and peptide bonds. Since most alkaloids are basic (alkaline) chemicals, the name of the class explicitly refers to this. Opium was the first alkaloid isolated from plant that was used as analgesic and narcotic drug, then morphine was extracted from opium which is until these days is permitted to be used for extreme pains, also caffeine **39**, strychnine **40**, quinine **41** and coniine **42** are an active alkaloid (28).



1.3 Essential oil

Essential oil is not a specific compound; it is a mixture of low molecular weight components. More than 300 compounds may be found in it, of which most of the components belong to the terpenes group. The second contributors belong to the phenolic group, while aliphatic (alkanes and alkenes) compounds are often found in a trace amount (29). EOs are flammable, which is how they got their name. They are also volatile with a distinct odor and less than one unit of density apart from a few rare instances (cinnamon, sassafras, and vetiver). Aside from that they are insoluble in water but soluble in organic solvents. Different portions of aromatic plants contain essential oils. They may be found in flowers (pink, orange, lavender), flower bud in case of (clove and bracts in case of ylang-ylang), leaves (in case of mint, eucalyptus, bay leaf, thyme, sage, savory, pine needles), rhizomes (sweet flag and ginger), roots (vetiver), seeds (coriander and carvi), fruits (anise, fennel, and citrus epicarps), and wood and bark (in sandalwood, cinnamon, and rosewood) (30). The glandular hairs, secretory ducts, and oil cells of plants may hold essential oils. They may sometimes be attached to sugars in the form of glycosides, so they need to be released in these conditions by hydrolyzing the glycosidic bond (31). EOs are crucial to plant life. They assist in regulating water, attracting pollinating insects and fruit-dispersing animals in addition to helping protect plants against herbivores, insects, and microbes. Moreover, it’s a very worthy product for human beings; they have been utilized as raw materials in a variety of businesses, including those in the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume sectors (32). Economically speaking, there is a lot of interest in the usage of essential oils in the perfume, soap, detergent, and cosmetics industries. With some kinds of aromatic plants being in high demand on the market, there is no doubt that the global output of essential oils used in the creation of perfumes has expanded. Salvia, lavender, and thyme concrete chemotypes are particularly recognized for producing exquisite and innovative fragrances. To this purpose, production

technique and judicious choice of raw materials are key components for raising the caliber of the finished good. Earlier, the main use of essential oils as therapeutic agents may be was in aromatherapy. Essential oils are most frequently applied topically in diluted forms, frequently in conjunction with a carrier oil as part of massage therapy. After adding a few drops to steaming water, using an atomizer, or using a humidifier, they can also be inhaled. They can also be used as compresses, ointments, and creams. To get the therapeutic benefits of these chemicals, oral administration of essential oils via encapsulation or other controlled release techniques has been proposed. They can also be taken as soft capsules, which allow for more precise dose and prevents a number of negative effects, or as tea with sugar. However, it's likely that using this method will increase the toxicity of the essential oils (33). The feature of several essential oils that can be attributed to their potential therapeutic effects against several disorders is their capacity for cytotoxicity. The primary ways through which essential oils exert their cytotoxic effects on cells are cell death promotion by activating apoptosis and/or necrosis processes, cell cycle arrest, and loss of function of vital organelles. As they are lipophilic and the molecular weight of their components is low this enables them to pass cell membranes, change the composition of membranes, and enhance membrane fluidity, which causes leakage of ions and cytoplasmic molecules. This change on membrane can induce cell death by lowering ATP synthesis, affecting the pH gradient, and reducing mitochondrial potential (34).

1.3.1 Extraction methods of EO

Several methods were developed to extract EOs, the purpose of all these different techniques is to produce EO with high quality, in addition, to obtaining oils with acceptable yields. It is particularly important to choose the appropriate method that does not destroy the therapeutic properties, where some solvents may affect them. Moreover, the final product's intended use also determines which method will be used (35).

In general, extraction techniques are divided into two main categories, the conventional extraction techniques, and the green extraction techniques.

1.3.1.1 Conventional extraction

Conventional extraction uses water or organic solvents under atmospheric pressure. These techniques are distinguished with their simplicity, wide accessibility, and low

cost. The solvents penetrate the solid plant matter and solubilize the polar comparable chemicals inside, so selection of the suitable solvent is essential (36). This type includes steam distillation, hydro diffusion, hydro distillation, cold pressing, and solvent extraction.

1.3.1.1.1 Steam Distillation

The plant in this method is injected into the alembic without water maceration, then exposed to the steam that flows from the bottom to the top of the alembic. Then the essential oil-filled vapor passes through a "swan-neck" column followed by condensation. Finally, it will be collected in a Florentine flask. The idea behind this method is to get the total vapor pressure to equal ambient pressure at around 100 °C so that the volatile substances, which are boiling at temperatures range from 150 to 300 °C, can be evaporated at a temperature similar to that of water (37).

1.3.1.1.2 Hydro Diffusion

It is a steam diffusion, which differs in the way steam is introduced into the still's container, where the steam is introduced from the top of the generator. This technique is primarily employed with dried plants that have been harmed by boiling. Low pressure or vacuum can be used to process this method which allows to lower the temperature than 100 °C, it has the benefit of gathering hydrosol (essential water) and EO separately. Moreover, because of its quicker processing time and higher oil production with less steam utilized, the hydro diffusion technique is more effective than steam distillation (38, 39).

1.3.1.1.3 Hydro Distillation

This method is considered the simplest, easiest, and oldest method of extracting oil. Clevenger apparatus which consists of heating source, a vessel, a condenser, and decanter

used in this method, where the plant is immersed in water inside the vessel, heated for 3-6 hours, then vapor from the vessel is converted to liquid by the condenser, that collected in the decanter where the EO is separated with little amount of water, and dried with anhydrous sodium sulfate (Na_2SO_4) (39).

1.3.1.1.4 Cold pressing

The cold pressing method occurs without heating, EO is extracted by mechanical methods, especially from the rinds of fruits belonging to the citrus family, such as lemons, oranges, and grapefruits. The result from this method is in the form of juicy oil, so a separation process is used to separate oils from water. The disadvantage of this method is that these oils spoil faster than other oils. Its simplicity, natural, safe, eco-friendly, traditional, and less expensive cost are considered advantages for this method. Other advantages of using this method include the preservation of certain temperature-sensitive phenolic compounds and lipophilic phytochemicals like antioxidants, which serve to keep us healthy and prevent disease (39, 40).

1.3.1.1.5 Solvent Extraction

This technique has been used for fragile or sensitive flower materials that cannot withstand the heat or steam distillation. The plant is dissolved after being submerged in a solvent bath and extracted by using moderate heat. The solvents may be alcohol, hexane, ethanol, petroleum ether, and methanol. The choice of extraction solvents is a crucial factor in this method, and the specialists avoid solvents that can impair the extraction procedure or react with the extract. The obtained extract is filtered then concentrated. The resulting concentrate consists of EO and other compounds, so it is mixed with alcohol to extract the oil followed by distillation at low temperature. This method is used in the perfume industry, while it is not safe to be used for food applications where it may contain a residue from the solvent that has been used (38, 41, 42).

1.3.1.2 Green extraction

Humans are facing challenges to protect themselves and the environment. The current international industries and economies heavily depend on fossil fuel supplies, but these resources are going to be depleted, so there is a need to go toward green methods. Chemistry is a component of this dilemma, from an ecological and economic chemistry standpoint, green extraction of natural resources may be one of the solutions for humanity's transition from the past to the future. Green extraction aims to produce extracts with higher extraction efficiency and a better quality while consuming less energy globally, using less solvent overall, having a smaller negative impact on the

environment, spending less money, and producing less waste. While the conventional methods are not always safe where the extracts may be contaminated with solvents, in addition to producing copious amounts of waste due to use of water and solvents in large scales. Additionally, it is considered as time and energy consuming (43). Ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE) are innovative methods for extraction listed under green extraction.

1.3.1.2.1 Ultrasound-assisted extraction (UAE)

UAE offers an environmentally friendly, clean extraction with several benefits offered by ultrasonic assisted extraction. In addition, compared to other innovative extraction procedures, ultrasound is comparatively inexpensive, versatile, and adaptable. Acoustic cavitation, which occurs because of the formation of cavitation bubbles is the primary factor that drives the extraction impacts of sonication. Two different forms of cavitation bubbles exist. When a medium is subjected to ultrasound, two types of cavitation are created: transient (inertial) cavitation and stable (noninertial) cavitation. Numerous transitory cavitations violently collapse, changing the temperature and pressure. In the case of EO, these changes result in the rupture of EOs glands, which encourages the release of EO from the plant (37, 44).

1.3.1.2.2 Supercritical fluid extraction (SFE)

This method uses CO_2 as a supercritical fluid (SF). It is readily detachable from extracts, has high purity, cheap cost, and is only slightly non-flammable, non-explosive, and non-toxic. In addition, CO_2 has low surface tension and viscosity with two to three times the diffusivity of other fluids. When temperature and pressure are increased past the critical points established for a particular liquid or gas, an SF manifests itself. CO_2 has a critical temperature (32 °C) and pressure (7.4 MPa) which are considered low, therefore, count as an advantage. The distinctive feature of SFs is that their density varies when pressure and temperature changes, a slight increase in pressure could result in a huge rise in fluid density, which would affect the solvating ability of SF, consequently, SFE's key benefit is selectivity. SFE by CO_2 is used in EOs extractions because it has polarity like pentane therefore; it is convenient for lipophilic substances (45).

1.3.1.2.3 Microwave-assisted extraction (MAE)

MAE has been demonstrated to have several benefits, including a reduced need for solvent, higher extraction efficiency, and a cheaper price. Electric field, which produces heat, and magnetic field, are the two oscillating perpendicular fields that make up microwaves. Two key factors which are ionic conduction and dipole rotation, govern the mechanism of microwave heating of samples, where both are directly influenced by polar materials or solvents (46). This technique involves grinding the sample materials and soaking them in a designated solvent. The mixture is then put into a microwave. Because of the disruption of plant cells caused by elevated temperatures, the volatile EOs released from the oil glands were then directed toward the condenser (39).

1.4 Biological activities of essential oils

Biological activity, in other words, is a substance's effects on living things, either good or bad. The diverse composition of EOs gives rise to a wide range of bioactivities, consequently, they can participate in the treatment of a number of illnesses, because their biological activities have been reported in much research such as anticancer, anti-microbial and antioxidant activities (47, 48).

1.4.1 Antioxidant activity

Antioxidants are "molecules able to react with radicals" or have reducing capacity to prevent the oxidative stress brought on by radicals (49). Free Radicals (FR) or Reactive Oxygen Species (ROS) can start aggressive oxidation events inside the cells as well as at the surface of the cell membranes. The mechanisms in the body (incomplete catabolism, energy production, hepatic detoxification, etc.) and the external environment (cigarette smoke, contaminated air, meals, medications, well/tap water, etc.) both contribute to the development of FR (50). Many disorders are related to ROS such as cancer, atherosclerosis, stroke, trauma, heart attack, asthma, age pigments, etc. ROS split into two types, oxygen centered radicals (superoxide anione $O^{\bullet -}_2$, hydroxyl radical $OH^{\bullet}$, alkoxyl radicals $RO^{\bullet}$ and peroxyl radicals $ROO^{\bullet}$) and oxygen centered non radicals (hydrogen peroxide H_2O_2 and singlet oxygen $^1O_2$), also there are reactive species contain nitrogen such as nitric oxide $NO^{\bullet}$, nitric dioxide $NO^{\bullet}_2$, and peroxynitrite $OONO^-$ (51).

Enzymatic and non-enzymatic systems work together to protect against oxidant assault; the enzymatic defenses include glutathione peroxidase, catalase, superoxide dismutase, DT diphorase and tetrameric selenoprotein, while the non-enzymatic defenses include ascorbate (vitamin C), α-tocopherol (vitamin E), flavonoids, ceruplascoenzyme Q, ferritin, bilirubin, taurine, melatonin, carotenes, zinc, manganese, and cysteine (52, 53).

Due to the significance of oxidative stress in disease, antioxidant capabilities also play a crucial role in various biological activities of EOs. These characteristics are caused by the natural ability of some of their constituents, especially phenols, to inhibit or postpone aerobic oxidation of organic matter. However, the distillation process used to extract oil from the raw material has a limit on the amount of phenolics that can be found in the final matrix because many of these compounds are nonvolatile. However, there are EOs that exhibit antioxidant activity that are phenol-free. This is a result of some terpenoids and other volatile components' radical chemistry. It is required to briefly discuss essential oils' composition in order to explain the mechanism of the antioxidant activity shown by those substances. Despite the considerable chemical diversity that has been observed, terpenoids (monoterpene, sesquiterpene, or diterpene) and phenylpropanoids are the two structural families that best describe the primary constituents of typical essential oils. Phenolic chemicals are members of the phenolic family and are found in both the terpenoid and phenylpropanoid families. Phenolic compounds, whether they are natural (for example, tocopherol) or synthetic (for example, BHA), operate as antioxidants because of their high reactivity with peroxyl radicals, which are eliminated through formal hydrogen atom transfer. A second peroxyl radical will "wait" for the resultant phenoxyl radical due to its stability, which will cause a very quick radical–radical interaction to quench it instead of continuing the radical chain. Other terpenoids in essential oils can react with peroxyl radicals quickly, but the interaction results in a reactive alkyl radical (from the terpene hydrocarbon skeleton), which when combined with oxygen creates a peroxyl radical that continues the oxidative chain. In other words, nonphenolic terpenoids, especially unsaturated ones, would undergo autoxidation in a manner similar to that of unsaturated lipids. When some EO components are combined with an oxidizable substance, such as unsaturated lipids, both the lipids and the EO substances will undergo autoxidation and experience similar deterioration. In other words, co-oxidation between the potential antioxidant (the EO components) and the substrate to be protected (the lipid) will occur.

Because the essential oil reacts with chain-carrying peroxyl radicals to produce reactive species that can further the oxidative chain, there is no hope that it will provide any protection. One may anticipate that when a natural EO is employed to protect a material, the more potent antioxidant components would predominate, and that this protection would account for the majority of the oil's overall oxidative protection. Although there have been many recorded exceptions, this is generally true. In reality, the intricate interactions between components and the oxidizable material to be protected are what ultimately determine an antioxidant's overall efficacy. In general, either synergistic or antagonistic action is anticipated, depending on the precise EO composition and the experimental setup. Overall, caution should be used before presuming that an EO's antioxidant property is solely due to one particular distinctive component. However, taking into account the composition of the oil can allow for an approximate prediction of its antioxidant potential: good antioxidant behavior can be expected from EOs having a large content in phenolics and a modest content in unsaturated terpenes; even greater protection could come when the oil contains both a lot of phenolics and a lot of cyclohexadiene-like components. When combined with edible fats, oils that contain little to no phenolic and cyclohexadiene-like components or only a small amount of them are likely to provide only minimal protection (49).

1.4.2 Anti-microbial activity (bacteria and fungi)

Many diseases and infections can be brought on by bacteria, viruses, fungi, parasites, and protozoa. Although bacteria are the most prevalent infectious agents. Tuberculosis, leprosy, treponematosis, brucellosis, glanders, actinomycosis, and plague are all bacterial diseases (54). HIV, Ebola, and Marburg hemorrhagic fevers are just a few of the thirty-nine new diseases that have been discovered since 1967, according to the WHO. Due to a confluence of biological mutations, increasing antibiotic resistance, and underdeveloped healthcare systems, "century-old threats" like influenza and malaria continue to spread. A more interconnected world, marked by greater mobility of people, animals, and things has made health hazards more acute (55).

Antibacterial drugs are now commonly referred to as antibiotics. Bacteria may be naturally resistant to some antibiotics, but they can also develop resistance to them through chromosomal gene changes and horizontal gene transfer. Antibiotic resistance is one of the biggest hazards to human health. According to the most recent World

Economic Forum Global Risks assessments (56). *Escherichia coli and Klebsiella* are types of bacteria commonly found in livestock, which are the most typical causes of bloodstream infections in patients as well as the most typical causes of urinary tract infections. *Staphylococcus aureus*, the most frequent cause of skin infections and the second-most frequent cause of bloodstream infections in patients (57).

There are very few treatment options for infections caused by antibiotic resistant bacteria. Furthermore, it is quite uncommon to find new and improved antibiotics as a result. It's important to investigate other options that might work to treat these severe bacterial infections. Plant extracts have been found to be promising treatments for illnesses and antimicrobial therapy, where there is plenty of prospective for developing therapeutic plant phytochemicals as bio-enhancers of antibiotic (58, 59).

EOs have been shown to have antibacterial activity in numerous studies, this activity is back to their hydrophobicity, where they can partition into the lipids of the bacterial cell membrane, causing the structure to be disturbed by increasing its permeability. Due to the loss of cellular components or essential molecules or ions, cell death may occur. It has been discovered that EOs with notable antibacterial activity contain a high concentration of phenolic compounds (Carvacrol, eugenol, and thymol) (60). It has been found that these components have an antibacterial impact on a variety of bacteria, including *Escherichia coli, Bacillus cereus, Listeria monocytogenes, Salmonella enterica, Clostridium jejuni, Lactobacillus sake, Staphylococcus aureus, and Helicobacter pyroli* (60).

One of the key contributors in the spread of diseases is the microbial contamination of food. Despite the availability of numerous trustworthy preservation techniques, the issue of food spoilage and microbial poisoning is still not sufficiently under control. A rapidly expanding market exists for moderate food preservation methods that are both safe and kind to the environment in many nations across the world. The attraction of fresh food products is negatively impacted by conventional methods of food preservation, and artificial preservatives are progressively being prohibited. Food makers are looking for new, more natural substitute ingredients or chemicals that can sufficiently ensure the safety of their goods in the retail supply chain by serving as an antibacterial agent in order to satisfy the expanding consumer demands. Since many plants' volatile oils are known to have antibacterial properties, they may serve as a

chemical barrier against plant diseases. Pathogens can easily enter wound sites left by various herbivores, for instance. When volatile oil glands that cover leaves are injured, the glands burst, causing the oil to spill over the lesion. Thus, the presence of antibacterial action in the oil would be extremely advantageous to the plant. In fact, the vast majority of aromatic and therapeutic plants resist many of the most prevalent ailments. Essential oils appear to be a suitable alternative In instead of antibiotics or artificial preservatives. But, the difficulty is in defining more precisely the threshold for each essential oil, the minimum inhibitory concentration, any possible future residue from their use, any potential allergenicity or toxicity, their efficacy in vivo, and ultimately their cost-effectiveness. As a result, a number of natural products will be available that are risk-free, efficient, and devoid of the negative side effects associated with the use of antibiotics and other chemical preservatives in the food supply (61).

Compared to bacterial infections, fungal infections are caused by eukaryotic organisms, making it more challenging to detect them and administer the proper therapeutic therapy. Because human cells lack chitin, the cell wall of fungus may be thought of as the primary target for selectively harmful antifungal drugs. Due to their accumulation in the lipophilic hydrocarbon molecules of the cell's lipid bilayer, EOs constituents may operate as antifungal agents. This buildup also makes it simpler for other EOs constituents to enter the cell's interior. The diversity in water solubility and lipophilic characteristics of the EOs may help to explain the varied activity (62).

1.4.3 Anticancer activity

Cancer is a fatal disease that ranks among humanity's most important healthcare concerns and demands a proactive treatment strategy. There will be 21 million cases of cancer worldwide by 2030, according to predictions. It is among the biggest causes of death and disease (63).

Cancer is uncontrolled cell growth that occurs because of altered cell function. The cell's accumulation of several genetic and epigenetic alterations may cause this deviation. An individual explanation cannot be responsible for all of this, but it can be said that the interplay of many risk factors plays a significant role in the development of cancer (64).

For cancer treatments there were many synthetic drugs, and several methods to achieve cures such as chemotherapy, surgery, and radiotherapy. The prime concern with these therapies is the side effects of chemotherapy medications, which have been proven to have detrimental effects on the body and suppress the immune system. Additionally, medication resistance diminishes the efficacy of treatment. New treatments and medications for cancer are desperately needed. Given that herbal medicine has been shown to be safe and to have few or no side effects, especially when compared to synthetic medications, there is currently a great deal of interest in researching plants that can be used to prevent and treat cancer (65-67). A high dietary intake of fruits and vegetables, as well as whole grains, is highly associated with lower risk of cancer, according to a large body of evidence from population and laboratory research. More than 5,000 distinct phytochemicals, primarily categorized as phenolics, carotenoids, vitamins, alkaloids, nitrogen-containing compounds, organosulfur compounds, and essential oils, have reportedly been discovered in fruits, vegetables, cereals, and other plants. Essential oil components have garnered the most curiosity and interest among the tremendous structural diversity of phytochemicals due to their extensive range of bioactivities. These dietary substances are thought to have cancer-preventing properties because they stimulate cellular defense mechanisms like the detoxifying and antioxidant enzyme systems and inhibit anti-inflammatory and anti-cell growth signaling pathways, which results in cell cycle arrest and/or cell death (68).

It has been reported that oxidative stress might damage DNA, increasing the rate of cell mutation and encouraging the development of cancer. Additionally, reactive oxygen species may particularly activate signaling pathways and hence influence cellular growth, angiogenesis, and metastasis, which in turn influences the development of tumors. As a result, chronic inflammation has been connected to a number of processes that contribute to the development of cancer, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis. So, EOs and the elements included within them may be effective against a variety of cancer cells, according to several studies. It is challenging to pinpoint a specific mode of action for EOs because of their highly varied compositions. A chemical may, in fact, affect one form of tumor but not another. In fact, an EO's biological action is typically correlated with its chemical makeup, specifically the primary functional groups of substances (alcohols, phenols, terpene compounds and ketone). Because different molecules may

interact with the big chemicals in a synergistic manner, the less common compounds could also be significant. For instance, limonene or linalyl acetate, which are reported to be concentrated in bergamot, cannot duplicate the effects of bergamot essential oil on caspase-3 activation, PARP cleavage, DNA fragmentation, cell shrinkage, cytoskeletal changes, as well as necrotic and apoptotic cell death (29).

1.5 *Aloysia citrodora*

Aloysia citrodora palau also called lemon verbena belongs to the verbena family, its origins back in South America but Africa, Europe, and the Mediterranean region are all seeing an increase in its cultivation, it's a flowering plant that is characterized as perennial, emits a strong lemon odor, *Aloysia triphylla, Lippia citriodora, Verbena triphylla* and *Lippia triphylla* are synonyms for *Aloysia citrodora*. Its height ranges from 3-7 meters, it has pointy, lance-shaped leaves that are pale green in color. Once dried, they retain their flavor and aroma well (69).

The distinctive flavor and scent of the *A. citrodora* essential oil accounts for a substantial portion of the plant's use, especially as food additives. Additionally, the herb was utilized for a range of culinary, aromatic, and cosmetics including herbal soaps and potpourris. It has been used in traditional medicine for gastrointestinal and antispasmodic purposes as well as against bronchitis and cardiac diseases. It was also used for diuretic, antipyretic, sedative, carminative, expectorant, against headache, antihistaminic, and emmenagogue actions (69, 70).

Recently it has been reported some pharmacological effects, such as analgesic effect, where the active ingredient was identified as acteoside (verbascoside), which also showed a mild sedative effect when taken orally (71). The biological activities of essential oils in general back to their chemical composition, sesquiterpenoids and their derivatives, which are a part of the *A. citrodora* EO, are thought to be responsible for many of the biological activities of this oil, including its anti-inflammatory, antibacterial, anti-asthmatic, and antifungal properties. This makes it an interesting herb for research (72).

Apigenin **43** and scutellarein **44** are the most abundant flavones found, along with geranial, which is a monoterpene found primarily in many aloysia essential oils. Oxygenated monoterpenes have been discovered as the predominant category in the

majority of the described profiles of Aloysia species, as well as other monoterpene hydrocarbons, oxygenated sesquiterpenes, and sesquiterpene hydrocarbons including limonene, geraniol, 1,8-cineole, β-caryophyllene, and spathulenol (71, 73).

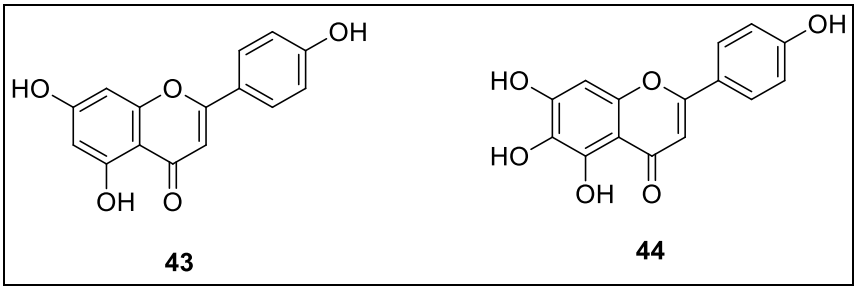


Figure 1.1

Aloysia citrodora plant.

1.6 *Origanum syriacum*

It is also known as za'atar, Lebanese oregano, Syrian oregano, and Bible hyssop. It belongs to the families Labiatae (Lamiaceae) and Mentheae. Origanum species are indigenous to the Mediterranean region and are gathered once or twice annually during the flowering period. It is one of the most important stable herbs in the Arab world due to its low cost, pleasant flavor, and wide range of culinary applications. *O syriacum* is a

perennial plant with branched, hairy, woody branches and creeping, woody roots, which grows to a height of 60 to 90 cm (about 2.95 ft) (74).

It is not only used in culinary recipes, but it was also used traditionally as an effective painkiller for joint discomfort, and chewing the leaves soothes tooth and gum pain. Rubbed on the chest, it cures bronchitis, benefits the liver and stomach, and has a potent anthelmintic effect (75).

The ingestion of this plant has also been demonstrated to have no negative effects on animals in toxicity experiments, furthermore, recently it has been found to possess several biological attributes, including anti-inflammatory, anti-cancer, neuroprotective, antioxidant, and antihelminthic actions, which making it a desirable natural agent for the discovery of new drugs (76).

The major components of Origanum EO are thymol and/or carvacrol. γ-terpinene, p-cymene, linalool, terpinen-4-ol and sabinene hydrate come in second place. The genotype, climate, type of soil, time of harvest, conditions of drying and storing, extraction methods and stage of plants growth all have an impact on its composition (77, 78).

Figure 1.2

Origanum syriacum plant.

1.7 Objectives of the Study

This study involves extracting the essential oils of *Aloysia citrodora* and *Origanum syriacum* from their dried leaves, analyzing their components using GC-MS, evaluating their bioactivities, and determining the synergistic effects of particular biological activities.

The specific objectives of this study are:

1. Extract the essential oil of *Aloysia citrodora* and *Origanum syriacum*.
2. Utilize the GC-MS technique to examine the chemical makeup of the EOs from the leaves of *Aloysia citrodora* and *Origanum syriacum*.
3. Analyzing the chemical differences between *Aloysia citrodora* taken from Qalqilya versus *Aloysia citrodora* collected from Jericho.
4. Investigating the antioxidant properties of the EOs of *Aloysia citrodora* from Qalqilya and *Origanum syriacum* from Jerusalem using DPPH assay.
5. Assessing the antibacterial activities of *Aloysia citrodora* EO from Qalqilya and *Origanum syriacum* EO from Jerusalem by evaluating MIC using broth-micro dilution method.
6. Investigating the anticancer activates of *Aloysia citrodora* EO from Qalqilya and *Origanum syriacum* EO from Jerusalem utilizing MTS assay against B16-F1, HeLa, 3T3, Hep G2, MCF-7 cancer cell lines and LX-2 as normal cell line.
7. Assessing the synergistic effects of *Aloysia citrodora* and *Origanum syriacum* EOs anticancer activity.

Chapter Two

Experimental Part

2.1 Materials and methods

2.1.1 Chemicals and reagents

The materials utilized in this study were of analytical grade required no additional purification. DPPH (1,1-Diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Germany), Methanol (Lobachemie, India), Trolox ((s)-(-)-6-hydroxy-2,5,7,8- tetramethychroman-2-carboxylic acid) (Sigma-Aldrich, Denmark), drying agent $CaCl_2$, Dimethyl sulfoxide (DMSO) 10% (Riedeldehaen, Germany), RPMI-1640 medium (Roswell Park Memorial Institute-1640 medium) (Sigma-Aldrich, R0883, UK) and Trypsin EDTA which was purchased from Sigma-Aldrich.

2.1.2 Instrumentation

Gas Chromatography Mass Spectrometry (GC-MS) (QP-5000 GC-MS Shimadzu, Japan), Electronic balance (Wagl, AS 220/C/2, Radwag, Poland), hydro distillation apparatus, 96-well plates (Greiner bio-one, North America), UV-Vis (Ultraviolet-Visible) spectrophotometer (Jenway 7315, England), micropipettes (Finnpipette, Finland) and CO_2 incubator (ESCO, 2012-74317, Singapore), Inverted biological microscope (MRC, 2017-170529, China), Microplate Absorbance (Bio Tek, 1903217-2019, USA).

2.1.3 plants collection and preparation

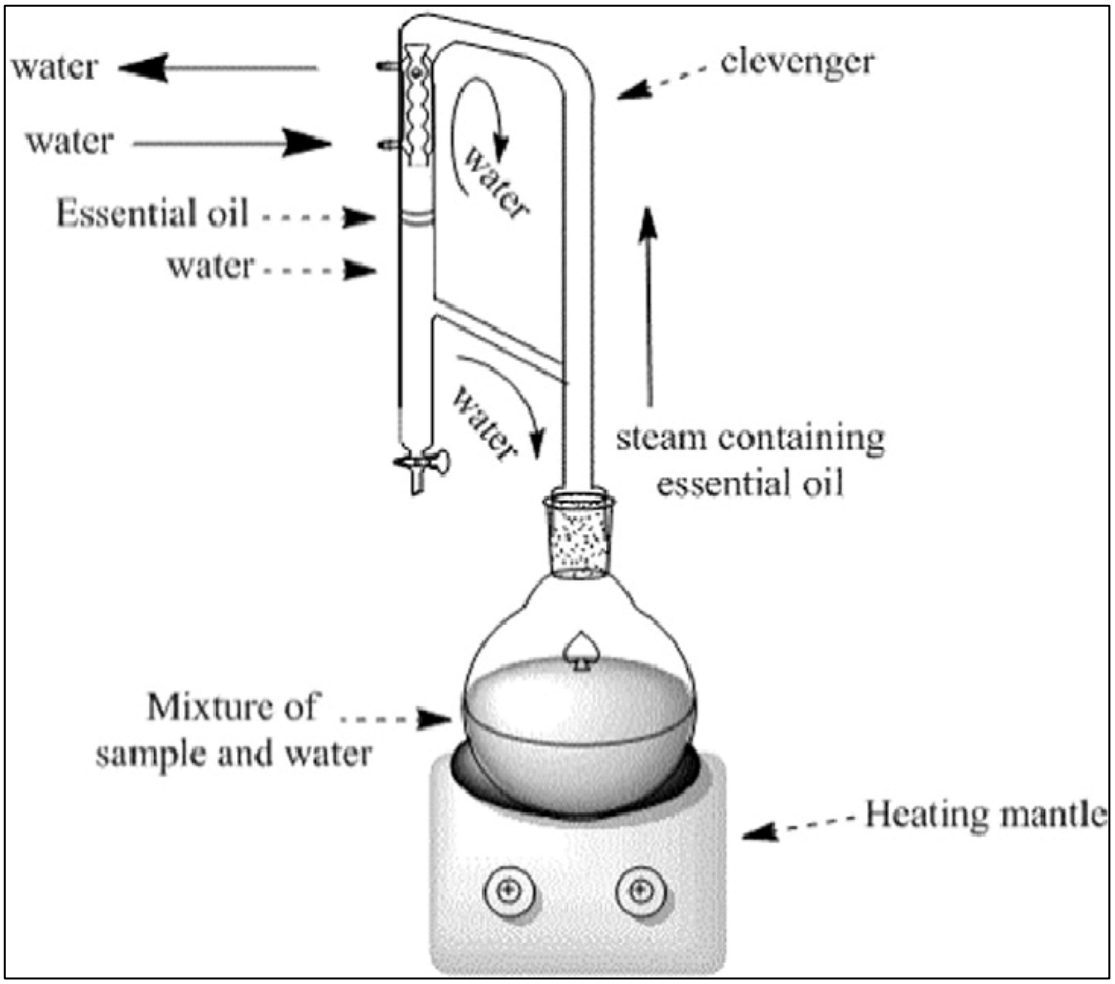
Origanum syriacum was collected from Al-Quds and *Aloysia citriodora* Palau leaves were harvested in two locations in Palestine in June 2022: Jericho, the world's deepest place, and Qalqilya 10 km from the Mediterranean. After identification of plants, voucher specimens have been deposited at An Najah National University's Department of Pharmacy's Herbarium under the collective code Pharm-PCT-2779P. The plants were washed with distilled water then dried in the shade for a week at room temperature (25±3 °C) and humidity (55±4 RH). After being dried the leaves were separated from the stems and coarsely ground, then stored in a paper bags for further isolation process.

2.1.4 Extraction of *O. syriacum* and *A. citrodora* essential oils

Hydro-distillation was used for extraction the EOs, 100g of the dried grinded leaves were put in 500 mL volumetric flask, and distilled water was added to it. The flask was subjected to Clevenger apparatus (**Figure 2.1)** for 3 hrs. Calcium chloride was used to dry the extracted EO before it was put into a small, clean glass vials with a tight lid and identified with the name of the plant, the date, and the sample code. The vials were then kept in the refrigerator at 4 °C for storage.

Figure 2.1

Clavenger apparatus.



2.2 Identification of the chemical composition of *O. syricum* and *A. citrodora* EOs by gas chromatography-mass spectrometry (GC–MS)

The Perkin Elmer Elite-5-MS fused silica capillary column (30 m 0.25 mm, film thickness of 0.25 m) was utilized for the separation and identification of *O. syricum and*

A. citrodora EOs components. Helium was employed as the carrier gas at a standard flow rate of 1.1 mL/min. The injector's temperature was set at 250 °C, with a ramp of 4.0 °C/min to 280 °C, an initial hold of 5 minutes, and a temperature of 50 °C. The solvent delay ranged from 0 to 4.0 minutes, and the whole running time was 62.5 minutes. For a mass range of 50.00 to 300.00 m/z, an MS scan took between 4 and 62.5 minutes.

Both mass spectral data and the calculated retention indices (RI) were utilized in the identification of the compounds. The calculation of linear-temperature-programmed RI was done from the equation: $RIx = 100n + 100\ (x) - (n)\ (n+1) + (n)$ where (x) is the retention time of the analyzed compound (x) and (n) and $(n+1)$ are retention times of n-alkanes (leaving the chromatographic column before and after the compound under consideration.

Comparing the mass spectra of *O. syricum* and *A. citrodora* EOs to reference spectra from the NIST's MS Data Centre and connecting their Kovats and retention indices to those stated in the literature, allowed for the identification of the chemical components of both species (79-81). The percentage areas for each component from the EOs of *O. syricum* and *A. citrodora* were calculated in quantitative calculations.

2.3 DPPH radical method for evaluation of antioxidant activity

The antioxidant activity of *O. syricum and A. citrodora* was determined by utilizing The free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay using literature procedure (82). It is based on a mechanism whereby the antioxidant reduces the violet DPPH radicals through a transfer of hydrogen atoms, changing the color to stable light yellow DPPH molecules. To test the antioxidant activity, a UV-Vis spectrophotometer is used to quantify the residual violet DPPH radicals at a wavelength of around 515–520 nm (83).

A stock solution with concentration of 100 μg/mL was prepared from each EO by dissolving 10 mg of the oil in 100 mL methanol followed by preparing a solution of trolox which is the reference product on the same way. Six dilutions were prepared by collecting quantities from the stock solution (EO and Trolox) that were 0.5, 1.0, 2.0,

5.0, 8.0, and 10.0 mL, and bringing them up to 10 mL with methanol using a 10 mL volumetric flask

(VF), working solutions with the following concentrations: 5, 10, 20, 50, 80, and 100 μg/ml were created respectively. Using 100 mL VF, 2 mg of DPPH was dissolved in 100 mL of methanol to create the freshly synthesized DPPH solution, which had a concentration of 0.002% w/v. Each EO dilution was combined with 1 mL of the methanolic DPPH solution. The final working volume was 3 mL after the addition of 1 mL of methanol. The blank control of the series concentrations was made by dissolving DPPH in methanol in 1:2 ratios without the EO. All of those solutions were then allowed to sit at room temperature for 30 minutes in darkness. Following the incubation period, the absorbance of those solutions was determined using a UV-Vis spectrophotometer at a 517 nm wavelength. Methanol was used to zero the spectrophotometer.

Using the following equation, the antioxidant activity of the EOs was calculated in terms of inhibition percentage:

$$In\% = \frac{A\ blank - A\ sample}{A\ blank} \times 100$$

Equation (2.1): Inhibition% of antioxidant activity (84)

Where A blank represents the absorbance of the control reaction (which contains all reagents excluding the test compound) and A sample represents the absorbance of the test compound.

2.4 Antibacterial and antifungal activity tests

The American Type Culture Collection (ATCC) provided six reference bacterial strains for this study: *K. pneumoniae* (ATCC 13883, UK), *E. coli* (ATCC 25922), *P. vulgaris* (ATCC 8427), *S. aureus* (ATCC 6538P, USA) *Pseudomonas aeruginosa* (ATCC 9027, USA) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) as well as *C. Albicans* (ATTC 90028, USA) as a fungal strain.

2.4.1 Preparation of bacterial and fungal suspensions

Both bacterial and fungal strains were cultured 24 hours before use. This formed the bacterial suspension. Using normal saline as a blank, the optical densities of each solution were measured with a spectrophotometer at = 620 nm. The turbidity of the bacterial suspensions was corrected to 0.5 McFarland turbidity standard (optical density 0.08 to 0.1), yielding a suspension with 1.5×10^8 colony-forming units (CFU) per milliliter. In addition, the turbidity of *Candida albicans* was adjusted to 0.5 McFarland solution (optical density of 0.12 to 0.15) at a concentration of 1×10^6 - 5×10^6 CFU/mL. Then, 100 µL of each bacterial and fungal suspension was mixed to 10 mL of Mueller Hinton Broth media to make stock solutions for each strain. Using the broth microdilution procedure, these stock solutions were then applied to the experiment.

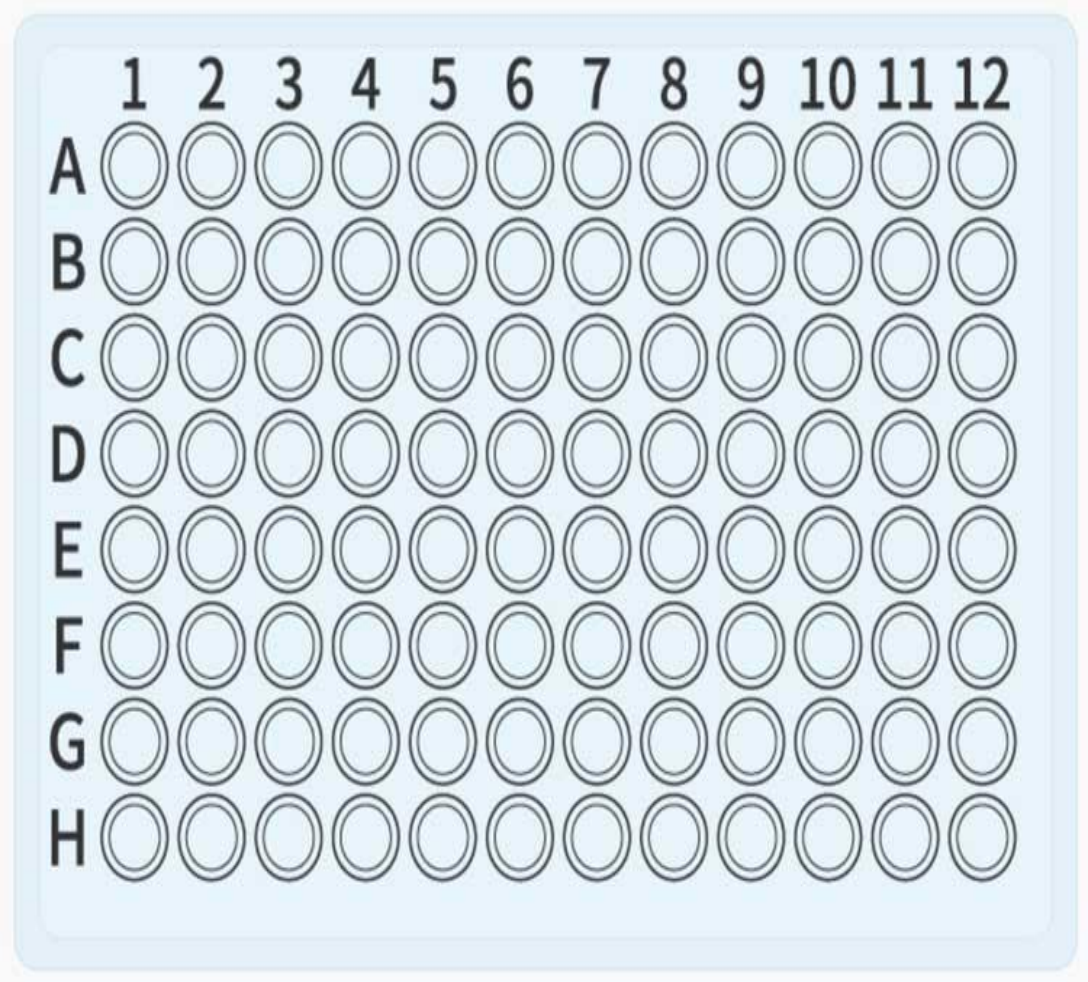
2.4.2 Anti-bacterial and anti-fungal assay

200 µL of the EO was combined with 150 µL DMSO and 150 µL Mueller Hinton Broth media, this solution was put under UV for 10-15 minutes. 96 microplate wells were filled with 50 µL Mueller Hinton Broth media using a pipette, and the wells for the first column received 50 µL of EO solution. 50 µL of the solution from well number 1 was then pipetted into well number 2 using a multichannel pipette, where it was mixed to achieve a 2-fold serial dilution. Up until well number 10, this process was repeated. Then 50 µL of each type of bacterial and fungal strain suspension were added to the corresponding labeled row for the wells 1–11. As a positive control for microbial development, well 11 contained solely Mueller Hinton Broth media and the microbial suspension without EO. Likewise, the growth medium was the only thing in microwell number 12; no test microorganisms were added to it. It was classified as a negative control. All of the inoculation plates were then incubated at 35 °C for about 24 hours. The ensuing turbidity in the microwells suggested bacterial proliferation.

The minimum inhibitory concentration (MIC) of the EO was investigated which is the lowest concentration at which no discernible bacterial growth in that microwell was observed. To manage the sensitivity of the investigated microorganisms, all established tests were conducted twice.

Figure 2.1

96 micro-wells plate for anti-bacterial and anti-fungal assay



2.5 Anti-cancer activity test

Five cancer cell lines in addition to normal cell line were utilized in this work, breast cancer (MCF-7), hepatocellular carcinoma (Hep G2), skin cancer (B16-F1), fibroblast (3T3), cervical adenocarcinoma (HeLa), and human hepatic stellate cell lines (LX-2).

Dulbecco's Modified Eagle's Medium (DMEM) media is used to culture the cancer cell lines for 24 hours, Cells have been grown in a humidified atmosphere with 5% CO_2 at 37°C. Cells were seeded at 5000 cells/well in a 96-well plate. Cells were treated with various concentrations 500, 250, 125, 62.5, 31.25, 15.625 µg/mL of the *A. citrodora* EO, *O. syriacum* EO and their mixture for 48 hr. The Cell-Tilter 96® Aqueous One Solution Cell Proliferation (MTS) assay was used to assess cell viability as directed by

the manufacturer (Promega Corporation, Madison, WI). At the end of the treatment, 20 μL of MTS solution was added to every 100 μL of medium, and the wells were incubated at 37 °C for two hours. The absorbance was checked at a wavelength of 490 nanometers.

Chapter Three

Result

3.1 GC–MS characterization of *A. citrodora* and *O. syriacum* essential oils

Utilizing GC-MS technique, the EOs extracted from the leaves of *A. citrodora* and *O. syriacum* were quantitatively and qualitatively evaluated. The *A. citrodora* EO collected in Jericho included 37 compounds, representing 87% of the total chromatographic area, meanwhile the EO collected in Qalqilya contained 31 compounds, accounting for 98% of the EO (Table 3.1), and the GC-MS chromatogram is shown in Figure 3.1. The components that were most predominant in the *A. citrodora* EO from Jericho were α-curcumene (26.94%), spathulenol (13.69%), geranial (10.79%), caryophellene oxide (8.66%), neral (7.59%) and ß-caryophyllene (6.14%); while the most abundant compounds identified in the *A. citrodora* EO gathered from Qalqilya were geranial (37.00%), neral (29.00%), α-curcumene (7.76%), ß-caryophyllene (6.00%), and bicyclogermacrene (2.79%).

Eleven compounds were identified in *O. syriacum* EO gathered from Jerusalem, representing 99.87% of the total mass of EO. Most of the EO consists from Carvacrol (79.46%), while thymol (15.87%) is considered to be the second most important ingredient. Cuminol also makes up 3.25 % of the EO. Oxyganetd monoterpenes is the major group of terpenes that occupy *O. syriacum* EO. The chemical structure, retention index (RI) and retention time (RT) with their concentration (%) are represented in Table 3.2 and the GC–MS chromatogram is described in Figure 3.2.

Figure 3.1

GC-MS chromatogram of Aloysia citrodora essential oil from Jericho and Qalqilya

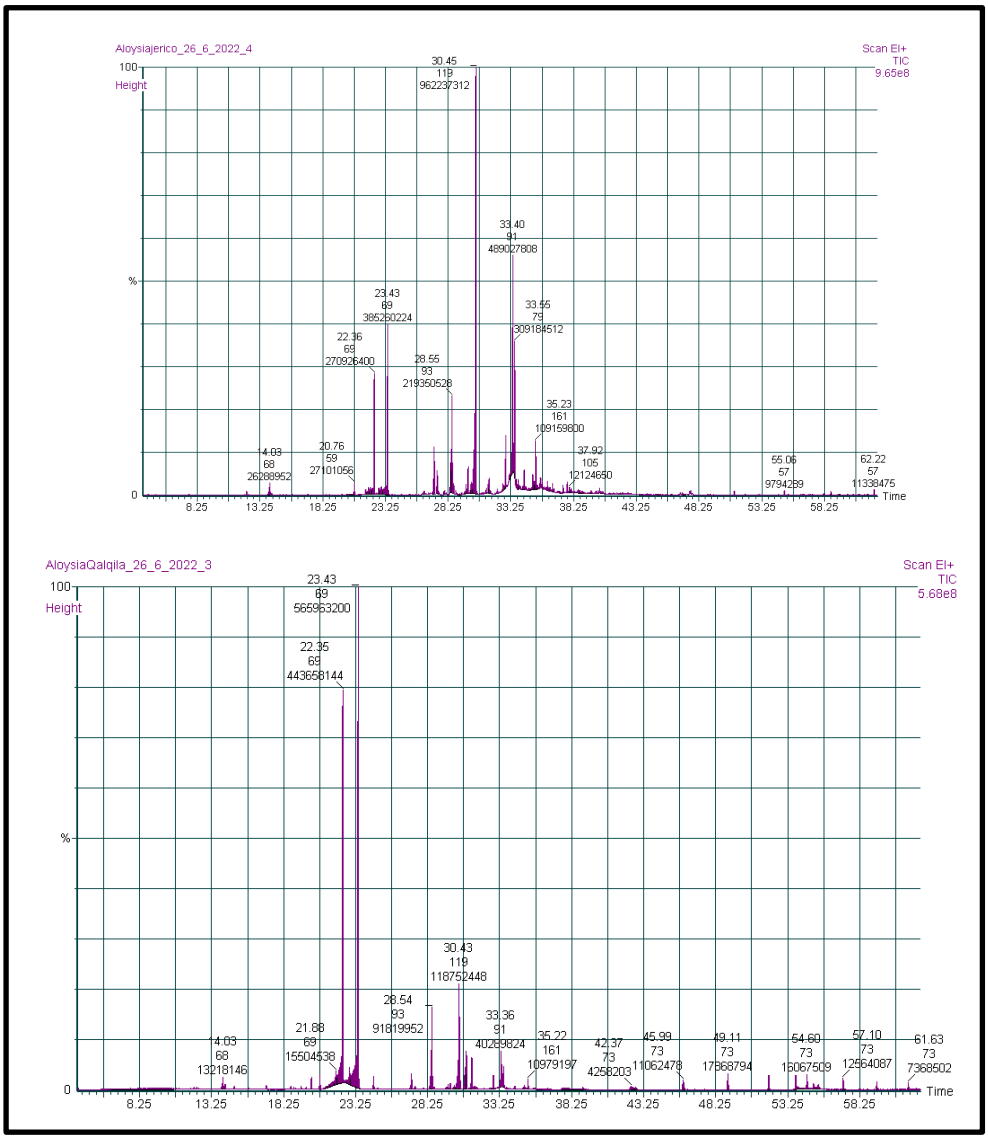


Table 3.2

Origanum syriacum essential oil chemical composition. RT: retention time; RI: retention index.

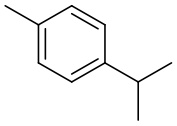
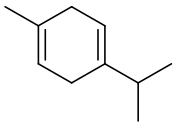
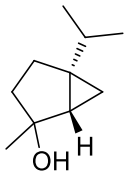
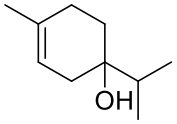
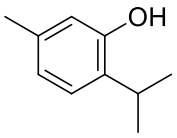
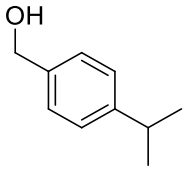
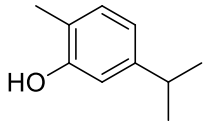
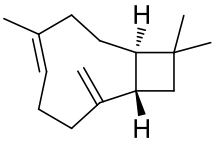
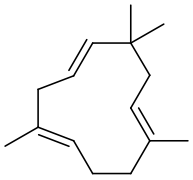
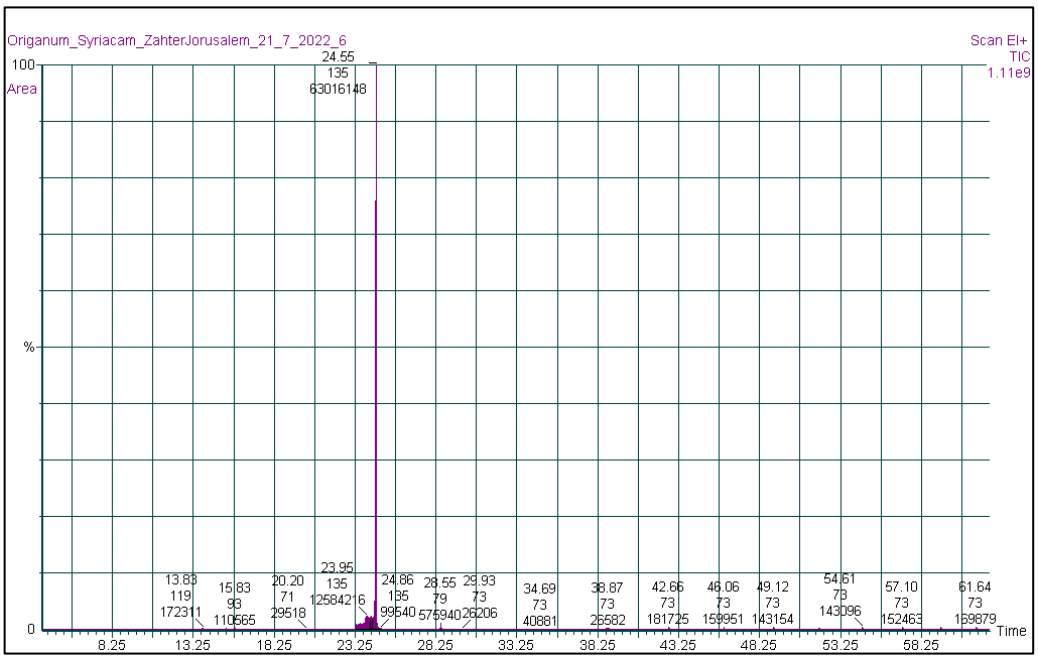
No.	Compounds	Compounds structures	% Area	RI	RT
1	p –Cymene		0.22	1021	13.83
2	γ-Terpinene		0.11	1056	15.288
3	trans-4-thujanol		0.14	1069	15.833
4	Terpinen-4-ol		0.04	1178	20.205
5	Thymol		15.87	1280	23.951
6	Cuminol		3.25	1288	24.226
7	Carvacrol		79.46	1297	24.547
8	β-caryophyllene		0.73	1418	28.548
9	α -caryophyllene		0.06	1459	29.684
	Total %		99.87		
	yield		1.6% v/w		
	Monoterpene Hydrocarbon		0.33		
	Oxygenated monoterpenes		98.76		
	Sesquiterpene hydrocarbons		0.78		

Figure 3.2

GC-MS chromatogram of Origanum syriacum essential oil



3.2 Antioxidant Activity of *A. citrodora* and *O. syriacum* essential oils

The antioxidant capabilities of A. citrodora and O. syriacum EO's were assessed utilizing DPPH radical scavenging and reduction power activities. For each of the EOs, the antioxidant half-maximal inhibitory doses (IC50) (giving 50% inhibition) were calculated. Both oils were found to exhibit effective in scavenging the DPPH radicals with IC50 values of 31.35 ± 0.33; while it was 9.29±0.52 µg/mL for A. citrodora EO and O. syriacum EO, respectively (Table 3.2, Figure 3.3).

Table 3.3

Aloysia citrodora EO, Origanum syriacum EO and trolox inhibition activity versus DPPH.

Concentrations (µg/mL)	**Trolox**	***A. citrodora* EO**	***O. syriacum* EO**
0	0 ± 0.00	0 ± 0.00	0 ± 0.00
5	58.71 ± 0.26	12.25 ± 0.4	26.09 ± 0.4
10	93.9 ± 0.33	17.39 ± 0.4	48.22 ± 0.4
20	93.01 ± 0.33	20.16 ± 0.4	89.46 ± 1.27
50	93.34 ± 0.33	43.08 ± 0.4	93.02 ± 0.23
80	93.67 ± 0.33	90.51 ± 0.4	95.78 ± 0.23
100	93.67 ± 0.68	92.49 ± 0.00	96.97 ± 0.6
IC_{50}	4.3 ± 0.58	31.35 ± 0.33	9.29 ± 0.52

Values are the mean ± SD (n = 3/group). DPPH is 1,1-diphenyl-2-picrylhydrazyl, and IC_{50} is the half maximum inhibitory concentration.

3.3 Anticancer activity

The3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was used in this study to assess the cytotoxicity of *A. citrodora*, *O. syriacum* EO's and their mixture (1:1; *w*:*w*) on the cell proliferation of breast cancer (MCF-7), hepatocellular carcinoma (Hep G2), skin cancer (B16-F1), fibroblast (3T3), cervical adenocarcinoma (HeLa), and human hepatic stellate cell lines (LX-2). Cells were subjected for 24 hours to increasing doses of the tested EOs (0, 15.65, 31.25, 62.5, 125, 250, 500 µg/mL). At 250 µg/mL, the inhibitory effect of EOs on cell viability varied from 75-91% (Figure 3.4-3.7). The IC_{50} values were calculated from figure 3.6-3.11 and demonstrated in Table 3.4.

Figure 3.3

DPPH Inhibition% by trolox, Aloysia citrodora EO and Origanum syriacum EO.

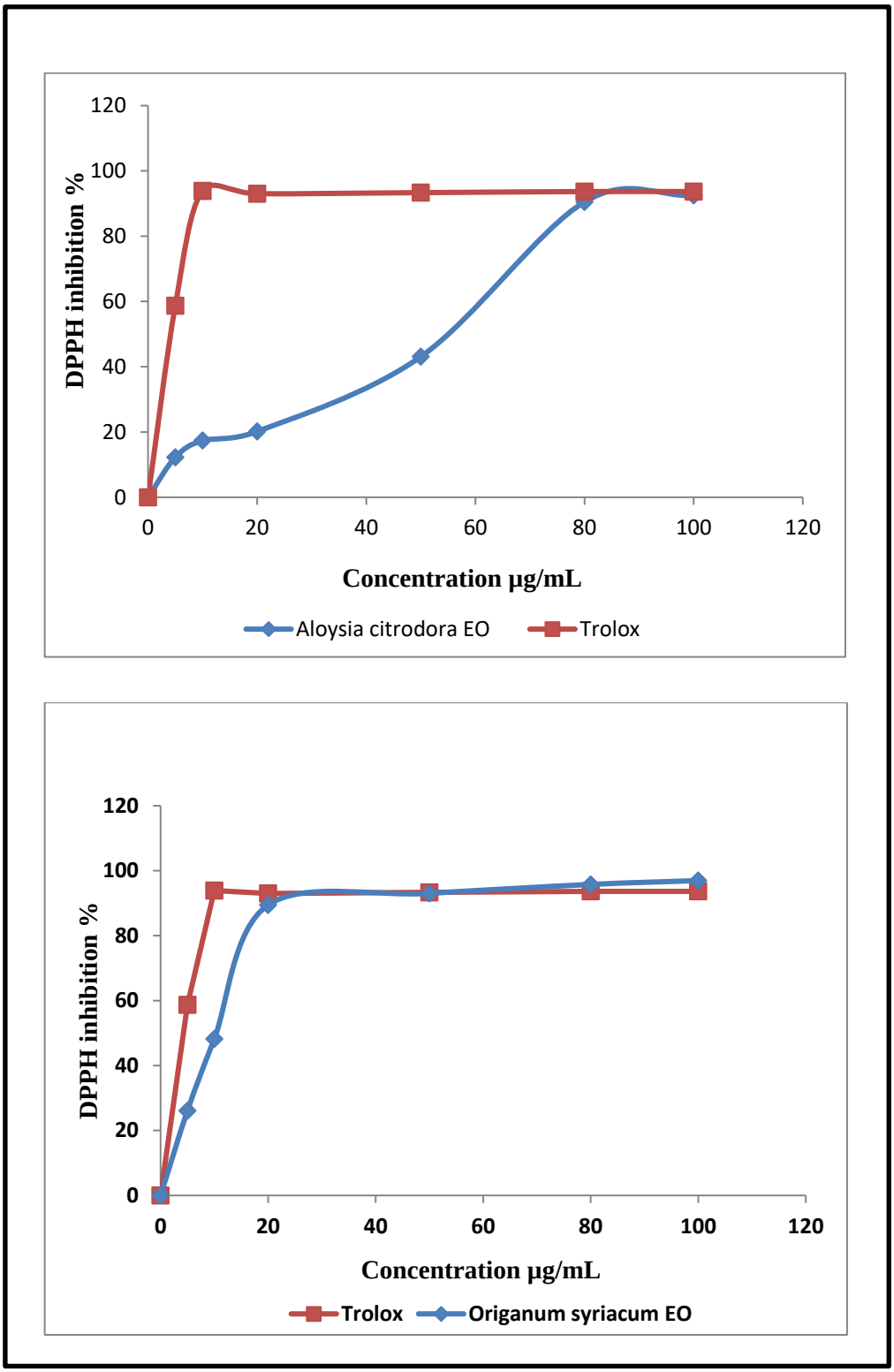


Figure 3.4

Percentage Inhibition of B16-F1 and Hep G2 cancer cell lines by A. citrodora EO, O. syriacum EO and mixture EOs at 460 nm

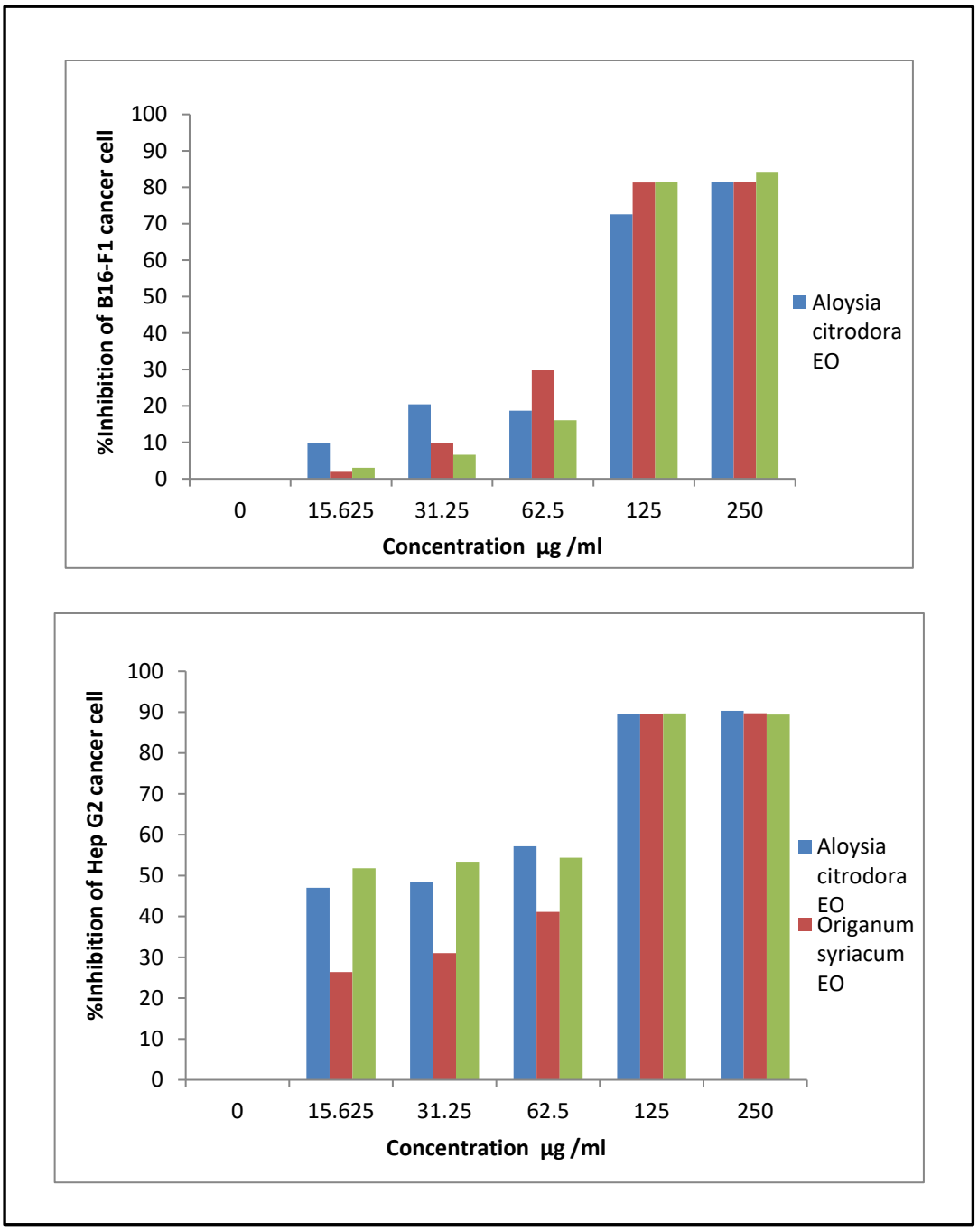


Figure 3.5

Percentage Inhibition of MCF-7 and 3T3 cancer cell lines by A. citrodora EO, O. syriacum EO and mixture EOs at 460 nm

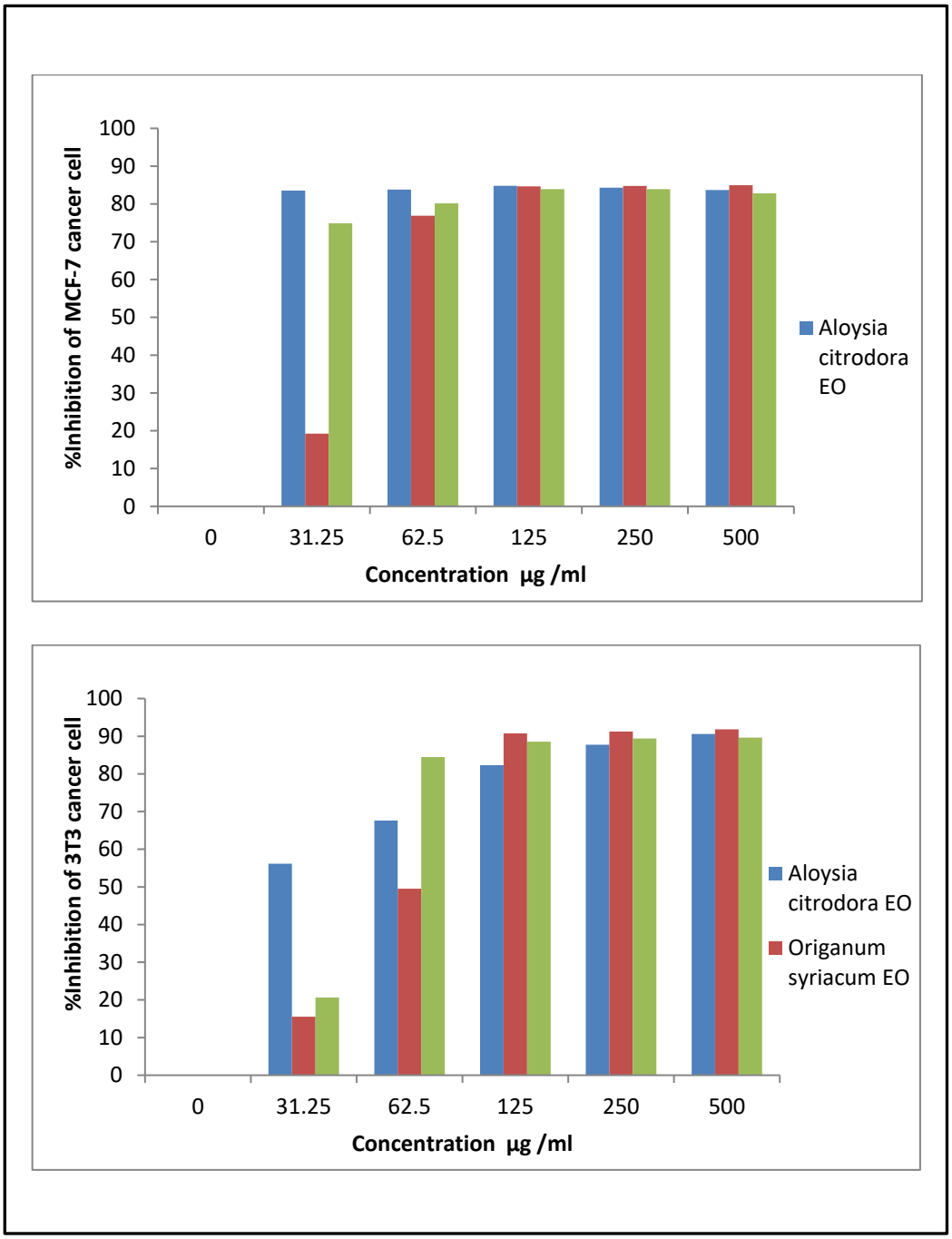


Figure 3.6

Percentage Inhibition of HeLa cancer cell by A. citrodora EO, O. syriacum EO and mixture EOs at 460 nm

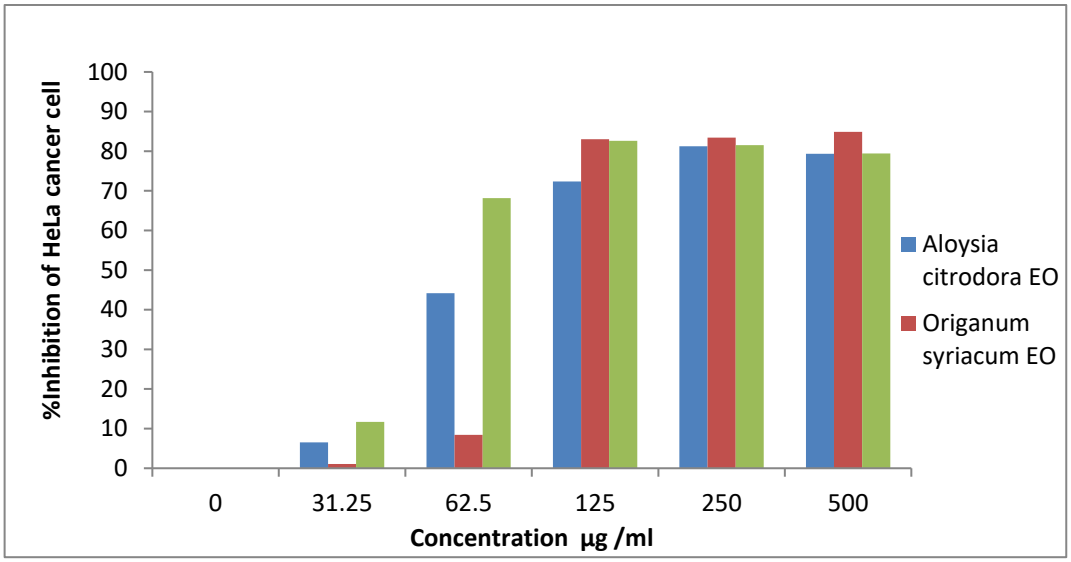


Figure 3.7

Percentage Inhibition of LX2 normal cell by A. citrodora EO, O. syriacum EO and mixture EOs at 460 nm

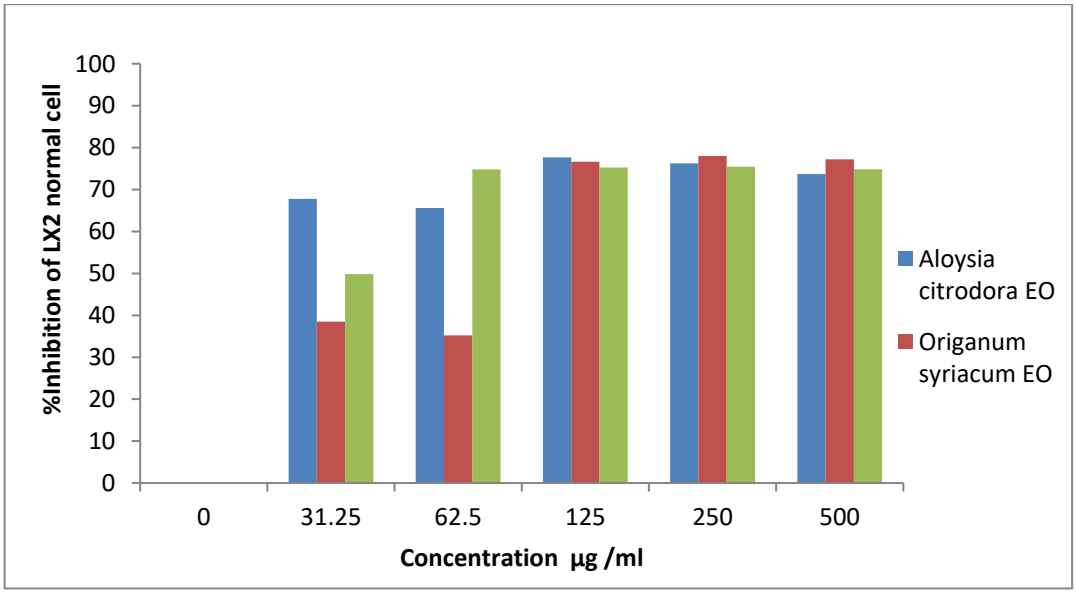


Table 3.4

IC_{50} values of Aloysia citrodora EO, Origanum syriacum EO and mixture oil against B16-F1, HepG2 MCF-7, 3T3, HeLa and LX2 normal cell

Essential Oil/cell line	IC_{50} µg/ml					
	B16-F1	HepG2	MCF-7	3T3	HeLa	LX2
Aloysia citrodora EO	87.6 ± 3.17	22.8 ± 4.73	13.5 ± 1.41	23.9 ± 13.0	67.3 ± 5.23	25.3 ± 4.74
Origanum syriacum EO	70.1 ± 3.32	39.2 ± 6.72	32.5 ± 1.20	38.5 ± 9.61	84.9 ± 3.41	52.7 ± 8.98
Mixture EOs	80.9 ± 8.93	21.2 ± 0.07	16.1 ± 0.99	27.0 ± 4.81	43.1 ± 2.76	29.5 ± 0.28

3.4 Anti-microbial activity

The minimum inhibitory activity (MIC) of both investigated EO's against the selected six bacterial and one fungus strains (**Table 3**) was determined using the broth micro-dilution assay. The *O. syriacum EO* gathered in Jerusalem area showed antibacterial activity against all the tested microbes with MIC values ranging from 48.7-25000 µg/mL, while *A. citrodora EO* showed only moderate antimicrobial properties with MIC values ranging from 3125-100000 µg/mL as demonstrated in **Table 3.5**

Table 3.5

Minimum inhibitory concentration values (µg/ml) for Aloysia citrodora and Origanum syriacum EO against a variety of bacteria and fungi.

				Bacteria			Fungus
ATCC Number	Clinical strain	ATCC 6538P	ATCC 25922	ATCC 13883	ATCC 8427	ATCC 9027	ATCC 90028
MIC (µg/ml)	MRSA	*S.aureus*	*E. coli*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Candida albicans*
A. citrodora	3125	3125	25000	25000	6250	100000	3125
O. syriacum	195	48.7	97.5	97.5	48.7	25000	48.7

Chapter Four

Discussion

4.1 The chemical composition of *Aloysia citrodora* and *Origanum syriacum* essential oils

37 compounds, accounting for 87% of the total chromatographic area, were found in the *A. citriodora* EO collected in Jericho, whereas 31 compounds, accounting for 98% of the EO collected in Qalqilya. α-curcumene (26.94%), spathulenol (13.69%), geranial (10.79%), caryophellene oxide (8.66%), neral (7.59%) and β-caryophyllene (6.14%) were main constituents of the *A. citriodora* EO gathered in Jericho; whereas geranial (37.00%), neral (29.00%), α-curcumene (7.76%), β-caryophyllene (6.00%), and bicyclogermacrene (2.79%) were the major compounds identified in the EO collected from Qalqilya. Thymol, α-cuprenene, and bicyclogermacrene were only found in EO from Qalqilya, whereas Muurolol, cederene, bourbonene, and humulene epoxide were only found in EO from Jericho. Oxygenated monoterpenes (71.92%) and sesquiterpene hydrocarbons (20.66%) were the most abundant constituents of essential oil from Qalqilya, while sesquiterpene hydrocarbons (44.50%) and oxygenated sesquiterpens (32.11%) were the most abundant constituents of essential oil from Jericho. The qualitative and quantitative differences observed between the two EOs can be linked to the climatic and geological differences between the two places. The results of the EO from Qalqilya are in accordance with existing literature as geranial and Neral are reported as two of the main components of Citriodora species essential oil (85). Saada et al. reported the detection of 48 compounds from the EO of. *A citriodora* areal parts collected from different locations in Tunisia, with geranial (24.85-27.41%), neral (14.81-18.73%), limonene (6.34-7.80%), and curcumenene (4.31-5.22%) being the principal constituents (86). Fitsiou et al. identified 43 chemicals in the *A. citriodora* collected in Greece, with geranial (26.40%), neral (17.16%), nerol (8.00%), and geraniol (5.70%) being the most abundant (87).

There were 11 identified components in *O. syriacum* EO, making up 99.87% of the whole EO. The most prevalent components were carvacrol (79.46%), thymol (15.87%) and cuminol (3.25%). β-caryophyllene (0.73%), p-cymene (0.22%), trans-4-thujanol (0.14%) and γ-terpinene (0.11%) were found in EO, albeit they are all present in modest

proportions compared to terpinen-4-ol (0.04%) and α-caryophyllene (0.06%), which are regarded to be the components with the lowest abundance. The three classes of detected chemicals, oxygenated monoterpenes were found to constitute the primary group, making up 98.76% of the content of all EO. Carvacrol (79.46%) is the major compound in this group. β-caryophyllene (0.73%) and α-caryophyllene (0.06%) represent the components of sesquiterpene hydrocarbons group (0.78%). M. Shehadeh et al. reported the composition of *O. syriacum* EO collected from different regions in Palestine, 17 components were identified, the dominant compounds were thymol (5.9–39.87%), α-terpinene (12.97–36.8%), carvacrol (13.64–21.40%) and γ-terpinene (12.97–36.80%) (88). AL-Mariri et al identified 15 compounds of *O. syriacum* gathered from different locations in Syria. ß-myrcene (21.93%), carvacrol (19.20%), anisaldehyde (7.57%), thymol (7.40%), γ-terpinene (5.27%), and sabinene (4.43%) were the major components (89). *O. syriacum* from Turkey was studied by Bayraktar et al., they revealed that its EO consist from 17 compounds, representing 98.76%. The major constituents were thymol (42.18%), carvacrol (33.95%), cymene (8.87%) and γ-terpinene (8.21%) (90). The types of soil, humidity, climate, light intensity, and temperature of the native geographic region may all have a role in the variances in EO composition.

4.2 Antioxidant activity

With an IC_{50} value of 31.35 ± 0.33 µg/mL for *A. citrodora* and 9.29 ± 0.52 µg/mL for *O. syriacum*, *O. syriacum* EO outperformed *A. citrodora* EO but was less effective than the positive control (Trolox; IC_{50} = 4.3 ± 0.58). The high antioxidant activity of the *O. syriacum* EO could be attributed to the high content of oxygenated monoterpenes especially thymol. According to Loizzo et al., *O. syriacum* essential oil that collected from Baskinta Mountain in Lebanon, showed substantial activity (IC_{50} =1.7±0.01 µg/mL). The antioxidant activity may be primarily related to the essential oil's high amount of phenolic components (91). Viuda-Martos et al. discovered that *O. syriacum* EO from Egypt has a high antioxidant activity, with an IC_{50} value of 6.66 µg/L, compared to the antioxidant properties of ascorbic acid and BHT with IC_{50} values of 0.42 and 0.53 µg/L, respectively (92). The presence of carvacrol, a key component of *O. syriacum*, is linked to antioxidant action.

Fitsiou et al. found that in the DPPH experiment, *A. citriodora* had strong radical scavenging activity with an IC_{50} value of 6.30 ± 0.20 µg/ml (87). With an IC_{50} value of 11.33 ± 0.01 µg/ml, Hosseini et al. found that EO of *A. citriodora* harvested in Iran had higher antioxidant activity than BHT (85). This is attributed to the phenolic component of lemon verbena EO, suggesting its potential use as an electron donor to scavenge free radicals.

4.3 Anticancer activity

The growth inhibitory effects of essential oils from *A. citriodora* leaves, *O. syriacum* leaves, and a 1:1 mixture of both oils were studied on five human cancer lines: B16-F1, Hep G2, MCF-7, 3T3, and HeLa cancer cell lines, as well as the LX2 normal cell line. *A. citriodora* displayed stronger growth inhibitory effects on all cell lines, with IC_{50} ranging from 13.5 ± 1.41-87.6 ± 3.17 µg/mL, when compared to preserved essential oil of *O. syriacum* EO (IC_{50} ranging from 32.5 ± 1.20-84.9 ±3.41µg/mL). As indicated in **table 3.4**, *A. citriodora* exhibited the greatest cytotoxicity on MCF-7, followed by HepG2 and 3T3 with IC_{50} values of 13.5 ± 1.41, 22.8 ± 4.73 and 23.9 ± 5.23 µg/mL. Skin cancer (B16-F1) and cervical (HeLa) cell lines were the least sensitive to both essential oils (IC_{50} ranging from 67.3 ± 5.23-87.6 ± 3.17 µg/mL). With the exception of the cervical cell line (HeLa), where the growth inhibitory activity was enhanced with an IC_{50} value of 43.12.76 µg/mL, the 1:1 (w:w) combination of both essential oils had no effect on growth inhibitory activity on other cell lines. The *A. citriodora* EO, which is rich in oxygenated monoterpenes (geranial and neral) and sesquiterpene hydrocarbons (20.66%), was shown to be much more active and potent against all cancer cell lines, with the exception of B16-F1, than *O. syriacum* EO, which is high in thymol and carvacrol and poor in sesquiterpenes.

Rashid et al. investigated the antiproliferative efficacy of *A. citrodora* EO against several cell lines. The IC_{50} ranged between 402 and 633 µg/mL against cancer cell lines, and 1990 µg/ml against the normal cell line Vero. MCF-7 (IC_{50} = 540 ± 40 µg/ml) indicating modest action in comparison to our data (93). Fitsiou et al investigated the cytotoxic effect of *lippia citrodora* EO collected in Athenes/Greece on various cell lines (Hepatocellular carcinoma Hep G2, Breast adenocarcinoma MCF-7, Colon adenocarcinoma Caco2, Leukemic monocytes THP-1 and Malignant melanoma A375)

and discovered EC values ranging from 9.1-111µg/mL, with A375 cell line showing the greatest sensitivity with an EC_{50} value of 9.1 µg/mL and THP-1 cell line showing the lowest sensitivity with an EC_{50} value of 111 µg/mL. With Hep G2 and MCF-7 the IC50 was 74 and 89 µg/mL respectively (94). The IC_{50} of *Lippia citrodora* EO was 77.8 ± 1.5 µg/ml according to Spyridopoulou et al against murine DA3 breast cancer cells, which show an agreement with our results (95). Najar et al evaluate the IC_{50} of *Aloysia citrodora* using five different cancer cell lines (MCF7, T47D and MDA-MB-231 which they are a human breast adenocarcinoma, human chronic myelogenous erythroleukaemia (K562) and human neuroblastoma cell line (SH-SY5Y). The calculated IC_{50} were from 29.3–119.2 ppm, the MCF-7 cell line was the least sensitive, with an IC_{50} of 119.2 ppm, whereas K562 showed the highest sensitivity to *Aloysia* EO (IC_{50} = 29.3 ppm) (96).

In all cancer cell lines, *O. syriacum* EO inhibited cell proliferation at high concentrations compared to low doses. IC_{50} values of *O. syriacum* EO against MCF-7, 3T3 and Hep G2 (32.5 ± 1.20 µg/mL, 38.5 ± 9.61 µg/mL and 39.2 ± 6.72 µg/mL, respectively) annotate a higher cytotoxicity compared with B16-F1 (IC_{50} = 70.1 ± 3.32 µg/mL) and HeLa (IC_{50} = 84.9 ± 3.41 µg/mL). Our results show an improvement in the antiproliferative activity on Hep G2 compared to Abd EL-Moneim et al's evaluation of the IC_{50} of *O. syriacum* oil from Egypt against Hep G2, which was 93.72 µg/mL (97). Its antiproliferative activity was also studied on the human colon cancer cell HCT 116 by Öztürk et al (IC_{50} = 15 µg/mL), it was collected from Turkey (98). IC_{50} value of *O. syriacum* EO from Jordan on MCF-7 was 122.80 ± 5.84 µg/mL according to Al-Kalaldeh et al (99).

This study examined the synergistic effects of both *A. citrodora* and *O. syriacum* EOs against the various cancer cell lines used in this investigation. The results of IC_{50} in B16-F1 (80.9 ± 8.93 µg/mL), MCF-7 (16.1 ± 0.99 µg/mL) and 3T3(27.0 ± 4.81 µg/mL) cancer cell lines were between the IC_{50} values of *A. citrodora* EO and *O. syriacum* EO on their own, while In HepG2 (21.2 ± 0.07 µg/mL) and HeLa (43.1 ± 2.76 µg/mL) cancer cell lines the IC_{50} values of mixture oils were a little bit lower than both oils when they are on their own. These results indicate that the combination of the EOs does not show a significant enhancement on the anticancer activity.

The inhibition of cell growth of LX2 normal cell line by the *A. citrodora* (IC_{50} = 25.3 ± 4.74 µg/mL) and *O. syriacum* (IC_{50} = 52.7 ± 8.98 µg/mL) EOs in addition to the mixture of both oils (IC_{50} = 29.5 ± 0.28 µg/mL) was relatively high at all concentrations. Therefore, they show low selectivity toward cancer cells against normal cells.

4.4 Antimicrobial activity

The antimicrobial activity of *Aloysia citrodora* and *Origanum syriacum* essential oils was tested in a broth microdilution assay against two gram-positive bacteria, *S.aureus* and MRSA, as well as the yeast *C. albicans* and four gram-negative bacteria, *E. coli*, *P. vulgaris, P. aeruginosa*, and *k. pneumonia*. The findings demonstrate that *O. syriacum* EO has superior antibacterial capabilities than *A. citrodora* EO against all tested strains. (**Table 3.5**). Significant antibacterial activity was shown by *O. syriacum* EO against *S. aureus, P. vulgaris, and C. albicans*, with MIC values of 48.7 µg/mL for all three species, followed by *klebsiella pneumonia* and *E. coli* with MIC values of 97.5 µg/mL. With MIC values of 3.125 mg/mL, *A. citrodora* EO demonstrated moderate antibacterial activity against *C. albicans, S. aureus,* and MRSA. *P. aeruginosa* was the most resistant bacterium to both essential oils, with MIC values of 25.00 mg/mL for *O.syriacum* EO and 100.00 mg/mL for *A.citrodora* EO.

According to their MIC values, plant materials can be categorized based on their antimicrobial activity, strong antibacterial activity is indicated by MIC values less than 500 while MIC values between 600 and 1500 µg/mL represent moderate activity but greater than 1600 µg/mL indicates poor antibacterial activity. Hosseini M, et al assessed the antimicrobial activity of lemon verbena gathered from Iran utilizing four gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Shigella dysenteriae*) and three gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes,* and *Bacillus cereus*) MIC values were from 1250 to 2500 µg/mL, MIC of *E. coli* was 2500 µg/mL, *P.aeroginosa* 2500 µg/mL and *S. aureus* 1250 µg/mL (85). Oukerrou et al. examined the antibacterial activity of *Aloysia citrodora* EO extracted from different regions of Morocco. The MIC values against *E. coli* ranged from 2.84 to 8.37 mg/mL, while it was between 7.43 mg/mL and 12.12 mg/mL against S. aureus (72). According to M. Shehadeh et al the antimicrobial of *O. syriacum* that

collected from Jerusalem, Qalqilya, Bethlehem and Tulkarem was investigated using *Staphylococcus aureus,* MRSA, *Enterococcus faecium*, *Escherichia coli* and *Pseudomonas aeruginosa*. MIC values of were ranging from 97-25000 µg/mL. MIC of *O. syriacum* that collected from Jerusalem on *Staphylococcus aureus* and *Enterococcus faecium* was 97 µg/mL, while MIC of MRSA was 6250 µg/mL, but *Pseudomonas aeruginosa* and *Escherichia coli* had MIC >25,000 µg/mL (88). Al Hafi et al reported a significant anti-microbial activity of *Origanum syriacum* against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans microbs* with MIC values of 0.4, 0.8, 0.4 and 0.8 mg/mL, respectively (100).

4.5 Conclusions

In conclusion, this study examined the chemical profile of *Origanum syriacum* collected from Jerusalem, and *Aloysia citrodora* gathered from Jericho and Qalqilya. 11 compounds were identified in *Origanum syriacum*. carvacrol, thymol and cuminol were the major ones. 37 phytochemicals were in *Aloysia citrodora* from Jericho whereas *Aloysia citrodora* from Qalqilya consist from 31 components. The difference in the composition of Aloysia from different regions was demonstrated. α-Curcumene, geranial, neral and ß-caryophyllene were a mutual major components in *A. citrodora* from both regions, Spathulenol and caryophellene oxide found only as a major constituents in *A. citrodora* from Jericho while bicyclogermacrene was only in *A. citrodora* from Qalqilya. Both essential oils have an antioxidant activity; *O. syriacum* outperformed the antioxidant activity of *A. citrodora* but less effective than trolox. MCF-7, Hep G2, B16-F1, 3T3 and HeLa cell lines were used in addition to the normal cell line (LX-2) to evaluate the anticancer activities. When compared to *O. syriacum's* essential oil, *A. citriodora's* essential oil had more growth-inhibitory effects on all cell lines. The combination of *A. citrodora* and *O. syriacum* EOs doesn’t show an enhancement on the anticancer activity against the cancer cell lines. *A. citrodora* EO was outperformed by *O. syriacum* EO in terms of antibacterial activity.

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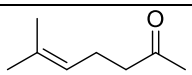
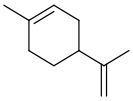
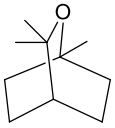
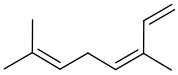
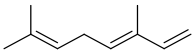
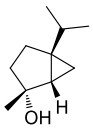
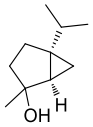
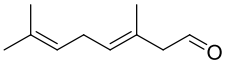
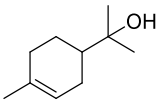
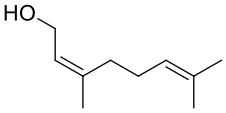
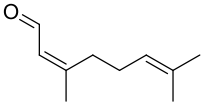
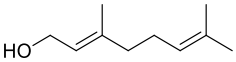
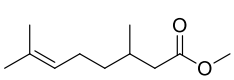
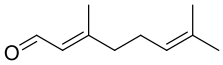
Appendices

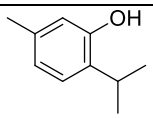
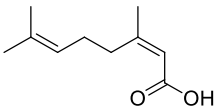
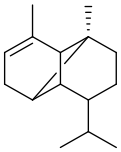
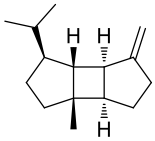
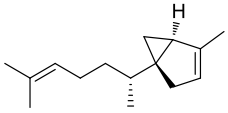
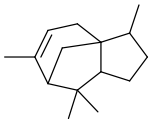
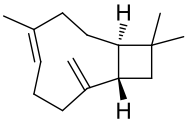
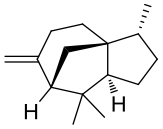
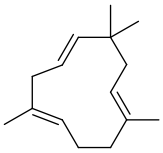
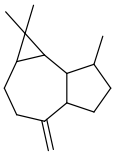
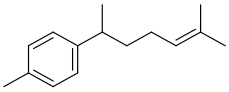
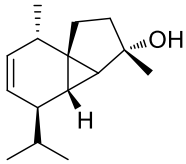
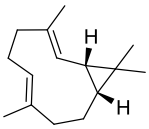
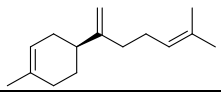
Appendix A

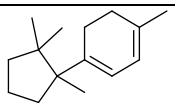
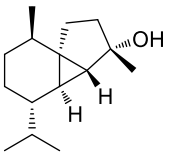
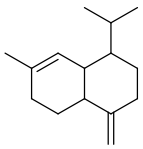
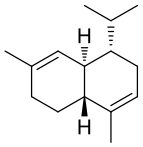
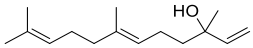
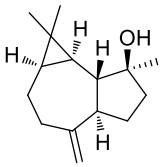
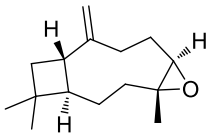
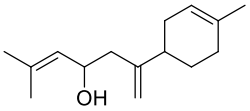
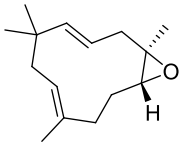
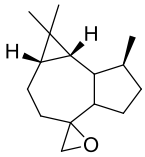
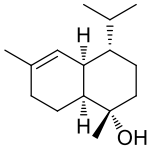
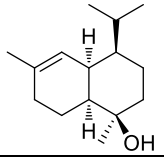
Tables

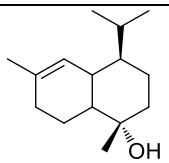
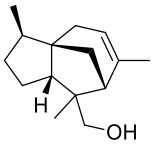
Table 3.1

Aloysia citrodora essential oil chemical composition. RT: retention time; RI: retention index.

No.	Compounds	Compounds structures	% Area (Aloysia Jerico)	% Area (Aloysia Qalqilya)	RI	RT
1	6-Methylhept-5-en-2-one		0.22	-	984	12.197
2	Limonene		0.74	0.86	1026	14.027
3	1,8-Cineole		0.09	0.32	1030	14.187
4	cis-Ocimene		-	0.05	1034	14.377
5	trans-Ocimene		-	0.22	1045	14.818
6	cis-sabinene hydrate		0.06	0.12	1068	15.848
7	cis-4-thujanol		-	0.28	1097	17.054
8	isogeranial		0.06	0.91	1177	20.19
9	α-Terpineol		0.76	1.06	1192	20.755
10	Nerol		0.44	1.01	1228	22.076
11	Neral		7.59	29.00	1236	22.356
12	Geraniol		-	1.26	1249	22.821
13	Methyl citronellate		0.49	-	1251	22.921
14	Geranial		10.79	37.00	1265	23.421

15	Thymol		-	0.96	1295	24.496
16	Neric acid		0.22	-	1351	26.347
17	α-Ylangene		3.00	1.13	1374	27.133
18	Bourbonene		1.47	-	1382	27.383
19	Sesquithujene		0.25	-	1399	27.958
20	Cedrene		2.19	-	1415	28.453
21	*β*-caryophyllene		6.14	6.00	1419	28.553
22	*β* -Cedrene		0.63	0.16	1422	28.693
23	α-caryophyllene		0.62	0.10	1454	29.679
24	all-Aromadendrene		1.68	0.41	1459	29.834
25	α-curcumene		26.94	7.76	1479	30.454
26	Epi-cubebol		0.09	-	1494	30.934
27	Bicyclogermacrene		-	2.79	1494	30.939
28	*β* -Bisabolene		0.33	-	1505	31.269

29	α-Cuprenene		-	2.31	1507	31.319
30	Cubebol		-	0.23	1514	31.539
31	γ-Cadinene		0.98	-	1515	31.539
32	α-Cadinene		0.28	-	1536	32.175
33	Nd		0.44	-	1551	32.61
34	E-Nerolidol		3.29	0.32	1558	31.84
35	Spathulenol		13.69	2.63	1577	33.4
36	Caryophyllene oxide		8.66	1.52	1583	33.555
37	Atlantol		-	0.23	1607	34.315
38	Humulene epoxide II		1.27	-	1607	34.321
39	Nd		0.25	-	1615	34.486
40	Nd		0.13	0.16	1627	34.836
41	alloaromadendrene epoxide		0.96	0.24	1633	35.001
42	τ-Muurolol		-	0.72	1642	35.221
43	α-Muurolol		3.06	-	1642	35.231

44	α-Cadinol		0.66	0.12	1655	35.616
45	8-Cedrene-13-ol		0.43	0.10	1661	35.766
46	Nd		0.60	-	1675	36.156
47	Nd		0.52	-	1690	36.587
	Total %		100	99.98		
	Yield		0.18% v/w	0.25% v/w		
	Monoterpene Hydrocarbon	0.74	1.13			
	Oxygenated monoterpenes	20.5	71.92			
	Sesquiterpene hydrocarbons	44.51	20.66			
	Oxygenated sesquiterpenes	32.11	6.11			
	others	1.12	0.16			

جـامعـــة النجاح الوطنية
كليـــة الدراســـات العليـــا

زيت أوريجانوم سيرياكوم وألويزيا سيترودورا العطري: خصائص كيميائية، نشاط مضاد للجراثيم، مضاد للأكسدة ومضاد للسرطان

إعداد
لينا تيسير حمدان

إشراف
د. نواف المحاريق
د. نضال جرادات

قدمت هذه الرسالة استكمالا لمتطلبات الحصول علي درجة الماجستير في الكيمياء، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2022

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الملخص

الأهداف: الاعتقاد بأن بعض النباتات لديها القدرة على علاج العديد من الأمراض دون آثار جانبية هو أحد الأسباب التي تجعل الاهتمام بالنباتات الطبية يتزايد يوما بعد يوم. في منطقتنا، تم استخدام نباتين شائعين هما أوريجانوم سيرياكوم "الزعتر" وألويزيا سيترودورا منذ فترة طويلة في العديد من العلاجات التقليدية. تهدف هذه الدراسة إلى استكشاف الملامح الكيميائية للزيوت الأساسية للأوريجانوم سيرياكوم التي تم جمعها من القدس وألويزيا سيترودورا التي جمعت من منطقة أريحا و قلقيلية ، بالإضافة إلى دراسة خصائصها المضادة للأكسدة والمضادة للميكروبات والمضادة للسرطان. تعد دراسة التأثير التآزري لخليط الزيوت على تكاثر الخلايا السرطانية هدفًا آخر.

الطريقة والاجراءات: باستخدام طريقة الاستخلاص بالتقطير المائي، تم إنتاج الزيت الأساسي للأوريجانوم سيرياكوم و الزيت الأساسي للويزيا سيترودورا, وتم تحديد المكونات الكيميائية للزيوت الأساسية نوعا وكميا باستخدام جهاز (GC–MS), و تم تقييم النشاط المضاد للأكسدة عن طريق تثبيط الجذور الحرة لمركب (DPPH), تم استخدام اختبار (MTS) لفحص النشاط المضاد للسرطان. تم استخدام طريقة (broth micro dilluton) لاختبار النشاط المضاد للبكتيريا.

النتائج: تم تحديد 11 مركباً في زيت الأوريجانوم الأساسي، (79.46%)Carvacrol، thymol (15.87%) و (3.25)cuminol % هم المركبات الأكثر وفرة. 37 مركبا موجودا في زيت الويزا

الأساسي من أريحا ، والمركبات الأكثر وفرة هي ß–caryophyllene (6.14%), neral (7.59%), caryophellene oxide(8.66%), geranial (10.79%), spathulenol (13.69%) α–curcumene (26.94%)، في حين أن 31 مركبا تم تحديدها في زيت الويزا الأساسي من قلقيلية، ß–caryophyllene (6.00%) وbicyclogermacrene (2.79%) كانت المكونات الرئيسية. كانت قيمة IC_{50} للأوريجانوم 9.29 ± 0.52 µg/mL بينما كانت قيمة IC_{50} للويزا 31.35 ± 0.33 µg/mL. كانت قيم IC_{50} لزيت الويزا الأساسي مقابل خطوط الخلايا السرطانية بين 13.5 ± 1.41 و 87.6 ± 3.17 µg/mL، بينما تراوحت قيم IC_{50} للأوريجانوم من 32.5 ± 1.20 – 84.9± 3.41 µg/mL. تراوحت قيم MIC لزيت الأوريجانوم الأساسي من 48.7 – 25000 µg/mL ، بينما تراوحت قيم زيت الويزا بين 3125 و 10000 µg/mL.

الخلاصة: إن النشاط المضاد للأكسدة والمضاد للبكتيريا للزيت الأساسي للأوريجانوم يفوق نشاط زيت الويزا الأساسي. كان لزيت الويزا الأساسي تأثير في تثبيط نمو جميع خطوط الخلايا السرطانية أكثر من زيت الأوريجانوم الأساسي. لم يظهر خليط الزيوت (1: 1 w: w) تحسنا في النشاط المضاد للسرطان.

الكلمات المفتاحية: زيت الويزا سيترودورا العطري، زيت أوريجانوم سيرياكوم الأساسي، الملامح الكيميائية، مضادات الأكسدة، مضاد للسرطان، مضاد للجراثيم، تأثير تآزري.