



**An-Najah National University**  
**Faculty of Graduate Studies**

**UNVEILING THE CHEMICAL PROFILING,  
ANTIOXIDANT, ANTICANCER AND ANTIBACTERIAL  
ACTIVITIES OF ESSENTIAL OILS DERIVED FROM  
FENNEL (*FOENICULUM VULGARE*), *M. FRUTICOSE*  
AND *ALYOSIA CITRIODORA***

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**This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree  
of Master of Chemistry, Faculty of Graduate Studies, An-Najah National  
University, Nablus, Palestine.**

**2023**

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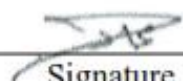
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## **Dedication**

To my dear family, my mother, father, sisters and brothers for their patience and encouragement, and my lovely wife, and to my friends and colleagues.

WITH ALL LOVE AND RESPECT

## **Acknowledgement**

Thanks first go to ALLAH. I would then express my sincere thanks and gratitude to my supervisor, Dr. Nawaf Al-Maharik and Dr. Derar Smadi for their guidance, encouragement and help during this study. My special thanks to all who have helped me in this study, including instructors and lab technicians. In addition, my deepest thanks, respect and appreciation to my dear mother, father, wife, sisters and brothers for their patience and encouragement. To my friends and colleagues and everyone who has wanted me to succeed progress and develop, and who have shared their emotions with me.

## Declaration

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

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**By  
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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:**

**Moataz Abu Alrub**

**Signature:**



**Date:**

**17/09/2023**

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**Abstract**

**Background:** Essential oils (EOs) are defined as natural plant products that have been applied for their benefits since ancient times such as local anesthetics, sedatives, anti-inflammatory, antimicrobials, and flavoring agents. This research aimed to describe the chemical constituents, antioxidant and antimicrobial activities and cytotoxicity effects for three types of essential oil which are extracted from *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare*.

**Methodology:** The three extracted EOs chemical profiles were characterized by gas chromatography coupled to mass spectrometry (GC-MS), while the antioxidant ability of the investigated EO was established utilizing the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method. Moreover, the broth micro dilution assay was used to determine the antibacterial activity against several microbial strains. In addition, the cytotoxic activity of the EO was evaluated using the MTT assay against two types of cancer cell lines.

**Results:** The GC-MS investigation revealed that *Micromeria fruticosa* essential oil contains mainly pulegone (81.77%), caryophellene (2.95%), isomenthone (2.1%), Piperitenone oxide (1.78%), and trans-anethole (1.36%), while *Foeniculum vulgare* essential oil primary chemicals were trans-anethole (93.69%), fenchone (3.93%), sylvestrene (0.83%), and methyl chavicol (0.57%). For *A. citrodora* essential oil the essential components were trans-anethole(18.91%), neral (8.12%), geranial (10.35%),

1,8-cineole (7.46%), limonene(6.51%), alpha-curcumene (6.50%), beta-caryophellene (3.38%), methyl chavicol (2.91%) and fenchone(2.33%). All oils were founded to exhibit effective in scavenging the DPPH radicals with IC<sub>50</sub> values of 10.11±0.20, 12.022±0.18 and 19.49±0.11 µg/mL for *A. citrodora*, *M. fruticosa* and *F. vulgare* EO, respectively, compared to Trolox the standard compound which had IC<sub>50</sub> equal to 3.09±0.23. The *A. citrodora* EO showed most potent antibacterial activity against all the tested microbes with MIC values ranging from 3.125-25 µg/mL except *Pseudomonas aeruginosa* which was resistant, while *M. fruticosa* EO showed only moderate antimicrobial properties with MIC values ranging from 6.25-25 µg/mL, for antifungal effect the most potent against *Candida albicans* was *A. citrodora* oil with MIC equal to 3.125µg/ml followed by *M. fruticosa* and *F. vulgare* oils with MIC equal to 6.25µg/ml. On the other hand *F. vulgare* oil was not active against the tested bacterial strains. For cytotoxicity the results showed a potent anti-cancer effect when the two oils from *M. fruticosa*, *F. vulgare* were mixed in (1:1) ratio, both essential oils seemed to have higher inhibitory effect on epidermoid carcinoma (A431) compared to skin cancer (B16-F1) cell lines. On the other hand *A. citrodora* EO from different rejoin in Palestine effects on B16F10 melanoma cell line, the results showed that it is the most inhibitory effect.

**Conclusion:** This study can be considered as evaluable investigation of the phytochemicals and chemical profiling of the EO of *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare*. The collected results demonstrate that these three plants can be considered as a rich origin of bioactive natural compounds in treating oxidative stress conditions, microbial infections and some types of carcinoma, so further validation for these three types of essential oils must be carried out especially in vivo tests to evaluate the medicinal potentials.

**Keywords:** Essential oils, GC-analysis, antioxidant, antimicrobial, cytotoxic.

# Chapter One

## Introduction

### 1.1. Medicinal plants

Plants have a widespread assortment of applications. Natural goods are still used by humans since they first became aware of their environment. The existence of educated and civilized man on Earth is thought to have lasted between two and three million years, during the majority of which he has battled to survive. After countless years of practice, he has honed the skill to discriminate between helpful and harmful plants under close inspection. Ever since, every society has used plants as an important source of medicine (1).

Ancient civilizations in Egypt, Assyria, China, and the Indies used medicinal plants to treat various ailments. Records from these societies include literature, myths, folktales, medical treatises, epic poetry, manuscripts, copper plates, and palm leaves. The Neanderthal man's grave in Iraq revealed plants with medicinal properties. Chaulmoogra oil from *Hydnocarpus gaertn* was one of the first reports of medicinal plant use. Chinese Emperor's pharmacopoeia recorded the use of leprosy between 2730-3000 B.C., and ancient Egyptian tombs confirmed the use of castor and opium seeds (2). Hippocrates' *Materia Medica* contains 400 herbal formulations, while Plin, a botanist, compiled the *Historia planetarium*, which lists 500 plants. Dioscoridies, the Greeks' significant pharmaceutical collection, contains the canonical text (3). Afterwards of him In 37 books, Pliny the Elder (23–79 AD) authored "Natural History." Along with his "Galanicals" collection of medical formulas, Galen also wrote about 30 volumes on pharmacology(4).

Chinese medicine utilizes medicinal preparations, with the first recorded mention of *Nong Ben Cao Jin* in the written record. Li Shizhens' "*Ben Cao Gang Mu*" is considered the complete pharmacopoeia with 1894 entries. Western authors have written on herbal treatments, with benzoic acid being the first chemical isolated from a plant in 1560. Indian medicines, primarily based on plant-based medications, are derived from the *Rig Vedas* and *Ayurveda's* (5). In addition to incorporating indigenous *Ayurveda* treatment, Muslim rulers of India introduced their ancient medical system. Immune stimulants, antibiotics, and antitumor drugs extracted from plants have been developed and introduced throughout the past 50 years, and this has resulted in a substantial improvement in the

control of many diseases. The use of traditional medicines has increased significantly and become more popular during the past ten years. These are employed not just in developing nations where traditional medical care predominates in the National Health Care System, but also in nations where poor people in such nations receive primary healthcare (6).

The ready availability, affordability, and socio-cultural justifications of traditional medicines are the most crucial elements in their continued usage. In some nations, the use of herbal remedies has a long history, and the populace, particularly in rural regions, places more trust in traditional medical practices. The fact that most medicinal herbs have been employed for treating illnesses for a long duration is convincing proof that many of the plants prescribed for use in medicine are actually safe, although scientific toxicological investigations are still required (7). In the nineteenth century, German chemist Friedrich Wohler accidentally created urea, marking the beginning of synthetic compounds and the first organic synthesis in recorded history. Western science shifted towards synthetic lines and less attention to herbal remedies, but eventually returned to using herbal remedies due to their lower cost, more frequent use, and less negative side effects. Many plants have medical qualities, and ethnopharmacology is an interdisciplinary field of research. Herbal medicines, or phototherapeutic agents, are standardized formulations with complex plant materials as active constituents. However, these treatments lack direct pharmacological impact, making them unsuitable for urgent care (8,9).

## **1.2 Plants Secondary Metabolites**

Herbal remedies' medicinal effects are based on plant chemistry. One can more fully understand a plant's potential medical advantages by being aware of its chemical composition. Modern chemistry has provided an explanation for the importance of important plant metabolites in essential biological activities such as cell division and development, storage, reproduction and respiration. These pathways are made up of the components that go into processes like photosynthesis, citric acid cycle or the Krebs, glycolysis and related pathways. Examples of tiny molecules that are primary metabolites include sugars, amino acids, tricarboxylic acids, polysaccharides, proteins, and nucleic acids. Eventually all living cells create similar fundamental chemicals (10).

The term "secondary plant metabolites" refers to a range of chemicals that plant cells make through metabolic channels descended from the original metabolic channels. The phrase "secondary metabolite" was initially used by Albrecht Kossel, who was awarded the 1910 Nobel Prize in physiology or medicine (11). A scientific basis for the use of plants in conventional medicine by many historical cultures may be found in the varied biological effects of secondary metabolites. Since they have been described as antifungal, antiviral, and antibiotic, they can protect plants against infections. They also have large UV-absorbing compounds, which shield leaves from serious light damage. It has been shown that several plants used as feed grasses, such as lucerne or clover, exhibit estrogenic characteristics and alter an animal's capacity for reproduction (12). Various groups of secondary plant metabolites have been formed according to the chemical makeup of these compositions. As a starting point for a study of the key component groups regarded to have therapeutic efficacy, the nature of secondary plant metabolites will be explained in this chapter. Each section begins with an overview of the composition, botanical distribution, and pharmacological underpinnings of a specific class of secondary plant metabolites, followed by representational molecular images. The following are some portions of secondary plant metabolites; Alkaloids, Lipids, Terpenes, Saponins, Phenolics, Carbohydrates, and more.

### **1.2.1 Phenolic compounds**

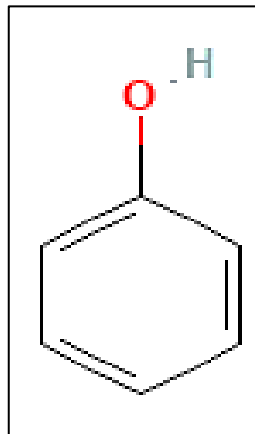
The greatest group of secondary metabolites found in plants are likely phenols. They range in complexity from unpretentious compounds with one aromatic ring to extremely complex polymeric substances, but they all have one thing in common: the existence of one or more phenol groups (Figure 1.1). They are abundant in plants and have a considerable impact on the flavor, color, foods, aroma of many herbs, and beverages. Pharmacologically, several phenolics are prized for their anti-inflammatory activity like quercetin or anti-hepatotoxic qualities like silybin. Some, like genistein and daidzein, have phytoestrogenic properties, while others, like naringenin, have insecticidal properties (13,14). Many phenolic compounds, particularly flavonoids, are powerful antioxidants and free radical scavengers. Phenolic compounds can be categorized based on place of biosynthesis or their structure.

Depending on their structural characteristics, phenolic compounds can be divided into:

- Simple phenolics
- Coumarins
- Tannins
- Flavonoids
- xanthones and Chromones
- Lignans
- Stilbenes

**Figure 1.1**

*Example of phenol*

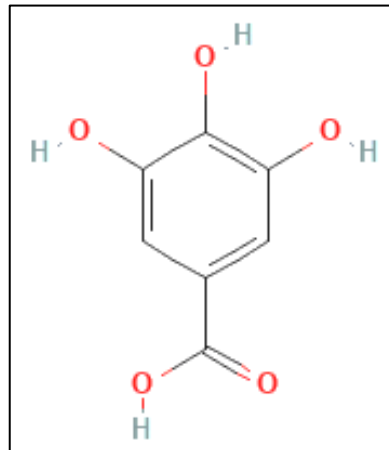


**1.2.1.1 Simple phenolic compounds**

Plants produce phenolic acids in abundance; whereas free phenols are uncommon, gallic acid, the parent substance of the gallotannins, is quite common (Figure 1.2). Gallic acid is well recognized<sup>o</sup> for its astringent effects, but it also exhibits a wide range of additional activities in vitro, including bronchodilatory, choleric, antibacterial, anti-inflammatory, antifungal, antiviral, anticancer, antianaphylactic, and antimutagenic effects. Additionally, it prevents insulin from degrading and encourages smooth muscle relaxation (14).

**Figure 1.2**

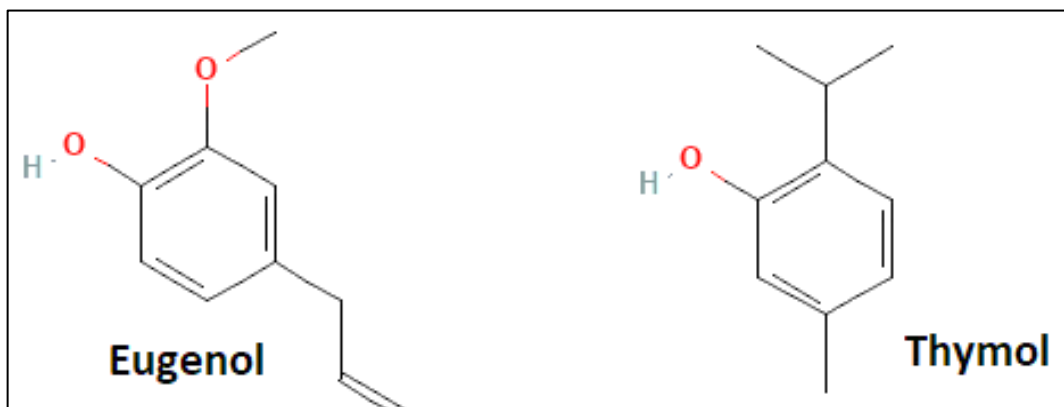
*Gallic acid*



Simple phenolic compounds are thought to be responsible° for many plants° pharmacological effects, including the antibacterial and diuretic properties of *Arctostaphylos° uva-ursi* (15). Because of the existence of capsaicinoids, which are straightforward phenolic compounds, *Capsicum* spp. exhibited rubefacient, analgesic properties, and circulatory stimulant (16). Additionally, the action of simple phenolics° is thought to be responsible for the cholagogue activity of *Cynara scolymus*, the anthelmintic activity of *Dryopteris filix-mas*, the anti-inflammatory analgesic activity of *Filipendula ulmaria*, as well as the diuretic activities of *Solidago virgaurea* and anticatarrhal (17). Simple phenolic examples are shown in Figure 1.3.

**Figure 1.3**

*Examples of simple phenolic compounds*

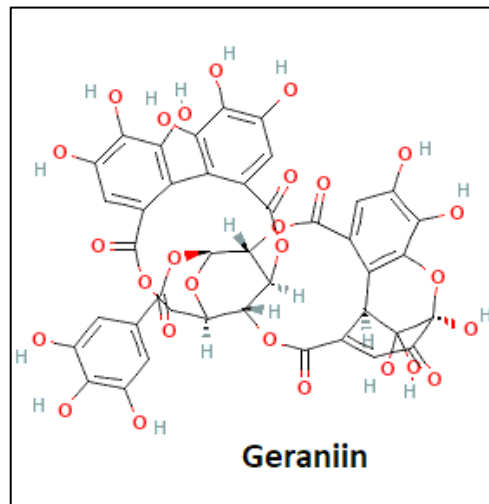


### **1.2.1.2 Tannins**

Polyphenols called tannins have the capacity to precipitate protein. For many years, these substances have been utilized to turn unprocessed animal hides into leather. Through the process of crosslinking, tannin molecules strengthen the protein's defense against microbial and fungal attack. However, many chemicals that are now classified as tannins due to their biosynthetic origin and structure have little to no potential to produce leather (17). Condensed tannins and hydrolysable tannins are the two main categories of tannins. Gallic and hexahydroxy diphenic acids are two phenolic acid molecules that are joined by ester bonds to a central glucose molecule to generate hydrolyzable tannins. ellagitannins and Gallotannins, which are made up of units of ellagic acid and gallic acid respectively, are the two main forms of hydrolysable tannins (18). Condensed tannins, also known as proanthocyanidins, are compounds with oligomeric flavonoid precursor-based structures. These compounds' linkages between hydroxylation patterns, flavonoid units, stereochemistry of the pyran ring's carbons 2, 3, 4, and the different effects of additional substituents. Some medications contain condensed and hydrolyzable tannins, such as those found in the leaves and bark of *Hamamelis virginiana* and *Camellia sinensis* (tea) (19). Tannin-containing medications have been used as antidiarrheal medications and as antidotes for heavy metal and alkaloid poisoning. The main ingredient in tea, epigallocatechin-3-gallate, has been demonstrated to have antiangiogenic properties in mice. Cranberry juice has a long history of usage as a urinary antiseptic(20). Figure 1.4 presented a few instances of hydrolysable tannins:

## Figure 1.4

*Examples of hydrolysable tannins*

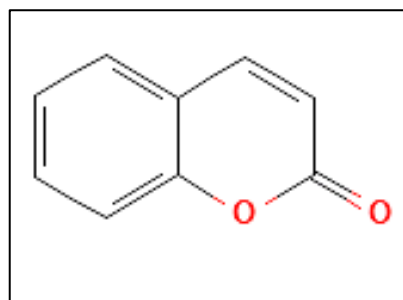


### 1.2.1.3 Coumarins

Benzo-pyrone, the lactone of O-hydroxycinnamic acid, from which coumarins are derived. The number of isolated natural coumarins is around 1000. More than 30 different families and 150 distinct species of organisms have been reported to have coumarin. Sweet clover, also known as melilot, Tonka bean, and Sweet Woodruff are the three plants that contain the most coumarin (21). Common coumarins found in both their free form and as glycosides in plants are aesculetin, umbelliferone, and scopoletin. *Datura stramonium* (Solanaceae), *Atropa belladonna*, *Ruta graveolens* (Umbelliferae), *Daphne mezereum* (Thymeliaceae), and specific *Aesculus hippocastanum* (Hippocastanaceae) and Rosaceae are among the plants high in coumarins. The most significant biological activity described for coumarins include anticoagulant, anti-inflammatory, anti-Alzheimer's, and anti-cancer (22). Examples of coumarins are shown in Figure 1.5

## Figure 1.5

*Coumarin main nucleus.*

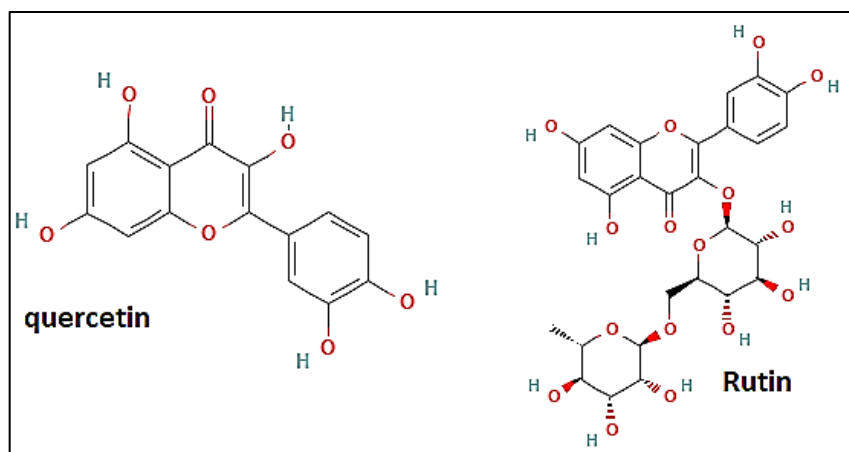


### 1.2.1.4 Flavonoids

The majority of phenols that occur in nature are flavonoids. Nearly 500 of these compounds out of the more than 2000 that are already known occur in the free state. Flavonoids have chroman rings with an aromatic ring in position 2, 3 or 4 as part of their structural skeleton. According to the degree of oxidation of the core ring, flavonoids can be categorized into several classes (ring C). Flavones are the most frequent of them, flavonols, and anthocyanins. Flavones and their relatives are frequently yellow (Latin flavus, yellow). Although they are found throughout nature, they are more prevalent in juvenile tissues and in higher plants where they are found in the cell sap. The Rutaceae, Polygonaceae, Compositae, Umbelliferae, and Leguminosae all have a lot of them (23, 24). Examples of flavonoids are shown in Figure 1.6.

**Figure 1.6**

*Examples of flavonoids*



### 1.2.1.5 Chromones and xanthenes

Though not of significant medicinal significance, some of these substances which are derivatives of benzo-pyrone are worth mentioning. eugenin from the khellin from mustard seeds and the clove plant. The active ingredients in the fruits of Ammi visnaga, furanochromones, are more complicated. Xanthenes are primarily found in the Guttiferae and Gentianaceae, although they are also rarely found in the Moraceae and Polygalaceae families of plants. The highlanders of Malawi and its neighbors utilize Polygala nyikensis to treat a variety of skin conditions with a fungus origin. Due to the presence of xanthenes, the plant's root has recently been demonstrated to exert its antifungal effect (25-27).

### **1.2.1.6 Stilbenes**

A heterogeneous collection of plant species contains a tiny but widely dispersed category of secondary metabolites known as stilbenes, which are primarily found as heartwood components. They play a significant role in the heartwood of trees belonging to the *Eucalyptus* (Myrtaceae), *Pinus* (Pinaceae), and *Madura* genera (Moraceae) (28). The most prevalent stilbene in nature is resveratrol, a para-hydroxylated substance. Resveratrol is found in the Myrtaceae, *Picea*, the Vitaceae, *Pinus*, and the Fabaceae and exhibits estrogen-like activity (29).

### **1.2.1.7 Lignans**

Lignans are dimeric chemicals found in Asteraceae (such as *Achillea lingulata*) that are essentially created by the union of two molecules of a phenylpropene derivative(30). There are four main subtypes of lignans: derivatives of 3,7-dioxabicyclo (3.3.0)-octane or bisepoxylignans, derivatives of tetrahydrofuran or monoepoxy lignans, and dibenzylbutane derivatives. Numerous of these substances demonstrated antibacterial and antifungal properties (25).

### **1.2.2 Alkaloids**

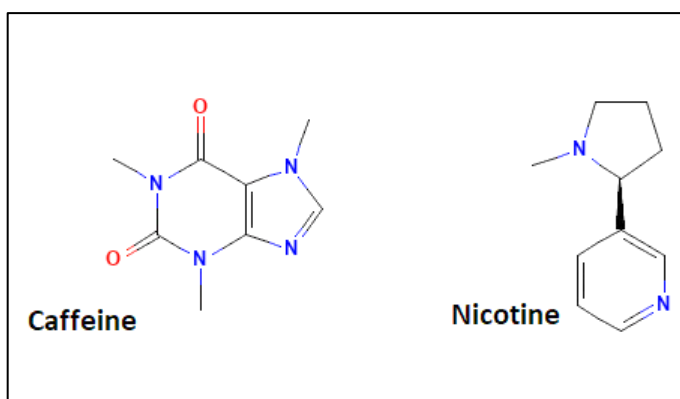
Alkaloids are organic substances that include a heterocyclic ring containing at least one nitrogen atom. They do not represent a homogeneous set of chemicals from any point of view, whether physiological, biochemical, or chemical, which makes their definition problematic. There is no single definition that applies to all alkaloids, except from the fact that they are all nitrogen-containing substances. Alkaloids can be categorized into many categories based on their fundamental chemical makeup. Alkaloids can be divided into several categories, including acridones, carbolines, aromatics, ephedra, ergots, indoles, imidazoles, indolizidines, bisindoles, oxindoles, manzamines, quinozolines, quinolines, phenylethylamines, phenylisoquinolines, purines, piperidines, pyrrolidines, pyrrolidines, pyrroloindoles, pyrrolizidines, simple tetrahydroisoquinolines and pyridines (31). Lower plants do not frequently have alkaloids. Fungi have been shown to contain sulfur-containing alkaloids and lysergic acid derivatives, such as the gliotoxins. The ephedra, *Taxus*, and lycopodium alkaloids are among those documented for their therapeutic usefulness in relation to pteridophytes and gymnosperms. The angiosperms have an unequal distribution of alkaloids.

### 1.2.2.1 Nicotine

The addictive substance in tobacco is nicotine, which is present in the tobacco plant (*Nicotiana tabacum*) and other *Nicotiana* species. Nicotine has sedative characteristics. Additionally, it is exceedingly poisonous, and excessive dosages can result in respiratory paralysis (Figure 1.7). A ganglion cholinergic receptor agonist, nicotine has complicated pharmacological effects that are mediated by binding to receptors in the brain, neuromuscular junction, autonomic ganglia, and adrenal medulla (32).

### Figure 1.7

*Examples of alkaloids*



### 1.2.2.2 Caffeine

Numerous botanically unrelated species, such as kola (*Cola acuminata*), mate (*Ilex paraguariensis*), guarana (*Paullinia cupana*), and coffee (*Coffea* spp.), contain caffeine (Figure 1.7). The chlorogenic acid is bonded to caffeine in fresh coffee beans. The process of roasting releases the components that give coffee its aroma, including caffeine. The cardiovascular, respiratory, and central neurological systems are all stimulated by caffeine, which also has diuretic properties (33).

### 1.2.2.3 Vinblastine

Vinblastine was isolated from *Catharanthus roseus* G. (Figure 1.7) and has been used as a disinfectant as a treatment for high blood pressure and diabetes. Vinblastine is nevertheless crucial for fighting cancer. Along with the other vinca alkaloids vincristine, vindesine, and vinorelbine, it is utilized in therapeutic settings in the US and Europe (34).

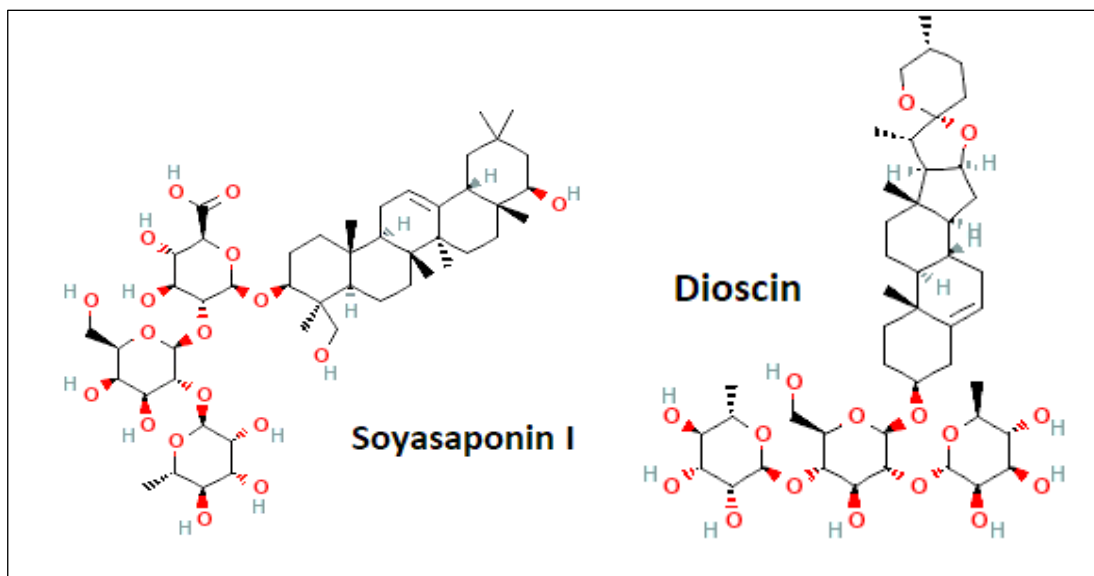
### 1.2.3 Saponin

When a triterpenoid (triterpenoidal saponins) or steroid (steroidal saponins) is connected to a carbohydrate unit (oligosaccharide chain or a monosaccharide), the resulting chemical is called a saponin (Figures 1.8). These sugar units can be made of uronic acids, hexoses, or pentoses, among other things. These substances are soap-like and are able to reduce surface tension due to the hydrophobic-hydrophilic asymmetry. In aqueous solutions, they produce foam, and in vitro, they hemolyze blood erythrocytes. The genin or sapogenin refers to the aglycone component of the saponin molecule. More than 500 plant species from at least 90 different families have been reported to contain saponins; these compounds have been separated from all parts of plants, including stems, leaves, roots, flowers, bulbs, and fruits though they are often concentrated in the roots of many species, including *Dioscorea villosa* (wild yam), *Digitalis purpurea* (foxglove), *Gentiana lutea* (gentian (Korean ginseng)), *Eleutherococcus senticosus* (Siberian ginseng) (35). There are several pharmacological effects that saponins have shown. Some saponins have analgesic, anticancer, sedative, piscicidal, molluscicidal, and spermicidal effects.

As an expectorant and antitussive, glycyrrhizin from *glycyrrhizae radix* (from *Glycyrrhiza glabra*, Fabaceae) is beneficial. Additionally, it is used to treat chronic hepatitis and cirrhosis. Some saponins, such as those from *Bupleurum falcatum*, have anti-inflammatory effects (Apiaceae). In Korean medicine, *phytolacca americana* roots are thought to have anti-inflammatory qualities. Numerous other saponins have also been shown to have similar effects. For instance, aescin from horse chestnut (*Aesculus hippocastanum*) has been shown to be 600 times more effective than rutin at reducing rat paw edema (36).

**Figure 1.8**

*Example of triterpenoidal saponin and steroidal saponin.*



### 1.2.4 Terpenes

The most numerous and varied class of secondary chemicals found in plants are terpenes. The word “turpentine”, which itself is derived from the old French word *ter(e)binth*, which means “resin,” gives rise to the name “terpene”. Chemically speaking, they are all formed from variously constructed 5-carbon isoprene units. A prefix in the name specifies the number of terpene units as follows: Terpenes are categorised based on the number of isoprene units in the molecule (37).

#### 1.2.4.1 Hemiterpenes

They are made up of just one isoprene unit. The oxygen-containing derivatives of isoprene, such as isovaleric acid from *Vaccinium myrtillus* and angelic acid obtained from *Angelica archangelica*, are thought to be the only hemiterpenoids (38).

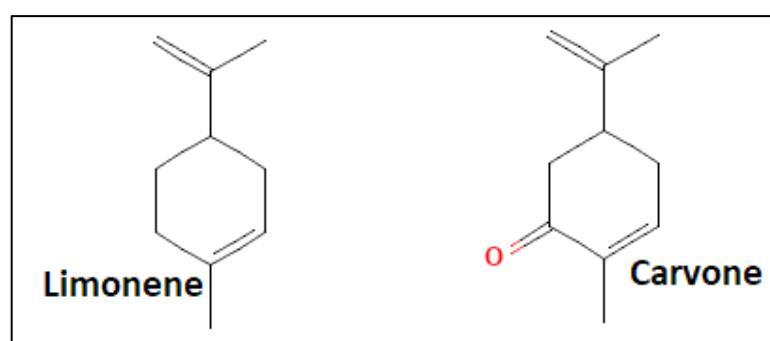
#### 1.2.4.2 Monoterpenes

They have the chemical formula  $C_{10}H_{16}$  and are made up of two isoprene units (see Figure 1.9). They are crucial elements of volatile oils or essential oils derived from plants. Members of specific plant families, such as the Pinaceae, Rutaceae, Lamiaceae, and Apiaceae, from which many essential oils are commercially derived, tend to include monoterpenes. Some of these substances, like geraniol, are practically universal and may be discovered in trace levels in the volatile secretions of the majority of plants.

Unsaturated hydrocarbons (like limonene), alcohol esters (like linalyl acetate), alcohols (like linalool), ketones, and aldehydes (like citronellal) are further categories for monoterpenes (e.g., Carvone). There are many common medical applications for monoterpenes and other volatile terpenes. Camphor and menthol are two substances that are used as anti-inflammatory, anti-irritants, and analgesics. Monoterpenes have a long history of use as anthelmintics. Several monoterpene glycosides appear to have a vaso dilating effect on the femoral vascular bed and coronary arteries (39).

### Figure 1.9

*Examples of monoterpenes.*

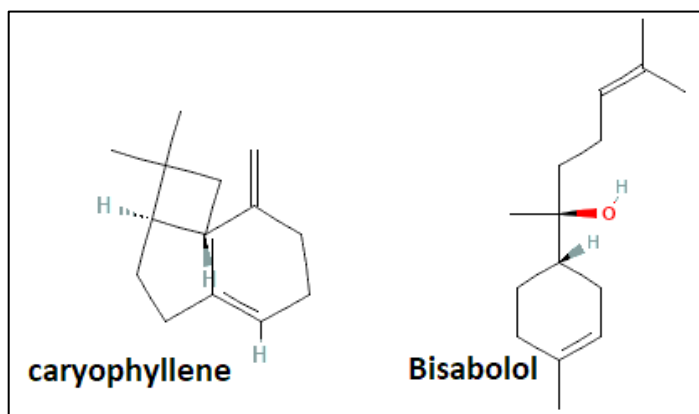


#### 1.2.4.3 Sesquiterpenes

They have the chemical formula  $C_{15}H_{24}$  and are made up of three isoprene units (see Figure 1.10). Sesquiterpenes can be categorized into more than 200 different structural kinds based on their biogenetic origins, and thousands of such molecules are known. According to their structural similarities, these substances can be neatly divided into three major groups: acyclic (like farnesol), monocyclic (like bisabolol), and bicyclic (e.g., caryophyllene). Sesquiterpene lactones have been found to have antifungal, antibacterial, and antiprotozoan properties. At quantities comparable to those of the amoebic medication metronidazole, sesquiterpenes from *Vernonia colorata* inhibit *Entamoeba histolytica*. The cardiotoxic effects of *Arnica montana* flowers are caused by helenialin and a group of similar chemicals. Clinical uses for the rhizome of *Atractylodes macrocephala* (Asteraceae) include diuretic, analgesic, and anti-inflammatory properties. Atractylenolide I and Eudesma-4(14)-7(11)-dien-8-one are two active substances that are associated with the activity. Because sesquiterpenes are present, other closely related medicinal plants are also utilized for the same purposes (40).

## Figure 1.10

### Examples of sesquiterpenes



#### 1.2.4.4 Diterpenes

Their chemical formula is  $C_{20}H_{32}$  and they are made up of four isoprene units (see Figure A.1 appendix A). Acyclic and macrocyclic chemicals are subclasses of diterpenes. In addition, the amount of ring systems that are present determines how macrocyclic diterpenes are categorized. Diterpenes can have fused 5- and 7-membered rings or 6-membered rings as their ring structure. A lot of diterpenes also include extra ring structures. These are side substitutions that happen in esters or epoxides (41). The active components of several therapeutic plants are diterpenoids. A diterpene, vitamin K1 was initially found in plants in 1929 and functions as an antihemorrhagic agent. “Carotenes” refers to both the related chemicals and vitamin A, a diterpenoid. Furanoditerpenes are responsible for the bitter properties of *Jateorhiza palmata* (calumba root). Both *T. scorodonia* (wood sage) and *Teucrium chamaedrys* (wall germander), both members of the Labiatae family, produce neoclerodane-type diterpenes. They serve as diaphoretics and antirheumatics in herbal medicine (42). The pharmacological effects of diterpenes, like those of all terpene groups, include antibacterial, analgesic, anti-inflammatory, antiprotozoal, antifungal, and antineoplastic actions (17). A few diterpenes from the Ericaceae family plant *Kalmia latifolia* exhibit antifeedant effects on gypsy moths. The diterpenoid acids known as gibberellins, which were originally isolated from the fungus of the genus *Gibberella* but are also present in higher plants, have a notable impact on seedling growth (22), (see figure A.1 appendix A).

#### **1.2.4.5 Sesterterpenes**

terpenes with five isoprene units and 25 carbons are uncommon compared to the other sizes, (the prefix sester- implies two and a half, or half to three). Geranyl farnesol, which was discovered from the *Camellia japonica* (camellia) and seed oils of *Camellia sasanqua* (sasanqua), family Theaceae, is an example of a sesterterpenoid (43). In mouse leukemic M1 cells, geranyl farnesol had cytotoxic properties (44).

#### **1.2.4.6 Triterpenes**

They have the chemical formula  $C_{30}H_{48}$  and are made up of six isoprene units (see Figure A.3 appendix A). The primary component of shark liver oil, the linear triterpene squalene, is produced by the reductive coupling of two molecules of farnesyl pyrophosphate. Over 4000 triterpenoids have been identified, making up a large fraction of the fatty compounds found in all plants.

Both plants and animals produce these substances as precursors to steroid hormones. Steroids and triterpenes can be found alone, coupled with other substances, or as free molecules. Triterpene and steroid structures can be split into around 40 primary categories (10). *Boswellia carterii*'s oleo-gum resin contains two triterpenes known as  $\beta$ -boswellic acids (oleanane-type triterpene) and  $\alpha$ -boswellic acids (ursane-type triterpene) which have been found to have anti-rheumatic and anti-inflammatory effects (45). The isolated quassinoids from *Quassia amara* are one class of substances that exhibit a variety of intriguing biological activity. These are the byproducts of triterpenes breakdown and modification. As an insecticide, bitter tonic, and thread worm enema, quassia is employed in traditional medicine. Sesquiterpenes, which have seven isoprene units and the formula  $C_{35}H_{56}$ , Tetraterpenes, which have eight isoprene units and the formula  $C_{40}H_{64}$ , Polyterpenes, and Norisoprenoids, which have lengthy chains of several isoprene units, are further terpenes (see Figure A.2 appendix A).

#### **1.2.5 Lipids**

Essential oils, Fixed oils, sterols, waxes, phospholipids, fat-soluble vitamins (such as vitamins A, K, D, and E), and other naturally occurring molecules are included in the category of molecules known as lipids. In addition to being hormones and vitamins, lipids also serve as essential structural elements of all biological membranes, energy storage,

and fuel for cellular processes(46,47). Recent investigations have shown that several of the phytochemicals in this family, known as lipids, have pharmacological effects despite being main plant metabolites.

#### **1.2.5.1 Fixed oils**

Fixed oils are made up of glycerol-esterified high molecular weight aliphatic long-chain fatty acids, such as stearic, oleic acids, and palmitic. While fats are rich in solid glycerides like glycerin stearate, fixed oils have a considerably higher percentage of liquid polyunsaturated glycerides like glycerin oleate (46). *Linum usitatissimum*, a member of the Linaceae family, is used to produce flax, linseed, and its oil. Some fixed oils contain polyunsaturated fatty acids, which are strong antioxidants and anti-inflammatory agents because they minimize the excretion of lipid peroxidation products. To lower the chance of developing atherosclerosis and cardiovascular disease, they are employed as preventative measures (48).

#### **1.2.5.2 Waxes**

Wax is a type of lipoidal material made primarily of long aliphatic chains, some of which may contain functional groups. They might have hydroxyl groups, just like primary and secondary long-chain alcohols, which are typically found as esters. Others have carboxylic or aromatic functional groups, amide or ketonic functional systems, or unsaturated bonds. Conversely, synthetic waxes are made of long-chain hydrocarbons (paraffins or alkanes) devoid of functional groups. Since they are fatty acid esters, they resemble fixed oils and fats with the exception that their alcohol is not glycerin. *Simmondsia chinensis* seeds produce jojoba wax, a liquid wax made up of straight-chain esters of alcohols and fatty acids (49). Jojoba wax can be used to treat a variety of skin disorders because of its anti-aging, wound-healing, and anti-inflammatory properties. To improve drug absorption, jojoba wax has also been applied to topical treatments. Additionally, it is utilized in skincare items and cosmetics including moisturizers and sunscreens (50).

### **1.2.5.3 Essential oils**

Essential oils are complex combinations of relatively low molecular weight volatile aromatic chemicals. The presence of two or three primary components at relatively high concentrations (20–70%) compared to other components present in trace amounts characterizes them despite the fact that they may have up to 60 components. For instance, the two main components of *Origanum compactum* essential oil are thymol (27%) and carvacrol (30%). Up to 68% of the *Coriandrum sativum* essential oil is made up of linalol, which is the main component. Other examples include *Cinnamomum camphora* essential oil with 1,8-cineole (50%) as primary constituent, *Artemisia herba-alba* essential oil with menthone (19%) and menthol (59%) as major constituents, and *Mentha piperita* essential oil with menthone (19%) and menthol (59%) as major constituents. Typically, The biological characteristics of the essential oils are mostly depending on these factors (51). They are used for a variety of significant medicinal purposes, including antiseptic, antibacterial, sedative, analgesic, spasmolytic, anti-inflammatory, and locally anesthetic treatments. Additionally, they serve as scents in food preservation and embalming (52).

### **1.2.6 Carbohydrates**

All living things on our planet have carbohydrates. Carbohydrates are the precursor to all phytochemicals and, by extension, all animal biochemicals because they are the earliest product of photosynthesis. More than any other sort of natural molecule, carbohydrates are found in nature. Cellulose, a polymer of glucose that serves as the primary structural component of plants, is the most prevalent organic compound on Earth. Although they are primary metabolites, carbohydrates are linked together through glycosidation in many secondary metabolites. Mucilages and gums are made from simple sugar and uronic acid polymers (53). Carbon, hydrogen, and oxygen are the three components that make up carbohydrates, and these last two are often present in the same ratios as in water. Monosaccharides, disaccharides, oligosaccharides, and polysaccharides are the four chemical classes into which they are divided. Monosaccharides range in carbon atom count from three to nine, but those with five and six (hexoses,  $C_6H_{12}O_6$ , pentoses, and  $C_5H_{10}O_5$ ) are the ones that accumulate in plants the most. Depending on the amount of saccharide units involved, monosaccharide condensation produces the other varieties. In addition to their structural roles and crucial biological in plants, certain of their constituents exhibit therapeutic properties, such as mucilage. Mucilage, a viscous, sticky

substance made by practically all plants and some microbes, thickens plant membranes for protection. Additionally, it aids in seed germination and the storage of food and water. It is made up chemically of an exopolysaccharide and a polar glycoprotein. Mucilage is employed as a demulcent in medicine. The two main sources of mucilage are *Linum usitatissimum* (flax seeds) and cacti (and other succulent plants). Due to its demulcent properties, the extract from the mucilaginous root of the marshmallow plant (*Althaea officinalis*), which is usually used to manufacture marshmallows, has been used as a cough suppressant. Due to its mucilaginous composition, the inner bark of *Ulmus rubra* (the slippery elm) is also employed as a demulcent. When mucilage comes into touch with mucous membrane surfaces or skin, it predominantly works as a local demulcent or emollient. Here, they create a layer of “slime” that calms and covers exposed or inflamed digestive tract surfaces. They are often used to treat inflammatory gastrointestinal problems, particularly when there is ulceration. Their degree of hydrophilicity and relative indigestibility have a significant impact on bowel behavior (54).

### **1.3 Extraction methods for essential oils**

The essential oils from aromatic plants can be extracted from a variety of plant parts. The qualities and components required for the purposes determine the extraction method to be used. In some cases, using ineffective extraction methods can destroy and affect the biological activity of chemical compounds found in essential oils, leading to things like active component loss, discoloration, off-flavoring, and in some cases, physical changes in the essential oils themselves. To achieve a high production yield for the premium essential oils, efficient and effective extraction necessitates minimal expense and less time-consuming procedures. The extraction of essential oils from various plant components can be done using a variety of techniques. These methods can be classified into two groups: modern techniques and traditional techniques.

#### **1.3.1 Traditional extraction techniques**

##### **1.3.1.1 Hydro distillation**

The first and most fundamental technique for extracting oils is hydro distillation, which Avicenna discovered. By immersing the plant materials directly in water inside the vessel and then boiling the mixture, the plant materials are first used to extract essential plant oils by hydro distillation. The apparatuses are made up of a heating source, a condenser

to turn vapor into liquid, an alembic-shaped vessel, and a decanter to collect the condensate and separate the essential oils from the water (55). This extraction method is unique and used frequently for extractions requiring hydrophobic natural plant material with a high boiling point, such as those involving wood or flowers. This procedure enables the extraction of essential oils at a controlled temperature without overheating because the oils are encircled by water. Azeotropic distillation serves as the foundation for the extraction theory. Under atmospheric pressure and during the extraction process, oil molecules are present together with any additional solvents and/or water (heating). The main advantage of this extraction method is the ability to isolate plant components below 100°C (56).

#### **1.3.1.2 Steam distillation**

For temperature-sensitive substances, such as resins, hydrocarbons, and oils, that are insoluble in water and may decompose near their boiling temperatures, steam distillation is a type of separation or distillation procedure. A mixture of compounds can be distilled using steam distillation at a temperature much lower than the boiling point of each individual constituent. On the other hand, by heating plant materials with steam produced by a steam generator, these compounds are volatilized at a temperature near 100°C under atmospheric pressure in the presence of steam or boiling water. Heat is the main factor that determines how well plant structures break down and rupture, releasing aromatic compounds or essential oils in vapor form (57). When the steam cools, it condenses into water. The essential oil is then extracted from the distillate/hydrosol layer on the water's surface by decanting it from the top. The benefits of steam distillation, at its most basic level, include being a relatively inexpensive process to operate and the well-known characteristics of the oils produced by this method (58).

#### **1.3.1.3 Cold expression**

Oil is expeller-pressed at low temperatures and pressure using the cold expression technique. The oil produced using this process is guaranteed to be 100 percent pure and retain every aspect of the plant. It is a mechanical extraction technique that lowers and minimizes heat throughout the batching of the raw materials. Essential oils are mostly extracted using this method from flowers, seeds, citrus oils, and plants such as tangerine and lemon (59). Scrubbing is done in this process to get rid of the oil-containing plant's

outer coat. The material from the pulp and the essential oil from the vesicles are then extracted by crushing the entire plant. The essential oil rises to the surface of the substance and is removed from it by centrifugation (60). These oils have a short shelf life after being produced. According to reports, oil created in this fashion has a higher concentration of the fruity aroma than oil made in any other way.

#### **1.3.1.4 Hydro diffusion**

In contrast to steam distillation, the steam in this method is injected into the alembic from top to bottom. The vapor combination, which includes Eos, is immediately condensed beneath the plant support through a perforated tray. The method for separating EOs is the same as earlier distillation procedures. This method can increase yield while reducing steam usage and distillation time when compared to steam distillation (61).

#### **1.3.1.5 Solvent extraction**

Another method that is frequently used in industry to extract edible oil or other chemicals is solvent extraction. It has been used in a variety of industrial sectors, including food engineering, hydrometallurgy, waste treatment, and pharmaceuticals. A procedure called solvent extraction uses a chemical solvent to remove liquid from a sample of solid-liquid. The type of solvent could either be polar or nonpolar. ethanol, Hexane, chloroform, methanol, petroleum ether, acetone, and diethyl ether are examples of solvents that are frequently used in industry (62). Because it can recover a significant amount of oil, the solvent extraction method is widely utilized around the world. In the industry, the solvent extraction method can produce a high yield with up to 99% recovery. However, the oil produced is of lower quality than oil that has been pressed, and the chemical solvent used is dangerous to people. In addition, the chemical solvent makes the extraction process more likely to result in a fire or explosion (63).

#### **1.3.2 Modern extraction techniques**

In order to extract essential oils from plants, efforts were made while keeping in mind the ideas of economically sensible, high-efficiency, quality production., and environmentally friendly.

### **1.3.2.1 Microwave-assisted extraction (MAE)**

A contactless heat source like a microwave can produce more efficient and targeted heating. Instead of taking many hours as with the traditional distillation procedure, microwaves can finish the distillation in a matter of minutes. According to the necessary protocols, plant materials are treated to a microwave reactor with or without water or organic solvents at various microwave treatment intensities(61). Because of its distinct heating mechanism (based on friction), high efficacy under normal settings, higher extraction yield, cost-effectiveness, good selectivity, and shorter extraction durations, this technology is currently receiving a lot of interest from researchers. The use of MAE for essential oil extraction has been the subject of numerous investigations (64).

### **1.3.2.2 Ultrasound-assisted extraction**

The development of ultrasound-assisted extraction is similar to that of MAE in that it aims to increase efficacy while shortening extraction times. This cavitation effect is greatly dependent on various factors, including incubation time, frequency and intensity of ultrasound, and temperature in UAE, where there are fewer chances of thermal breakdown and essential oils' quality and flavor remain better. The collapse of cavitation bubbles generated through ultrasonication increases the rate at which essential oils are released (65,66).

### **1.3.2.3 Supercritical fluid extraction (SFE)**

A supercritical fluid (SF) develops when pressure and temperature are raised above critical values for a particular liquid or gas. In the supercritical zone, the line separating liquid from gas dissolves, giving rise to a homogeneous fluid. The diffusivity and density of supercritical fluids set them apart from liquids and gases. The density of SFs varies when temperature and pressure values change, unlike the density of liquids. As a result, a little increase in pressure can cause a significant increase in fluid density, which is followed by a change in the SF's solvating power. A multicomponent mixture can be broken down into its individual components because to this phenomena. As a result, selectivity is the main advantage of supercritical fluid extraction. The application of this method could aid in the extraction of natural compounds that could degrade at high temperatures. Due to its high extraction yield, reduced extraction time, and ability to

recover the solvent used due to the SF's volatility, this approach is both cost-effective and ecologically friendly for the extraction of essential oils and other natural products(67).

#### **1.3.2.4 Subcritical water extraction**

The turbo distillation process is similar to traditional water distillation, except in turbo distillation, a stainless-steel stirrer is used to continuously agitate the mixture at a sufficient speed. With tough-to-extract compounds and coarse raw materials, this method works well (spices, woods). Turbo distillation, when compared to aqueous distillation, shortens distillation times and uses less energy while also halting the deterioration of volatile components. In reality, it is a sort of green extraction based on water distillation (68). Using the turbo distillation extraction technique, essential oils can be extracted from portions of plants and other things that are challenging to remove. The essential oils and flavonoids were extracted simultaneously utilizing turbo extraction-distillation in a study by Mnayer et al (69).

### **1.4 Uses of essential oils in biology**

#### **1.4.1 Pharmacological applications**

##### **1.4.1.1 Anticancer mechanism of essential oils**

The majority of cancer chemotherapies involve highly cytotoxic medicines that target populations of growing cells. These medications non-discriminatory nature causes severe negative effects in healthy cells. Cancer prevention and therapy benefit greatly from natural essential oils and the compounds they contain. The chemo preventive characteristics of essential oils are due to a number of processes, including antioxidant, antiproliferative, and anti-mutagenic effects, which improve detoxification and the synergistic action of their constituent parts. Reactive oxygen species are directly linked to the cause of oxidation and inflammation, both of which can result in cancer. Oxidative stress, which raises the mutation rate inside cells and encourages neoplastic transformation, can result in mitochondrial DNA damage. In addition, reactive oxygen species (ROs) regulate cellular proliferation, angiogenesis, and metastasis to precisely activate signaling pathways and advance tumor development (70). The results of some of the research that demonstrate the anticancer efficacy of EOs against different malignancies are reported in Table 1.1.

**Table 1.1***Anticancer activity of EOs against various cancer cell lines*

<b>Cancer</b>	<b>Species</b>	<b>Major essential oil constituents</b>	<b>Reference</b>
Lung	citrifolia Morinda	nordamnacanthal, L-scopoletin, $\beta$ -morindone, 9-H-pyrido [3,4-b] indole, $\beta$ -thujene, $\alpha$ -copaene	(71)
Breast	Boswellia sacra	$\alpha$ -thujene, $\alpha$ -pinene, boswellic acid, myrcene	(71)
Colon	Limettioides Citrus	triacontane, d-limonene, $\beta$ -myrcene, sabinene	(72)
Liver	Citriodorus Thymus	thymol, borneol, camphor, 3, 7-dimethyl-1,6-octadiene-3-ol, 1-methyl-4-[ $\alpha$ -hydroxy-isopropyl] cyclohexene	(73)

**1.4.1.2 Essential oil as an antioxidant agent**

Free radicals and other reactive oxygen species oxidize biomolecules, which ultimately results in molecular changes that can cause diseases including diabetes, asthma, and Parkinson's disease as well as long-term conditions linked to aging, arteriosclerosis, and cancer (74). Additionally, as frequently demonstrated by physicochemical techniques, essential oils have impressive antioxidant and free radical scavenging properties (Table 3). cinnamon, Basil, nutmeg, clove, thyme, and oregano are a few medicinally significant plants that have essential oils that have been shown to have antioxidant and radical-scavenging capabilities (75). The chemical components have a major role in determining the antioxidant characteristics. For example, the presence of thymol and the content of carvacrol (36.5 and 29.8%, respectively) in thymus is largely responsible for the antioxidant activity.

**Table 1.2***Reported antioxidant activities of essential oils of different plant families*

<b>Plant</b>	<b>Family</b>	<b>Major essential oil constituents</b>	<b>Antioxidant assay</b>	<b>Reference</b>
<i>Salvia reflexa</i>	Lamiaceae	E-caryophyllene, phytol, caryophyllene oxide, palmitic acid.	DPPH radical, Reducing power, Metal chelating of Fe <sup>2+</sup> , scavenging,	(76)
<i>Cotinus coggygria</i>	Anacardiaceae	α-pinene, myrcene, α-terpineol, sabinene, cymene	DPPH radical, Reducing power, Metal chelating of Fe <sup>2+</sup> , scavenging,	(77)
<i>Origanum vulgare</i>	Lamiaceae	thymol, carvacrol, caryophyllene, 3-carene, p-cymene,	DPPH radical, scavenging,	(78)
<i>Ocimum basilicum</i>	Lamiaceae	methyl chavicol, linalool, eugenol, trans-α-bergamotene, eucalyptol	scavenging, DPPH radical, TBHQ inhibition, β-carotene bleaching assay	(79)

**1.4.1.3 Essential oil as an anti-diabetic agent**

A diabetes condition called hyperglycemia results from an inability to either make insulin or use it to control normal blood glucose levels. Due to the fact that both α-glucosidase and α-amylase are involved in the digestion of carbohydrates, inhibition of both enzymes is a key component in the therapy of type 2 diabetes mellitus. Long-chain carbohydrates are broken down into disaccharides by α-amylase, whereas disaccharides and starch are converted to glucose or mono saccharides by α-glucosidase. Thus, by blocking the enzyme, carbohydrate breakdown can be slowed, which ultimately results in less glucose being absorbed into the bloodstream (80). The enzyme activity is inhibited by essential oils by binding to the active site of the enzyme (glucosidase or amylase) and acting as an inhibitor to create an enzyme-inhibitor complex. Mechanism of enzyme (α-glucosidase and α-amylase) inhibition by essential oil (81). The antidiabetic potential of several essential oils and their constituents has been studied, including those from the plants *Syzygium aromaticum*, *Cuminum cyminum* (82), *Thymus kotschyanus*, *Oliveria decumbens*, *Zataria multiflora*, and *Trachyspermum ammi* (83).

### 1.4.2 Antimicrobial application

EOs and their components are essential for having antibacterial properties. The hydrophilic or hydrophobic nature of the essential oil, its chemical components, and the organism being targeted are the three key factors that determine the antibacterial action of the oil (84,85). Due to their hydrophobic nature, EOs penetrate through the cytoplasmic membrane and cell wall, disrupting the structure of the latter and making them more permeable. The permeability of the membrane allows macromolecules and other cellular components to flow out, which causes cell death (86). In bacteria, membrane permeabilization is accompanied by ion loss, a decrease in membrane potential, the failure of the proton pump, and ATP pool depletion. EOs can coagulate the cytoplasm, which can harm proteins and lipids. Aldehydes, alcohols, and phenols are the primary causes of EOs' antibacterial activity. One of the main components of EOs, terpenoids, has methyl groups or oxygen atoms that can be removed or localized from particular enzymes to increase their activity. Generally, it has been found that peptidoglycan layer, which is present outside the outer membrane, causes EOs to be more active in gram-positive bacteria than gram-negative bacteria. Table 1.4 summarizes the several experiments that have shown that EOs is effective against a variety of plants and human pathogenic bacteria, fungi, and viruses.

**Table 1.3**

*Antimicrobial activities of essential oils*

Essential oil	Action	Target microorganism	Reference
Osmophloeum Cinnamomum	Antibacterial	Salmonella sp, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis.	(87)
Artemisia judaica, A. biennis, A.absinthium	Antifungal	T.mentagrophytes, Pythium debaryanum, Trichophyton rubrum; T. roseum;	(88)
Citrus essential oil	Antiviral	SARS-CoV2	(89)

### 1.4.3 Pesticides applications of essential oils

Pesticides are a broad category of substances that have a variety of diverse effects, including insecticides, herbicides, rodenticides, nematicides, algicides, avicides, bactericides, and fungicides (90). Chemical pesticides are very toxic, environmentally

harmful, expensive, and have a number of other drawbacks. Research is being done to develop new pesticides that are less toxic, behave less negatively toward the environment, and have better specificity of action. In this regard, several botanicals have historically been employed for the management of storage pests, particularly in the Mediterranean area and Southern Asia. However, the significance of essential oils only became apparent in the 1990s after it was discovered that they had fumigant and contact insecticidal activities against a variety of pests (91). Essential oils have many uses in plant protection, including herbicidal, insecticidal, fungicidal, and nematocidal properties. Table 5 lists a few of these more recent investigations.

**Table 1.4**

*Pesticidal applications of essential oils from different plant species*

<b>Botanical source of essential oil (species)</b>	<b>Pesticidal Action</b>	<b>Activity against</b>	<b>Reference</b>
Coleus barbatus	Antifeedant	third instar larvae of Spilosoma oblique	(92)
camphora Cinnamomum	insect repellent and Insecticidal	Aphis gossypii Glover	(93)
zeylanicum Cinnamomum	Acaricide	Tyrophagus putrescentiae mites and Dermatophagoides spp.	(94)

#### **1.4.4 Other biological activities of EOs**

Various medicinal and aromatic plants bearing essential oil have been found to have a number of pharmaceutical and biological properties, including antifungal, antibacterial, antiviral, anticancer, antimutagenic, antidiabetic, anti-inflammatory, antiprotozoal, analgesic, antipyretic, hepatoprotective, antihyperlipidemic, antidiarrheal, neuroprotective, pesticidal and diuretic activities (95).

#### **1.5 Aloysia citrodora**

Lemon verbena, also known as Aloysia citrodora, is a species of flowering plant that is native to South America and belongs to the verbena family Verbenaceae. Lemon bee brush is another frequent name for this plant (96). It was introduced to Europe by the Spanish and Portuguese in the 17th century, where it was raised for its oil (97). Lemon verbena is a perennial shrub or subshrub that can reach heights of 2-3 meters (7–10 feet). The leaves are glossy, pointy, and 8 centimeters (3 in) long. They feel slightly rough to

the touch and have a powerful lemon aroma when bruised (thus the Latin specific epithet *citrodora*, or lemon-scented) (98). Additionally, tinctures and essential oils made from this plant are frequently used to treat cutaneous conditions. Research on *A. citrodora* has showed its powerful biological effects, including its antibacterial, antifungal, and anti-inflammatory capabilities (99, 100). Its leaves are frequently used in numerous food preparations, including drinks, fish and poultry meals, salad dressings, vegetable marinades, puddings, jams, and sorbets (101). They are also highly regarded for their distinctive lemony aroma. The plant is rich in phenolic substances such as phenylpropanoids, lignans, flavonoids, and tannins, as well as a variety of other non-phenolic chemicals, primarily verbascoside (102, 103). Moreover, geraniol and neral and citral isomers<sup>o</sup> dominate the essential oil of *A. citrodora* leaves (104, 105), despite<sup>o</sup> the fact that multiple studies have shown that the genotype, environmental factors, and growing circumstances all have an impact on the oil's composition (106), (see Figure A.3 appendix A).

### **1.6 *Micromeria fruticosa***

*Micromeria fruticosa* or as well known White *Micromeria*, is a subspecies *serpyllifolia* (Lamiaceae), is an aromatic herb (107). That is common in Palestine and other eastern Mediterranean countries. It has a lovely minty scent that, in the sweltering summer, gives the impression of cooling (108, 109). The aerial parts of the plant, which are known in Palestinian culture as Duqat Adas, Ishbit esh-shai, Qurnya, and thyme-leaf savory in English, are used in folk medicine (110). It has a long history of use in traditional medicine as an anti-inflammatory and for the treatment of wounds, skin and eye infections, diarrhea, abdominal pain, colds, headaches, and hypertension. Aside from their CNS depressive and analgesic properties, *M. fruticosa serpyllifolia* VOs also have antibacterial, antifungal, antioxidant, insecticide and anticonvulsant properties (111, 112), (see Figure A.4 appendix A).

### **1.7 *Foeniculum vulgare***

The name *Foeniculum vulgare* must be credited to Philip Miller because he did so in the eighth edition of his “Gardeners Dictionary” in 1768, which is in compliance with the international standards as established at Cambridge. *Foeniculum vulgare* Mill is how the name of this plant is written from that point forward. Due of its flavor, it is a medicinal

plant from the *Umbelliferae* (Apiaceae) family that has been used and known by people for centuries. Nearly all nations practiced cultivation of it (113). It has more than 100 names in addition to the common name “fennel”. It is a well-known and traditional herb having a lengthy history of medical usage. Studies have shown that *F. vulgare* is capable of efficiently treating a wide range of infectious diseases with bacterial, fungal, viral, mycobacterial, and protozoal origins (114-116). It has anti-inflammatory, cytoprotective, hepatoprotective, anticancer, hypoglycemic and oestrogenic properties (117-120). Limited clinical trials and animal studies indicate that long-term usage of *F. vulgare* is not hazardous. Fennel can be eaten on a regular basis in a variety of ways, including fresh in salads and snacks, cooked in stews, boiling, grilled, or baked foods, or even used to make herbal teas or alcoholic beverages. Due to the valuable nutritional makeup of fennel, which includes the presence of important fatty acids, a diet containing the recommended amount of fennel could have possible<sup>o</sup> health benefits (121), (see Figure A.5 appendix A).

## Chapter Two

### Methodology

#### 2.1 Materials

##### 2.1.1 Chemicals and reagents

The used materials in this project 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-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, Denmark), drying agent CaCl<sub>2</sub>, Dimethyl sulfoxide (DMSO) 10% (Riedel dehaen, 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<sub>2</sub> incubator (ESCO, 2012-74317, Singapore) Inverted biological microscope (MRC, 2017-170529, China), Microplate Absorbance (Bio Tek, 1903217-2019, USA).

##### 2.1.3 Plant collection and preparation

*Aloysia citrodora* leaves were collected from Jericho-Palestine while *Micromeria fruticosa* leaves and *Foeniculum vulgare* seeds were collected from Jenin-Palestine. Each collected plant parts were shade-dried during two weeks, and then crushed into fine powder using electronic grinder, after that each powder was kept in well closed container for further use.

##### 2.1.4 Extraction of essential oils

Hydro-distillation was used for extraction the Eos from *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare* plant material after following steps in previous protocols (122), In a 500 mL volumetric flask, 100g of the dried, ground leaves were put, and it was filled with distilled water. Dean Stark apparatus (see Figure A.6 appendix A)

was used on the flask for three hours. The extracted EO was dried with calcium chloride before being placed in a tiny, clean glass vial with a tight cover and labelled with the plant's name, the date, and the sample code. The vials were then stored at 4°C in the refrigerator, (see Figure A.6 appendix A).

At the end of the distillation process, the organic phase which contains the essential oil is separated from the hydrosol (aqueous phase) with ether followed by gentle removal of the solvent by evaporation.

## **2.2 Identification of the chemical composition of *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare* EO by gas chromatography-mass spectrometry (GC-MS)**

For the separation and identification of EO components for each tested plant, a fused silica capillary column (30 m 0.25 mm, film thickness of 0.25 m) by Perkin Elmer Elite-5-MS was employed. As the carrier gas, helium was used at a normal flow rate of 1.1 mL/min. With a ramp of 4.0°C/min to 280°C, a 5-minute initial hold, and a temperature of 50°C, the injector's temperature was set at 250°C. The total running time was 62.5 minutes, while the solvent delay varied from 0 to 4.0 minutes. An MS scan took between 4 and 62.5 minutes for masses between 50.00 and 300.00 m/z (123).

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:  $RI_x = 100n + 100(x - (n) - (n+1) + (n))$  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).

## **2.3 DPPH inhibitory assay for evaluation of *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare* EO antioxidant activity**

Each EO was initially prepared as a 100µg/ml methanolic stock solution, and subsequent serial dilutions were made at various concentrations between 2 and 80µg/ml. One millilitre of each concentration was combined with one millilitre of 0.002% DPPH (a free radical solution made by dissolving two milligrammes of DPPH in one hundred millilitres of methanol), and all obtained samples were incubated for 30 minutes at room

temperature in a dark container. The absorption was then measured at 517 nm. For the positive control drug Trolox, these procedures were repeated. After combining the DPPH solution with methanol in a 1:1 ratio to create the blank solution, its absorbance was measured to calculate the percentage of DPPH inhibition. The following equation was used to finish the computations (124).

$$\text{DPPH inhibition \%} = \frac{(\text{ABI} - \text{AS})}{\text{ABI}} \times 100\% \dots\dots\dots (1)$$

ABI: stated for blank absorbance.

AS: stated for sample absorbance.

#### **2.4 Antimicrobial activity assay for *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare* EO**

The essential oil of the three tested plants was assessed for antimicrobial activity using previously prescribed protocol (125). However, five common bacterial species from the American Type Culture Collection (ATCC) were used to test the essential oil's antibacterial effects: *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, with the ATCC numbers 25922, 13883, 12453, 9027, and 25923, respectively. *Methicillin-resistant Staphylococcus aureus* (MRSA) was employed in the current experiment, along with a clinical isolate from An-Najah National University Hospital. Each essential oil's antifungal ability was also examined in relation to the growth of *Candida albicans* (ATCC 90028). To determine the Minimal Inhibitory Concentration (MIC) of each tested essential oil, broth microdilution was utilized. The essential oil was first prepared in DMSO at a concentration of 200 µg/mL. Two-fold serial micro-dilutions were done 10 times (10 wells) in sterile Mueller-Hinton Broth (MHB). The dilutions were carried out in 96-well plates in aseptic conditions. Those mentioned 10 wells contain a gradient of concentrations of the essential oil (50 µg/mL to 0.1 µg/mL) mixed with prepared bacterial solutions. The other two remaining wells were used as controls; one was a positive growth control that contained media alone and bacteria, while the other one was a negative growth control that contained media alone. Then micro-well plates were incubated at 37 °C for 18–24 h. concerning the used fungal strain, *C. albicans*, the exact procedure was employed, but the media used was RPMI, and the incubation time was up to 48 h. The

lowest concentration of the EO that inhibited the growth of the microbe is considered MIC. To assess the method (126).

## **2.5 Cytotoxicity evaluation for *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare***

Based on 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay(127), in this study to assess the cytotoxicity of essential oil extracted from *A. citrodora*, *M. fruticosa*, *F. vulgare* and their mixture (1:1; w:w) on the cell proliferation of skin cancer (B16-F1) and (A431) cell line. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble blue compound formazan, depending on the viability of cells.

All cancer cells were incubated in 96-well plates for 24 hours in the presence or absence of essential oil. 20 $\mu$ L of MTT solution (Sigma) (5 mg mL<sup>-1</sup> in PBS) were added to each well. The plate was incubated for 4 h at 37°C in a CO<sub>2</sub>-incubator. 100  $\mu$ L of medium was removed from every well without disturbing the cell clusters. 180  $\mu$ l methanol/DMSO solutions (50:50) were added to each well, and the preparations were thoroughly mixed on a plate shaker with the cell containing formazan crystals.

## Chapter Three

### Results

#### 3.1. GC–MS identification of essential oil from *Micromeria fruticosa* leaves

The essential oil of *M. fruticosa* leaves from Jenin-Palestine yielded 3.5%, with 25 components detected, accounting for 97.73% of the total content. These compounds could be put into four groups: oxygenated monoterpenes (90.72% of the total content), sesquiterpene hydrocarbons (4.16%), monoterpene Hydrocarbons (1.76%), and Oxygenated sesquiterpenes (1.09%). The primary chemicals were pulegone (81.77%), caryophellene (2.95%), isomenthone (2.1%), Piperitenone oxide (1.78%), and trans-anethole (1.36%), (see Table B.1 appendix B).

#### 3.2. GC–MS identification of essential oil from *Foeniculum vulgare*

The EO yield of *Foeniculum vulgare* seeds oil was 4.5% (see Table B.2 appendix B). The essential oil in this study from fennel (*Foeniculum vulgare*) had 25 compounds, which made up 99.97% of its total content. These compounds could be put into two groups: oxygenated monoterpenes (98.66% of the total content) and monoterpene hydrocarbons (1.31%). The primary chemicals were trans-anethole (93.69%), fenchone (3.93%), sylvestrene (0.83%), and methyl chavicol (0.57%). Other chemicals were discovered in less than 0.2% of the overall oil sample, (see Table A.3 appendix B).

#### 3.3 *Aloysia citrodora* leaves essential oil GC–MS identification

The components according to GC-MS analysis were shown in (see table A.3 appendix B). that were most predominant in the *A. citrodora* essential oil from Jericho were trans-anethole(18.91%) which was the major compound, neral (8.12%), geranial (10.35%), 1,8-cineole (7.46%), limonene (6.51%), alpha-curcumene (6.50%), beta-caryophellene (3.38%), methyl chavicol (2.91%) and fenchone (2.33%), (see table B.3 appendix B).

#### 3.4 Antioxidant (free radical scavenging) Activity of *M. fruticosa*, *A. citrodora* and *F. vulgare* essential oils

The antioxidant capabilities of three extracted EO's were measured using DPPH radical scavenging and reduction power activities. For each of the EOs, the antioxidant half-maximal inhibitory doses (IC<sub>50</sub>) (giving 50% inhibition) were calculated. All oils were found to exhibit effective in scavenging the DPPH radicals with IC<sub>50</sub> values of

10.11±0.20, 12.022±0.18 and 19.49±0.11 µg/mL for *A. citrodora*, *M. fruticosa* and *F. vulgare* EO, respectively, compared to trolox the standard compound which had IC<sub>50</sub> equal to **3.09±0.23** (Table 3.1), see Figure A.7 appendix A).

**Table 3.1**

*M. fruticosa*, *A. citrodora* and *F. vulgare* EO and trolox free radical scavenging effect

Concentrations (µg/mL)	Trolox	<i>F. vulgare</i> EO	<i>A. citrodoram</i> EO	<i>M. fruticose</i> EO
0	0	0	0	0
2	51.55±0.55	17.5±0.5	17.5±0.5	23.5±0.81
5	69.85±0.35	20.5±0.5	45.95±0.41	42±0.5
10	96.04±0.05	45.5±0	55.86±0.5	55±0
20	95.85±0	52.05±0	74.7±0	60.05±0.5
40	95.85±0.15	59.5±0.05	74.85±0.05	70±0
50	97.37±0.22	63.5±0.5	74.85±0.18	71.05±0
80	99.1±0.6	72.5±0	74.85±0.28	71.05±0.05
<b>IC<sub>50</sub></b>	<b>3.09±0.23</b>	<b>19.49±0.11</b>	<b>10.11±0.20</b>	<b>12.022±0.18</b>

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

### 3.5. Anti-microbial activity of *M. fruticosa*, *A. citrodora* and *F. vulgare* essential oil

The minimum inhibitory activity (MIC) of the three investigated EO's against the selected bacterial strains and fungus strain (Table 3.2) was determined using the broth micro-dilution assay. The *A. citrodora* EO showed most potent antibacterial activity against all the tested microbes with MIC values ranging from 3.125-25 mg/mL except *Pseudomonas aeruginosa* which was resistant, while *M. fruticosa* EO showed only moderate antimicrobial properties with MIC values ranging from 6.25-25µg/mL as demonstrated in Table 3.2. For antifungal effect the most potent against *Candida albicans* was *A. citrodora* oil with MIC equal to 3.125µg/ml followed by *M. fruticosa* and *F. vulgare* oils with MIC equal to 6.25µg/ml. On the other hand *F. vulgare* oil was not active against the tested bacterial strains.

**Table 3.2**

*Minimum inhibitory concentration values (µg/ml) for A. citrodora, M. fruticosa and F. vulgare EO against a variety of bacteria and fungi*

ATCC Number	Clinical strain	Bacteria					Fungus	
		ATCC 6538P	ATCC 25922	ATCC 13883	ATCC 8427	ATCC 9027	ATCC 90028	
MIC (µg/ml)	MRSA	<i>S.aureus</i>	<i>E. coli</i>	<i>Klebsiella pneumonia</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	
<i>A. citrodora</i>	6.25	6.25	12.5	12.5	3.125	R	3.125	
<i>M. fruticosa</i>	25	25	12.5	12.5	6.25	25	6.25	
<i>F. vulgare</i>	NA	NA	NA	NA	NA	NA	6.25	

**Values are Minimum inhibitory concentration values (MIC), NA: Not Active**

### **3.6. Anticancer activity for *M. fruticosa*, *A. citrodora* and *F. vulgare* essential oils**

The results were collected basing on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay in this study to assess the cytotoxicity of essential oil extracted from *M. fruticosa*, *F. vulgare* and their mixture (1:1; w:w) on the cell proliferation of skin cancer (B16-F1) and epidermoid carcinoma (A431). The results as shown in figure A.8 appendix A, showed a potent anti-cancer effect when the two oils from *M. fruticosa*, *F. vulgare* were mixed in (1:1) ratio, both essential oils seemed to have higher inhibitory effect on epidermoid carcinoma (A431) compared to skin cancer (B16-F1) cell lines. On the other hand, as shown in figure A.9 appendix A, effects of *A. citrodora* EO from Jericho on B16F10 melanoma cell line at different concentrations

## Chapter Four

### Discussion

#### 4.1. The chemical composition of *Aloysia citrodora*, *Foeniculum vulgare* and *Micromeria fruticosa*

Previous studies reported the yield varies from 1.3% to 5%, depending on the geographical origin, year of harvest, climate and soil(128). In this study the yield of essential oil of fennel (*Foeniculum vulgare*) was 4.5% and had 25 compounds, which made up 99.97% of its total content. These compounds could be put into two groups: oxygenated monoterpenes (98.66% of the total content) and monoterpene hydrocarbons (1.31%). The primary chemicals were trans-anethole (93.69%), fenchone (3.93%), sylvestrene (0.83%), and methyl chavicol (0.57%). The combination of a high concentration of t-anethole and a concentration of fenchone less than 5% allows us to classify the tested wild fennel EO as a sweet variety as suggested in European Pharmacopeia 2015 (129). There are several previous studies addressing the variance of fennel essential oils in various countries, all of which corroborate the presence of trans-anethole as a key constituent ranging from 35-84% (130). Also it was reported in another study that the predominating components in the EO of cultivated fennel obtained in Egypt were pinene (32.82%), estragole (15.33%), fenchone (5.91%), t-anethole (14.84%), and Limonene (13.92%) (131). The predominant component in the EO of wild fennel was trans –anethole (6.22%), whereas Fenchone was discovered at a low level (0.78%). The main constituents of EOs of 56 samples of wild fennel (*Foeniculum vulgare* Mill.) collected in different wild localities of Sicily were estragole (34-89%), followed by fenchone (3.3-27.2%), alpha-pinene (1.6-20.9%), while the trans-anethole level was ranging from 0.1-36% (132). In several previous studies the yield varies from 1.3% to more than 5%, depending on the geographical origin, year of harvest according to European Pharmacopeia reported the yield is usually 2% (v/w) in sweet fennel and 4% in bitter fennel (129, 133).

On the other hand, in this study 25 components were detected in the essential oil of *Micromeria fruticosa*, accounting for 97.73% of the total content, which can be classified into four groups: oxygenated monoterpenes (90.72% of the total content), sesquiterpene hydrocarbons (4.16%), monoterpene Hydrocarbons (1.76%), and Oxygenated

sesquiterpenes (1.09%). The primary chemicals were pulegone (81.77%), caryophellene (2.95%), isomenthone (2.1%), Piperitenone oxide (1.78%), and trans-anethole (1.36%). This result in our research was confirmed by a prior research found that pulegone is a crucial component, with levels varying from 50.22-81.51% depending on harvesting time in a research carried out on investigating of seasonal Variations in the Chemical Compositions of Essential Oils of Selected Aromatic Plants Growing Wild in Turkey(134). In another study of Chemical compositions in *M. fruticosa* essential oil using the GC–MS method, the main components of the plant essential oil were carvacrol (56.03%), *trans* caryophyllene (10.41%), *p*-cymene (9.61%) and  $\gamma$ -terpinene (6.87%) which also confirmed our results(135). Furthermore, In the essential oil derived from *M. fruticosa*, the most abundant compounds were carvacrol (63.23%),  $\gamma$ -terpinene (18.94%), and *p*-cymene (8.31%) (136). Furthermore, the composition of the essential oil from aerial parts of *Micromeria fruticosa* from Turkey, GC-MS analysis of the essential oil resulted in the identification of twenty-three constituents, representing 97.04% of the oil. The major compounds detected in the essential oil were carvacrol (60.39%),  $\gamma$ -terpinene (12.95%), and *p*-cymene (9.61%)(137). The majority of prior studies discovered that carvacrol is a key component with a level of more than 80%, observed that carvacrol was the predominant component, accounting for 67.5%-88.3% of the total oil of *Micromeria fruticosa* collected in several locations around Greece with no discernible seasonal variations. The other main components were *p*-cymene (0.7%-17.4%),  $\gamma$ -terpinene (0.1%-6.9%), and caryophyllene oxide (0.9%-2.5%) (138).in addition, in another research the yield of Za'atar (*Micromeria fruticosa*) essential oils were found to be 2.5–3.4% at extraction process temperatures of 100–175 °C. The essential oils contained 79.5–86.2% carvacrol (139).

Beside that in this study the components that were most predominant in the *Aloysia citrodora* EO harvested from Jericho were trans-anethole (18.91%) which was the major compound, neral (8.12%), geranial (10.35%), 1,8-cineole (7.46%), limonene (6.51%), alpha-curcumene (6.50%), beta-caryophellene (3.38%), methyl chavicol (2.91%) and fenchone (2.33%). This confirmed previous results showed that *Aloysia citrodora* essential oil plant contained many active natural compounds especially, 1,8-cineole, geraniol, limonene in its essential oil (140). Also our results were matched with previous findings that showed the *A. citrodora* essential oils from the wild population were mainly

composed of sabinene (5.7-15.1%), limonene (21.7- 37.7%), neral (11.2-12.1%), geranial (14.8-17.5%), and  $\beta$ - caryophyllene (2.3-4.2%) (141).

#### **4.2 Antioxidant (free radical scavenging) Activity of *A. citrodora*, *M. fruticosa* and *F. vulgare* essential oils**

The antioxidant activities of the three different essential oils were evaluated by measuring the scavenging activity of these EO's toward the stable 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) free radicals, the antioxidant half-maximal inhibitory doses ( $IC_{50}$ ) (giving 50% inhibition) were calculated. All oils were found to exhibit effective in scavenging the DPPH radicals with  $IC_{50}$  values of  $10.11 \pm 0.20$ ,  $12.022 \pm 0.18$  and  $19.49 \pm 0.11$   $\mu\text{g/mL}$  for *A. citrodora*, *M. fruticosa* and *F. vulgare* EO, respectively, compared to trolox the standard compound which had  $IC_{50}$  equal to  $3.09 \pm 0.23$ . Previous investigations have revealed that *F. vulgare* essential oil has antioxidant properties that are, in certain situations, equivalent to those of the reference antioxidants -tocopherol and butylated hydroxytoluene (BHT). Additionally, it has been suggested that using *F. vulgare* essential oil as a simple, accessible, and secure source of natural antioxidants might enhance the oxidative stability of fatty foods while they are in storage (128, 142, 143). In a study, the essential oils of *F. vulgare* demonstrated pro-oxidant activity, the ability to inhibit 5-lipoxygenase, and a nearly 50% increase in the ability to scavenge free radicals (144).

Also Previous research demonstrated that the essential oil of *Micromeria fruticosa* is a good source of potent and natural antioxidants(145). Also it was reported that the inhibiting effect of *Micromeria* oils was stronger than the synthetic BHT, particularly at lower concentrations, as  $IC_{50}$  concentration of EOs for the reduction of DPPH radicals was 1.28 ml/ml which was lower than  $IC_{50}$  calculated for BHT (1.31 ml/ml)(146). *Micromeria fruticosa* the main component of its essential oil, pulegone compound, which has previously been reported in other studies on medicinal plants containing this natural compound to exert significant antioxidant activity through scavenging effect of free radicals, can be used to explain antioxidant activity (147, 148).

On the other hand, when tested using several samples from various rejoin in Palestine, the essential oil from *Aloysia citriodora* leaves had strong DPPH (free radical) scavenging activity (149). As these natural structures possess free radical scavenging properties, the

presence of trans-anethole and other compounds that have been described in prior studies as natural antioxidant chemicals may be responsible for this powerful antioxidant activity (150, 151). An investigation also conducted by found that this plant oil has potent antioxidant activity compared with the positive control (BHT), with IC<sub>50</sub> values of  $11.33 \pm 01$  and  $27.43 \pm 04$  µg/mL, respectively (152).

#### **4.3 Antimicrobial Activity of *A. citrodora*, *M. fruticosa* and *F. vulgare* essential oils**

After applying the minimum inhibitory activity (MIC) of the three investigated EO's against the selected bacterial strains and fungus strain using the broth micro-dilution assay. The *A. citrodora* EO showed the most potent antibacterial activity against all the tested microbes with MIC values ranging from 3.125-25 µg/mL except *Pseudomonas aeruginosa* which was resistant, while *M. fruticosa* EO showed only moderate antimicrobial properties with MIC values ranging from 6.25-25µg/mL, for antifungal effect the most potent was *A. citrodora* oil with MIC equal to 3.125µg/ml followed by *M. fruticosa* and *F. vulgare* oils with MIC equal to 6.25mg/ml. On the other hand *F. vulgare* oil was not active (NA) against the tested bacterial strains.

When the antibacterial properties of the essential oils from *Micromeria fruticosa* and *Satureja thymbra* were compared in earlier studies, the oil from *M. fruticosa* shown the highest antimicrobial activity (153). Additionally, the micro dilution method was used to investigate the essential oils of *Micromeria fruticosa* and *Teucrium polium* plants against *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922 and *Samonella typhimurium* ATCC 14028. *M. fruticosa* essential oil proved efficient against *E. coli* and *B. cereus*. On *B. cereus*, the *T. polium* essential oil worked(5). Tests have been done on *E. coli*, *S. epidermidis*, *B. subtilis*, *S. aureus*, *S. typhimurium*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, and *C. albicans* against the essential oil and the major components, carvacrol and trans-caryophyllene. Trans-caryophyllene only showed action against *C. albicans*, whereas essential oil and carvacrol had considerable activity against all bacteria with the exception of *P. aeruginosa*. Carvacrol and the essential oil both exhibited potent anti-mycobacterial properties (154).

In a prior investigation, *A. citriodora* essential oil shown a substantial level of antibacterial activity against both Gram-positive and Gram-negative bacteria. The essential oil of *A. citriodora* leaves has a substantial antibacterial activity, as evidenced

by the fact that MICs ranged between 2.84 and 8.37  $\mu\text{g/ml}$ , which supported our findings (155). With MICs of 2500, 2500, and 1250  $\mu\text{g/mL}$ , respectively, another study that examined the antibacterial activity of this plant oil using the micro dilution method discovered that it had antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus* (152).

With a MIC of 3.125  $\mu\text{g/ml}$ , the oil from *A. citrodora* showed the best antifungal effectiveness against *Candida albicans*, followed by oils from *M. fruticosa* and *F. vulgare*, which both had a MIC of 6.25  $\mu\text{g/ml}$ . On the other hand, the examined bacterial strains were unaffected by *F. vulgare* oil. An examination of additional plants that contained trans-anethole, which looked to have antifungal effects, previously validated these findings (156, 157).

#### **4.4 Cytotoxic Activity of *A. citrodora*, *M. fruticosa* and *F. vulgare* essential oils**

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) In this work, the cytotoxicity of the two tested EOs and their combination (1:1; w: w) on the cell proliferation of skin cancer (B16-F1) and A431 epidermoid carcinoma was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) tetrazolium (MTT) assay. When the two oils from *M. fruticosa* and *F. vulgare* were combined in a ratio of 1:1, the findings as shown in figure A.8 appendix A, demonstrated a significant anti-cancer impact. Both essential oils appeared to have a larger inhibitory effect on epidermoid carcinoma (A431) cell lines than skin cancer (B16-F1) cell lines. On the other hand, *A. citrodora* EO from Jericho in Palestine had an inhibitory impact on the B16F10 melanoma cell line, according to the data. Due to their antiproliferative actions on some cancer cell lines, *M. fruticosa* leaves were formerly thought to be a unique treatment tool that might be used in conjunction with traditional chemotherapy to lessen negative side effects and drug resistance (158). Furthermore, in previous studies the results showed that the cytotoxic effect of the essential oil of *A. citriodora* was high on P815 and moderate on MCF7 and on VERO cell lines, so it will be considered as anticancer medicinal plant (155). An investigation conducted found that *A. citriodora* essential oil exerted a dose-dependent cytotoxic effect on P815, MCF7, and VERO tumor cell lines, with  $\text{IC}_{50}$  ranging from 6.60 to 79.63  $\mu\text{g/mL}$  (159).

Furthermore, the essential oil of fennel (*Foeniculum vulgare*) exhibited in vitro anticancer activity as reported in previous researches (160). Additionally, fennel oil had

stronger cytotoxic effects on the MCF-7 cancer cell line and had inhibitory effects on bacterial development. Additionally, the essential oil drastically lowered BCL-2 gene expression while considerably decreasing Bax gene expression (161). The antioxidant and antibacterial properties of extracts and essential oils from *Foeniculum vulgare* varied significantly (p 0.05) in a prior study (162).

#### **4.5 Conclusion**

This study can be considered as evaluable investigation of the phytochemicals and chemical profiling of the essential oil derived by hydro distillation from *Micromeria fruticosa*, *Aloysia citrodora* and *Foeniculum vulgare*. The basic components in the extracted essential oil were identified using gas chromatography (GS/MS). The gathered data show that these three plants can be regarded as a rich source of bioactive natural compounds for treating oxidative stress conditions, microbial infections, and some types of carcinoma. Therefore, additional validation for these three essential oil types must be carried out, especially in vivo tests to assess the medicinal potentials.

## List of Abbreviations

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<b>Abbreviation</b>	<b>Meaning</b>
<b>DPPH</b>	2,2-diphenyl-1-picrylhydrazyl
<b>IC<sub>50</sub></b>	half maximal inhibitory concentration
<b>Trolox</b>	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
<b>A<sub>B</sub></b>	absorbance of the blank solution
<b>A<sub>ts</sub></b>	absorbance of the tested sample solution
<b>MCF7 cells</b>	human cervix adenocarcinoma cell line
<b>DMSO</b>	dimethyl sulfoxide
<b>MTT</b>	3- (4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide
<b>ATCC</b>	American Type Culture Collection
<b>B16-F1</b>	Skin cancer

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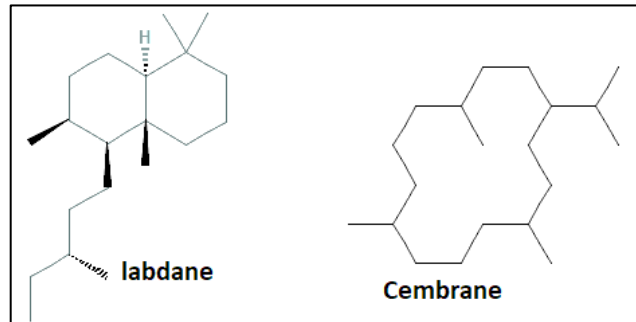
## Appendices

### Appendix (A)

#### Figures

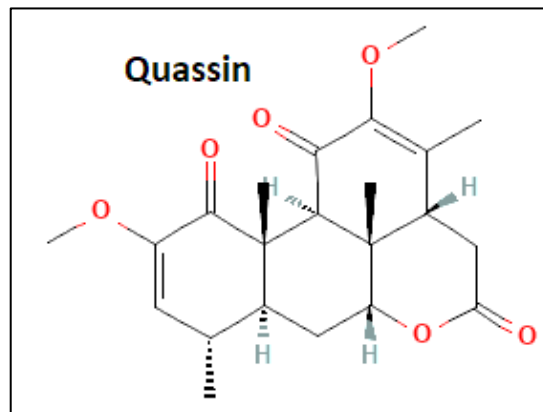
**Figure A.1**

*Examples of diterpenes*



**Figure A.2**

*Example of triterpene*



**Figure A.3**

*Aloysia<sup>o</sup> citrodora*



**Figure°A.4**

*Thymbra° spicata*



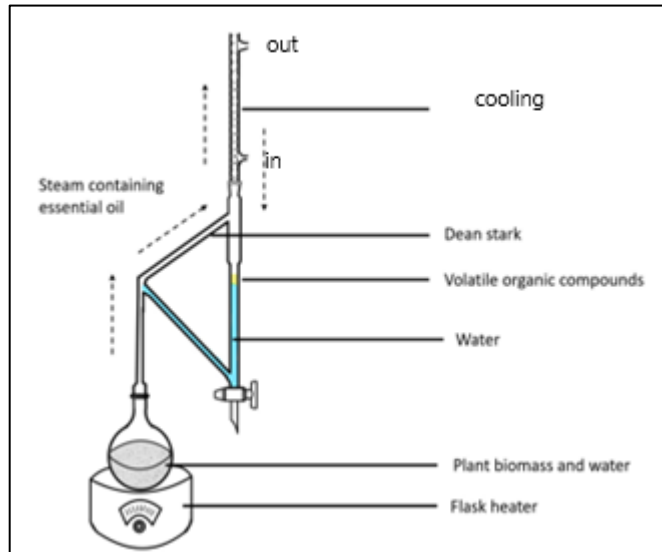
**Figure°A.5**

*Foeniculum° vulgare*



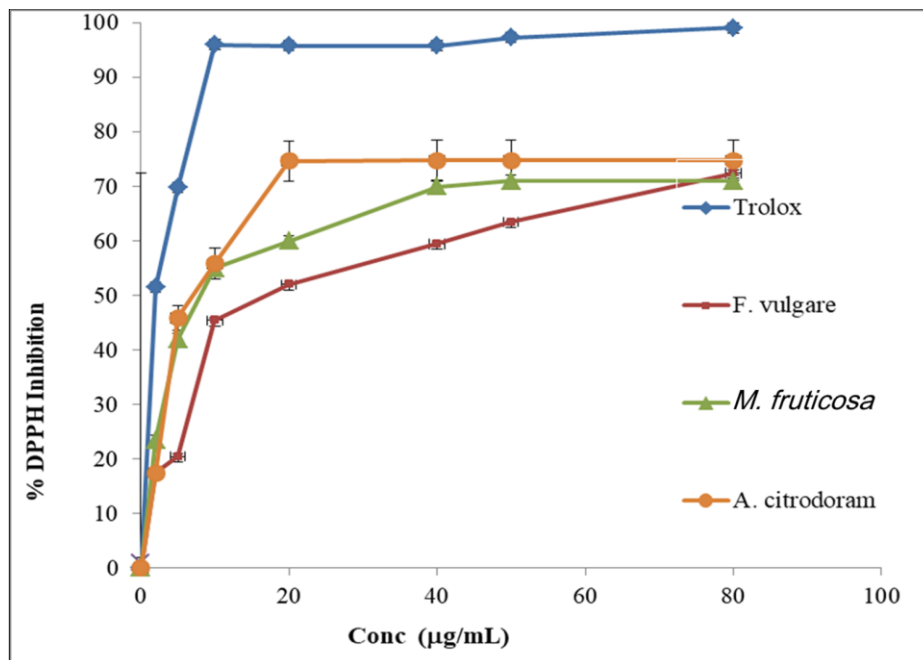
**Figure A.6**

*Dean Stark apparatus*



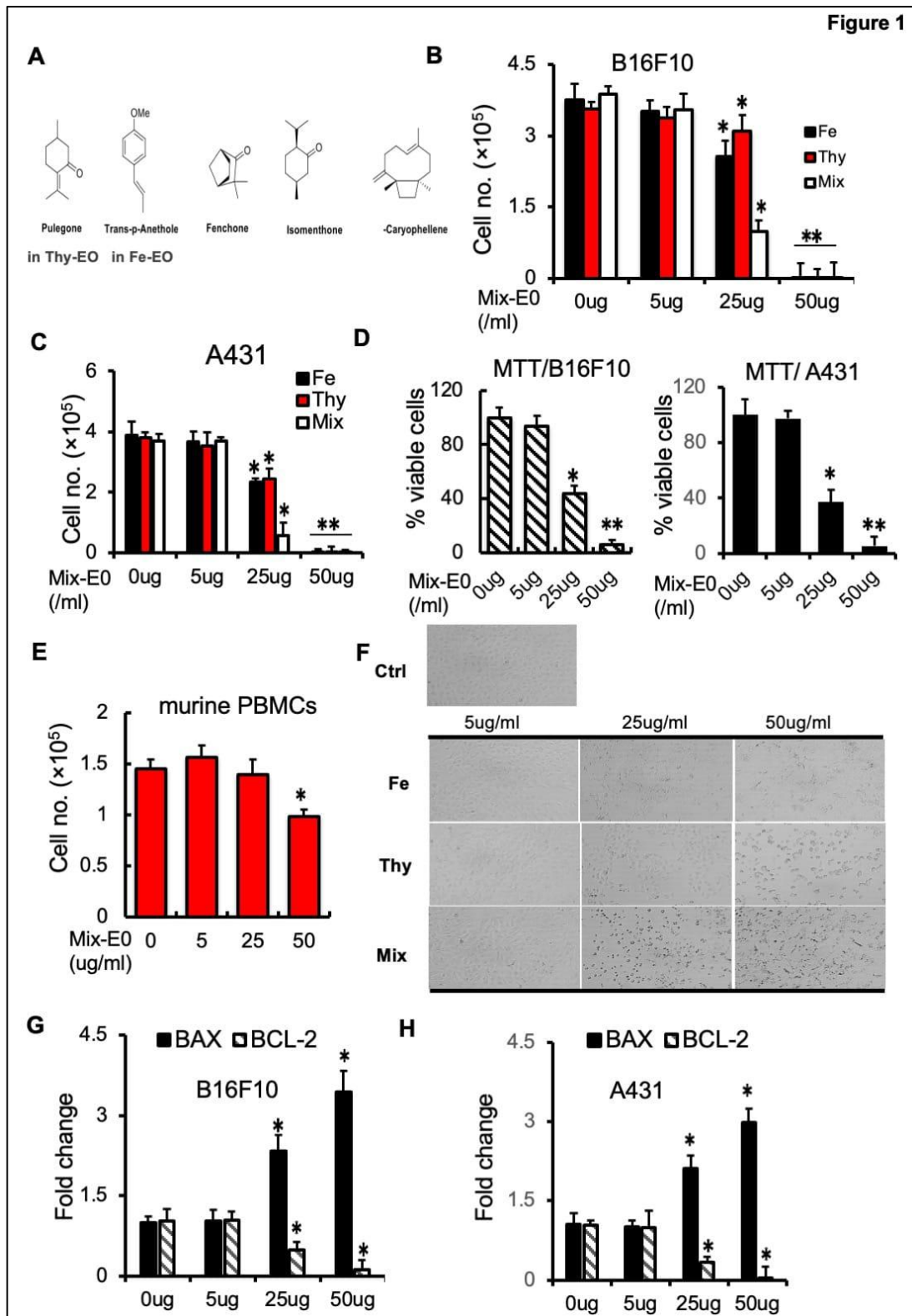
**Figure A.7**

*M. fruticosa*, *A. citrodora* and *F. vulgare* EO and trolox free radical (DPPH) scavenging effect



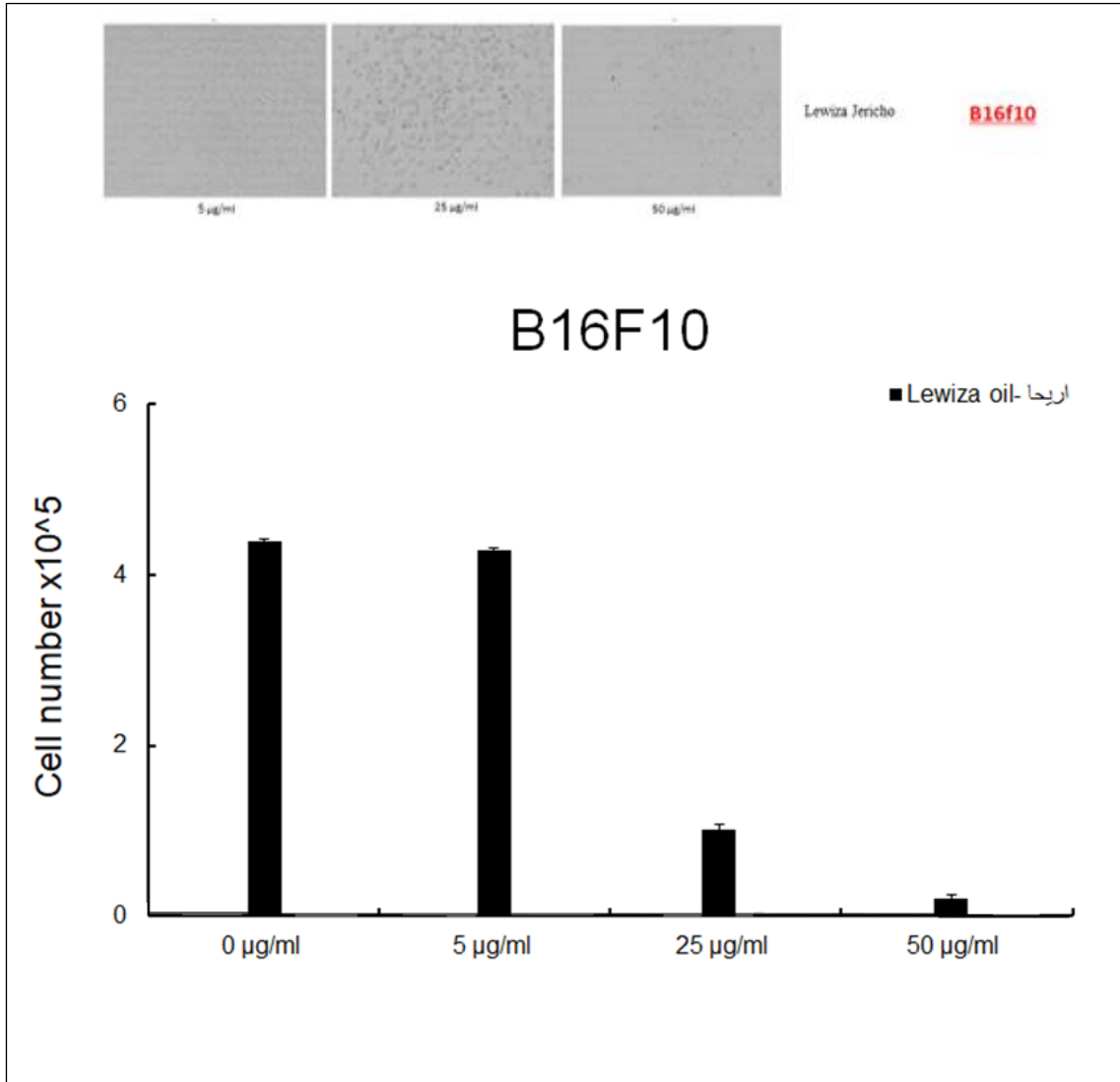
**Figure A.8**

*M. fruticosa* (Thy) and *F. vulgare*(Fe) EOs effects on B16F10 melanoma cell line, A431 epidermoid carcinoma using MTT assay



**Figure A.9**

*Effects of A. citrodora EO from Jericho on B16F10 melanoma cell line at different concentrations*

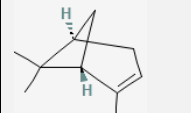
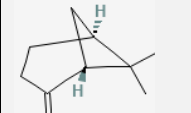
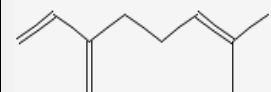
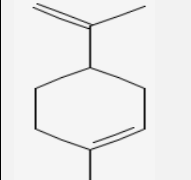
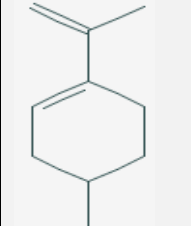
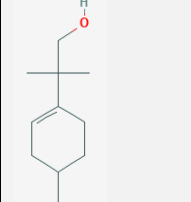
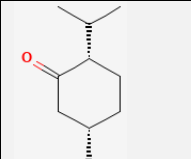
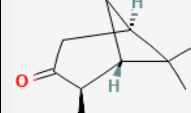
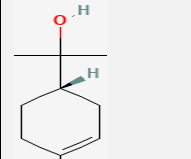


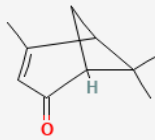
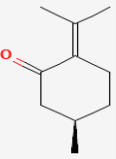
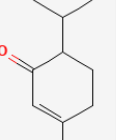
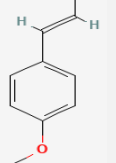
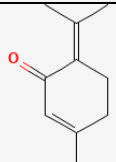
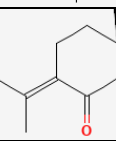
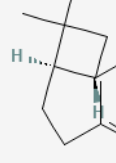
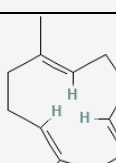
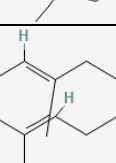
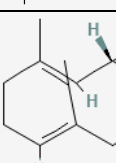
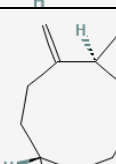
## Appendix B

### Tables

**Table B.1**

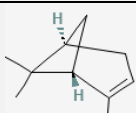
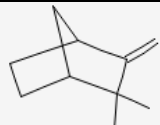
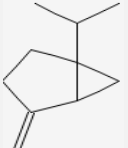
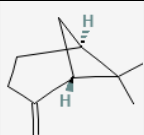
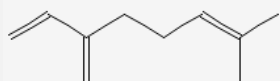
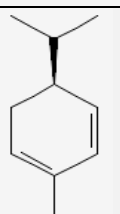
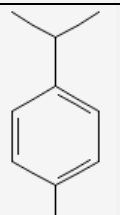
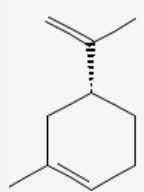
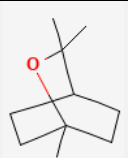
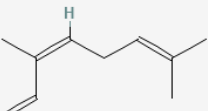
*Micromeria fruticosa* essential oil chemical composition by GC-MS analysis

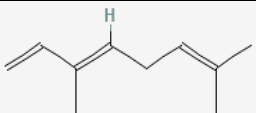
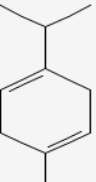
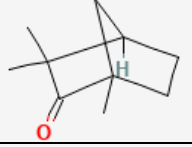
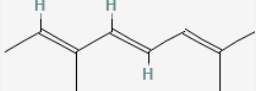
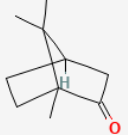
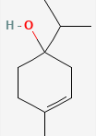
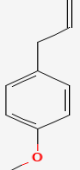
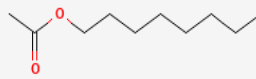
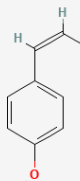
Compound	Structure	R.T	RI	area	Content%
alpha-Pinene		9.78	931	466975	0.12
Beta-Pinene		11.73	974	1338466	0.33
Myrcene		12.32	987	458939	0.11
Limonene		14.02	1026	3476595	0.86
<i>p</i> -Mentha-3,8-diene		15.81	1063	1358796	0.34
<i>p</i> -Mentha-3-en-8-ol		19.05	1149	5545595	1.38
Isomenthone		19.56	1162	8748732	2.17
trans-3-Pinanone		19.96	1172	5104956	1.27
□-Terpineol		20.8	1193	1167582	0.29

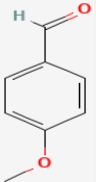
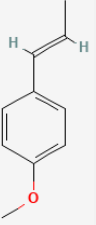
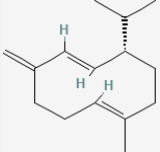
Verbenone		21.24	1205	3086970	0.77
Pulegone		22.46	1239	329259584	81.77
Piperitone		22.91	1251	655228	0.16
Trans-Anethole		24.05	1289	5480765	1.36
Piperitenone		25.83	1336	4192985	1.04
Piperitenone oxide		26.59	1358	7157261	1.78
alpha-caryophellene		28.56	1418	11876279	2.95
Beta-caryophellene		29.68	1454	723042	0.18
Germacrene D		30.48	1480	2962117	0.74
Bicyclogermacrene		30.94	1495	1165526	0.29
Caryophyllene oxide		33.53	1582	4380476	1.09

Total identified				402671661	97.73
Monoterpene Hydrocarbons					1.76
Oxygenated monoterpenes					90.72
Sesquiterpene hydrocarbons					4.16
Oxygenated sesquiterpe					1.09

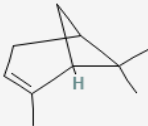
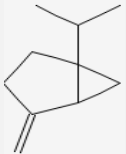
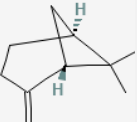
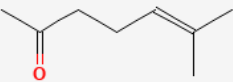
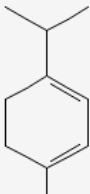
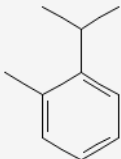
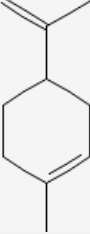
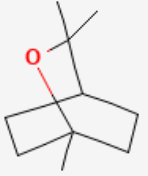
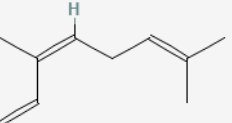
**Table B.2***Foeniculum vulgare* seeds essential oil chemical composition by GC-MS analysis

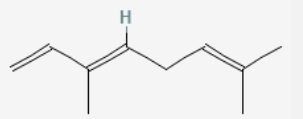
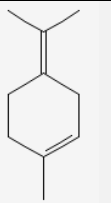
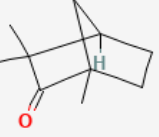
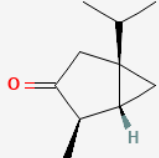
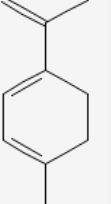

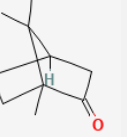
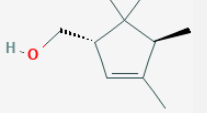
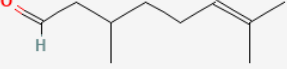
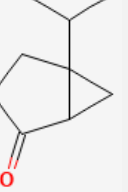
Compound	Structure	R.T	RI	area	Content%
$\alpha$ -Pinene		9.79	931	9922612	0.06
Camphene		10.51	947	2406774	0.01
Sabinene		11.56	970	1706302	0.01
$\beta$ -Pinene		11.75	974	14626314	0.08
Myrcene		12.35	987	14030112	0.08
Phellandrene		13.04	1003	18356128	0.10
<i>p</i> -Cymene		13.84	1022	9043268	0.05
Sylvestrene		14.11	1028	146991280	0.83
1,8-Cineole		14.13	1030	1787574	0.01
Cis-Ocimene		14.4	1035	18439344	0.10

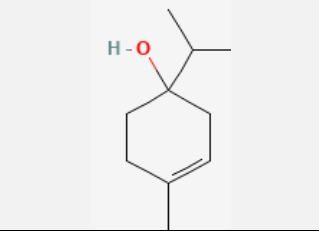
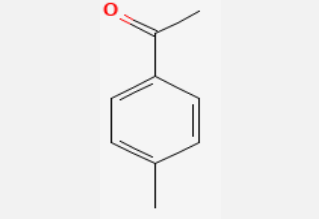
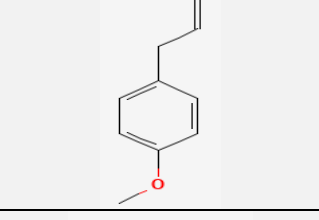
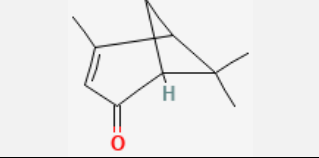
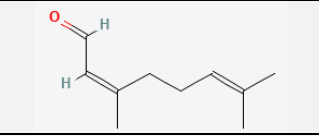
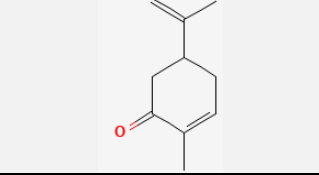
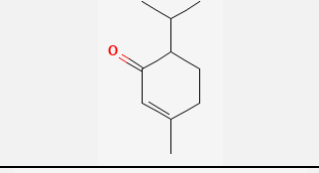
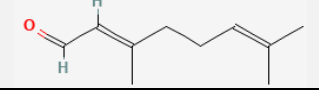
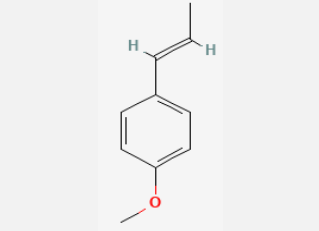
Trans-Ocimene		14.83	1045	603612	0.00
□-Terpinene		15.31	1056	16404660	0.09
Fenchone		16.71	1089	695880192	3.93
Allo-Ocimene		18	1126	1159722	0.01
missing		18.52		1658631	0.01
Camphor		18.87	1144	18827058	0.11
Terpinen-4-ol		20.23	1178	1421560	0.01
Methyl chavicol		20.92	1196	101463000	0.57
Octanol acetate		21.34	1207	1850079	0.01
endo-Fenchyl acetate		21.6	1215	5444257	0.03
exo-Fenchyl acetate		22.1	1229	28539864	0.16
ND		22.36	1236	443113	0.00
Cis-p-Anethole		22.89	1251	3302672	0.02

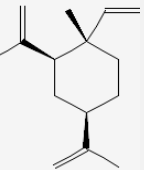
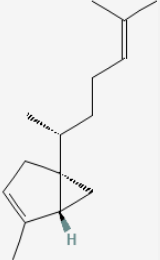
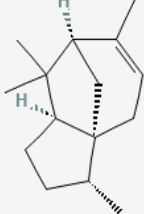
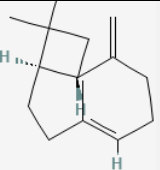
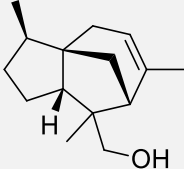
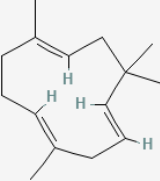
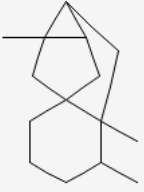
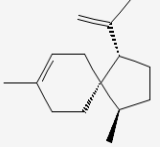
<i>p</i> -Anisaldehyde		23.02	1254	660390	0.00
Trans-Anethole		24.43	1294	1658323200 0	93.69
Germacrene D		30.5	1480	2615847	0.01
Total				1770081636 5	100.00
Monoterpene hydrocarbons					1.31
Oxygenated monoterpenes					98.66
Sesquiterpene hydrocarbons					0.01
Oxygenated sesquiterpes					0.00

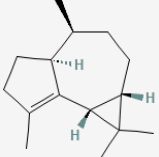
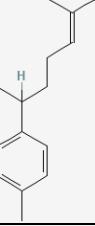
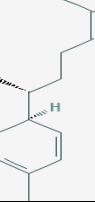
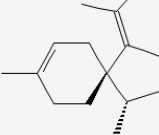
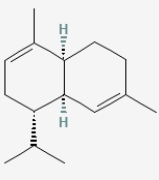
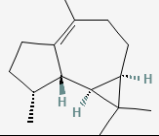
**Table B.3***Aloysia citrodora* essential oil chemical composition by GC-MS analysis

Compounds	Compounds structures	R.T	RI	% Area
Alpha-pinene		9.79	931	0.30
sabinene		11.55	970	0.42
beta-pinene		11.74	974	0.19
6-methylhept-5-en-2-one		12.12	982	1.15
alpha-terpinene		13.5	987	0.05
o-cymene		13.83	1014	0.51
limonene		14.07	1021	6.51
1,8-cineole		14.16	1027	7.46
cis-ocimene		14.38	1029	0.21

trans-ocimene		14.82	1034	0.30
gamma-terpinene		15.3	1045	0.36
terpinolene		16.44	1056	0.06
fenchone		16.52	1083	2.33
alpha-thujone		17.2	1085	0.51
1,3,8-p-menthatriene		17.37	1090	0.03
alpha-campholenal		18.09	1101	0.33
verbinol		18.52	1105	0.22
exoisocitral		18.74	1123	0.11
camphor		18.86	1135	0.08
trans-alpha-necrodol		18.94	1140	0.23
citronellal		19.1	1143	0.17
sabina ketone		19.31	1145	0.05

terpinen-4-ol		20.21	1150	0.63
p-methyl acetophenone		20.34	1155	0.25
methyl chavicol		20.86	1178	2.91
verbenone		21.42	1181	0.10
endo-fenchyl acetate		21.59	1195	0.07
exo fenchyl acetate		22.09	1210	0.19
neral		22.38	1214	8.12
carvone		22.57	1228	0.40
piperitone		22.92	1237	0.43
geranial		23.45	1242	10.35
trans-anethole		24.13	1251	18.91

acetomesithlene		24.9	1266	0.06
alpha-ylangene		27.14	1285	1.08
anisyl methylketone		27.263	1307	0.18
beta-elemene		27.9	1374	0.18
sesquithujene		27.98	1378	0.05
funebrene		28.47	1382	0.63
beta-caryophellene		28.57	1385	3.38
beta-cedrene		28.71	1397	0.32
α-caryophellene		29.7	1399	0.23
ishwarane		29.85	1415	0.59
beta-acoradiene		30.11	1418	0.15

gurjunene		30.31	1423	0.11
alpha-curcumene		30.46	1454	6.50
alpha-zingiberene		30.88	1459	0.43
Beta-alaskene		30.96	1468	0.53
alpha-muurolene		31.04	1474	0.06
viridiflorene		31.14	1483	0.07
alpha-cuprenene			1492	
delta-amorphene			1495	
delta-cadinene			1497	
spathulenol			1501	
syn-anti-anti helifolen-12-ol a			1508	
6-methyl-6-(3- methyl phenyl) heptan-2-one			1512	
cubenol			1518	
valeranone			1584	
acorenone B			1593	
			1633	



جامعة النّجّاح الوطنيّة  
كلّيّة الدّراسات العليا

المسح الدوائي والكيميائي للزيوت العطرية المستخلصة  
من أوراق اللوزة وأوراق الزعيتان وبذور الشومر

إعداد

معتز أبو الرب

إشراف

د. نواف المحاريق

د. ضرار صمادي

قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا  
في جامعة النجاح الوطنية في نابلس - فلسطين.

2023

## المسح الدوائي والكيميائي للزيوت العطرية المستخلصة من أوراق اللوزة وأوراق الزعيتمان وبذور الشومر

إعداد

معتز أبو الرب

إشراف

د. نواف المحاريق

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### الملخص

تشكل النباتات الطبية نافذة مهمة لدى الباحثين في مجال العقاقير الطبية للعمل على تطوير بدائل طبيعية فعالة لعلاج العديد من المشاكل الصحية مثل الإجهاد التأكسدي والسرطان والالتهابات البكتيرية والفطرية دون أن تحدث هذه البدائل الأعراض الجانبية التي تظهر خلال استعمال الأدوية الكيميائية وفي هذا البحث تم تسليط الضوء على ثلاث نباتات طبية وهي أوراق اللوزة والزعيتمان وبذور الشومر لاستخلاص الزيوت العطرية باستخدام جهاز التقطير ببخار الماء ومن ثم إتباع مجموعة من الفحوصات الكيميائية لتحديد نوعية وكمية المركبات الفعالة في هذه الزيوت العطرية. وبعدها تم فحص فاعلية كل زيت على حدا ضد مجموعة مختارة من الخلايا السرطانية والسلالات البكتيرية والفطرية بالإضافة الى اختبار قدرتها في تثبيط الشوارد الحرة المسببة للإجهاد التأكسدي.

وقد كشفت نتائج GC-MS أن زيت *Thymbra spicata* يحتوي بشكل أساسي على بوليغون (81.77%)، كاريوفيلين (2.95%)، أيزومنتون (2.1%)، أكسيد بييريبتينون (1.78%)، و-trans-anethole (1.36%) بينما *Foeniculum vulgare* كانت المواد الكيميائية الأولية للزيوت العطرية عبارة عن ترانس أنيثول (93.69%)، فينشون (3.39%)، سيلفسترين (0.83%)، وميثيل شافيكول (0.57%). بالنسبة للزيت العطري *A. citrodora*، كانت المكونات الأساسية عبارة عن trans-anethole (18.91%)، (8.12%) neral، (10.35%) geranial، (7.461%)، cineole (8%)، الليمونين (6.51%)،

alpha-curcumene(6.50%)، بيتا كاريوفيلين (3.38%)، ميثيل شافيكول (2.91%)، فينشون (2.33%) . تم إثبات أن جميع الزيوت فعالة في تنظيف جذور DPPH بقيم IC50 من 0.20 ± 10.11 و 0.18 ± 12.022 و 0.11 ± 19.49 ميكروغرام / مل لـ *A. citrodora* و *M. fruticosa* و *F. vulgare* EO ، على التوالي. كما أظهر نبات *A. citrodora* EO أقوى نشاط مضاد للجراثيم ضد جميع الميكروبات المختبرة بقيم MIC تتراوح من 3.125-25 ملجم / مل باستثناء *Pseudomonas aeruginosa* الذي كان مقاومًا ، بينما أظهر *M. fruticosa* EO خواصًا معتدلة لمضادات الميكروبات مع قيم MIC تتراوح بين 6.25-25 ميكرو/مل، بالنسبة للتأثير المضاد للفطريات، كان أقوى زيت سيترودورا مع MIC يساوي 3.125 ميكرو/مل، يليه زيوت *M. fruticosa* و *F. vulgare* مع MIC يساوي 6.25 ميكرو/مل. من ناحية أخرى، لم يكن زيت *F. vulgare* فعالاً ضد السلالات البكتيرية المختبرة. بالنسبة للسمية الخلوية، أظهرت النتائج تأثيرًا قويًا مضادًا للسرطان عندما تم خلط اثنين من الزيوت من *M. fruticosa* ، *F. vulgare* بنسبة (1 : 1) ، ويبدو أن كلا الزيتين الأساسيين لهما تأثير مثبط أعلى على سرطان البشرة (A431) مقارنةً بـ خطوط خلايا سرطان الجلد (B16-F1). من ناحية أخرى، أظهرت النتائج أن تأثير *A. citrodora* EO من ارتباطات مختلفة في فلسطين على خط خلايا سرطان الجلد B16F10، هو التأثير الأكثر تشبيهاً.

وهكذا يمكن اعتبار هذا البحث على هذه الزيوت العطرية الثلاثة تحدياً بالمستقبل للصناعات الدوائية الطبيعية التي قد تصبح الخيار الأفضل للعديد من المرضى مقارنة بالعقاقير الكيميائية المرتبطة بالكثير من الآثار الجانبية على الجسم.

**الكلمات المفتاحية:** المسح الدوائي؛ الزيوت العطرية؛ أوراق اللوزة، وأوراق الزعيتان؛ بذور الشومر مضادات الأكسدة، مضادات الميكروبات، السامة للخلايا.