An-Najah National University

Faculty of Graduate Studies

VARIATION IN THE CHEMICAL COMPOSITION AND BIOACTIVE COMPONENTS OF *MYRTUS COMMUNIS* ESSENTIAL OILS FROM TWO PALESTINIAN REGIONS

By

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This Thesis was Defended Successfully on 5/2/2023 and approved by

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Dedication

I dedicate this letter to myself and say well done.

To my husband, who was the first to suggest that I join the master's program and who always supporting me.

To my mother and sisters who always wish me to reach the top.

To the soul of my father and father-in-law who will be in my heart forever.

I dedicate this work

Acknowledgments

I want to start by thanking God for giving me the opportunity I needed to reach my objective.

Secondly, I would like to say thank you to Dr. Nawaf Al-Maharik & Dr. Nidal Jaradat for all of their helpful recommendations, and support.

Thirdly, a lot of thanks for Ms. Linda Arar, Ms. Fatima Hussen and Mr Abedalrazeq for their helpful.

Finally, all thanks, appreciation and love to my husband, mother, sisters, and brothers for always standing by me and for their continuous support.

Declaration

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

VARIATION IN THE CHEMICAL COMPOSITION AND BIOACTIVE COMPONENTS OF *MYRTUS COMMUNIS* ESSENTIAL OILS FROM TWO PALESTINIAN REGIONS

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

Student's Name: _____

Signature: _____

Date: 5/2/2023

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VARIATIONS OF THE CHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITIES OF *MYRTUS. COMMUNIS* ESSENTIAL OILS FROM TWO REGIONS IN PALESTINE

By
Sumayyah Salim Mohammad Jaber
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Abstract

Background: One of the reasons why interest in medicinal plants is growing daily is the idea that some plants have the ability to treat a variety of illnesses without having any negative side effects. *Myrtus* has long been used in many conventional treatments in our region.

Objectives: In this study, the chemical profiles of the *Myrtus* essential oils (EO) that were gathered in Jericho and Jenin will be investigated, along with their antioxidant, anti-amylase, antimicrobial, and anticancer effects.

Methodology: *Myrtus* essential oils (EO) were created via hydro distillation extraction, and the chemical constituents of the EO were characterized qualitatively and quantitatively using GC-MS.

Results: Forty one chemicals were found in Jericho EO, with cis-4-Thujanol (27.37%), 1, 8-Cineole (24.32%), Myrtenol (12.97%), Myrtenal (12.46%), and trans-4-Thujanol acetate (9.48%) being the main contributors. There were 37 different chemicals in Jenin EO, with 1,8-cineole (31.55%), linalool (21.65%), Trans-4-thujanol acetate (11.26%), α-pinene(10.22%), Myrtenal(6.78%) and □-terpineol(4.35%) being the most prevalent.

The antioxidant activity of the EOs was assessed by blocking DPPH free radicals. Jericho and Jenin ethyl acetate extracts outperformed the positive control Trolox, which exhibited an IC_{50} of 10.25±1.02 □g/mL, and demonstrated dose-dependent free radical scavenging capabilities with IC_{50} values of 8.55±2.31 and 3.60±0.35 □g/mL, respectively. Jenin EO has higher antioxidant activity comparison to the EO from Jericho is presumably brought on by larger amounts of oxygenated monoterpenes, such as 1, 8-cineole and □-pinene. The most effective α-amylase inhibitory agents were *Myrtus* EO and extract from Jenin, with IC_{50}

values of 950.48±2.54 and 795.43±1.88µg/mL, respectively, whereas EO and extract from Jericho had no effect.

The antitumor activity was tested using the MTS assay. Four cancer cell lines—the human cervical (HeLa), breast (MCF-7), mouse embryo fibroblasts (3T3), and normal hepatic (LX-2) cell line were employed as normal cell lines to test the anti-proliferative activity. Jenin EO has IC_{50} values between 215.25±1.07 and 597.01±3.11 □g/mL, while Jericho EO has values between 644.47±2.89 and 914.54±3.05 □g/ml.

The antibacterial activity was examined using the microdillution technique. Gram-positive bacteria are more sensitive to both EOs than Gram-negative bacteria. The two EOs have less antifungal efficacy against C. albicans than any other extract under study.

Keywords: Bioactive Components; Chemical Composition; Essential Oil; Myrtus; Palestinian.

Chapter One
Introduction

In spite of the widespread use of synthetic and bio-ecological-based pharmaceuticals, plants continue to be one of the most significant sources of medicine, and millions of people still rely on herbal medicine for their treatments and cures. Since ancient times, more than half of the world's population has turned to medicinal plants to treat a variety of illnesses, and this tendency is becoming more and more prevalent in global health. There is a rising demand for fresh and "natural" based treatments and solutions in western countries due to the development of nutritional supplements and the expansion of cosmetics. The potential to lower illness risk and promote healthy aging in many natural items is now being explored [1].

Almost every culture makes use of medicinal plants as a source of health care. Making sure medicinal plants and herbal medicines are safe, effective, and of high quality has only recently been a top priority in both developed and developing nations. Bioactive compounds originating from plants can be regulated and evaluated in order to monitor and study their health. A new era in human healthcare may be brought about through herbal remedies [2].

1.1 A brief overview of medicinal plants history

Once Adam and Eve had spent time in heaven, they were unaware of disease or pain; however, when they were ejected, they discovered suffering and disease. Since the beginning of time, people have looked for medications in nature to treat their illnesses. The usage of the therapeutic plant began spontaneously, as it does in the case of animals [3].

The truth is that at that time, knowledge about the causes of illnesses was few, and any knowledge of which herbs may be employed as a preventative measure was based only on personal experience. Over time, it became clear that some medicinal plants might be used to treat particular diseases, and as a result, the use of those medicinal plants gained empirical support and turned into explicatory facts. Up until the introduction of iatrochemistry in the 16th century, plants had been employed for both treatment and defense [3].

Throughout history, man has examined nature for two primary purposes: food for surviving and medicinal plants for treating pain and illness. As a total cure for illnesses, ancient cultures employed herbs or mixes of them known as therapeutic corpus. Ebers papyrus was one of these components. In about 1550 B.C., the E. papyrus explained the medicinal herbs used in Egyptian society. Dr. George Ebers, a German Egyptologist, bought the papyrus in Egypt in 1872, examined it, and described it as of great value [3, 4].

Since ancient times, essential oils, aromatic plants and herbs have been employed in traditional medicine, food preservation, religious rituals, and cosmetics. Plant extracts are increasingly being employed as sources of pharmacological substances in food supplements, in order to meet modern customer needs for healthy, safe, and high-quality materials [5].

In the Middle East, Latin America, Africa, and Asia, traditional medicine is used by more than 85% of the population. They heavily rely on natural remedies for their medical demands. The population of the European Union is approximately 100 million. Up to 90% of people still utilize complementary, herbal, or traditional therapies [2, 6].

1.2 Herbal medicine and drug development

The earliest known instance of medicinal herbs being used to make medications is found on a Sumerian clay slab from Nagpur, which is thought to be around 5000 years old. Over 250 distinct plants, some of which contained alkaloids like poppies, henbane, and mandrake, were represented by 12 medication preparation formulas [7].

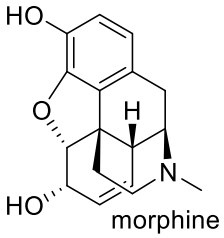
A Chinese treatise on roots and grasses called "Pen T'Sao," published by Emperor Shen Nung in 2500 BC, lists 365 medicines (dried sections of medicinal plants). The large yellow gentian, camphor, Theae folium, Podophyllum, ginseng, cinnamon bark, jimson weed and ephedra are among the treatments that are still used today [8].

India possesses a huge number of medicinal plants and the ability to satisfy the demand for them globally. The principal healthcare systems in Indian society include Ayurveda, folk medicine, Siddha, Unani and Naturopathy, all of which are completely reliant on natural resources. The market for herbal medications has grown at an unbelievable rate as a result of a global renaissance in conventional and alternative healthcare systems, and medicinal plants are therefore very important economically [9].

Traditional Arabic and Islamic Medicine (TAIM) have a long history and were formerly utilized in many important texts found in libraries all throughout Europe. Numerous Arab nations have studied the TAIM plants, including Syria, Morocco, Yemen, Egypt, and others. Recent studies by ethno pharmacologists on the potential uses of plant species in the Mediterranean region show that 250–290 plant species from different families are still in use [10].

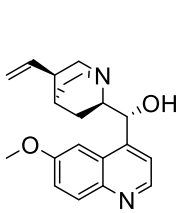
1.3 Drugs derived from plants

H.E. Merck of Darmstadt, Germany, was the first to extract morphine **1** and other alkaloids in 1826. (Kaiser, 2008After that, attempts were done to chemically synthesize natural products to speed up production. at cheaper prices and higher quality. In 1853, the first natural substance synthesized by chemical synthesis was salicylic acid [4].



morphine

Several bioactive natural compounds, mainly alkaloids (e.g., quinine **2**, caffeine **3**, nicotine **4**, codeine **5**, atropine **6** , colchicine, cocaine **7**, capsaicin **8**), were separated from their natural sources during the succeeding decades of the nineteenth century[4].



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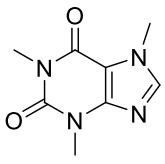

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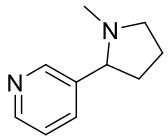

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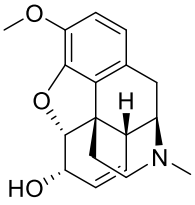

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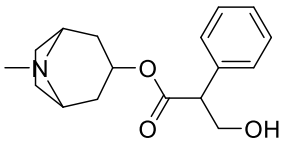

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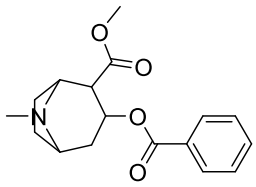

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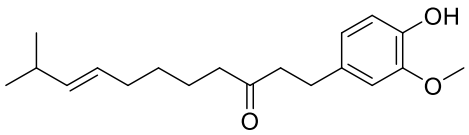

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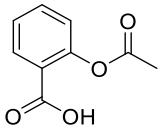
Phase II in relation to Artemisone/Artemifon (BAY 44-9585): First isolated from the Chinese plant Artemisia annua in 1971, this is a semi-synthetic antimalarial derivative

of artemisinin (Asteraceae). In collaboration with Medicines for Malaria Venture, BAY 44- 9585 (artemisinin) is being developed (MMV) [11].

During the years 2000–2006 plant-based pharmaceuticals in all, including rare molecules like Galanthamine HBr (Reminyl1), Miglustat (Zavesca1), and Nitisinone, were registered or sold (Orfadin1) [11].

Plant-based medications and many herbal treatments influence immune function and are known to have a wide range of immune modulatory effects. Aspirin **9**, a salicyclic acid derivative, has long been used to treat inflammation-related disorders, and several plant-based treatments have also been shown to be effective against immunological problems [11].



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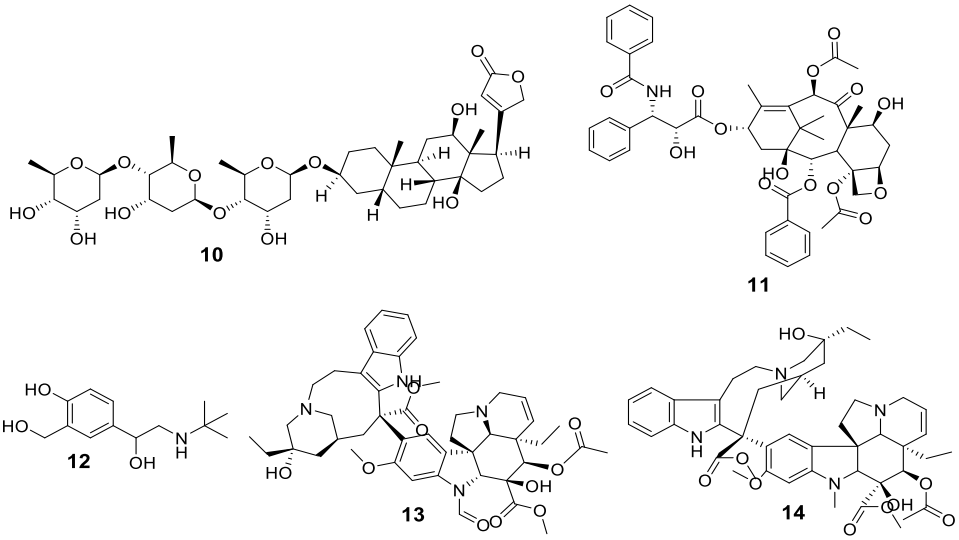
Common 'Western' prescription medications derived from plant components include Digoxin 10, Taxol 11, Salbutamol 12, Vincristine 13, Morphine 1, and Vinblastine 14. Additionally, Hippocrates was the first to describe the pain-relieving qualities of aspirin, a pharmaceutical substance extracted from the willow tree's bark and one of the most popular over-the-counter drugs available today [6] .



Cancer is a major public health issue, with a predicted 6 million new cases reported each year around the world. After cardiovascular illnesses, it is the second most

common reason for death. Chemotherapy is still the most usual treatment for a variety of malignancies. Herbal medicines are gaining popularity as potential anticancer therapies due to the availability of materials, low cost, little or no side effects, broad applicability, and clinical effects, all of which have stimulated scientific study, these some plants have anticancer properties. Adiantum venusutum, Abelmoschus moschatus, Aspidosperma tomentosum, Anemopsis californica, Alangium salviifolium, Acorus calamus, Aegle marmelos [12].

1.4 A Palestinian perspective

In addition to the continental rift valley, Due to its unique geographic location between three continents, Palestine has a lot of mountains in the north, a lot of wilderness in the south, and a lot of mountains in the middle [13]. It is also located on the Mediterranean shore. Geographic variance results in a wide range of soil and temperature conditions, which contributes to biodiversity. As a result, Palestine is known for having a large number of medicinal plants that have been consumed for a long time [14]. Testing of pharmacologically active chemicals in plants, on the other hand, began in the late 1960s [15].

The Palestinian hills, valleys, and deserts are home to around 2600 vegetation types from various families, with some more than 700 of them famous for their usage as medicinal herbs or botanical pesticides [16].

1.5 Secondary metabolites

Secondary metabolites in plants are natural chemicals present in certain plants that help them survive and reproduce in the area. Secondary metabolites are formed from primary and intermediary metabolism's building blocks [17]. The kingdom of plants as a whole is able to produce approximately 200,000 different natural compounds especially for defense and reproductive activities[18]. Several of these substances are important to humans as medicines, nutraceuticals, colorants, tastes, and perfumes, making them ideal choices for metabolic engineering [19].

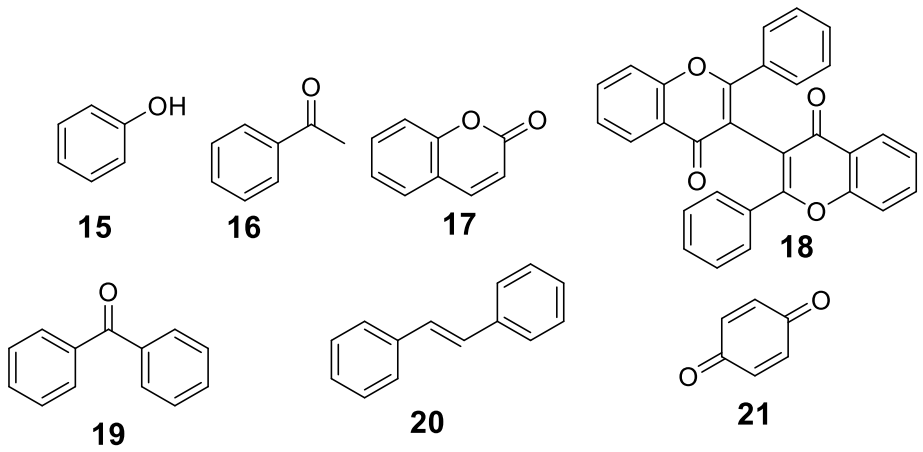
Classes of secondary metabolites

Chemical structure, composition, solubility in various solvents, and the mechanism by which they are created are all used to classify secondary metabolites. Phenolic compounds, alkaloids, and Terpenoids are the three major groups in the main classification system [20, 21]**.**

1.5.1 Phenolic compounds

Phenolic chemicals are a large group of secondary metabolites that have a variety of roles in plant defense and survival. Because their structure varies so much, they've been divided into groups based on the arrangement of their basic skeletons [22]. Chemical substances known as phenolic compounds have an aromatic hydrocarbon and a hydroxyl group connected immediately. Phenol is the most basic member of this class. The number of carbons in the molecule is the most essential factor for classifying phenolic compounds [23]. Phenolic compounds are used by plants for a variety of reasons, including their rapid oxidation and antioxidant properties. They serve as deterrents to plant growth, and seeds accumulate high concentrations of phenols, which serve as a filter, blocking oxygen from reaching the embryo and delaying germination [24].

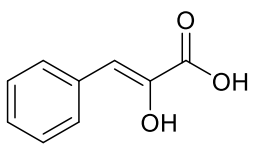
Simple phenols **15**, acetophenones **16**, phenylacetic acids, coumarins **17**, flavonoids, acidic phenols, biflavonyls **18**, benzophenones **19**, xhantones, stilbenes **20**, quinones **21,** hydroxycinnamic acids and betacyanins are all phenolic chemicals. This group also includes lignans, neolignans, tannins, and phlobaphenes [23].



1.5.1.1 Phenolic acid

The first class of phenolic compounds is phenolic acid. They are present in many plant-based meals, with seeds, fruit skins, and vegetable leaves containing the highest concentrations [25] .

Benzoic and cinnamic acids, which have 6- and 9-carbon skeletons, respectively, are the two most basic phenolic acids found in nature. A carboxylic group that is joined to the benzene ring in these compounds is coupled to one or more hydroxyl or methoxy groups[26]. Foods frequently contain hydroxycinnamic acids 22, which are produced from cinnamic acid and exist as basic esters of quinic acid 23 or glucose 24. The most prevalent soluble hydroxycinnamic acid (a combination of quinic acid **23** and caffeic acid **26**) is chlorogenic acid. Hydroxybenzoic acids, on the other hand, are generated from benzoic acid and have C1-C6 structure. They are present in soluble form (conjugated with sugars or organic acids) [27].



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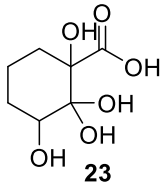

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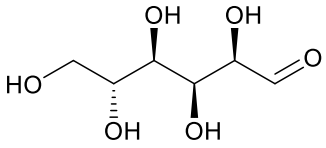

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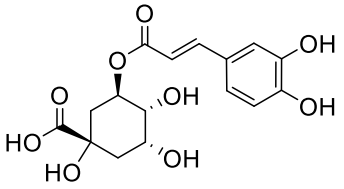

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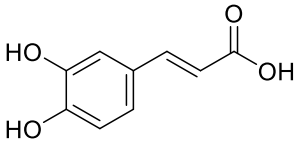

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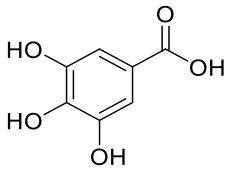
In small proportions, red fruits, onions, and black radish contain hydroxybenzoic acids, which are similar to hydroxycinnamic acids [25]. Gallic acid **27** and Ellagic acid **28** are these chemicals' most popular derivatives.



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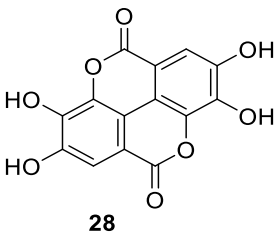

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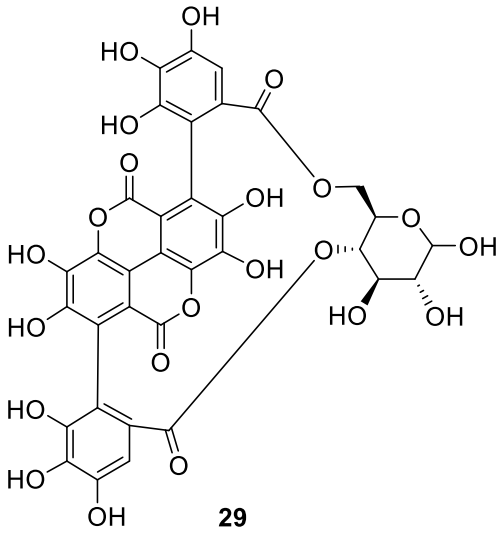
1.5.1.2 Tannins

Tannins are phenolic molecules with a high molecular weight that are found in the leaves, bark, and wood of plants. They can bind to proteins to create soluble or insoluble tannin-protein complexes. Based on their chemical makeup and properties, tannins are divided into Hydrolysable (HT) and Condensed Tannins (CT). Generally speaking, plants produce less hydrolysable tannins than CTs [28].

HTs (e.g. punicalin **29**) can create hazardous chemicals for ruminant animals, such as pyrogallol. Toxic compounds from diets containing more than 20% HT can result in increased mortality in sheep and cattle, liver necrosis, kidney damage with proximal tuberal necrosis, bleeding gastroenteritis-like disorders, and kidney damage [28].

CTs (e.g. procyanidin B2 **30**) in moderate amounts have been shown to improve ruminant protein synthesis and metabolism by reducing rumen breakdown of dietary protein and improving small intestinal absorption of amino acids. As a result, the CT may be able to bypass the rumen and enabling lower digestive system digesting [28].

Leather production has been the principal use for tannins for years, and it still is today. Animal hides' collagen proteins are bound together by tannins, which also increase the leather's flexibility and resistance to microbes [29].



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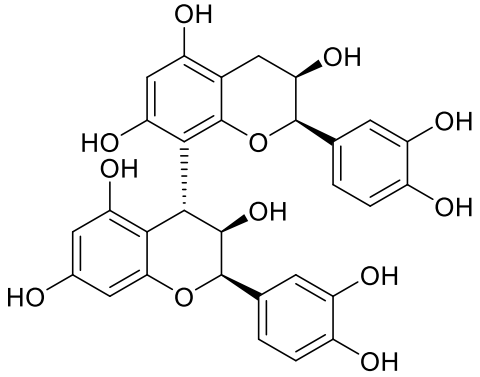

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1.5.1.3 Flavonoids

In the vacuoles of the full order of green plants, flavonoids are polyphenolic secondary metabolic products with a low molecular weight. Flavonoids are bioactive compounds

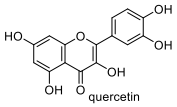
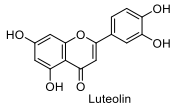
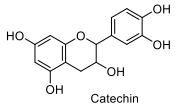
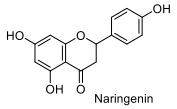
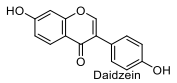
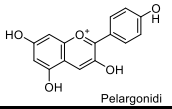
found in plants, animals, and bacteria. Flavonoids are known to be produced in particular places in plants for a long time and they are responsible for flower color, aroma, and fruit dispersion by attracting insects; they also help seed, mushroom, and seedling fertilization, development and growth. Plants are shielded from biotic and abiotic stress by flavonoids, which also serve as UV filters [30].

Subclasses of flavonoids

The structure of most flavonoids is C6-C3-C6, which links two benzene rings with an oxygen-containing heterocycle called a pyrene ring (C). The degree of central heterocyclic ring saturation divides flavonoids into two classes. Examples of saturated flavonoids include Flavanones, dihydroflavonols, and flavan-3-ols, whereas anthocyanidins, flavones, flavonols, and isoflavones have a C2=C3 unsaturation. Flavonoids can also be grouped according on their molecular size, despite the fact that this is the most used classification. The degree of substituents on the first or the second rings, such as hydroxy, methoxy, and alkyl, is another important aspect of flavonoid structure [31].

Table 1.1

The several flavonoid subclasses and some of their dietary sources.

Class	Food sources	Example	Strucure
Flavonols	Apple, Tea, Onions	Quercetin	 quercetin
Flavones	Olive oil, Red pepper	Luteolin	 Luteolin
Flavanols	Nectarine, Cocoa	Catechin	 Catechin
Flavanones	Orange, Lemon	Naringenin	 Naringenin
Isoflavones	Peanots,Soyabean	Daidzein	 Daidzein
Anthocyanin's	Strawberries, plums	Pelargonidin	 Pelargonidin

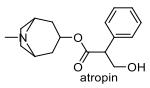
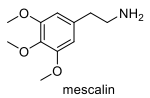
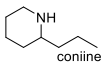
1.5.1.4 Alkaloids

A class of substances known as alkaloids has many different biological effects, including an ability to fight malaria. They are a type of chemical compounds with a wide range of structures that are generated from amino acids and contain a nitrogen atom in a heterocyclic ring [32].

Plants create a complex mixture of alkaloids, with one or more dominant components. Alkaloids differ significantly from one part of the plant to another, and some parts may not have any at all. Alkaloids are found in animals, bacteria, and fungi. Alkaloids have specific physiological and toxicological characteristics that primarily influence the central nervous system at various levels. For these reasons, they are drug-like substances [23].

They can be found freely in plants, though they mostly exist as salts. True alkaloids, Protoalkaloids, and pseudo alkaloids are different types of alkaloids.

Table 1.2
Classes of Alkaloids

Class	Example	Structure
True alkaloids	Atropin	 atropin
Protoalkaloids	Mescalin	 mescalin
Pseudo alkaloids	Caoinine	 coniine

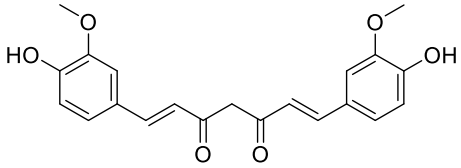
1.5.1.5 Terpenoids

Isoprenoids, generally referred to as terpenes, are the most prevalent and varied class of naturally occurring compounds. Although larger families of terpenes, including sterols and squalene, can be found in animals, they are mostly found in plants. They regulate the flavor, color, and odor of plants [23, 33, 34].

Terpenes, which are concoctions of volatile molecules with distinct scents, are present in the flowers and fruits of numerous plants, including those with the scents of lemon,

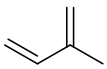
mint, eucalyptus, ginger, and great basil [23]. They serve as natural antioxidants for plant protection that is direct or serve as signals for animal and natural enemy defense that is indirect [35].

Terpenes were frequently used in conventional herbal medicine. One of these terpenes is curcumin 31, which possesses diuretic, anti-inflammatory, antibacterial, anticancer, antioxidant, antiplasmodial, astringent, and other properties. Additionally, curcumin has become a staple in healthy diets, which has made it possible for several medical investigations [34].



31

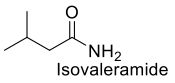
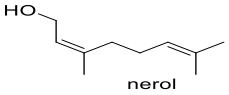
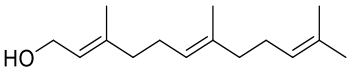
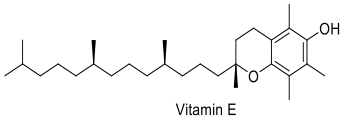
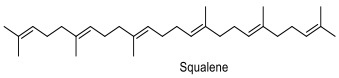
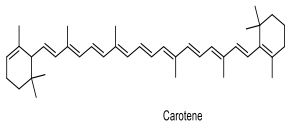
They're also called isoprenoids because the isoprene **32** molecule is the main structural component that connects them. The amount of isoprene units in them determines their classification. Hemiterpenes, which have five carbons and one isoprene unit in their structure, are the most basic of all the classes. The most well-known hemiterpene is isoprene, a volatile byproduct of tissues exposed to sun radiation. There are two groups in monoterpenes, three in sesquiterpenes, four in diterpenes, six in triterpenes, eight in tetraterpenes, and over ten in polyterpenes [23, 33].



32

Table 2.2

Terpene classes based on the amount of isoprene units.

Class	number of units of isoprene	The structure's carbon atom number	Examples
Hemiterpene	1	5	 Isovaleramide
Monoterpenes	2	10	 nerol
Sesquiterpenes	3	15	 Farnesol
Diterpenes	4	20	 Vitamin E
Triterpenes	6	30	 Squalene
Tetraterpenes	8	40	 Carotene
Polyterpnes	9>	40>	Rubber

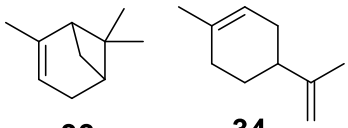
1.5.1.5.1 Hemiterpenes

The tiniest and most fundamental terpenoids are known as hemiterpenoids. The volatile hydrocarbon isoprene is the most prominent and well-known hemiterpene **32**, which is released by the leaves of numerous trees. [36].

1.5.1.5.2 Monoterpenes

Monoterpenes are the smallest terpenes. They are regarded as the main element of essential oils, perfumes, and several structural isomers and are derived from various flowers, fruits, and plants. α-pinene **33**, which gives pine trees their aroma, and limonene **34**, which comes from citrus plants, are two examples of the kinds of monoterpenes that can be found in natural odors. One of monoterpenes' major goals is to bring pollinators or inhibit other animals from eating plants. They could possibly be linked to the plants' flowering process. Steam distillation is used to separate them from

their plant sources, and their boiling points range from 150 to 185 degrees Celsius. Monoterpenes are purified by fractional distillation at low pressures or another method to produce a crystalline derivative [34].



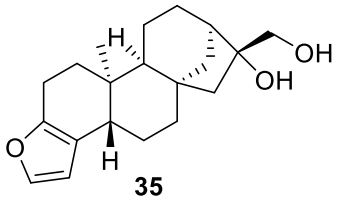
33 **34**

1.5.1.5.3 Sesquiterpenes

Sesquiterpenes are significantly larger and more stable than monoterpenes, and they contain the chemical formula $C_{15}H_{24}$. Steam distillation or extraction is used to separate them, and then they are refined using vacuum fractional distillation or gas chromatography. Sesquiterpenes are organic substances that are found in fungi, plants and insects and are used as a defense mechanism or to find females. Sesquiterpenes are necessary for the production of plant growth hormones and signaling mechanisms that respond to the environment. It is how plants react to various environmental circumstances. To maintain the stoma closed, it controls ion channels and water exchange across the plasma membrane [34].

1.5.1.5.4 Diterpenes

Diterpenes are a class of organic chemicals having the molecular formula $C_{20}H_{32}$. Diterpenes are chemical compounds that have physiological activity. Examples include vitamin A activity and plant growth hormones, which regulate plant fertilization, flowering, and the shift from asexual to sexual reproduction. Over 650 diterpenoids have been found in the huge and diverse genus Euphorbia plants, which includes flowering plants. Diterpenes have many therapeutic benefits, including anti-inflammatory, cytotoxic, and anticancer effects. They are present in tumor-promoting agent phorbol and anticancer drugs like taxol. Cafestol **35** and kahweol **36** are alcohols from diterpenes discovered in coffee bean oil. These chemical structures are nearly identical, with the exception of an additional double bond in kahweol's structure. According to research, coffee has been found to lower the risk of depression in women, chronic prostatitis in men, hypertension, diabetes, and many cancers [34].



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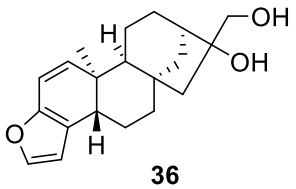

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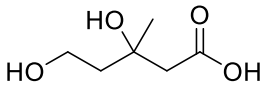
1.5.1.5.5Triterpenes

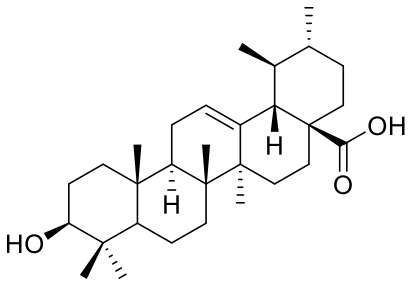
Triterpenes, which include steroids and sterols, have a six-isoprene unit composition and the chemical formula $C_{30}H_{48}$; all triterpenes have squalene as their biological precursor. Plants, animals and fungus all create triterpenes. They are generated from mevalonic acid **37** and act as steroid precursors in both plant and animal organisms. Saponins are emulsion-like compounds found in the skins of many plants that in the human digestive system serve as great detergents. Steroid saponins have chemical structures that are equivalent to hormones generated by the human body [34].



37

Some studies have found that using triterpenes reduce blood sugar levels and get rid of sweetness-inhibiting compounds in sugary and high-calorie foods. They can help persons with diabetes. Saponins have detoxifying qualities, as well as renal diuretic and wound healing properties [34].

Examples of triterpenoids include ursolic acid **38**, and betulinic acid **39.**



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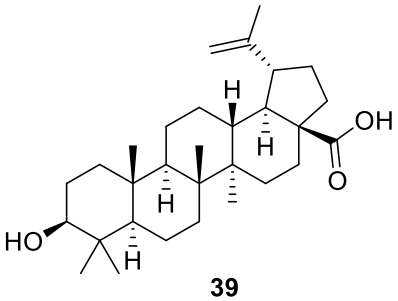
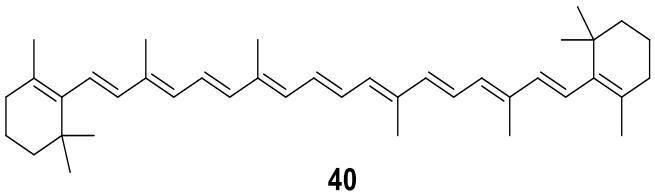


39

1.5.1.5.6 Tetraterpenes

Tetraterpenes, also referred to as carotenoids, are compounds with the chemical formula $C_{40}H_{56}$ and can be classified as terpenes due to their isoprene units (e.g. carotene **40)**. Because most carotenoids are highly unsaturated, they are difficult to extract and

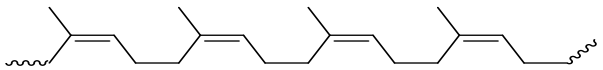
purify. The majority of the red, yellow, or orange fat-soluble plant and animal pigments are produced by these enzymes, which are present in a wide range of fungi, bacteria, and plants. Tetraterpene beta-carotene is a component of the yellow coloring in carrots. Because it is a source to vitamin A and other terpenoids that improve vision, it is essential for mammals [34].



40

1.5.1.5.7 Polyterpenes

Polyterpenoids are terpenoids with more than 40 carbons and more than 8 isoprene units. The natural rubber **41** (cis-configuration) molecule is the most well-known of these compounds. Long vessels called as laticifers have been found to contain rubber, which provides protection against pests and aids in wound repair [37].



41

1.5.2 Essential oils

The aromatic oily liquids known as essential oils, also referred to as volatile natural aromatic oils, are extracted from a variety of plant components, including leaf, peels, branches, flowers, buds, and seeds [38].

They are mostly the source of a plant's characteristic smell. There are around three thousand essential oils recognized, 10% of which have economic significance in the industries related to cosmetics, food, and drugs. Therefore, they are regarded as generally safe by the US Food and Drug Administration. Since essential oils are helpful when applied fresh, their protective benefits usually fade soon. This is most likely because of their extreme volatility, a quality that might be enhanced by the creation of formulations that can keep active ingredients on the skin for extended periods of time. Other organic products, like vanillin, may lengthen the duration of protection, boosting the repellent effects of specific essential oils [38, 39].

1.5.2.1 Sources and Chemical Composition

Hydrocarbons (terpenes and sesquiterpenes) and oxygenated chemicals make up the complex combinations of volatile organic molecules known as essential oils (EOs), which are formed as secondary metabolites in plants (alcohols , lactones, esters, ethers, ketones, aldehydes, phenols and phenol ethers) [38, 39].

Terpenes are the most prevalent type of chemical molecule found in essential oils. Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) make up the majority of essential oils. Essential oils contain low concentrations of diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}). Essential oils contain terpenoids (a type of terpene that contains oxygen) [38], as mentioned in table 1.3 .

1.5.2.2 Extraction methods for essential oils

Essential oils are made from various fragrant plant components and have many uses in the fields of cosmetics, pharmaceuticals, and food safety. The manufacturing process and methods used to extract essential oils are based on the characteristics and elements that must be present in the botanical extract. The method of extraction used is the most crucial factor in assuring the quality of essential oils, as poor extraction techniques can damage phytochemicals included in aromatic oils and alter their function. Some of the disadvantages include the loss of medicinal components, impact on stains, odor, and physical differences to essential oils. There are two types of extraction techniques: traditional procedures and innovative ones. Innovative methods, like ultrasonic and microwave enhanced techniques, have increased the efficiency of the extraction process in terms of time needed for isolating the essential oils and energy loss, along with production yield and essential oil content [40].

1.5.2.2.1 Traditional procedures

1.5.2.2.1.1 Cold pressing

The oldest technique for obtaining essential oils is called cold pressing (CP), and it was in use long before people learned how to distill. This method has the advantage of producing little to no warmth during the process, but it results in low yields. By applying pressure or roughness to the oil glands, rupturing them, ejecting the oil, and washing it away with water, the mechanical cold pressing technique extracts essential

oils from fruit peel. The most widely used piece of commercially available equipment for CP oil extraction is the in-line extractor from FMC (Food Machinery Corporation, Chicago, Illinois). the method's drawbacks due to the low output extraction and low purification attained [41].

1.5.2.2.1.2 Distillation

1.5.2.2.1.2.1 Steam distillation

Steam distillation is the most used technique for obtaining plant essential oils. 93% of essential oils are extracted by steam distillation, and the remaining 7% are extracted using other techniques. In fact, the plant sample is heated by steam or immersed in boiling water. Plant cell rupture and breakdown are mostly caused by the application of heat. As a result, aromatic molecules or essential oils are emitted from plant matter. The temperature of the heating element must be high enough to decompose plant material and produce aromatic compounds or essential oils [38].

1.5.2.2.1.2.2 Hydrodistillation

The simplest and oldest way of extracting oil is hydrodistillation, which was also the first to create extraction through the crystallizer. The first plant extract refined using this approach was rose. The process starts with the plant parts being submerged in water inside the alembic (vessel), and then the entire combination is boiled. The equipment includes a heating source, a container (Alembic), a condenser to turn vapor from the container into liquid, and a separator to collect residue and filter essential oils from water. This extraction method is used most frequently when extracting hydrophobic biological plant material with a high boiling point, such as wood or flowers. It is regarded as a novel method for extracting plant materials. Essential oils may be extracted using this method at a controlled temperature without overheating because the oils are encircled by water. This extraction method's main advantage is its ability to isolate plant components below 100°C [40].

1.5.2.2.1.2.3 Hydrodiffusion

The main difference between steam distillation and hydrodiffusion extraction is how steam is supplied into the still container. This technique is applied when the dried herb has been dried and is not harmed by boiling temperatures. For hydrodiffusion, vapor is

supplied from the head of the plant material; whereas, steam is introduced during steam distillation from the bottom. Low pressure or vacuum can also be used to operate the operation, bringing the heating value under 100 ° C. Because the processing takes less time and produces more oil while consuming less steam, the hydrodiffusion method outperforms steam distillation [38].

1.5.2.2.1.3 Solvent Extraction

Solvent extraction is a method that can be used to extract volatile essential oils (e.g., from blossom). The plant substance in a solvent bath, dissolved during this procedure. After the essential oil has been extracted, the liquid combination (which also contains other compounds) is put through filtration and distillation. Hexane, ethanol, petroleum ether, methanol, and alcohol are all frequent extraction solvents. The main advantage of extraction from distillation is that it operates at a lower temperature, which lowers the risk of chemical changes brought on by distillation's use of high temperatures. Because diffusion speeds are influenced by temperature, solvent extraction is both quick and affordable, and it is possible to accelerate the process by using hot solvents. Because the solvent used to create the essential oil will leave a small amount of residue, it cannot be used in food applications. Alcohol-containing solvents are safe to consume and are referred to as "food grade". The perfume industry frequently use this strategy [42].

1.5.2.2.2 Innovative procedures

1.5.2.2.2.1 Supercritical Fluid Extraction (SFE)

In order to extract and isolate EOs from aromatic plants, supercritical fluid extraction (SFE) has emerged as the most popular technique. This method extracts effectively and quickly, simply mild temperatures are required, lack cleanup procedures, and uses no hazardous organic compounds. Due to its non-explosive nature, lack of toxicity, availability, and ease of removal from derived goods, CO_2 is a great solvent for separating and isolating EOs from plants. SFE efficiency is influenced by a variety of factors, such as extraction time, temperature, pressure, and flow rate. In situations like this, where numerous variables may affect the outcome, modeling and optimizing processing parameters using methods like response surface methodology (RSM) is a viable strategy to boost process efficacy [43].

1.5.2.2.2.2 Ultrasonic microwave-assisted extraction (UMAE)

A non-contact heating element is the microwave that can aid to speed up energy transfer, startup, and reaction to heating control, as well as start reducing on the equipment, operations, and thermal difference. Microwave-assisted extraction (MAE) is a critical, long-term technology for attaining green analytical chemistry (GAC's) goals. It has quickly become one of the most popular techniques for separating high-value compounds from solids [44].

Now, extraction or distillation can be completed in a few of minutes rather than hours thanks to microwaves, which has several advantages (for example, high reproducibility, reduced energy and solvent use, more efficient techniques, and higher product purity) [45].

Another type of MAE known as ultrasonic microwave assisted extraction can help improve the extraction's mass transfer mechanism (UMAE). When microwave and ultrasonic waves are used together, the plant cell is ruptured and the active compounds are eluted into the extraction solvent, which improves the mass transfer mechanism. Extraction is quicker and consumes less solvent as a result. Vegetable oil, polysaccharides, and tomato lycopene have all been extracted from diverse plants using the UMAE method [46].

1.5.2.2.2.3 Microwave Solvent-Free Extraction (SFME)

Because there is increasing concern about how petroleum-based solvents affect the environment and the human body, a more environmentally friendly technology, known as solvent free microwave extraction (SFME), was created with great success in recent years using same values to those of MAE. The major benefits of employing SFME are lower handling and pollution prices due to the streamlined manipulation technique, quick clean-up, and labor savings. Both in the laboratory and in industry, they would be critical considerations [45].

The plant cell's internal mass and heat can go to the exterior thanks to SFME, whereas in conventional breakups, these two access phenomena take place in the opposite manner. Because of the volumetric heating effect, the temperature rises in a considerably shorter time, depending on the heating rate and the material being irradiated's dielectric loss factor. SFME just takes a few minutes to heat and less than 30

minutes to evaporate water and excerpts from the same source. SFME utilizes only around 0.5 kWh of energy for regular performance, compared to more than 4.5 kWh for conventional methods [45].

1.5.2.2.2.4 Extraction Assisted by Ultrasound

Ultrasound-assisted extraction (UAE) is a time-saving extraction technology that increases yields and, in some cases, extracts quality. Several research have looked at how ultrasound can be used to enhance bioactive molecule extractions to enhance the industrial extraction of bioactive compounds from plants, oils from beans, and proteins from soybeans. As a result, UAE has extracted several compounds from various matrices in recent years, with a focus on the food industry's commercial manufacturing of bioactive compounds [47, 48].

Ultrasound is made up of mechanical waves that travel across an elastic material. Sound and ultrasound have different wave frequencies; while ultrasound waves have frequencies between 20 kHz and 10 MHz, sound waves range from 16 Hertz to 16–20 kilohertz., which are higher than human audible range but lower than microwave range [48].

With ultrasound, there are fewer adverse effects on chemicals that can be extracted, less need for organic solvents (because it works with solvents that are usually regarded as safe), and shorter extraction times [42].

1.5.2.3 Quantitation and qualification analysis of EOs

There are numerous applications for gas chromatography. However, its primary and most widespread use is in the analysis and separation of multi-component combinations, such as those including solvents, hydrocarbons, and essential oils [49].

While GC separates thermally stable and volatile substitutes in a sample, GC-MS fragments the analyte that needs to be identified depending on mass [50].

The flame ionization sensor and the electron capture sensor in gas chromatography can quantitatively identify chemicals present at very low concentrations (both of which have very high sensitivities). As a result, pollution research, detective testing, and general trace analysis are the next most prominent main applications. Due to its ease of use, sensitivity, and effectiveness in isolating components of mixtures, gas chromatography

is one of the most important tools in chemistry. It is frequently used to determine thermochemical constants such temperatures of solution and evaporation, vapor pressure, and activity factors as well as for quantitative and qualitative mixture testing, compound purifying, and other reasons [49].

1.5.3 Biological activity

1.5.3.1 Anti-oxidant activity

Hypertension, reperfusion, inflammation and consequent damage to different tissues, a cancerous tumor, diarrhea, and a central nervous system injury are just a few of the diseases caused by radicals in humans. Free radicals are caused by radioactivity, chemicals, and environmental toxins, poisons, deep fry and spicy meals, in addition to physical stress, and they deplete antioxidants of the immune system, modify expression of genes and produce atypical proteins. In food, pharmaceuticals, and even living systems, one of the most significant methods for producing free radicals is through the oxidation process [51].

Antioxidants are important species that can protect organisms from harm caused by oxidative stress caused by free radicals. In order to function as reductants, proton donors, and singlet oxygen quenchers, and metal chelators, phenolics must possess antioxidant properties. These properties are what give phenolics their ability to do so. Thus, the creation of natural antioxidants originating from botanical extracts, particularly herbal plants, is becoming increasingly interesting to the fields of preventative care and the food sector. Reactive oxygen species (ROS) are potentially reactive oxygen derivatives that are continuously made within the human body. The antioxidants in the body purify the ROS that are produced. Reactive oxygen species (ROS) generation that is too high or inadequate antioxidant protections, on the other hand, may rapidly influence and result in oxidative damage to a wide range of biomolecules, including DNA, lipoproteins, lipids and proteins. This oxidative damage has been connected to several chronic human illnesses, such as diabetes, cancer, cvd, arthritis, neurological disorders, and the aging process [51, 52].

By forming secondary reaction products in meals, oxidation reduces the nutritional quality and safety following food preparation and culinary application (deep-fried foods). The creation of a complex mixture of lipid hydro peroxides (LOOH) and chain-

cleavage products arises from the autoxidation of lipids in meals. Antioxidants in food are chemical substances that have the ability to donate hydrogen radicals, lowering food's lipid peroxidation and rancidity. They increase the food's lipid-rich foods' shelf life while preserving their sensory and nutritional quality [53, 54].

The general advise for consumers is to increase their consumption of antioxidant-rich foods. As a result, consuming these items (fruits, vegetables, teas, wine, medicinal plants, and their preparations) on a daily basis may be a smart way to support one's health [53].

1.5.3.2 Anti-microbial activity

Long before humans realized the existence of microbes, it was widely accepted that some plants have therapeutic qualities, and that they included what we now call antimicrobial principles [55].

Infectious infections are a major cause of death and morbidity in the general population, particularly in developing countries. As a result, In recent years, demand has increased on medical companies to create new antimicrobial drugs, particularly in light of the ongoing rise of microbes resistant to existing antibiotics. Since bacteria that were originally recognized to be sensitive to widely used antibiotics have frequently been reported to become offers a new to other pharmaceuticals on the market, it appears that bacterial species have the genetic ability to acquire and distribute resistance to presently offered anti-bacterial [56].

The study of medicinal plants' antibacterial properties has obviously become a growing pattern. The possibility to expand conventional medicine to the point where it is recognized as a competitive alternative to western healthcare systems now exists thanks to improvements in laboratory techniques, rising interest in the topic, and scientific confirmation of traditional use [57].

1.5.4 *Myrtus*

The Myrtaceae family includes the well-known medicinal plant known as *myrtle* (*Myrtus communis* L.), which has been utilized by people all over the world for medical purposes. The family Myrtaceae has 100 genera and about 3000 species. Hardwood bushes or trees in the Myrtus genus can reach heights of up to 5 meters [58] .*M.*

communis L. grows in sandy soil and mild climate. It is found in South America, the North Western Himalaya, Australia, and Southern Europe, North Africa, and Western Asia [59]. The plant is 3 m tall, with branches covered in small, aromatic green leaves and black, little fruits [60].

M. communis L. leaves have long been utilized extensively in traditional medicine by many communities due to its tonic and antiseptic characteristics. Leucorrhoea, rhinorrhoea, epistaxis, blindness, excessive sweating, peptic ulcers, haemorrhoids, irritation, bleeding, headache, palpitations, lung and skin illnesses are only a few of the conditions that have been traditionally treated with this herb [59, 61]. *M. communis* leaves are utilized to treat wounds and digestive and urogenital disorders. It has been suggested by clinical and experimental studies that it has a wider range of therapeutic and pharmacological effects. The extensive use of myrtle in traditional medicine and the inclusion of its products in the drug companies have prompted the need for more research into the herb's various features, including its phytochemical, pharmaceutical, and toxicology aspects. Yellowish green or yellow. It has been discovered that the composition of essential oil obtained from myrtle leaves, berries, and flowers depends on the place of production, the time of year during which it is harvested, and the method used to extract it [60]. The principal constituents of the essential oil extracted from the leaves were discovered to be α-pinene, myrtenyl acetate, 1, 8-cineole, linalool, limonene and α -terpinolene in the majority of locations [59, 62, 63]. The leaves often include tannins, coumarins, and derivatives of flavonoids as quercetin, catechin, and myricetin. Italian, Sardinian, Corsican, Tunisian, Algerian, Greek, Cypriot, Montenegrin, Croatian, and Iranian M. communis EOs' chemical compositions were studied [59, 61, 63]. To our best knowledge, the phytochemical components of the *M. communis* growing in Palestine have never been investigated. Due to its importance in the perfume and favour business, this study was designed to study the phytochemical nature of M. communis's essential oil growing in two different locations namely in Jericho and Jenin, as well as to evaluate its in vitro antioxidative, metabolic enzymes inhibitory and antibacterial effects.

Figure 1.1

Picture of Myrtus

1.5.5 Objectives of the research

The study's primary goal was to compare the chemical composition of *M. communis* oil and extracts which was collected from two geographical regions in Palestine (i.e., Jenin, Jericho). The EOs' antimicrobial, antioxidant, and enzymatic (antiamylase) properties have also been discussed and contrasted.

However specific objectives of the current thesis were:

- To have an essential oil of both Jericho and Jenin *Myrtus* leaves.
- To extract the two essential oil from Jericho and Jenin, and have four extracts, two of them are methanol extracts and the other are ethyl acetate extracts.
- iii. To determine the chemical characteristics of the *M.communis* Eos collected from several Palestinian localities
- To assess their antibacterial, antifungal and anticancer activities
- To conduct their antioxidant activity
- To test the enzymatic properties of these EOs as anti-amylase.

Chapter Two
Experimental Part

2.1 Instrumentation

The following pieces of equipment were used in this study: GC-MS method, uv spectrophotometer, an electronic balance (Wagl, AS 220/C/2, Radwag, Poland), laminar flow (MRC, BBS12HGs, Israel), the Clevenger apparatus method, 24-well and 96-well plates (Greiner bio-one, North America), Namyangju, South Korea), a shaking laboratory water bath (Lab Tech, BPXOP1001040, micropipettes (Finnpipette, Finland).

2.2 Chemicals

All of the chemicals were obtained from the Sigma Aldrich chemical firm and used directly. Methanol, dichloromethane, ether, distilled water, ethyl acetate, sulfuric acid (H_2SO_4), potassium permanganate ($KMnO_4$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Mueller-Hinton broth, Trolox and dimethyl sulfoxide (DMSO) are some of the substances that have been utilized.

2.3 Myrtus (Collection of plant and extraction)

In April 2021, *M. communis* leaves were gathered from two cities resembling two regions of Palestine: Jenin (north), Jericho, separated carefully, washed twice with distilled water, dried for 14 days at room temperature and in the shade. Grounded well, and kept in cotton bags for later use.

The Clevenger apparatus method was used to extract the EOs from the two samples of *M. communis* plant. About 500 g of the Jericho dry portion powder was placed in a 1 L round-bottom flask with this apparatus. The material in this flask was dissolved in around 500 cc of deionized water. After that, the flask was attached to the Clevenger apparatus, which was housed within the same apparatus. This equipment was used for an extraction procedure that lasted 10 minutes at 100°C. Three times were done this for the Jenin plant sample (650g). The resulting EO was gathered into a pristine beaker, chemically dried, and kept chilled at 2-8°C until usage. The average yield of separated EOs from dried parts from Jericho and Jenin was 01.31% and 1.15%, respectively.

2.4 Gas chromatography-mass spectrometry used to determine the phytochemical content of *Myrtus* EO (GC–MS)

Using the GC-MS method, the two EO sample chemical compositions were identified. Shimadzu QP-5000 GC-MS chromatograms were captured using a Rtx-5ms column with a 30 m length, 0.25 μm thickness, and 0.250 mm inner diameter. One mL/min of helium was employed as the carrier gas. 220°C was the injector temperature. The temperature of the oven was programmed to rise from 50°C (1 minute hold) to 130°C, then to 250°C, and was maintained at that temperature for 15 minutes. 290°C was the transfer line temperature. An electron ionization system with a 1.7 KV detector volt was employed for GC-MS detection. A mass range of 38-450M/Z was covered using a 0.5-second scan rate and a scan speed of 1000 amu/sec.

2.5 Test for free radical scavenging

EOs from the two samples' scavenging activity was evaluated using the Jaradat et al. method [64], [65]. The two samples' EOs were produced as a stock solution in methanol and Trolox at a concentration of 1 mg/mL. To create Twelve working solutions with the following concentrations: 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, and 100 g/mL, each of these stock solutions was diluted in methanol.

A freshly made DPPH solvent (0.002% w/v) was combined in a 1:1:1 ratio with methanol and each of the aforementioned working solutions. A control treatment solution was also created by combining the aforementioned DPPH solution in a 1:1 ratio with methanol. All of these solutions then underwent a 30-minute incubation period at room temp in a dark cabinet. The optical density of these solutions at the end of the incubation period was calculated uv spectrophotometer at a wave length of 517 nm with methanol as the control solution.

The following equation was used to calculate the antioxidant property of the EOs and the Trolox standard in terms of the proportion of DPPH activity that was inhibited:

$$In\% = \frac{A\ blank - A\ sample}{A\ blank} \times 100 \qquad Eq.1$$

Where A blank represents the absorption of the blank and A sample the absorbance reading of the sample.

Using BioData Fit edition 1.02, the antioxidant ½ maximal inhibitory concentration (IC50) for every one of the examined M. communis EOs and Trolox standard solution, as well as their standard deviations, were determined.

The following equation was used to translate the antioxidant activities of M. communis EOs at the various concentrations described above into terms of the antioxidant capacity of the Trolox standard:

$$\%\ inhibition\ according\ to\ Trolox = \frac{Trolox\ IC50}{volatile\ oil\ IC50} \times 100\% \qquad Eq.2$$

2.6 Test for α -amylase inhibition

The 3, 5-dinitrosalicylic acid (DNSA) technique was used to carry out the α-amylase inhibition experiment. The EOs of the four *M. communis* samples were separately dissolved in a minimum of 10% DMSO and then in a buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) to generate concentrations ranging from 10 to 1000 g/ml. 200 L of the extract were combined with a volume of 200 L of α-amylase solution (2 units/ml), which was then incubated for 10 minutes at 30 °C. After that, each tube received 200 µl of the starch solution (1% in water (w/v)), which was then incubated for 3 min. 200 µL of the DNSA reagent (12 g of sodium potassium tartrate tetra hydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3.5-dinitrosalicylic acid solution) were added to the reaction to stop it, and it was then heated for 10 minutes at 85–90 °C in a water bath. The mixture was diluted with 5 ml of distilled water after cooling to room temperature, and the absorbance at 540 nm was assessed using a UV-Visible spectrophotometer. By substituting 200 l of buffer for the plant extract, the blank was created with 100% enzyme activity. In the absence of the enzyme solution, a blank reaction was similarly constructed using the plant extract at each concentration. Acarbose was used to create a positive control sample, and the reaction behaved equally to the interaction with plant extract as previously reported. The following equation was used to compute the percent inhibition of α-amylase inhibitory activity: Plotting the extract concentration versus the percentage of α-amylase inhibition yielded the IC50 values [66].

$$\%\ \alpha\ amylase\ inhibition = \frac{Abs100\%\ control - AbsSample}{Abs100\%\ Control} \times 100 \qquad Eq\ .3$$

2.7 Antimicrobial evaluation

As well as being evaluated against the development of clinical isolates of *methicillin-resistant Staphylococcus aureus (MRSA)*, EOs samples of *M. communis* were also tested against the bacterial strains of *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus*. The growth of a clinical isolate of *Candida albicans* with a confirmed diagnosis was subjected to EOs' antifungal activity.

EO samples from two regions of Palestine were tested for antibacterial activity using the broth micro-dilution method described in the reference, with some changes Individual bacteria stains were cultivated for 12 hours in culture broth. The isolated *M. communis* EOs were all dissolved at a concentration of 132 mg/ml in 5% DMSO. After being filter-sterilized, the *M. communis* EOs solutions were serially micro-diluted (by two folds) eleven times in sterile nutritional broth. The dilution procedures were carried out in 96-well plates under aseptic conditions [67-69].

2.7.1 Test for bacteria

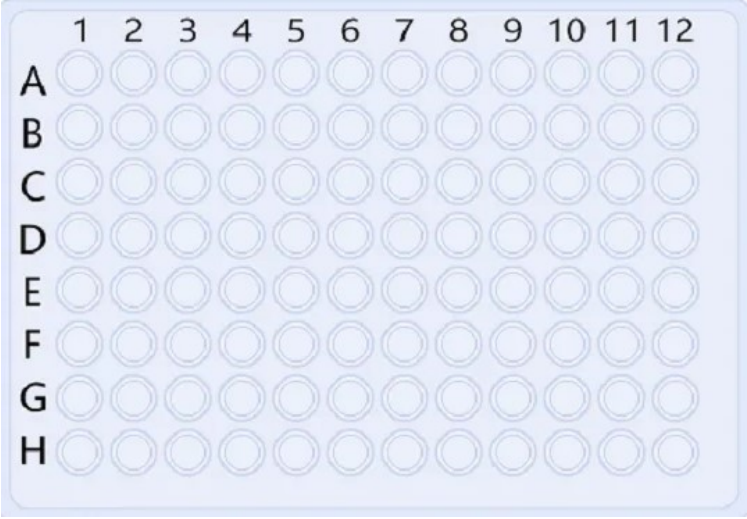
The concentration of such oils in the micro-wells used to measure the antibacterial effects of the obtained *M. communis* EOs ranged from 0.129 to 66 mg/mL. These plates' micro-well number 11, which served as a positive control to bacterial growth, contained nutritional broth free of EOs. However, the EOs-free nutrient broth in micro-well number 12 was not infected with any of the examined bacterial cells. This is an effective negative bacterial growth control. Microwells 1 through 11 were aseptically injected with the test microorganisms. The investigated bacterial pathogens had final microbial cell densities of approximately 5 105 colony-forming unit (CFU)/mL at the time of inoculation. In this investigation, the *M. communis* EOs were evaluated in duplicate on each of the bacterial cells that were included. At 35 °C, the inoculation plates were all incubated. The incubation period was roughly 18 hours long. The minimum inhibitory concentration (MIC) of the *M. communis* EOs under examination is the lowest concentration of *M. communis* EOs at which no discernible bacterial growth in that micro-well was observed. In concurrent trials, the MIC of gentamycin was also established in order to regulate the susceptibility of the test bacteria. Every established test was carried out in three copies [67-69].

2.7.2 Test for antifungal

The concentration of two oils ranged from 55 to 0.065 mg/mL in the micro-wells used to assess the antifungal effects of the collected *M. communis* EOs. These plates' micro-well number 11, which served as a positive control for fungi development, provided nutritional broth free of EOs. In contrast, the nutrient broth in micro-well number 12 did not contain any EOs and was not contaminated with the tested strain of *Candida albicans*. This is an effective negative control for microbial growth. The evaluated *Candida albicans* injected aseptically into microwells 1 through 11. The final *Candida albicans* concentrations at the time of injection ranged from 0.5 to 2.5 103 CFU/ml. The *M. communis* EOs were investigated in duplicate for their potential to inhibit. At 35 °C, the inoculation plates were all incubated. A 48-hour incubation period was experienced. The MIC of the *M. communis* EOs under examination is defined as the smallest amount of *M. communis* EOs at which no discernible fungal growth was seen in that micro-well. The amphotericin MIC was established in concurrent trials to regulate the resistance of the tested organisms. Every established test was carried out in three copies.

Figure 2.1

Anti-bacterial and anti-fungal test plate with 96 microwells



2.8 Tests for cytotoxicity and cell culture

In RPMI-1640 medium, HeLa and MCF-7 cancer cell lines from humans as well as 3T3 and LX-2 normal hepatic and cervical embryo fibroblasts from mice were cultivated (Sigma-Norwich, United Kingdom). After that, 10% fetal bovine serum was combined with 1% penicillin/streptomycin antibiotics (BI, India), 1% L-glutamine, and applied to

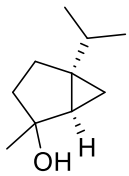
each type of the test cells (Sigma, UK). The cancer cells were cultivated at 37 °C in an environment that was humidified and contained 5% CO2 before being plated at a density of 2.6X104 cells per well in a 96-well plate. Following two days, the cells were exposed to EO at various doses for 24 hours (1000, 500, 250, 125, and 62.5 g/mL). The CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) method was used to calculate cell viability in accordance with the manufacturer's instructions. The well plates were then filled with 100 l of medium and 20 l of MTS solution, and they were incubated at 37 °C for 2 hours. 490 nm was used to measure the absorbance [70, 71].

Chapter Three

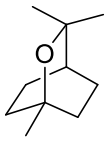
Results

3.1 Identification of the essential oil from M. communis by GC-MS

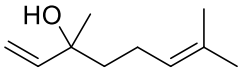
The two EO samples' chemical content was established using GC- MS. The components of *M.communis* EO that were determined to be the most prevalent were cis-4-thujanol **43** (27.37 %) and 1,8-Cineole **44** (24.32 %) in totally 41 components in Jericho leaves oil. The main components in the EO of *M. communis* were 1,8-Cinoele **44** (31.55%) and linalool **45** (21.65%) from a total thirty seven compounds founded in Jenin leaves oil, according to GC-MS analysis.



42



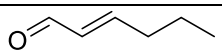
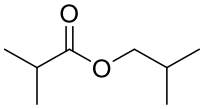
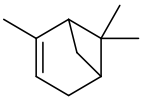
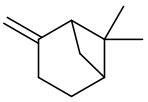
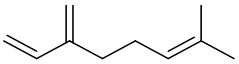
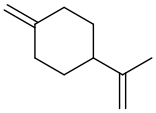
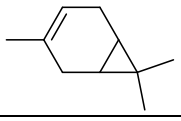
43

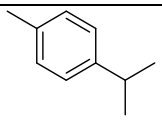
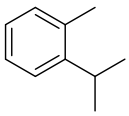
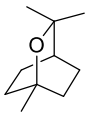
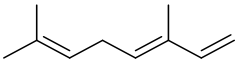
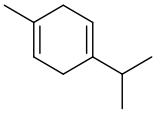
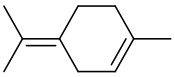
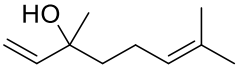
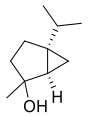
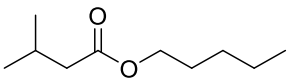
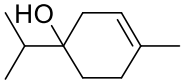
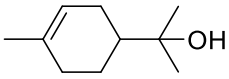
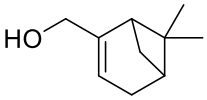
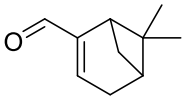
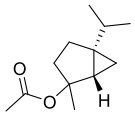
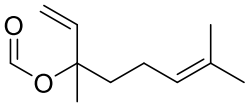
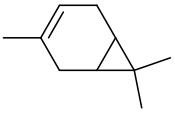
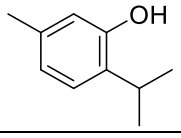


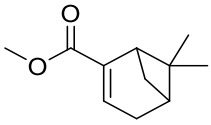
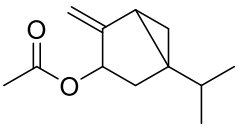
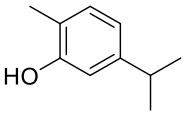
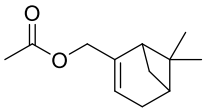
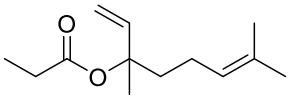
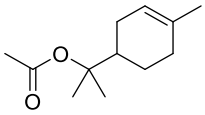
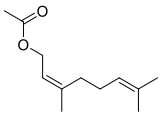
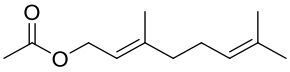
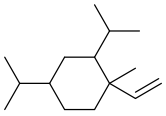
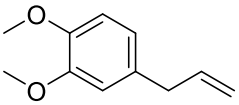
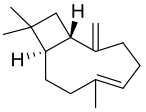
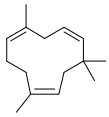
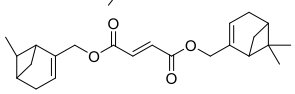
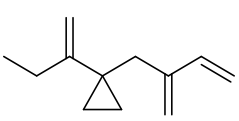
44

Table 3.1

The M. communis essential oil's chemical content from Jericho and Jenin leaves. RT: Retention time, RI: retention index

No	Name	Molecular formula	RT	RI	Percentage	
					Jericho	Jenin
1	Trans-2-hexenal		5.73	856	-	0.30
2	Isobutyl isobutyrate		7.99	889	0.14	0.50
3	α-Pinene		8.7	933	3.95	10.22
4	β-Pinene		10.49	976	0.05	0.09
5	Myrcene		11.07	990	0.21	0.38
6	Pseudo limonene		11.72	1006	-	0.23
7	δ-3-Carene		11.81	1008	0.04	0.21

8	p-Cymene		12.17	1017	-	0.05
9	o-Cymene		12.5	1025	0.17	1.34
10	1,8-Cineole		12.82	1033	24.32	31.55
11	Z-b-Ocimene		13.42	1047	0.28	0.71
12	d-Terpinene		13.88	1059	-	0.36
13	Terpinolene		14.98	1085	-	0.92
14	Linalool		15.65	1102	-	21.65
15	cis-4-Thujanol		15.65	1102	27.37	-
16	n-Amyl isovalerate		15.75	1105	-	0.46
17	Terpinen-4-ol		18.69	1181	0.24	0.32
18	-terpineol		19.28	1196	-	4.35
19	Myrtenol		19.29	1197	12.97	-
20	Myrtenal		19.36	1198	12.46	6.78
21	Trans-4-Thujanol acetate		21.26	1251	9.48	11.26
22	Linalyl acetate		21.33	1253	1.04	0.42
23	Trans-Sabinyl acetate		21.87	1296	0.01	0.10
24	nd	-	22.00	1271	0.01	-
25	nd	-	22.33	1281	0.01	-
26	Thymol		22.47	1285	0.02	-

27	Methyl myrtenate		22.78	1294	0.10	-
28	Sabinyl acetate		22.87	1296	0.17	0.10
29	Carvacrol		23.06	1302	0.11	-
30	nd	-	23.19	1305	0.01	-
31	nd	-	23.50	1315	0.01	-
32	nd	-	23.63	1319	0.02	-
33	Myrtenyl acetate		23.82	1324	0.62	2.89
34	nd	-	23.92	1327	0.01	-
35	nd	-	24.15	1334	0.02	
36	Linalool propanoate		24.38	1341	0.01	-
37	-terpinyl acetate		24.61	1348	1.77	1.30
38	Neryl acetate		25	1360	0.48	0.21
39	nd	-	25.49	1374	-	0.01
40	Geranyl acetate		25.65	1379	0.92	0.55
41	β-element		26.03	1390	0.05	0.03
42	Methyl eugenol		26.41	1402	0.70	0.37
43	β-Caryophyllene		27.01	1421	0.71	0.70
44	α-Caryophyllene		28.15	1458	0.40	0.22
45	Fumaric acid dimyrtenyl ester		28.37	1463	0.12	0.04
46	Cyclopropane, 1-(2- methylene-3-butenyl)-1- (1-methylenepropyl)		28.73	1476	0.02	-

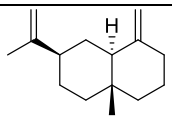
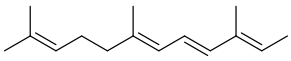
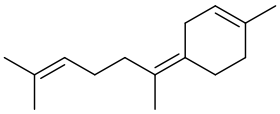
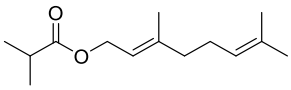
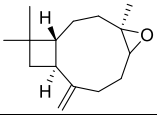
47	β-Selinene		29.22	1491	0.34	0.07
48	(E,E)-□-Farnesene		29.43	1498	0.33	0.07
49	β-bisabolene		29.77	1509	-	0.7
50	Geranyl isobutanoate		29.98	1516	0.02	0.01
51	Caryophyllene oxide		32.03	1585	0.22	0.07
Oil Yield					1.31	1.15
Total identified					99.93	99.54
Monoterpene hydrocarbons					4.72	14.57
Oxygenated monoterpenes					92.99	82.74
Sesquiterpene hydrocarbons					1.83	1.79
Oxygenated sesquiterenes					0.22	0.07
Others					0.17	0.37

Figure 3.1

M. communis oil GC-MS chromatogram from Jericho.

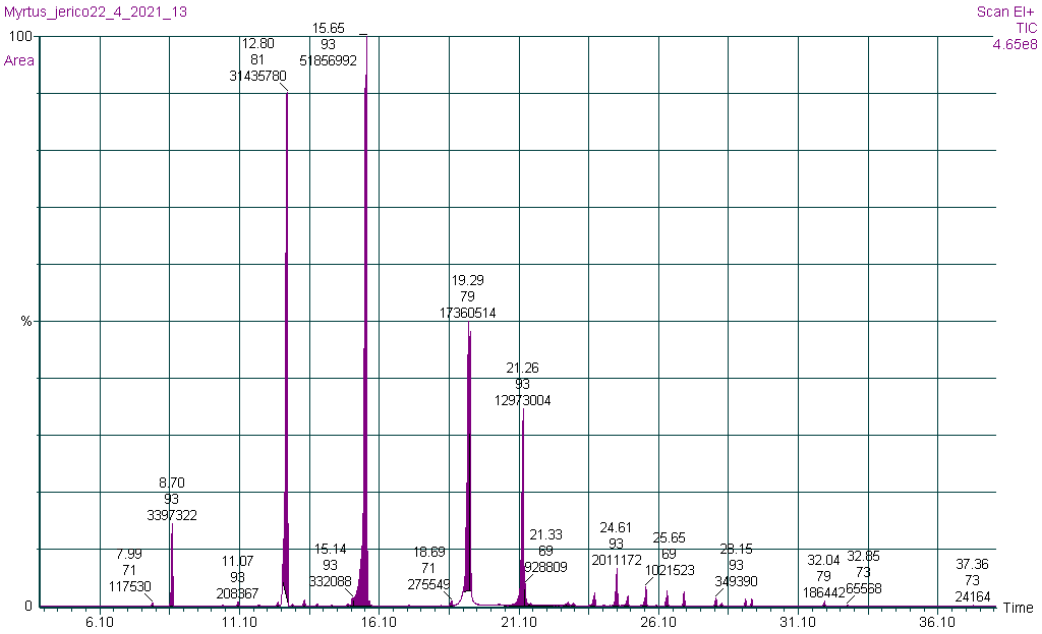
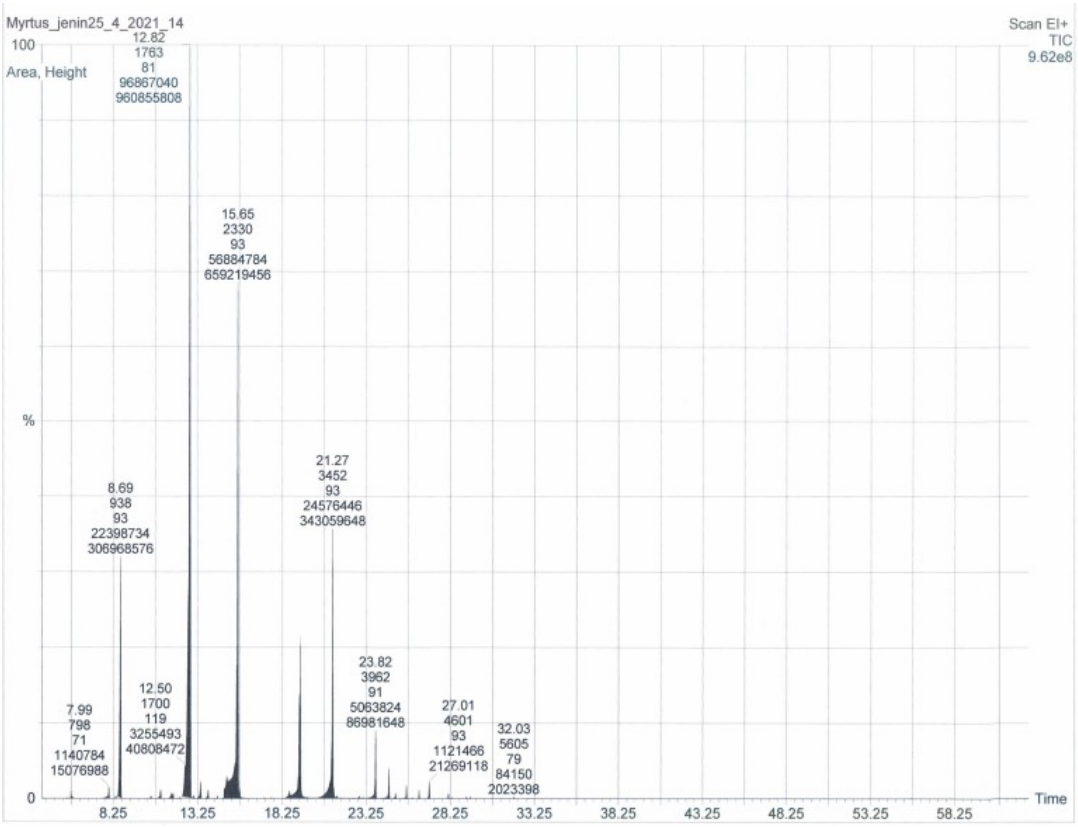


Figure 3.2

M. communis oil GC-MS chromatogram from Jenin.



3.2 Antioxidant activity of *M. communis*

The DPPH test method was used to evaluate the scavenging capacity of the EOs in the samples. The proportion of DPPH activity that was inhibited allowed the antioxidant activity of the EOs and Trolox standard to be calculated. The following equation was used to determine the standard deviations and antioxidant half-maximal inhibitory concentrations (IC50) for each of the examined *M. communis* EOs and Trolox standard solution:

$$\%\ inhibition\ according\ to\ Trolox = \frac{Trolox\ IC50}{volatile\ oil\ IC50} \times 100\% \qquad Eq.4$$

In the antioxidant activity test, samples of Jericho EO oil, Jenin EO oil, Jenin methanol extract, Jericho methanol extract, Jenin ethyl acetate extract, Jericho ethyl acetate extract, were used.

Table 3.2

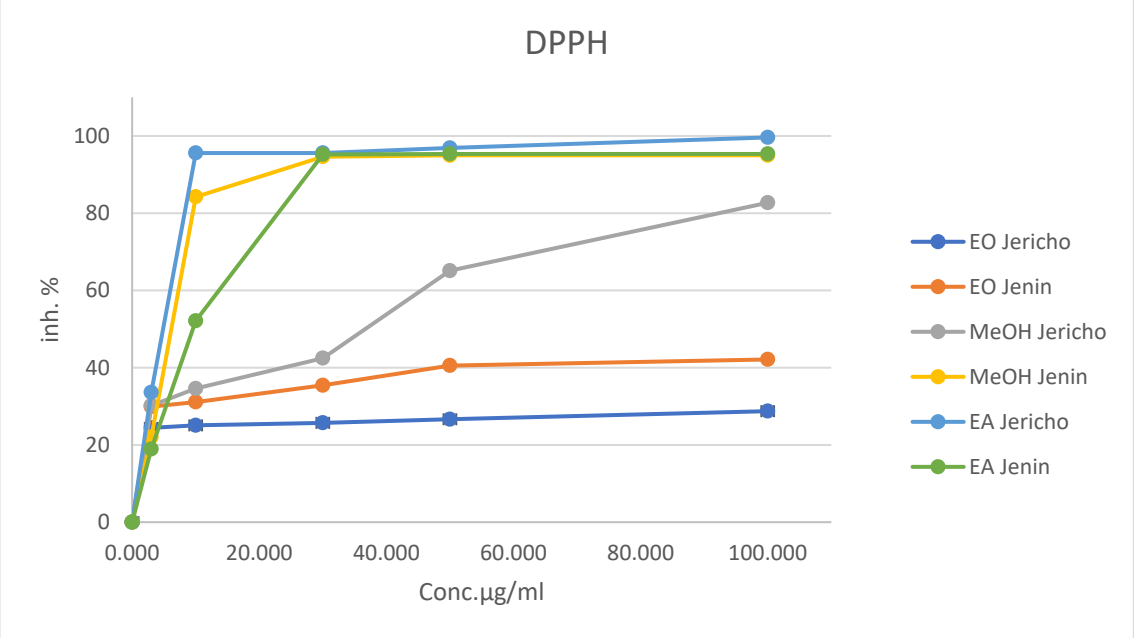
The inhibitory activity of M. communis essential oil, extracts and trolox against DPPH. IC_{50}=half maximal inhibitory concentration, DPPH = 1,1-diphenyl-2- picrylhydrazyl.

Concentration µg/ml	EO Jenin	EO Jericho	MeOH Jenin	MeOH Jericho	EA Jenin	EA Jericho	Trolox
3.00	29.92	24.41	21.98	30.19	18.94	33.59	0.01
10.00	31.10	25.07	84.23	34.59	52.14	95.59	40.44
30.00	35.43	25.72	94.62	42.45	95.20	95.59	95.90
50.00	40.55	26.64	94.98	65.09	95.33	96.89	93.03
100.00	42.13	28.74	94.98	82.70	95.33	99.61	94.26
IC50 (µg/ml)	Ni	Ni	4.86± 0.48	25.70± 0.45	8.55± 2.31	3.60± 0.35	10.25± 1.02

EOJenin: EO from Jenin; EOJericho: EO from Jericho; MeOHJenin: Methanol extract from Jenin; EAJenin: Ethyl acetate extract from Jenin; MeOHJericho: Methanol extract from Jericho; EAJericho: Ethyl acetate extract from Jericho.

Figure 3.3

Inhibition% of DPPH (1,1-diphenyl-2-picrylhydrazyl) by trolox and M. communis essential oil and extracts.



3.3 α-Amylase activity

The 3, 5-dinitrosalicylic acid (DNSA) technique was used to carry out the α-amylase inhibition experiment. The following equation can be used to compute the % inhibition of α-amylase stated as: Plotting the percentage of α-amylase inhibition versus extract concentration and IC50 values.

$$\%\ \alpha\ amylase\ inhibition = \frac{Abs100\%\ control - AbsSample}{Abs100\%\ Control} \times 100 \qquad Eq\ 5$$

Table 3.3

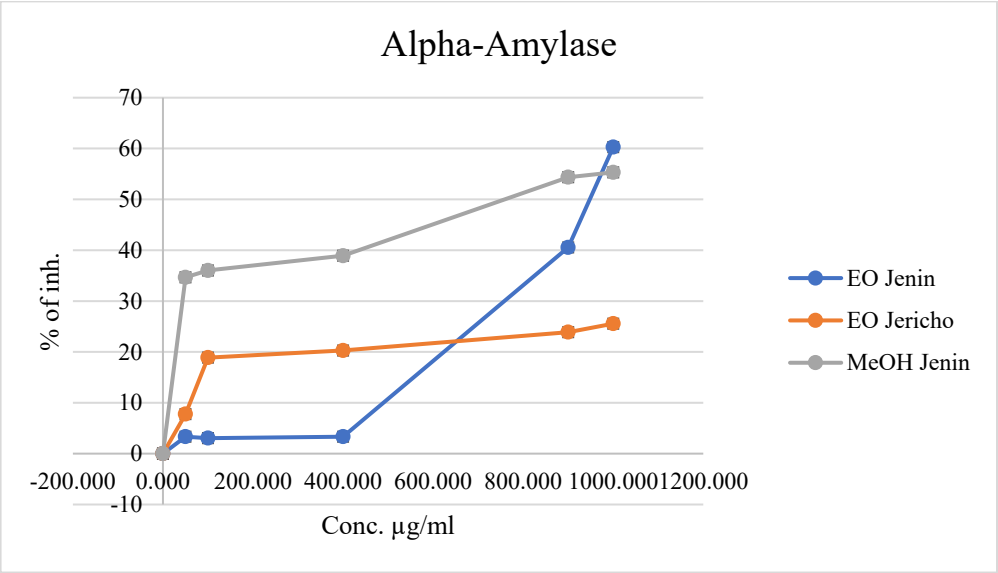
α-Amylase inhibition of M. communis essential oil and extracts. IC50=half maximal inhibitory concentration.

Concentration (µg/ml)	EO Jenin	EO Jericho	MeOH Jenin	MeOH Jericho	EA Jericho	EA Jenin
50.00	3.33	7.78	34.68	No inhibtion	No inhibtion	No inhibtion
100.00	3.06	18.89	36.02			
400.00	3.33	20.28	38.93			
900.00	40.56	23.89	54.36			
1000.00	60.28	25.56	55.31			
IC50 (µg/ml)	950.48±2.54	>1000	795.43±1.88			

EOJenin: EO from Jenin; EOJericho: EO from Jericho; MeOH Jenin: Methanol extract from Jenin; EAJenin: Ethyl acetate extract from Jenin; MeOH Jericho: Methanol extract from Jericho; EAJericho: Ethyl acetate extract from Jericho.

Figure 3.4

α-Amylase inhibition of M. communis essential oil and Jenin extract.



3.4 Anti-microbial activity

M. communis EO samples were examined for their ability to inhibit the development of clinical isolates of *methicillin-resistant Staphylococcus aureus* (*MRSA*), as well as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. A diagnostically confirmed clinical isolate of *Candida albicans* will be used to test the antifungal effectiveness of EOs.

The stronger antibacterial activity of the extracts on the tested bacterial strains is shown by the lower MIC value.

These results (Table 3.5) suggested that Jenin oil had a high anti-inhibitory action proteos and a moderate inhibitory effect against *MRSA, S.aureus, Klebi, E.coli* and *Candida*. Jericho oil had a high inhibitory effect against *S.auteus* and *E.coli* .

Table 3.4

Minimal inhibitory concentrations (mg/ml) for several M. communis extracts versus particular infections (bacteria and fungi).

Microbe	EO Jenin	EO Jericho	MeOH Jenin	EA Jenin	MeOH Jericho	EA Jericho
MRSA	1.13	0.135	0.195	0.78	0.78	0.097
S. aureus	1.13	0.27	0.195	1.56	0.78	0.097
K. pneumonia	1.13	0.135	12.5	12.5	25	1.56
E. coli	1.13	0.27	12.5	12.5	25	1.56
P. vulgaris	0.56	0.135	12.5	12.5	25	3.125
P. aeruginosa	18.1	17.3	25	12.5	25	3.125
C. albicans (fungi)	1.13	1.08	0.049	0.049	0.049	0.049

EOJenin: EO from Jenin; EOJericho: EO from Jericho; MeOHJenin: Methanol extract from Jenin; EAJenin: Ethyl acetate extract from Jenin; MeOHJericho: Methanol extract from Jericho; EAJericho: Ethyl acetate extract from Jericho.

3.5 Anti-cancer activity

Cytotoxicity Four different concentrations were evaluated for cytotoxicity using the MTS test of Jericho and Jenin oils versus HeLa, tumor cells and MCF-7 as well as against normal cells like Mouse Embryo Fibroblasts (3T3) and hepatic (LX-2). The IC50 of five different concentrations was calculated and presented in Table 3.5 of Jericho and Jenin oils as well as for Doxorubicin against the used cell lines, the Jenin oil has more cytotoxic activities than Jericho oil against HeLa, MCF-7 and 3T3 cell lines. Moreover the cell viability at 0.5 mg/mL were calculated versus HeLa, 3T3 cell lines MCF-7and presented in figure 3.6.

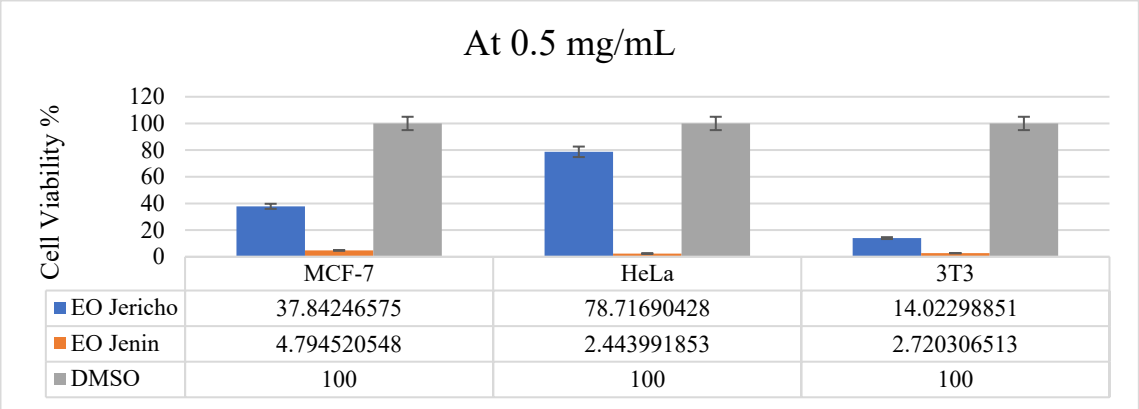
Table 3.5

The IC_{50} (µg/mL) for Jericho and Jenin oils against cells lines

Cells lines	IC_{50} (µg/mL)		
	EO Jericho	EO Jenin	Dox
HeLa	914.54±3.05	592.40±2.55	0.84±1.1
MCF7	762.45±2.25	597.01±3.11	0.37±0.22
3T3	644.47±2.89	215.25±1.07	1.21±1.0
LX-2	199.80±3.41	202.02±2.27	5.72±0.09

Figure 3.5

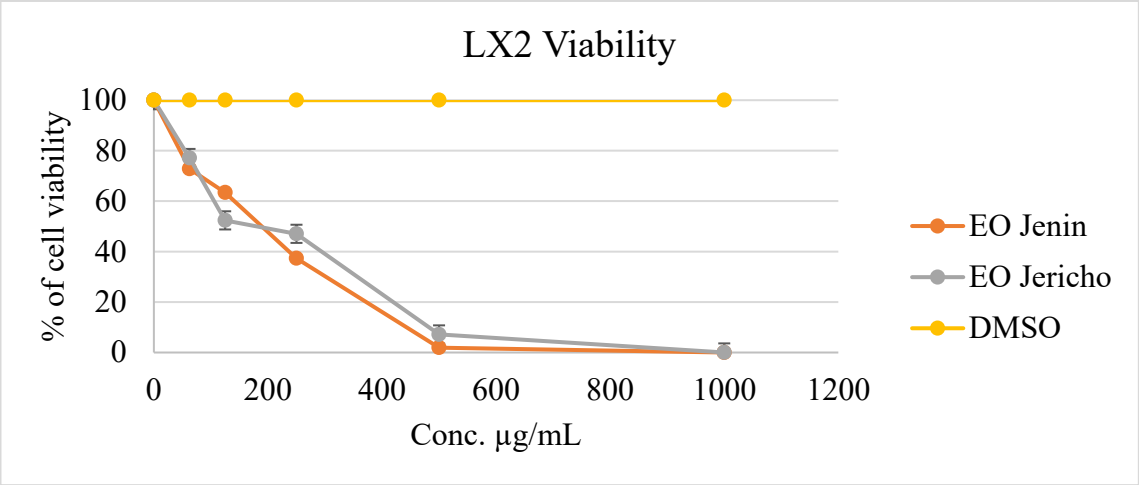
Cell viability percentages against MCF-7, HeLa, and 3t3 cell lines for the oils versus DMSO (negative control)



The vitality of LX-2 cells after exposure to various extracted oils was assessed using the MTS test in order to investigate the anti-fibrotic actions of these substances on the human hepatic stroma cell (HSC) line LX-2. Both of used oils showed significant antifibrotic activities with IC50 199.80±3.41 and 202.02±2.27 µg/mL for Jericho and Jenin oils respectively. Their cell viability percentage was presented in Figure3.6 in comparison with DMSO negative control.

Figure 3.6

Percentage Inhibition of LX2 normal cell by Jenin EO and Jericho EO at 460 nm.



Chapter Four

Discussion

4.1 The chemical composition of *Myrtus*.

With the discovered components presented in the order of their elution on the DB-5 column, together with their retention indices and percentages, Table 3.1 displays the chemical composition of the examined oils obtained from two districts in Palestine. While air-dried *M. communis* leaves from Jenin produced 1.15% EO, *M. communis* from Jericho produced 1.31% as pale yellow essential oil.

The yield obtained is regarded as being better than that from *M. communis* in Lebanon, where it yielded 1% [72], and better than that from Algeria, where it yielded 0.32% [62], and Morocco, where it yielded 0.68% [73]. The essential oil of *M.communis* from Jericho, the world's deepest location, included a total of 41 volatile components, the primary ones being cis-4-Thujanol (27.37%), 1,8-Cineole (24.32%), Myrtenol (12.97%), Myrtenal (12.46%), and trans-4-Thujanol acetate (9.48%). Only 37 components, however, were found in the EO of *M. communis* from Jenin, of which the major six were 1,8-cineole (31.55%), linalool (21.65%), Trans-4-thujanol acetate(11.26%), α-pinene(10.22%), Myrtenal(6.78%) and α-terpineol(4.35%).

According to Table 3.1, there are considerable differences between the two examined EOs that were collected from the two different locations in terms of both the quality and quantity of their constituent parts. This fluctuation in essential oil content and ratios of different components may be partially explained by variations in ecological factors, environmental circumstances such as water, nutritional stress, temperature, and geographical source [65, 74-76]. According to a review of the literature, *M. communis* leaves essential oil has been the focus of numerous prior studies from various countries, and the essential oil's composition appears to be greatly influenced by regional, seasonal, geographic, or genetic variations [65, 74]. Our analysis suggests that 1,8-cineole is the main component of *M. communis* oil, which is reasonably close to findings reported in previous research [65, 74] on the composition of essential oils from the Jenin region. On the other hand, the order of the numerous components as described in the literature is different. Therefore, only 2.86% and 0.62%, respectively, of Jenin

and Jericho EOs included myrtenyl acetate, which was present in large amounts in *M. communis* leaves EOs from Tunisia [77], Morocco [78], and Spain [79].

Additionally, according to certain research [64, 80, 81], 1,8-cineole was considered to be the second most prevalent component, whereas α-pinene was shown to be the most significant component [82-87], which we identified in Jericho EO at 3.95% and Jenin Oil at 10.22%. The unusual atmosphere and soil of Jericho, which is close to the Dead Sea, are the reason why Jericho myrtle leaf oil differs from all other oils mentioned in the literature.

4.2 Antioxidant activity of *Myrtus* EO

The DPPH assay, one of the most popular methods for determining antioxidant potential, was used to measure the antioxidant activities of both EOs as well as the methanol extracts of *M. communis* [88]. Figure 3.4 displays that *M. communis* collected from Jenin and Jericho in methanol extracts had comparable antioxidant capacity, with IC_{50} values of 4.86±0.48 and 25.70±0.45 μg/mL, respectively (Table 3.2). Jenin and Jericho Ethyl Acetate extracts outperformed the positive control Trolox, which exhibited an IC_{50} of 10.25±1.02 μg/mL, and demonstrated dose-dependent free radical scavenging capabilities with IC_{50} values of 8.55±2.31 and 3.60±0.35 μg/mL, respectively. The concentration of the free radical DPPH was slightly reduced by the essential oils of *M. communis*.

The essential oils of *M. communis* only included a little amount of phenolic compounds, which may be why methanol and ethyl acetate extracts shown considerably stronger antioxidant activity than the EOs. Our EOs' antioxidant capabilities are in line with research done in the past on *M.* communis EOs from Yemen, Morocco, and Italy. These EOs had IC_{50} values that ranged from 0.80 to 4.50 μg/mL [78, 89, 90] and showed only moderate activity. With IC_{50} values ranging from 200–693 μg/mL, EOs from Algeria [62, 91, 92] and Tunisia [93] demonstrated superior activity for scavenging DPPH. Additionally, the antioxidant activity of our methanol and ethyl acetate extracts was in line with earlier research on aqueous, ethyl acetate, and methanol extracts of plant leaves, which revealed strong antioxidant activity with IC_{50} values of 3.5, 9, and 11 μg/ml, respectively [94]. In Tunisia, Wannes et al. reported significant free radical scavenging properties of a methanolic extract of myrtle leaves, stems, flowers, and

seeds with IC_{50} values of 3, 90, 3, and 10 µg/ml, respectively. This extract had greater DPPH radical scavenging ability than the essential oils of the leaves (IC_{50} = 600 µg/ml), stems (IC_{50} = 2,000 µg/m. The observed antioxidant effect [95] may be due to the methanol extracts' ability to neutralize free radicals (DPPH), either through the transfer of an electron or a hydrogen atom. Accordingly, it has been noted that the antioxidant activity depends on the kind of phenolic compounds and the presence of hydrolysable tannins in addition to the overall amount of phenolics [93].

4.3 α-Amylase activity

A metabolic disease called diabetes causes elevated blood sugar levels. Diabetes can result in ketoacidosis and nonketotic hyperosmolar coma in addition to long-term issues such heart disease, stroke, kidney failure, foot ulcers, and eye impairment [96, 97]. Diabetes and antioxidants are intimately associated because antioxidants defend and sustain the functionality of cells against oxidative damage. It has been demonstrated that there is a strong association between dietary antioxidant consumption and defense against diabetes [97] since diet-derived antioxidants are essential for the prevention and treatment of many diseases[98].

The primary enzyme in saliva that breaks down 1,4-glycosidic bonds in starch is called α-amylase. Postprandial hyperglycemia is usually managed by suppressing it with the unfavorable synthetic α-amylase inhibitor acarbose [99, 100]. The existence of monoterpenes including Thymol, limonene, and α-pinene has been shown to have considerable inhibitory effects on the activities of α-amylase and α-glucosidase [100]. α-amylase inhibition appeared to be unsuccessful according to the testing of *M. communis* essential oils and extracts on the carbohydrate hydrolyzing activity of α-amylase in contrast to acarbose. Plant EO and extract from Jenin were the most active, with IC_{50} values of 950.48±2.54 and 795.43±1.88µg/mL, respectively, while EO and extract from Jericho had no effect. Ibrahim et al.[101] found that *M. communis* EO from Egypt had dose-dependent α -amylase inhibition activity, with the highest preventing activity being observed at 1000, 750, and 125 g/mL, 96.22.140, 83.20162, and 36.24146µg/mL, respectively, in comparison to the acarbose standard, which was 88.810.69, 78.950.917, and 23.160.190µg/mL

4.4 Anti-bacterial activity

The antibacterial activity of two essential oils (EOs) and four extracts was assessed against six bacterial strains (the most prevalent pathogenic species) and one fungus strain using the broth micro dilution method on Mueller-Hinton agar. The EOs and extracts had antibacterial activities, according to the results (table 3.4). Except for *P. aeruginosa*, where both *M. communis* essential oils from Jericho and Jenin exhibited equivalent levels of activity, *M. communis* essential oil from Jericho showed higher levels of antimicrobial activity against all of the bacterial strains tested (table 3.4). The Jericho essential oil showed stronger antibacterial activity against *MRSA, P. vulgaris*, and *K. pneumonia* with a MIC value of 0.135 mg/ml. The gram-negative bacteria P. vulgaris, on the other hand, was most vulnerable to the Jenin-derived *M. communis* EO (MIC = 0.56 mg/mL). According to data from the literature, *P. aeruginosa*, a Gram-negative bacteria, was the most resistant to *M. communis* extracts and EOs. A number of studies from all around the world evaluated *M. communis* EO and extracts against Gram-positive, Gram-negative bacteria, and fungi. According to Ghasemi et al., Iranian-origin *M. communis* EO showed more activity against *C. albicans* (MIC = 0.036 mg/mL) than *E. coli* (MIC = 10 mg/mL) [87]. *M. communis* leaves EO [102] from Tunisia, of which 1,8-cineole constitutes half of the EO, shown moderate antibacterial activity against all examined bacterial strains, including *E. coli* and *K. pneumoniae*, with MIC values in the range from 12.5 to 25 mg/ml. Hsouna et al. [103], on the other hand, reported that Tunisia's *M. communis* leaves EO, of which myrtenyl acetate (20.75%), 1,8-cineole (16.55%), and α-pinene were the major, exhibited high antibacterial activity against *E. coli* and *K. pneumoniae*, as well as *P. aeruginosa*, with MIC values of 2.5, 2.5, and 1.

According to Touaibia et al. [77], M. communis leaves EO from Algeria included limonene (12.93%), octadienol (12.85%), and α-pinene (10.01%) that had antibacterial activity against *E. coli, K. pneumonia,* and *P. aeruginosa* and MIC values of 1.125, 4.5, and 18 mg/mL, respectively.

The oxygenated terpenes present in myrtle oil, including 1,8-cineole, linalool, and -terpineol, have been shown by Randrianarivelo et al. [104] to have strong antibacterial properties. *M. communis* leaves EO from Tunisia showed interesting antimicrobial activity against Gram negative bacteria like *P. aeruginosa* and *E. coli* with inhibition

zones between 18 and 20 mm and moderate antibacterial activity against Gram positive bacteria but no antifungal activity against *C albicans* [105]. The main components of *M. communis* leaves EO are α-pinene (35.6%), 1,8-cineole (29.6%), linalool (6.87). On the other hand, Mahboubi et al. found that leaves EO from Iran with 1,8-cineole (36.1%), α-pinene (22.5%), linalool (8.4%), bornyl acetate (5.2%), and α-terpineol (4.4%) exhibited good antifungal effect against fungi with a MIC value of 8 mL/mL [106]. With MIC values of 2 L for *C. albicans* and 4 and 8 L/mL for bacteria, respectively, Yadegarinia et al. [106]demonstrated that Iranian myrtle essential oil, which includes α-pinene, limonene, 1,8-cineole, and linalool, was more efficient against *C. albicans* than *E. coli* or *S. aureus*. EO from *M. communis* leaves with high concentrations of α-pinene, 1,8-cineole, and linalool exhibited a strong inhibitory effect against *C. albicans*, according to Ebrahimabadi et al. [107], with MIC values ranging from 1.0 to 2.0 mg/ml.

According to research by Aboutabl et al. [108], *M. communis* leaves EO from Egypt, which has eugenol (35.5%), 1,8-cineole (27.2%), and limonene (21.8%), shows antifungal activity towards *C. albicans* with a MIC value of 100 L/ml. Together, these characteristics are referred to as the "essential oils versatility" [105]. The mechanism of action of EOs tends to depend on their chemical makeup, and their antimicrobial activity is not attributable to a single mechanism but rather is a cascade of reactions involving the entire bacterial cell. In general, EOs function to stop the development of bacterial cells as well as the generation of harmful metabolites. Most EOs have a stronger effect on Gram-positive bacteria than Gram-negative bacteria, and this effect is most likely caused by variations in the compositions of the cell membranes.

With MIC values ranging from 0.195 to 0.78 mg/mL, the methanol extracts of *M. communis* from Jenin and Jericho in Palestine shown higher antibacterial activity against *MRSA* and *S. aureus* than the EOs from both cities. With corresponding MIC values of 0.195 and 0.78 mg/mL, the methanol extracts from Jenin were four times more active than those from Jericho. Jericho ethyl acetate extract revealed considerable antibacterial activity with MIC values of 0.097 mg/mL for both *MRSA* and *S. aureus*. The ethyl acetate extract, in contrast to other extracts, showed antibacterial activity against the final four tested microorganisms. The two EOs from Jericho and Jenin with MIC values of 1.08 and 1.13 mg/mL, respectively demonstrated less antifungal activity against *C. albicans* than any of the other tested extracts. Our findings are consistent

with a portion of the data that have already been published in the literature. Methanol and plant leaf extract in aqueous form shown antibacterial efficacy against *MRSA*, *S. aureus*, and *P. aeruginosa* with MIC values of 0.781 mg/mL [109].

The antibacterial activity of a methanol crude extract of *M. communis* L was tested against 10 bacterial strains, including *S.aureus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*, and Mansouri et al. [88]discovered that the MIC ranged from 0.1-2 mg/mL, which is consistent with our findings. The soluble component of methanol in ethyl acetate was able to kill *S. aureus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa* with MIC values of 0.1, 0.1, 0.8, and 1.5 mg/mL, respectively. *M. communis* extract has active components that function as antimicrobials, which can be used to destroy pathogens. The leaves of *M. communis* are known to include polyphenolic substances, tannins, and flavonoids [109]. The inhibition zones of the Moroccan *M. communis* methanolic extract ranged from 19.66 0.57 to 12.33 0.57 mm at doses of 2.5-0.32 mg/disc. According to Messaoud et al., an Iranian plant leaf extract in methanol had an antibacterial impact against a variety of bacteria, including *S aureus* and *K. pneumoniae*, with MIC values ranging from 12.5 to 25 mg/mL 19. MIC ranged from 0.5 mg/ml to 45 mg/ml in a different research. These findings demonstrated the potent antibacterial properties of Myrtle plant 20.

4.5 Anticancer activity

There haven't been any published research on the potential cytotoxic effects of *M. communis* EO on the cancer cell lines 3T3 and LX-2 [87, 110-112]. HeLa, MCF-7, 3T3 fibroblast, and hepatic stellate cell lines were used as in vitro test subjects for the essential oils from *M. communis*. (LX-2) Cells were treated to the following concentrations of each of the EOs during 24 hours: 0, 15, 65, 31, 25, 62.5, 125, 250, and 500 μg/ml. EOs decreased cell viability by 75% to 91% at a concentration of 500 μg/ml. The proliferation of the 3T3, MCF-7, and HeLa cell lines was inhibited by Jenin EO, as shown in Table 3.5, with respective IC_{50} values of 215.25±1.07, 592.40±2.55, and 597.01±3.11 μg/ml. Jericho EO had IC_{50} values between 644.47±2.89 and 914.54±3.05 μg/mL against the same cell lines, with the 3T3 cell line being the most sensitive. The increased cytotoxicity of the Jenin EO in comparison to the EO from Jericho is presumably brought on by larger amounts of oxygenated monoterpenes, such as 1,8-cineole and α-pinene. After giving a commercial myrtle EO a 24-hour test, Scazzocchio

et al. observed no harm on HeLa cells [110]. After 72 hours, the HT29 cell line tested with *M. communis* EO from Yemen had an IC_{50} of 110 g/mL [111]. After 48 hours, Harassi et al. observed significant cytotoxicity of two Moroccan myrtle EOs against MCF7 and P815 cells, with IC_{50} values for MCF7 ranging from 4.0 to 6.25 μg/ml and for P815 from 53.9 to 260 μg/ml. Caputoi et al. reported that *M. communis* EO from Salerno, Italy, exhibited substantial cytotoxicity against SH-SY5Y cells, with an IC_{50} of 209.1 μg/ml. However, our IC_{50} number was greater than 20 g/ml, which meant that the essential oil did not meet the National Cancer Institute's standard, which stated that only naturally occurring compounds should meet the IC_{50}.

4.6 Conclusion

In this study, I extracted the essential oils from the leaves of Jericho and Jenin Myrtles using the Clevenger equipment method. I then collected two methanol extracts and two ethyl acetate extracts from both EO. Then the study looked at the chemical composition of myrtle that was gathered from Jenin and Jericho. A total of 41 substances were found in *Myrtus* from Jericho. The main ones were cis-4-Thujanol , 1,8-cineole, myrtenol, myrtenal, and trans-4-Thujanol acetate. There were 37 different substances found in the Jenin *Myrtus*, with 1,8 cineole, linalool, trans-4-Thujanol acetate, α-pinene, Myrtenal, and α-terpineol being the main ones. While the antioxidant activity of both essential oils was relatively moderate, Jericho and Jenin Ethyl Acetate extracts surpassed the positive control. Strong antioxidant activity was found in plant leaf extracts made with trolox and methanol. The most effective α-amylase inhibitory agents were *myrtus* EO and extract from Jenin, whereas EO and extract from Jericho had no effect. Gram-positive bacteria are more sensitive to both EOs than Gram-negative bacteria, but the methanol extracts from Jenin were four times more potent than those from Jericho. The antifungal activity of the two EOs against *C. albicans* was lower than that of any other studied extract. Jenin EO has higher antioxidant activity comparison to the EO from Jericho is presumably brought on by larger amounts of oxygenated monoterpenes, such as 1,8-cineole and α-pinene

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جامعة النجاح الوطنية

كلية الدراسات العليا

تباينات المكونات الكيميائية والأنشطة الدوائية للزيوت العطرية الأساسية لنبتة الآس من منطقتين في فلسطين

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

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الملخص

الخلفية: أحد أسباب تزايد الاهتمام بالنباتات الطبية يومياً هو فكرة أن بعض النباتات لديها القدرة على علاج مجموعة متنوعة من الأمراض دون أن يكون لها أي آثار جانبية سلبية، لطالما استخدم نبات الآس في العديد من العلاجات التقليدية في منطقتنا.

الأهداف: في هذه الدراسة، سيتم فحص الملامح الكيميائية لزيت الآس الأساسي (EO) الذي تم جمعه في أريحا وجنين، إلى جانب تأثيراته المضادة للأكسدة، ومضادات الأميليز، ومضادات الميكروبات، ومضادة للسرطان.

المنهجية: تم إنشاء زيت نبات الآس الأساسي (EO) عن طريق استخراج التقطير المائي، وتم تحديد المكونات الكيميائية لـــ EO نوعاً وكماً باستخدام GC–MS.

النتائج: تم العثور على واحد وأربعين مادة كيميائية في زيت اريحا الاساسي، أبرزها cis–4–Thujanol (27.37٪ (24.32) ٪1,8–Cineole (، 12.97) ٪Myrtenol ((12.46) ٪Myrtenal ،–trans (9.48%) 4–Thujanol acetate)، كما تم العثور على 37 مادة كيميائية مختلفة في جنين EO، منها 31.55) ٪1,8–cineole،(21.65) ٪linalool (، 11.26) ٪Trans–4–thujanol acetate (، α– 10.22) ٪pinene (، 6.78) ٪Myrtenal و α–4.35) ٪terpineol() وهي الأكثر وجودا.

النشاط المضاد للأكسدة في الزيوت الاساسية (Eos) تم تقييمه عن طريق DPPH، وتفوقت مستخلصات ethyl acetate extract لزيوت أريحا وجنين على trolox (IC50 من 10.25 ± 1.02 ميكروغرام / مل) والتي اظهرت قيم 2.31 ± 8.55 IC50 و 3.60 ± 0.35 ميكروغرام / مل ، على التوالى.

الزيت الاساسي لجنين اظهر نشاط مضاد للأكسدة أعلى مقارنةً بـــ الزيت الاساسي لأريحا، يُفترض أن يكون السبب أنه يحتوي على كميات أكبر من monoterpenes المؤكسد، مثل 1,8-cineole و-α pinene.

الزيت الاساسي لجنين كان من أكثر العوامل المثبطة لفاعلية α-amylase بقيم IC50 2.54 ± 950.48و 795.43 ± 1.88 ميكروغرام / مل على التوالي، في حين أن الزيت الاساسي و ethyl acetate extract وmethanol extract المستخلصه من أريحا لم يكن لهما أي تأثير.

تم اختبار النشاط المضاد للورم باستخدام مقياس MTS، واستخدمت أربعة عينات من الخلايا السرطانية هي: عنق الرحم البشري (هيلا)، والثدي (MCF-7)، والأورمات الليفية لجنين الفأر (T33)، والخلايا الكبدية الطبيعية (LX-2)، كخلايا طبيعية لاختبار النشاط المضاد للتكاثر. الزيت الاساسي لجنين اعطى قيم IC بين 215.25 ± 1.07 و597.01 ± 3.11 ميكروغرام / مل، بينما الزيت الاساسي لاريحا اعطى قيم بين 644.47 ± 2.89 و914.54 ± 3.05 ميكروغرام / مل.

النشاط المضاد للبكتيريا تم فحصه باستخدام تقنية microdillution, البكتيريا موجبة الجرام أكثر حساسية في كلا الزيتين الاساسيين من جنين واريحا EOs من البكتيريا سالبة الجرام. يحتوي كلا المستخلصين على فعالية مضادة للفطريات أقل من أي مستخلص آخر قيد الدراسة.

الكلمات المفتاحية: نبات الآس، زيت عطري، التركيب الكيميائي، المكونات النشطة بيولوجيا، فلسطيني.