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Biological Activities and Nutritional Composition of Essential Oil from *Rosmarinus officinalis* L. Leaves

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Dedication

I thank Allah for giving me the strength to carry on this project.

I dedicate my thesis to:

My father and mother, special thanks for helping me and always staying by my side.

My sister and my brothers and their wives and children, I love you all and I am blessed to have such a supportive family.

At last, I dedicate this research to some people in my life who touch my heart.

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List of Abbreviations

°C	Celsius
µl	Microliter
AAS	Atomic absorption spectrometry
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
AI	Adequate Intake
ATP	Adenosine triphosphate
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxytoluene
Ca	Calcium
Cm	Centimeter
Conc.	Concentration
D.W	Distilled water
DPPH	2, 2'-Diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
EU	European Union
Fe	Iron
G	Gram
G.A	Gallic acid
GAE	Gallic acid equivalents
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
Ib	Pounds
ICP-OES	Inductively coupled plasma optical emission spectrometry
K	Potassium
MF	Molecular formula
Mg	Magnesium
Mg	Milligram
mg GAE/g	Milligrams of Gallic acid equivalent /gram
mg/kg	Milligram/kilogram
Min	Minute
Ml	Milliliter
Mm	Millimeter
Mn	Manganese
MW	Molecular weight
Na	Sodium
NIST	National Institute of Standards and Technology
P	Phosphorus
P. aeruginosa	*Pseudomonas aeruginosa*
RE	Rosemary Extract
RNS	Reactive nitrogen species

ROS	Reactive oxygen species
Rt	Retention time
S. aureus	*Staphylococcus aureus*
TG	Triglyceride
UV/Visible spectrophotometer	Ultraviolet-visible spectrophotometer
WHO	World Health Organization

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Abstract

Herbal medicine in Palestine is very popular. One of the most used herbs is rosemary. However, its use is linked to inherited traditions more than its reliance on scientific research. It has become known that rosemary in particular contains secondary metabolites that have wide uses in folk medicine and in the food industry as a flavoring and preservative. In this scientific research, methanolic extracts of rosemary plant leaves from different locations were evaluated for volatile compounds using GC-MS analysis, antioxidant activities were estimated by DPPH and ABTS methods, antibacterial activities were examined by disk diffusion method, and mineral content of dry leaves, using an ICP-OES device. A number of volatile compounds have been identified in rosemary leaf extract (80%, and 90% at both room temperature and reflux conditions). The main constituents in all studied samples of rosemary were Eucalyptol and Camphor respectively. Phytochemical analysis of the rosemary leaves revealed the presence of phytochemical compounds such as cardiac glycosides, phenolic groups, coumarin, saponins, steroids, tannins, and terpenoids in all samples. The methanolic extracts of rosemary leaves of the samples studied which were collected from all regions, showed a scavenging capacity of range 73.25%-76.36%, using DPPH assay and 73.91%-88.82% using ABTS assay. Furthermore, total phenolic content (TPC) in which they presented higher values with rosemary leaves in Umm Lasfah village (876.7 mg GAE/g) has the highest percentage of total phenols, followed by Khilt Al-Adrah (728.3 mg GAE/g), Raqaa (693.3mg GAE/g), Hebron (652.8 mg GAE/g), and Bani Naim (616.7 mg GAE/g). The antibacterial studies using the disk diffusion method showed that the methanolic extracts (80%, and 90% at both room temperature and reflux conditions) of rosemary have an inhibition zone against *K. pneumonia*, *E. coli*, *and S. aureus*. The most interesting result was 80% methanolic extract at room temperature in all samples compared with the methanol control. The rosemary leaves collected were discovered to be rich in minerals, especially calcium and potassium in all regions tested. The antioxidant and antibacterial activities of rosemary indicate that this plant could be a promising antioxidant and potential antibacterial remedy. According to our knowledge, this study was the first to screen and evaluate phytochemical compounds in rosemary plant Palestinian for their antibacterial and antioxidant activities.

Chapter One

Introduction

Chapter One: Introduction

1. Introduction

1.1 Medicinal plants and traditional medicine.

The first written evidence for the use of medicinal plants dates back to 5,000 years ago and was found on a Sumerian clay slab from Nagpur, which contains more than 250 plants (Petrovska, 2012). Medicinal plants are currently in high demand, and their popularity is increasing due to their high efficacy, fewer side effects, and low cost (Oga *et al.*, 2016). It can be said that before history the first humans realized the use of medicinal plants as fuel, clothing, shelter, and food (Pal and Shukla, 2003). The World Health Organization (WHO) estimates that the use of medicinal plants worldwide exceeds the use of traditional medicines by two to three times (Tilburt and Kaptchuk, 2008). Traditional medicinal herbs are natural plant materials used for disease treatment on a local or regional level in the presence or absence of industrial processing (Jamshidi-Kia *et al.*, 2018). Traditional medicines have a great reservoir of medicinal plants. Traditional medicine is widely used in China, India, Japan, Pakistan, Sri Lanka, and Thailand (Dar *et al.*, 2017; Batool *et al.*, 2020). In France and Germany, it is estimated that 70% of all doctors regularly prescribe herbal medicines (Pal and Shukla, 2003).

Medicinal plants include a wide range of natural antioxidants and are used to cure a variety of diseases all over the world. Due to the positive benefits of natural antioxidants in the treatment and prevention of illnesses such as cancer, diabetes (Shirzad *et al.*, 2009), atherosclerosis, heart disease (Mohammad *et al.*, 2013), nephrotoxicity, hepatotoxicity, cognitive and vision loss (Bartlett and Eperjesi, 2007). There is a lot of interest in discovering natural antioxidants from plants. According to studies on medicinal herbs, the majority of them have substantial antioxidant activity (Rafieian-Kopaei *et al.*, 2013). Traditional medicine is considered part of the popular culture and religious beliefs in Palestine (Jaradat, 2005). Traditional medicine is commonly used in the West Bank regions of Palestine, particularly in rural areas; this may be due to political conflict and the high cost of traditional medicines (Jaradat *et al.*, 2017). Traditional remedies were empirically applied for many decades in Saudi Arabia, and throughout Asia, to treat various diseases. Therefore, medicinal herbs are frequently used in

traditional medicine and are commonly used as a routine treatment and home remedy (Dzoyem *et al.*, 2013; Mosleh *et al.*, 2014).

1.2 Flora in Palestine

Flora Palestine is rich in economic plants such as vegetables, field crops, fruit trees, and medicinal plants (Ighbareyeh *et al.*, 2021). Although Palestine is small in size, it is rich in flora due to its geographical position as a meeting point between Asia and Africa. The number of plant species found in historical Palestine is estimated to be about 2655 species, in which 1591 species were recorded in the West Bank (Al Sheikh and Mahassnehb, 2017). Palestinian areas face threats to biodiversity due to Israeli colonial activities. The plants most in danger are estimated to be about 600 species within the West Bank may be a worry (Al-Sheikh and Qumsiyeh, 2021).

1.3 Rosemary

Rosemary was named herb of the year by the International Herb Association (Begum *et al.*, 2013). *Rosmarinus officinalis* L. is a member of the Lamiaceae family and is often known as rosemary. It is native to the Mediterranean region and is grown in various European countries as well as the United States (Minaiyan *et al.*, 2011). The Latin term Rosmarinus which means sea dew is the source of the name rosemary (Ribeiro-Santos *et al.*, 2015). Rosemary is a perennial woody herb of 90 - 200cm in height, with small (2-4cm) pointed and hairy leaves and a small blue flower. The leaves of rosemary are dark green and elongated, while the flowers are white or purple (Hanson, 2016; Neves *et al.*, 2018). **Figure (1.1)** below shows the Rosemary plant.

Rosemary is commonly used as a spice in cooking and traditional medicine (Andrade *et al.*, 2018). It is also used in cooking as a flavor, food preservation (Berdahl and McKeague, 2015), cosmetics (Ghule & Ghule, 2020), and traditional medicine for its anti-inflammatory (Benincá *et al.*, 2011), diuretic, and antibacterial properties (Bozin *et al.*, 2007), as well as for the prevention and treatment of diabetes (Selmi et al., 2017), cancer (Moore *et al.*, 2016), and cardiovascular disease (Ribeiro-Santos *et al.*, 2015). Rosemary has been used in both culinary and medicinal uses for thousands of years due to its aromatic qualities and health benefits

(Ribeiro-Santos *et al.*, 2015). It is used to relieve stomach cramps and flatulence (Anadón *et al.*, 2008), as well as to improve appetite and gastric juice secretion (Veenstra & Johnson, 2021). It is beneficial for headaches and nerve issues (Heinrich *et al.*, 2006). It also alleviates muscular aches and joint troubles (Amin *et al.*, 2012). It is used to treat depression, migraines, and liver and digestive issues. An ointment prepared from the leaves of the plant is used to treat neuralgia, rheumatism, and eczema, and to heal small wounds (Charles, 2012).

Figure 1. 1: Rosemary plant

The medicinal value of rosemary is due to the high bioactive compounds present in the plant such as triterpenes, tricyclic diterpenes, phenolic acids, and essential oils (De Macedo *et al.*, 2020; Jeevalatha *et al.*, 2022). Some of the most active compounds in rosemary are caffeic acid, rosmarinic acid (RA), ursolic acid (UA), carnosic acid (CA), and carnosol (Miroddi *et al.*, 2014; Andrade *et al.*, 2018).

1.4 Biosynthesis of rosemary components

The terpenoids or mevalonate pathway produces diterpenes and so have a repeated 5-carbon backbone skeleton, isoprene unit(s) (Nagegowda & Gupta, 2020). Isopentenyl pyrophosphate and dimethylallyl pyrophosphate are two recognized isoprene-building components that polymerize head-to-tail to make the 20-carbon diterpene precursor (4 isoprene units) called geranylgeranyl pyrophosphate. The only diterpene class found in Rosemary is the abietane type (5–7), which is made of a six-membered tricyclic ring structure, one of which is fragrant (Habtemariam, 2016; Brückner et al., 2014).

1.5 Minerals in rosemary

The plant can accumulate several mineral elements such as calcium (Ca), phosphorus (P), potassium (K), sodium (Na), s iron (Fe) and zinc (Zn) that are necessary for human nutrition (Huang *et al.*, 2020). So heavy metal analysis is an important aspect of quality control to ensure that plant materials are not contaminated with toxic metals, such as cadmium, lead, aluminum, and mercury due to their negative impact on human health (Tchounwou *et al.*, 2012; Mosleh *et al.*, 2014). Based on study, the rosemary plant has the highest content of K and Ca minerals (Kiczorowska *et al.*, 2015; Hejaz *et al.*, 2022).

1.6 Pharmacological activities of rosemary

Phytochemicals found in medicinal plants include flavonoids, alkaloids, tannins, and terpenoids, which have antibacterial and antioxidant properties (Akhtar & Mirza, 2018; Gonelimali *et al.*, 2018). Rosemary extracts from dried rosemary leaves are of large interest to the food and drug industries because they have several health benefits, including antioxidant, antibacterial, anti-inflammatory, and anti-cancer properties (Johnson, 2011; Sabbobeh et al.,

2016; Lešnik *et al.*, 2021). As we aforementioned, there are many pharmacological activities of rosemary, however, we will concentrate on two of them:

I) Antimicrobial activity: Infectious illnesses are the leading cause of morbidity and mortality all over the world (Cohen, 2000). According to the World Health Organization, 55 million people died globally in 2011 (Liu *et al.*, 2017). The antimicrobial activities of some plant species that have been widely researched exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Gonelimali *et al.*, 2018). Many types of bacteria, including E. coli, Staphylococcus aureus, Candida albicans, and Saccharomyces cerevisiae, can be efficiently inhibited by rosemary extracts (Dai and Liu, 2021). Rosemary inhibits the growth of bacteria that cause illness and delays the growth of bacteria (Kloy *et al.*, 2020). Antimicrobial action is mostly caused by α-pinene, bornyl acetate, camphor, and 1,8-cineole present in medicinal plants (Genena *et al.*, 2008).

II)Antioxidant activity: Antioxidants are chemicals that aid in the protection of other molecules from oxidation (Singh *et al.*, 2004; Ginsburg and Maleky, 2020). There are two main types of antioxidants: water-soluble antioxidants, which are more effective in protecting aqueous solutions from oxidation, and fat-soluble antioxidants, which prevent lipid oxidation in lipid-containing foods by preventing free radicals from reacting with fatty acids, quenching oxygen, and/or chelating metal ions (Ginsburg and Maleky, 2020). A free radical is an atom or molecule with one or more unpaired electrons in a valency shell or outer orbit with the ability to exist independently (Mozaffarieh *et al.*, 2008; Phaniendra *et al.*, 2015). Free radicals and other reactive oxygen species (ROS) are produced by the human body's regular metabolic processes or by external sources such as X-rays, ozone, cigarette smoking, air pollution, and industrial toxins (Lobo *et al.*, 2010; Wansutha *et al.*, 2018). This unstable configuration generates energy, which is released through reactions with nearby molecules such as proteins, lipids, carbohydrates, and nucleic acids. The vast majority of free radicals that harm biological systems are oxygen-free radicals, also known as "reactive oxygen species" (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) (Rahman, 2007; Ray *et al.*, 2012). Rosemary is high in lipid-soluble antioxidants, particularly carnosic acid, which has been proven to be a powerful antioxidant (Birtić *et al.*, 2015). The principal antioxidant components in rosemary are phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, epiand iso-rosmanol, rosmadial and methyl carnosate (Pérez *et al.*, 2007).

1.7 Analytical methods for the analysis of Rosemary

1.7.1 Gas Chromatography (GC)

Chromatography is a technique that separates components in a mixture by the difference in partitioning behavior between mobile and stationary phases (Mukadam *et al.*, 2021). Gas chromatography (GC) was historically introduced originally by James and Martin in 1952. GC is a technique for separating, identifying, and quantifying volatile chemicals with boiling temperatures as high as 350^{o}C or 400^{o}C (Teonata *et al.*, 2021). The mobile phase in GC is gas, and the stationary phase is liquid or solid (Mukadam *et al.*, 2021). A gas chromatograph employs a path through a thin tube known as the column containing a stationary phase, through which the mobile phase containing different chemical constituents of a sample pass (mobile gas) (Kondeti *et al.*, 2014; Pravallika, 2016). In GC, the sample is initially injected in the injector and then vaporized in the chromatographic column, where it passes through the column with the flow of inert gas, resulting in the separation of the sample's components, which are recorded as a sequence of peaks as they exit the column and reach the detector. The various components of the sample are separated and eluted at different and specific times, which is referred to as retention time (Pravallika, 2016). There are a few types of GC such as gas chromatography-mass spectrometry (GC/MS), gas chromatography-olfactometry (GC/O), gas chromatography-flame ionization (GC/FID), and gas chromatography-time-of-Flight (GCTOF) (Sneddon *et al.*, 2007; Cajka, 2013; Teonata *et al.*, 2021). GC-MS is an important tool for identifying and quantifying organic compounds in environmental samples (Santos and Galceran, 2003).

1.7.2 Mass Spectrometry (MS)

Mass spectrometry is an analytical method used to find new compounds, determine amounts of known components, and determine a molecule's structural and chemical characteristics (Noriega *et al.*, 2021). It is a technique for determining the mass-to-charge ratio of ions. This is done by using a mass spectrometer, which is a device that is used to produce a spectrum of the masses of the ions (Finehout & Lee, 2004). Gas chromatography coupled with mass spectrometry (GC-MS) is considered the standard of excellence for the analysis of many compounds such as lipids, drug metabolites, and environmental contaminants (Garcia and Barbas, 2011).

1.7.3 Inductively coupled plasma optical emission spectrometry

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) is an analytical technique that is used to identify the atomic composition of a particular sample (Habte *et al.*, 2016). It is primarily used for the analysis of an element in different samples like environmental, industrial, plant, tissue, and pharmaceutical samples (Khan *et al.*, 2022). The ICP-OES principle uses the fact that atoms and ions can absorb energy to move electrons from the ground state to an excited state (Balaram, 2019). Samples are fused, and dissolved in a solvent before being aspirated into the plasma flame. The element-specific light emitted by the plasma is then viewed radially (through the side of the plasma) or axially (through the length of the plasma) plume and analyzed by a multi-element spectrometer and detector (Ghosh *et al.*, 2013; Khan *et al.*, 2022).

1.8 Problem statement and motivation of the study

A few studies about Palestinian herbs are available, but their composition, efficacy, and safety are still unexplored. Also, due to the undesirable side effects of orthodox synthetic medications such as toxicity and carcinogenicity and the emerging microbial resistance to available antimicrobial agents, attention has considerably increased to find out naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine.

There are rare studies in Palestine that have shown the impact of *Rosmarinus officinalis* L. on the general health status and enhancement of therapeutic properties (Jarrar *et al.*, 2010; Al-Maharik *et al.*, 2022). Investigating the availability degree of antioxidants, minerals, antibacterial, and other polyphenols was not carried out in Palestine. However, we propose to evaluate and compare the antioxidants, phytochemicals, anti-microbial, nutritional composition, and biological potential of rosemary leaves. *Rosmarinus officinalis* L. is chosen for this study because of its medicinal reputation among Palestinians and the common use by them. The lack of phytochemical composition of its volatiles, semi volatile, and minerals and the scarcity of pharmacological studies such as antioxidant and antimicrobial motivated this research.

1.9 Aim of the study

This study aims to screen Palestinian *Rosmarinus officinalis* L. secondary metabolites and minerals by using ICP-OES and to examine some of their claimed pharmacological activities. The tests will include anti-oxidant and anti-bacterial biological activities.

1.10 Objectives of the study

1. To extract leaves *of Rosmarinus officinalis* L. by methanol to analyze GC-MS technology using the electron impact (EI) mode.

2. To estimate the anti-oxidant activity of *Rosmarinus officinalis* L. leaves using a spectrophotometric method.

3. To estimate antimicrobial activity of the leaves on selected bacteria in comparison with positive controls.

4. To study the *Rosmarinus officinalis* L. leaves minerals content by ICP-OES.

Chapter Two

Literature Review

Chapter Two: Literature Review

2.1. General aspects

2.1.1. Rosemary classification.

- **Kingdom:** *Plantae*
- **Division:** *Magnoliophyta*
- **Class:** *Magnoliopsida*
- **Order:** *Lamiales*
- **Family:** *Lamiaceae*
- **Genus:** *Rosmarinus*
- **Species:** *Officinalis*
- **Binomial nomenclature:** *Rosmarinus officinalis* L. (Andrade *et al.*, 2018)

2.1.2. Origin and distribution

Rosemary is native to the Mediterranean region and widely planted in Europe, America, and Asia (Uritu *et al.*, 2018; Stefanaki & van Andel, 2021).

2.1.3. Production areas

Rosemary is cultivated in Algeria, China, France, Hungary, Italy, the Middle East, Morocco, Portugal, Russia, Serbia and Montenegro, Spain, Tunisia, Turkey, the United States, and to a lesser amount in India (Sasikumar, 2012; Datiles & Acevedo-Rodríguez, 2014).

2.1.4. Description of the plant

Rosemary is a perennial aromatic herb growing to a height of 60-200 cm (Sasikumar, 2012; Selvaraj *et al.*, 2022). known as Rosemary (English), Alecrim (Portuguese), and Romero (Spanish) in popular culture (Borges *et al.*, 2019). The leaves are opposite, leathery, sharply recurved, and have fringed edges. The leave is 4 cm in width and 1.0–2.5 cm in length. The upper side of the leaf is green, while the undersurface is grayish and woolly (Begum *et al.*, 2013). The small flowers of Rosemary can be light blue or lilac. the leaves and flowers have a distinct aromatic odor (Ribeiro-Santos *et al.*, 2015). In the Mediterranean environment, the flowering time occurs between May and June, while the fruiting period occurs between spring and summer (Borges *et al.*, 2019).

2.1.5. Rosemary varieties

The rosemary plant is available in almost 20 varieties. Following are the numerous varieties of rosemary: upright rosemary, creeping rosemary, pine-scented rosemary, arp rosemary, madalene hill rosemary, pink rosemary, dancing waters rosemary, golden rain rosemary, blue boy rosemary (Hameed and Mohammed, 2017).

There are just three species in the genus *Rosmarinus* includes *Rosmarinus Officinalis*, *Rosmarinus eryocalix*, and *Rosmarinus tomentosus*. Each of the three species is a diploid chamaephyte with 2n = 24 chromosomes. (Hernández *et al.*, 2016; Hammer and Junghanns, 2020).

2.1.6. Environmental requirements

2.1.6.1. Climacteric requirements

Temperature: The rosemary plant is hard and temperate that can tolerate frost very well. However, it cannot grow at temperatures below 3 °C. It grows best in places with average temperatures ranging from 20 to 25° C (Tigist *et al.*, 2016; Aziz *et al.*, 2021).

Soils: This plant grows in dry or mildly humid soil with a pH range of 5.5 to 8.0; it does not grow in high clay soils, and it can tolerate salinity to some extent (Tigist *et al.*, 2016; Borges *et al.*, 2019).

Irrigation: Irrigation is required during the transplanting process until the plants are fully established. Rosemary may be cultivated in rainfed conditions if the annual rainfall is above 500 mm, and under these conditions, planting should be done at the beginning of the rainy season. In irrigated conditions, depending on the availability of water, watering should be done once every three days for the first three weeks following transplanting. Thereafter, irrigation can be done once a week or every two weeks (Tigist *et al.*, 2016).

2.2. Cultivation practices

2.2.1. Plant Propagation

Rosemary may be propagated using cuttings, transplants, and seeds. It grows best from cuttings or transplants. while seed propagation is slower, their germination rate is typically just about 15% (Masabni and King, 2014; Lešnik *et al.*, 2021). The cutting from the mother plant is ideal for the vegetative propagation of Rosemary plants. The mother plants used to prepare the cuttings should be free of disease, and the branches should be at least 9 months old but no older than 1 year (Tigist *et al.*, 2016). The cuttings are first planted in sand beds in a protected

nursery. Watering regularly is required for good germination. Planting cuttings in the main field takes 45-50 days (Sasikumar, 2012).

2.2.2. Weed control

Weed control in rosemary cultivation is important for the production of good yields and high-quality produce. Automated or hand methods can be used to remove the weed. We must be cautious to protect the rosemary roots when using mechanical methods (http://projectcult.com/wp-content/uploads/2016/11/PRIRACNIK_EN_.pdf). Weed management in rosemary is accomplished by hand weeding (Sasikumar, 2012).

2.2.3. Diseases

Although rosemary resists most diseases, the rapid breakout of disease and pests may happen as a result of weather changes (Tigist *et al.*, 2016). The most main diseases that harm rosemary are collar and root rot, foliar necrosis/leaf spot, aerial blight, hook disease, and powdery mildew. To avoid disease transmission, examine the plants regularly and spray appropriate fungicides as needed. Disease incidence can be reduced by pruning plants to enhance air circulation within them (Masabni and King, 2014).

2.2.4. Harvesting

The harvesting time of rosemary varies based on soil fertility, geographical location, and climatic conditions (Tigist *et al.*, 2016). The first harvesting is carried out about eight months after planting (Sasikumar, 2012). Rosemary may be harvested three to four times annually (Berdahl and McKeague, 2015). Harvest rosemary branches approximately 15 cm above the ground, leaving growth points (nodes) below the cutting point. Plants will die if harvesting is not done correctly (Tigist *et al.*, 2016).

2.2.5. Post-harvesting handling

After harvesting, rosemary leaves can be dried before or after separating the leaves from the gathered branches. The quality of the final product is greatly affected when the leaves are mixed with foreign substances during the drying process, which may either decrease the market price of the product or cause the product to lose market acceptance. Rosemary should be dried at temperatures lower than 40 °C to avoid flavor loss due to essential oil volatilization and to keep a healthy green color (Tigist *et al.*, 2016). The best traditional method of drying in a well-ventilated room produced the highest quantity and purity of the antioxidant principles of rosemary (Lešnik *et al.*, 2021).

Rosemary can be sold directly to the market as fresh. It should then be packed suitably. Dry rosemary can be stored in glass or plastic containers as a food seasoning. the storage temperature should not exceed 18 degrees Celsius (http://projectcult.com/wp-content/uploads/2016/11/PRIRACNIK_EN_.pdf).

2.3. Food, Medicinal and therapeutic

2.3.1. Rosemary as food

Rosemary leaves have been used in their dried form in cooking for ages (Aguilar *et al.*, 2008; El-Sayed & Youssef, 2019). Both fresh and dried, are used as spices in soups, stews, sausages, roast lamb, fish, pickles, season fried chicken and poultry, or as herbal teas (Charles, 2012; Tigist *et al.*, 2016; Kloy *et al.*, 2020). The essential oil of rosemary can extend the shelf life of food (Yıldız, 2016). It improves product quality by lowering lipid peroxidation in foodstuffs like meat and fish products stored in refrigerators (Aziz *et al.*, 2022).

2.3.2. Rosemary as cosmetic

Rosemary oil is used in a variety of cosmetic products, including soaps, air fresheners, skin lotions, and deodorants (Aburjai & Natsheh, 2003). The oil helps with dandruff, as well as hair growth, and manages greasy hair (Sasikumar, 2012).

2.3.3. Medicinal uses

2.3.3.1. Traditional uses as medicine

Rosemary leaves have several traditional uses due to their antimicrobial and spasmolytic properties (Golshani & Sharifzadeh, 2014; Singh *et al.*, 2018). Orally administered to relieve dyspeptic symptoms (Begum *et al.*, 2013). In addition, it treats stomach cramps and flatulence and stimulates appetite (Prasad *et al.*, 2005), menstrual disorders (Amin et al., 2012), tiredness, headache, and the secretion of gastric juices. provides relieves pain in the joints and muscles (Sharangi & Guha, 2013). Also used as a hair rinse and mouthwash. The essential oil is used in aromatherapy to relieve congestion, as an inhalant, to treat tiredness and headaches, and to improve memory and focus (Charles, 2012; Begum *et al.*, 2013).

Rosemary possesses antifungal (de Sousa *et al.*, 2013), antiviral (Nasr-Eldin *et al.*, 2017), antibacterial (Roomiani *et al.*, 2013), anti-inflammatory (Daher & Kashour, 2008), anticancer, antithrombotic, analgesic, depressive, antiulcerogenic, and antioxidant properties (Mhiri *et al.*, 2018; De Macedo *et al.*, 2020).

2.3.3.2. Nutritional compounds

The difference in the content of macro- and micro-nutrients in rosemary can be largely attributed to differences in species, cultivars, growing conditions, harvest times, soil characteristics, climate, origin, and geographical parameters (Arslan & Özcan, 2008). Aromatic plants contain protein, fiber, volatile components, vitamins (A, C, and B), minerals (Ca, P, Na, K, and Fe), and chemical compounds known for their ability to prevent diseases (Ribeiro-Santos *et al.*, 2015).

2.4. Phytochemistry

The phytochemical concentration of rosemary Extract varies depending on the extraction procedure (Gird *et al.*, 2017). The chemical group's flavonoids, polyphenols, terpenoids, and volatile oils are among those found in RE (Veenstra and Johnson, 2021). The phytochemicals found in *R. Officinalis* extracts include Rosmarinic acid, Caffeic acid, Ursolic acid, Betulinic acid, Carnosic acid, and Carnosol (Andrade *et al.*, 2018). Rosemary essential oils comprise 1,8-Cineole, α-Pinene, Verbenone, Camphor, and Borneol, but their compositions can vary greatly (Satyal *et al.*, 2017).

Phenolic compounds: Phenolic compounds are secondary metabolites and are widely distributed in plants (Hassanpour *et al.*, 2011). Phenolic compounds are made up of an aromatic ring and several hydroxyl groups connected to them. There are five primary categories of phenolic compounds: flavonoids, phenolic acids, tannins, stilbenes, and lignans (Zhang *et al.*, 2022).

Phenolic acids: Phenolic acids are a type of secondary metabolite found across the plant kingdom that is involved in a variety of cellular activities which have a role in plant growth and reproduction (Pratyusha, 2022). It is also produced as a defense mechanism to maintain various environmental stresses. This type is divided into two subclasses, benzoic acids (C6-C1) and cinnamic acids (C6-C3) (Saibabu *et al.*, 2015).

The content of phenolic acids compounds in rosemary is salvianic acid, caffeic acid, and rosmarinic acid (Kheiria *et al.*, 2021). Another study showed that among the phenolic acids contained in rosemary extract such as rosmarinic acid, caffeic acid, chlorogenic acid, coumaric

acid, ferulic acid, vanillic acid, syringic acid, homovanillic acid, hydroxybenzoic acid (Senanayake, 2018).

Flavonoids: Flavonoids are responsible for the color and aroma of flowers. Flavonoids defend plants against a variety of biotic and abiotic stressors. Flavonoids protect plants from cold and drought, and they may also help with thermal acclimation and freeze resistance (Nisar, 2022). Nature's most prevalent flavonoids are anthocyanins, flavones, flavonols, flavanones, isoflavones, flavanonols, and other subclasses (Zhang *et al.*, 2022).

In *R. officinalis*, the most common Flavonoids are Luteolin −7-*O*-Rutinoxide, Luteolin-7-Glucoronide, Hesperidin, Luteolin, Apigenin, Hispidulin, Cirsimaritin, Genkwanin, and Salvigenin by methanolic extracts (Kheiria *et al.*, 2021).

Terpenoids: Terpenoids are a large and diverse class of naturally occurring compounds derived from five-carbon isoprene units. Terpenoids are classified as monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes depending on how many C5 units are present in the molecule. They are often present in plants and are the primary components of essential oils (Cox-Georgian *et al.*, 2019).

The content of Diterpenes compounds in Rosemary is Rosmadial, 7-CH_3-Rosmanol, Carnosol, Carnosic acid, and 12-CH_3- Carnosic Acid (Kheiria *et al.*, 2021). Rosemary extracts may also contain triterpenes and triterpenic acids such Betulin, Amyrin, Betulinic acid, Oleanic acid, and Ursolic acid (Senanayake, 2018).

Volatile compounds: Essential oils, also known as volatiles, are highly concentrated, volatile, hydrophobic, and odorous chemicals found in aromatic plants. These can be extracted from various parts of the plant. Gas chromatography and mass spectrometry may provide a detailed compositional characterization of the volatile chemicals in oils (Christaki *et al.*, 2012). The volatile components are present in the essential oil fraction of rosemary extract, which is a colorless or pale-yellow liquid. According to reports, the essential oils extracted from Rosemary have antibacterial, antifungal, and anticancer effects (Senanayake, 2018).

According to studies, the main volatile compounds responsible for the potent aroma of rosemary include α-Pinene, 1,8-Cineole, Camphene, Camphor, P-cymene, Myrcene,

Limonene, β-Caryophyllene, Borneol, Bornyl acetate, Verbenone, and α-Terpineol (Yılmazer *et al.*, 2016; Senanayake, 2018).

2.5. Role of rosemary in disease prevention (biological activity)

2.5.1. Anti-oxidant activity

Antioxidants play an important role in the prevention of many diseases such as cancer, diabetes mellitus, and inflammatory (Rani, 2017), and aging because they block or delay the oxidation process by limiting the onset or propagation of oxidative chain reactions (Moreno *et al.*, 2006).

Rosemary has high antioxidant activity (Xie *et al.*, 2017). it prevents lipid peroxidation, a harmful process produced by oxidative stress (Valenzuela *et al.*, 2003). Additionally, lowering the body's reactive species level (Aziz *et al.*, 2022). The essential oil and extract of rosemary have been proven to eliminate and prevent free radicals (Hamidpour *et al.*, 2017). Flavonoids and phenolic diterpenes (Carnosol, Carnosic acid, and Rosmanol) are the main components of rosemary that contribute to its antioxidative properties (Ho *et al.*, 2000). Several rosemary extracts have been shown to have antioxidative activity, with the best being the methanol extract (Charles, 2012).

2.5.2. Anti-inflammatory activity

Rosemary extracts containing Carnosic acid, Carnosol, Ursolic acid, and flavonoids play an essential role in anti-inflammatory illnesses such as spasmolytic, arthritic, and gout affections. The volatile odor components in rosemary, including 1,8-Cineole, Borneol, and Camphor play a role in its anti-inflammatory effects (Ribeiro-Santos *et al.*, 2015).

The anti-inflammatory effects of rosmarinic acid and an extract of R. officinalis were investigated in local inflammation in a rat carrageenin-induced paw edema model. The administration of rosmarinic acid and extract at a dose of 25 mg/kg decreased paw edema by more than 60% after 6 hours (Miraj, 2016). In vivo, rosemary essential oil and extract were found to strongly inhibit leukocyte migration. This lowered the number of leukocytes (White Blood Cells) at the inflammatory site, resulting in an anti-inflammatory response (Hamidpour *et al.*, 2017).

2.5.3. Anti-microbial activity

From the 1990s until 2014, Research into rosemary essential oil's anti-infectious properties has revealed that it possesses the strongest antimicrobial properties (Jiang *et al.*, 2011). When compared to 1,8-Cineole and α-pinene alone, the essential oil had greater antimicrobial action (Wong & Kitts, 2006; Andrade *et al.*, 2018).

2.5.3.1. Anti-bacterial activity

The rosemary inhibitory effect is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol (Lazarova-Zdravkova *et al.*, 2020). They interact with the cell membrane, affecting the production of fatty acids, genetic material, nutrients, altered electron transport, and leakage of cellular components (Desbois & Smith, 2010). Additionally, it caused an interaction with membrane proteins and a loss of membrane function and structure (Nieto *et al.*, 2018).

Fresh leaves, essential oils, and aqueous or alcoholic extracts all have varying degrees of bactericidal activity (Yahaya *et al.*, 2018). Gram-positive and Gram-negative bacteria are both sensitive, Because of the difference in the structure of the bacterial cell wall, rosemary was reported to be a more powerful inhibitor against Gram-positive bacteria (Nazzaro *et al.*, 2013). Rosemary has shown bacteriostatic and bactericidal effects against the bacteria that are responsible for food poisoning and food spoilage (Pawłowska *et al.*, 2020).

2.5.3.2. Anti-viral activity

Rosmarinic acid has an antiviral impact and can prevent the growth of viruses such as HIV (Bailly & Cotelle, 2005), hepatitis B (Tsukamoto *et al.*, 2018), and enterovirus 71 (Lin *et al.*, 2019). Rosmarinic acid and NO2 may combine to form 6-nitro and 6,6-dinitro rosmarinic acid. These two substances were used for HIV-1 integrase to prevent lymphocyte MT-4 cell viral replication (Dai and Liu, 2021).

2.5.3.3. Anti-fungal activity

Rosmarinus officinalis L. has numerous antifungal mechanisms (Ksouri *et al.*, 2017). It was discovered that the essential oil of the plant prevented the adhesion of Candida albicans via cellular denaturation and changing membrane permeability (De Oliveira *et al.*, 2019). Rosemary can even stop the growth of extremely resilient fungal biofilms. By coating nanoparticles with rosemary essential oil, a nanobiosystem was created that dramatically reduced the adhesion and biofilm growth of Candida fungal strains (Hamidpour *et al.*, 2017).

2.5.4. Anti-diabetic activity

Diabetes is a growing global problem. It is expected to affect 300 million people by 2025. Diabetes is frequently exacerbated by high oxidative stress; pancreatic β-cells are particularly vulnerable to reactive oxygen species, resulting in reduced insulin secretion and increased blood glucose levels (Bakırel *et al.*, 2008). According to one study, rosemary extract reduced blood glucose levels in diabetic, hyperglycemic, and prediabetic rabbits. By inhibiting lipid peroxidation and activating antioxidant enzymes, the extract also enhances insulin secretion (Hamidpour *et al.*, 2017).

2.5.5. Anti-obesity

Rosemary has a promising anti-obesity treatment potential. One study found that rosemary can help people lose weight by lowering fat absorption in the intestine by suppressing the action of pancreatic lipase. In rats fed a high-fat diet, carnosic-standardized rosemary extract decreases weight gain and improves plasma lipid and glucose levels (Aziz *et al.*, 2021).

2.6. Rosemary's impact on the human body

2.6.1. Rosemary's impact on carbohydrate metabolism

Numerous chemical compounds present in plants have insulin-like properties and can be utilized to treat diabetes. Rosemary contains a high concentration of phenolics, flavonoids, and terpenoids, which lower blood glucose levels and so help reduce hyperglycemia. According to Naimi et al., rosemary extract and rosemary extract polyphenols (Carnosic acid and

Rosmarinic acid), which are both present in considerable amounts in rosemary, it has insulin-like actions in insulin target cells as well as anti-hyperglycemia capabilities (Naimi *et al.*, 2017). When taking rosemary extract with a body weight of 200 ml/kg body weight showed similar results to glibenclamide (a drug that reduces glucose levels in the blood). These effects were associated with increased insulin secretion (Pawłowska *et al.*, 2020).

In humans, rosemary leaf powder is effective. Participants in the study (48 persons) were placed into three groups and given 2, 5, and 10 grams of rosemary leaf powder daily for eight weeks. The greatest impact was found at a dosage of 10 grams, which reduced blood glucose levels by 18.25%. Levels fell by 11.2% and 15.74% in the 2 g and 5 g groups (Labban *et al.*, 2014).

2.6.2. Rosemary's impact on lipid metabolism

The effects of rosemary on total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) fractions, and triglyceride (TG) concentrations in blood, have been reported.

Fonso et al., researchers looked at how an aqueous extract and its phenolic fractions affected changes in blood serum composition and tissue condition in rats with diet-induced hypercholesterolemia. The extract, given at doses of 70 and 140 mg/kg body weight, resulted in a significant increase in HDL cholesterol, a decrease in serum TG, and a decrease in the level of total cholesterol (Pawłowska *et al.*, 2020).

Chapter Three

Methodology

Chapter Three: Methodology

3.1. Data and sample collection

During the 2021 season, five samples of rosemary leaves were collected from different sites in the southern region of the West Bank, Hebron-Palestine (Fig. 3.1, Table 3.1). Leaves of *R. officinalis* were dried in the shade at room temperature. The dried samples were stored in airtight paper bags protected from light.

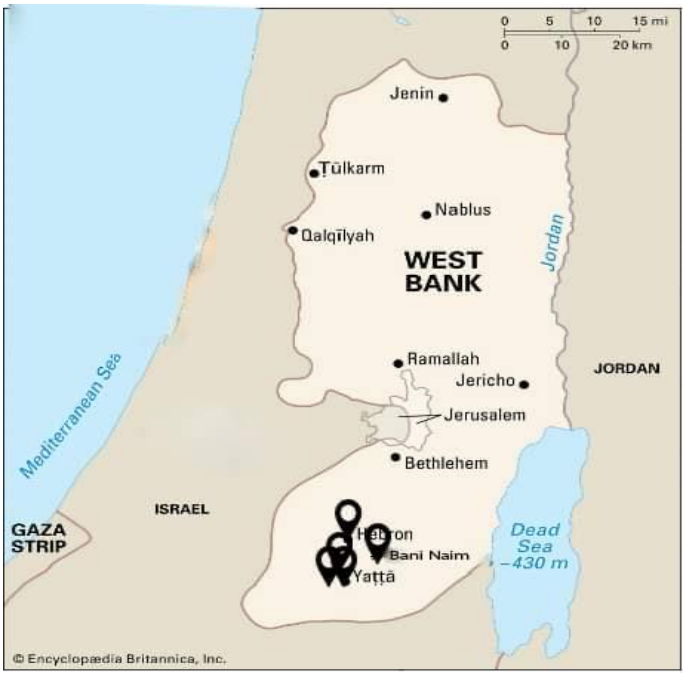


Figure 3. 1:Map showing the sites of collected *R. Officinalis* (Google maps).

Table 3. 1: Sample's location and harvesting time.

Hebron areas	Harvest	Weight before drying	Weight after drying	Storage
Raqah	16/2/2021	2.5 kg	875g	14/3/2021
Khilt Al-Adrah	24/3/2021	1.495kg	560g	8/4/2021
Umm Lasfah village	28/3/2021	1.600kg	720g	8/4/2021
Hebron	15/5/2021	1.710kg	900g	20/5/2021
Bani Naim	5/7/2021	1.625kg	520g	11/7/2021

3.2. Gas chromatography-mass spectrometry (GC-MS) analysis

3.2.1. Equipment

The following equipment was used GC vial and syringe filter.

3.2.2. Instrumentation

GC-MS (American), PerkinElmer company.

3.2.3. Procedure

The volatile compounds of rosemary were extracted (5 grams) with (50 ml) of methanol concentrations (80%,90%) and two extraction methods (i.e. at room temperature and with reflux apparatus) overnight as mentioned previously and analyzed using GC-MS. Used as a DB-5ms capillary column (30 m, 0.25 µm film thickness, 0.25µm capillary diameter) and the injection volume was 1 µL as identified by (Qawasmeh *et al.,* 2011) with minor changes. The oven temperature was maintained at 80 °C for 2 min and raised to 280 °C at the rate of 6 °C /min. The temperature of the injector was set at 280 °C. The carrier gas used is helium; the total-gas flow and velocity were maintained at 134.3 ml min^{-1} and 43.1 cm s^{-1}, respectively. The MS scan speed was 1000 amu s^{-1} and the molecular masses (m/z) of the compounds were between 50 and 500 m/z, which M/Z were acquired at 70 mv. Each sample analyzed was repeated 3 times. After being identified by the NIST05 mass spectral library, the compounds' mass spectra were compared to those found in the literature.

3.3. Phytochemicals

Phytochemicals tests of the samples were examined according to the procedure described by (Harborne, 1998; Mujeeb *et al.,* 2014). All materials used in the tests were provided by Hebron University, Hebron, Palestine.

3.3.1. Extract preparation

Rosemary samples (3 g) were extracted in 60ml methanol 80% for 24 h at 25 °C in a shaking incubator. Then the extracts were filtered through a filter paper and used to determine the phytochemicals of each sample as follows:

- **Test for anthocyanin:** In a test tube, 2 ml extract was added to 1 ml of (2 N) NaOH and heated for 5 minutes. the formation of bluish-green color indicates a positive for anthocyanin.
- **Test for Coumarin:** In a test tube, 1 ml extract was added to 1 ml of NaOH and kept in a boiling water bath for a few minutes, the presence of yellow color indicates positive coumarins.

- **Test for Saponins**: In a test tube, 5 ml of distilled water was shaken with 2 ml of extract, and the formation of foam indicates positive Saponins.
- **Test for Quinone:** In a test tube, 1 ml of conc. H_2SO_4 was added to 1 ml of extract. The presence of red color indicates a positive for quinines.
- **Test for glycosides:** In a test tube, 2 ml of the extract was added to the 2 ml of 50% H_2SO_4. After 5 minutes of heating in a water bath, 10 ml of Fehling's solution is added and boiled. The presence of red prick precipitate indicates a positive for glycosides.
- **Test for anthraquinones:** In a test tube, 1 ml of 10% NH_3 solution was added to 2 ml extract, which was mixed with benzene, the presence of red, pink, or violet color indicates positive for anthraquinones.
- **Test for cardiac glycosides:** In a test tube, 2 ml of glacial acetic acid, and 1 ml of conc. H_2SO_4 and a few drops of $FeCl_3$ were added to the 2 ml extract. The formation of the brown ring indicates a positive for glycosides.
- **Test for steroids:** In a test tube, 2 ml of $ChCl_3$, and 1 ml of H_2SO_4 were added to 1 ml extract, the appearance of a reddish-brown ring indicates positive for steroids.
- **Test for flavonoids:** In a test tube, a few drops of 1%NH_3 solution were mixed with 2 ml extract. The presence of yellow color indicates a positive for flavonoids.
- **Test for phenolic groups:** in a test tube, 2 ml of distilled water and a few drops of 10% $FeCl_3$ were added to 1 ml extract. The formation of blue or black color indicates positive for phenolic groups.
- **Test for tannins:** In a test tube, 1 ml of distilled water and 1-2 drops of $FeCl_3$, were added to 2 ml extract, the presence of green or blow black color indicates positive for tannins.
- **Test for terpenoids:** in a test tube, 2 ml of $CHCL_3$, and 3 ml conc. H_2SO_4 was mixed with 2 ml extract. The formation of a reddish-brown layer indicates a positive for terpenoids.
- **Test for phlobatannins:** In a test tube, 1 ml of 10% NaOH was added to the 2 ml extract. The formation of yellow color indicates a positive for phlobatannins.
- **Test for alkaloids:** In a test tube, 1 ml of 1% HCl was added to 2 ml extract then a few drops of Meyers reagent were added to the mixture. The presence of white precipitate indicates a positive for alkaloids.

3.4. Evaluation of the antioxidant activity

DPPH and ABTS were used to measure the antioxidant capacity by (Dowek *et al.*, 2020)

3.4.1. Extract preparation

Extract 1 g of each rosemary sample with 10 ml 80% methanol for 24 h at 25 °C in a shaking incubator. Then the extracts were filtered with filter paper and used for determination.

3.4.2. 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) assay

The capacity of leaves of rosemary to donate electrons was determined by bleaching a purple-colored methanol solution of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) with a UV-visible spectrophotometer. After the incubation period, the absorbance was measured at 517 nm.

Samples were prepared diluted (1:10), and 30 µl of the extract was added to 2 ml of DPPH solution prepared in a plastic cuvette. continuously all cuvettes were mixed and kept in dark for 1 h at room temperature. Finally, the absorbance of the samples and the control were measured at 517 nm using a UV-visible spectrophotometer.

The radical scavenging activity was determined as a percentage of DPPH discoloration using the following equation:

$$\textbf{DPPH Scavenging (\%)} = [1-(As/Ac)] * 100$$

As: ***Sample absorbance and*** **Ac:** ***Control absorbance***

3.4.3. [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) assay

The ABTS solution was made by combining 3 ml of ABTS stock solution (7 mmol, produced by dissolving 6.9 mg in 3 ml distilled water (DW) [each 18 mg of ABTS requires 5 ml D.W] with 3 ml of potassium persulfate solution (2.45 mmol, prepared by dissolving 10.5mg of $K_2S_2O_8$ with 15.9 ml D.W.). Then put it in the dark for 24 hours at room temperature.

The ABTS working solution was made by diluting the ABTS solution with D.W. until the final absorbance at 734 nm was 0.7000±0.02. In micro cuvettes, Samples were prepared diluted (1:10), and 15 µl of extract solutions were mixed with 1 ml of ABTS working solution. For control, 15 µl methanol (80%) was mixed. All cuvettes were mixed and kept at room temperature for 1 hour in the dark. The absorbance of plant extracts (A sample) and the methanol (A control) were measured at 734 nm using the UV/Visible spectrophotometer.

3.4.4. Total phenols

3.4.4.1. Preparing Gallic acid as a stock solution

Estimate the total phenols as described in (Qawasmeh *et al.*, 2012). Gallic acid was prepared by dissolving 250 mg of G.A. in 5 ml of 80% methanol and diluting it with distilled water to 50 ml. Different Gallic acid concentrations were prepared by adding six other volumes of G.A (50, 100, 200, 300, 500, and 1000 µl) in a cuvette, then adding methanol up to 10 ml.

3.4.4.2. Preparing Na_2CO_3

To prepare sodium carbonate (Na_2CO_3), 5g of Na_2CO_3 was dissolved in 20 ml of D.W. in a beaker, the mixture was heated to a boil, cooled, and filtered, and then distilled water was added up to 25 ml.

3.4.4.3. Procedure for total phenols

In plastic cuvettes, 20 µl of rosemary extracts were combined with 1.58 ml distilled water, and 150 µl of Folin Ciocalteu reagent, and all cuvettes were mixed. After mixing, we add 300 µl of Na_2CO_3 and kept it in the dark for 1 h and the absorbance of the resulting solution was measured at 760 nm. Blank is water and the assay were done in triplicate

3.5. Antimicrobial activity

3.5.1. Reagents

Muller Hinton agar and nutrient broth were kindly supplied by the Central Public Health Laboratory, Ministry of Health, Ramallah, Palestine.

3.5.2. Extract preparation

Two methods were used to extract the rosemary plant with different methanol concentrations (90,80%). In the first method, at room temperature, 5 g of the plant was weighed and 50 ml of methanol was added with stirring for 24 hours. The second method of extraction was carried out using the reflux device; weighing 5 grams of the plant with 50 ml of methanol, then heating it at 50 °C for 24 hours. The methods of extraction were applied to all concentrations of methanol (80%,90%).

3.5.3. Muller Hinton agar (MHA) preparation

Thirty-eight grams of Mueller Hinton agar powder in 1000 ml purified/ distilled water was suspended. The medium is boiled for a few seconds until the ingredients are completely dissolved. Sterilized by autoclaving at 121°C for 15 minutes. Cooled to 45-50 °C. Poured the liquid into a sterile petri dish and waited for the medium to solidify.

3.5.4. Nutrient broth preparation

Thirteen grams of Mueller Hinton agar powder in 1000 ml purified/ distilled water was suspended. The media is heated to dissolve it completely. Dispensed it into tubes and/or flasks as desired. Sterilized by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. We took a colony of bacteria (*Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa*) by means of the wire loop. And mixed with broth, then incubated at a temperature of 37 degrees Celsius for 24 hours.

3.5.5. Microbial strains

- ***Staphylococcus aureus***
- ***E. coli***
- ***Pseudomonas aeruginosa***

All the above strains were obtained from Princess Alia Governmental Hospital, Hebron.

3.5.6. Procedure

The antibacterial activity was examined according to the procedure described by (Jahiman *et al.*, 2021). In this study, the disk diffusion assay method was used for the anti-bacterial activities, were 3 different types of bacteria are used *(Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa).* The Mueller-Hinton agar used was poured into the petri dishes. The bacteria are cultured by getting the first colony and then the bacteria were put into the nutrient broth and incubated at 37 ºC for 24 hours (The function of the nutrient broth was to put the bacteria into the nutrient broth solution). Five disks were spread on the surface of the media in each plate. A rosemary extract sample (50 µl) was added to each disk. Each plate was incubated at 37 °C for 24 hours. The zone of inhibition was measured and the result was recorded.

3.6. Proximate and minerals analysis:

The amount of total ash, fiber, fat, and minerals were evaluated according to (Official, 2000; Estefan *et al.*, 2013).

3.6.1. Instrumentation

Muffle furnace (UK), GALLENKAMP company, while sox-therm (German), Gerhardt company, fiber analyzer (American), ANKOM 2000 company, and atomic absorption PERKIN ELMER AAnalyst100 company.

3.6.2. Ash estimation

The crucible was weighed and 1 gram of rosemary plant was added and placed in the Muffle Furnace oven at 550 °C. The obtained ash was calculated according to the following formula:

Ash % = [(weight of crucible with ash- the weight of crucible) / (weight of sample)] *100.

3.6.3. Fat estimation

Using Sox-therm and petroleum ether as the solvent, the crude fat of the rosemary sample was determined according to the following procedure: Rosemary sample in the thimble was weighed and placed in a beaker with boiling chips, 140 ml ether was added to the beaker. The mixture was boiled in a Sox-therm for 30 minutes. The volume of ether was decreased in the beaker to the bottom thimble. After waiting 80 minutes, we get rid of the ether by drying it for 1 hour at 100 °C. The fat was calculated according to the following formula:

Crude fat (%) = [(Weight of flask with fat − weight of empty flask)/ (Weight of original sample)] × 100

3.6.4. Fiber estimation

Crude fibers from each sample were estimated using a fiber analyzer, and the samples were treated with 1.25% sulfuric acid for 30 minutes of boiling, then washed with hot water. After that, the samples were treated with 1.25% potassium hydroxide base material for 30 min. of boiling and then washed with hot water. Finally, drying of the samples is done for 3 hours at 105 °C.

3.6.5. Minerals analysis (Mg, Na, Ca, K, Mn, P, and Fe)

The ash samples were mixed with 5 ml of 2N HCl and then filtered in a volumetric flask with up to 100 ml of distilled water. Finally, using Inductively Coupled Plasma Optical Emission

spectroscopy (ICP-OES), the absorbance of all samples was measured in comparison to the standard of each mineral.

3.6.5.1. Phosphate estimation

Ammonium vanadomolybdate (10 ml) was added to 10 ml of the previously extracted ash, and 100 ml of distilled water was used to dilute the mixture. The reagents produced a stable yellow color with phosphates. Finally, the absorbance of all the samples was measured at 410 nm with a spectrophotometer.

Chapter Four

Results

Chapter Four: Results

4.1. Gas chromatography-mass spectrometry (GC-MS) analysis

Different concentrations of methanolic extracts from rosemary leaves were tested by GC-MS and identified by comparing them with the NIST library. The GC-MS analysis revealed the presence of many volatile compounds in each rosemary sample with different values **Figures (4.1**, **4.2**, **4.3**, **4.4**, **4.5**, **4.6**, **4.7**, **4.8**, **4.9**, **4.10**, **4.11**, **4.12**, **4.13**, **4.14**, **4.15**, **4.16**, **4.17**, **4.18**, **4.19**, and **4.20).** All rosemary leaves despite their geographical site, were found to contain volatile compounds. Several major compounds were identified and their molecular formula, molecular weight, and retention time are summarized in **Tables (4.1**, **4.2**, **4.3**, **4.4**, **4.5**, **4.6**, **4.7**, **4.8**, **4.9**, **4.10**, **4.11**, **4.12**, **4.13**, **4.14**, **4.15**, **4.16**, **4.17**, **4.18**, **4.19**, and **4.20)**. **Figure (3.2)** is the blank methanol; the solvent of extraction.

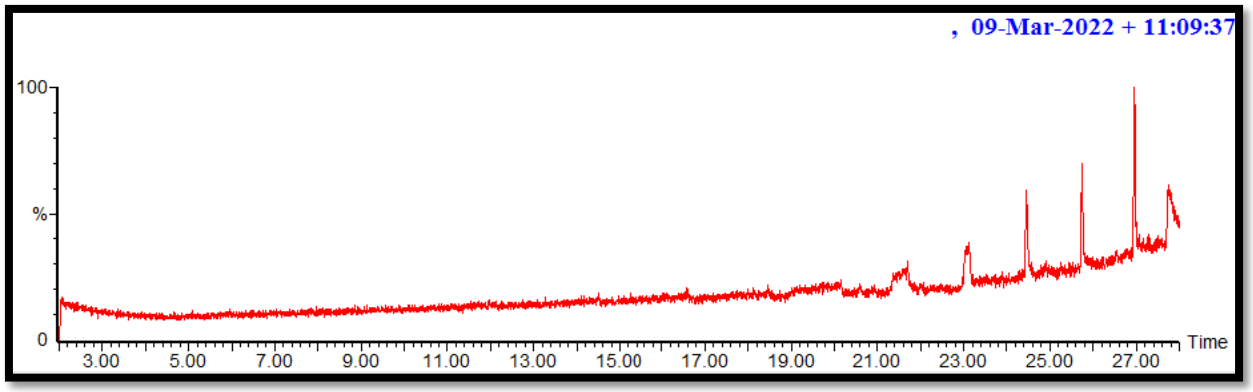


Figure 3.2: Blank of methanol

GC-MS analysis of Rosemary in the 80% methanolic extract at room temperature showed the identification of many compounds. Major volatile compounds detected in all samples were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. Table (4.1) and Figure (4.1) in the Raqaa region showed the presence of more components compared with other samples. In addition to the mentioned, also contained major volatile compounds detected ex Vitamin A aldehyde, Naphthalene, Beta-Humulen, Epicedrol, Isoparvifuran, Kokusaginine, (2(1H)-phenanthrenone, 3, 4, 4A, 9, 10, 10A-hexahydro-6-methoxy-1,1,4A-) and (Indolo[2,3-A] Quinalizine, 1, 2, 3, 4, 5, 6, 7, 12B- octahydro-12, 12B-Dimethyl). The other minor volatile compounds identified were shown in **Tables (4.1)**.

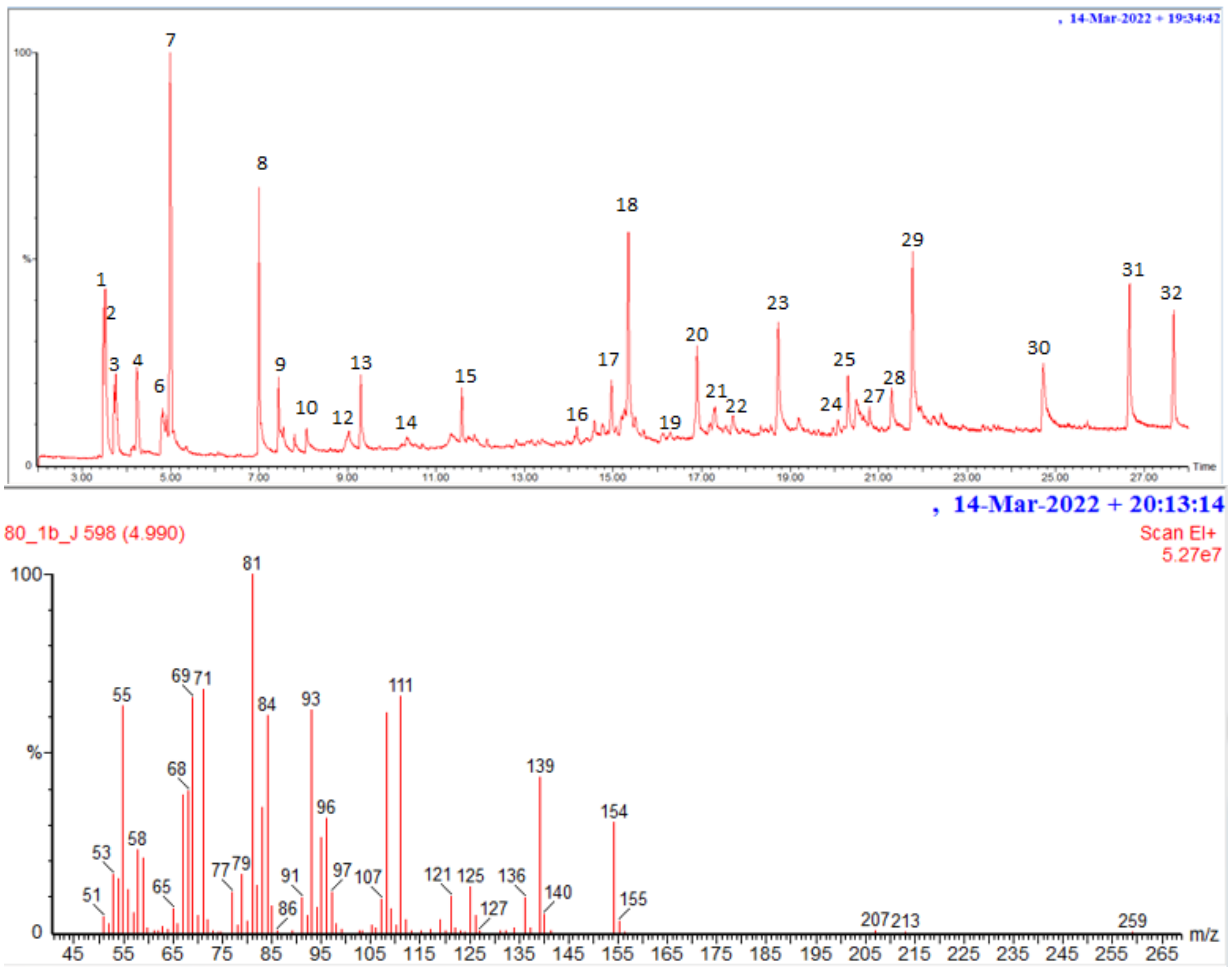


Figure 4. 1: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% at room temp. for rosemary leaves in the Raqaa region sample. The peak numbers of the components are listed in **Table (4.1)**.

Table 4. 1: Compounds detected in methanolic extracts 80% at room temp. of rosemary leaves in the Raqaa region sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.489	3-Carene	136	$C_{10}H_{16}$
2	3.539	α-Pinene	136	$C_{10}H_{16}$
3	3.729	Camphene	136	$C_{10}H_{16}$
4	4.254	1.3 cyclopentadene	122	C_9H_{14}
5	4.825	Benzene, 1 methyl-3-(methylethyl)	134	$C_{10}H_{14}$
6	4.905	D-Limonene	136	$C_{10}H_{16}$
7	4.990	Eucalyptol	154	$C_{10}H_{18}O$
8	6.990	Camphor	152	$C_{10}H_{16}O$
9	7.441	Borneol	154	$C_{10}H_{18}O$
10	7.556	Borneol chloride	172	$C_{10}H_{17}Cl$
11	8.076	Bicycle (3,1,1 hepta-3-(-N-2-one)	150	$C_{10}H_{14}O$
12	9.031	Cyclopropane carboxylic acid, 2,2- Dimethyl-3-(2-methyl-1-propen)	168	$C_{10}H_{16}O_2$
13	9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$
14	10.352	Cyclohexanmethanol	140	$C_9H_{16}O$
15	11.337	Vitamin A aldehyde	284	$C_{20}H_{28}O$
16	11.612	Humulen-(V1)	204	$C_{15}H_{24}$
17	14.989	Naphthalene	204	$C_{15}H_{24}$
18	15.324	Beta-Humulen	204	$C_{15}H_{24}$
19	16.279	Thunbergol	290	$C_{20}H_{34}O$
20	16.924	Aromadendrene oxide-(2)	220	$C_{15}H_{24}O$
21	17.290	Caryophyllene-(I3)	204	$C_{15}H_{24}$
22	17.720	Thujopsene	204	$C_{15}H_{24}$
23	18.735	Epicedrol	222	$C_{15}H_{26}O$
24	20.086	3,4 Diethylphenol	150	$C_{10}H_{14}O$
25	20.311	Lumisantonin	246	$C_{15}H_{18}O_3$
26	20.501	Methoxsalen	216	$C_{12}H_8O_4$
27	20.791	Menthol	156	$C_{10}H_{20}O$
28	21.296	Ambrosin	246	$C_{15}H_{18}O_3$
29	21.771	Isoparvifuran	254	$C_{16}H_{14}O_3$
30	24.723	Furo[2,3B]Quinoline, 4, 6, 7- trimethoxy **(**Kokusaginine**)**	259	$C_{14}H_{13}O_4N$
31	26.678	2(1H)-phenanthrenone, 3, 4, 4A, 9, 10, 10A-hexahydro-6-methoxy-1,1,4A-.	314	$C_{21}H_{30}O_2$
32	27.674	Indolo[2,3-A] Quinalizine, 1, 2, 3, 4, 5, 6, 7, 12B- octahydro-12, 12B-Dimethyl	296	$C_{20}H_{28}ON_2$

Table (4.2) and **Figure (4.2)** show 14 compounds found in rosemary leaves in 80% methanol at room temperature in the Khilt Al-Adrah region sample. The additional major compounds identified were Isoparvifuran and 2(1h)-pyridinone, 3, 4, 4A, 10, 10A-hexahydro-6-methoxy-1,1,4A. The other minor volatile compounds identified were shown in **Tables (4.2)**.

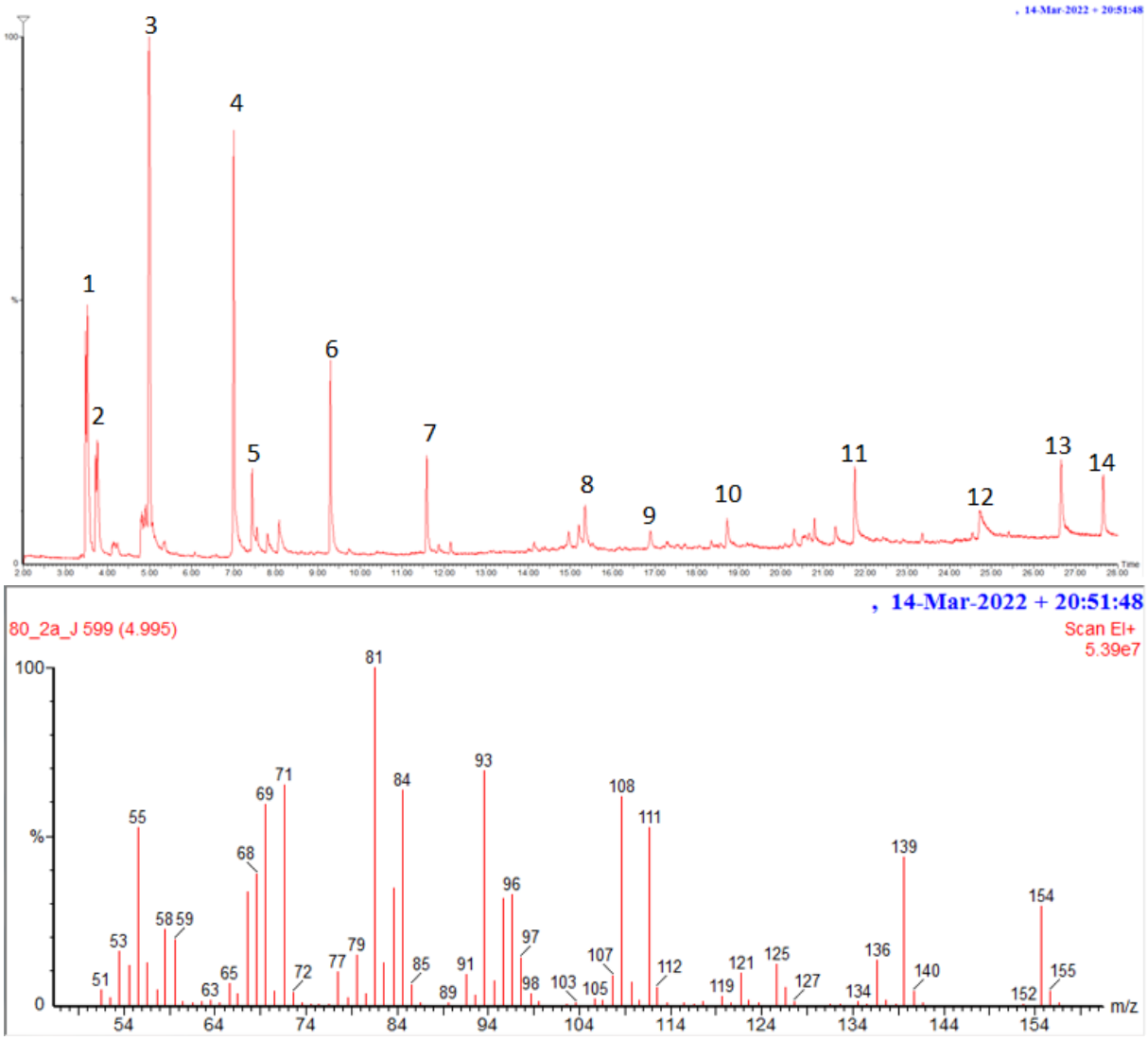


Figure 4. 2: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% at room temp. of rosemary leaves in the Khilt Al-Adrah region sample. The peak numbers of the components are listed in **Table (4.2)**.

Table 4. 2: Compounds detected in methanolic extracts 80% at room temp. of rosemary leaves in the Khilt Al-Adrah region sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.524	3-Carene	136	$C_{10}H_{16}$
2	3.764	Camphene	136	$C_{10}H_{16}$
3	4.995	Eucalyptol	154	$C_{10}H_{18}O$
4	7.005	Camphor	152	$C_{10}H_{16}O$
5	7.451	Borneol	154	$C_{10}H_{18}O$
6	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
7	11.587	Caryophyllene	204	$C_{15}H_{24}$
8	15.349	Beta-Humulene	204	$C_{15}H_{24}$
9	16.914	Geraniol	430	$C_{10}H_{18}O$
10	18.760	Longifolenealdehyde	220	$C_{15}H_{24}O$
11	21.761	Isoparvifuran	254	$C_{16}H_{14}O_3$
12	24.752	Furo[2,3B]Quinoline, 4, 6, 7- trimethoxy **(**Kokusaginine**)**	259	$C_{14}H_{13}NO_4$
13	26.658	2(1h)-pyridinone, 3, 4, 4A, 10, 10A-hexahydro-6-methoxy-1, 1, 4A-	314	$C_{21}H_{30}O_2$
14	27.659	indolo[2,3-A]Quinalizine, 1, 2,3,4,5,6,7,12B-Octahydro-12,12B-Dimethyl	296	$C_{20}H_{28}ON_2$

Table (4.3) and **Figure (4.3)** present the 12 compounds extracted from rosemary leaves in 80% methanol at room temperature in the Umm Lasfah village region sample. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.3)**.

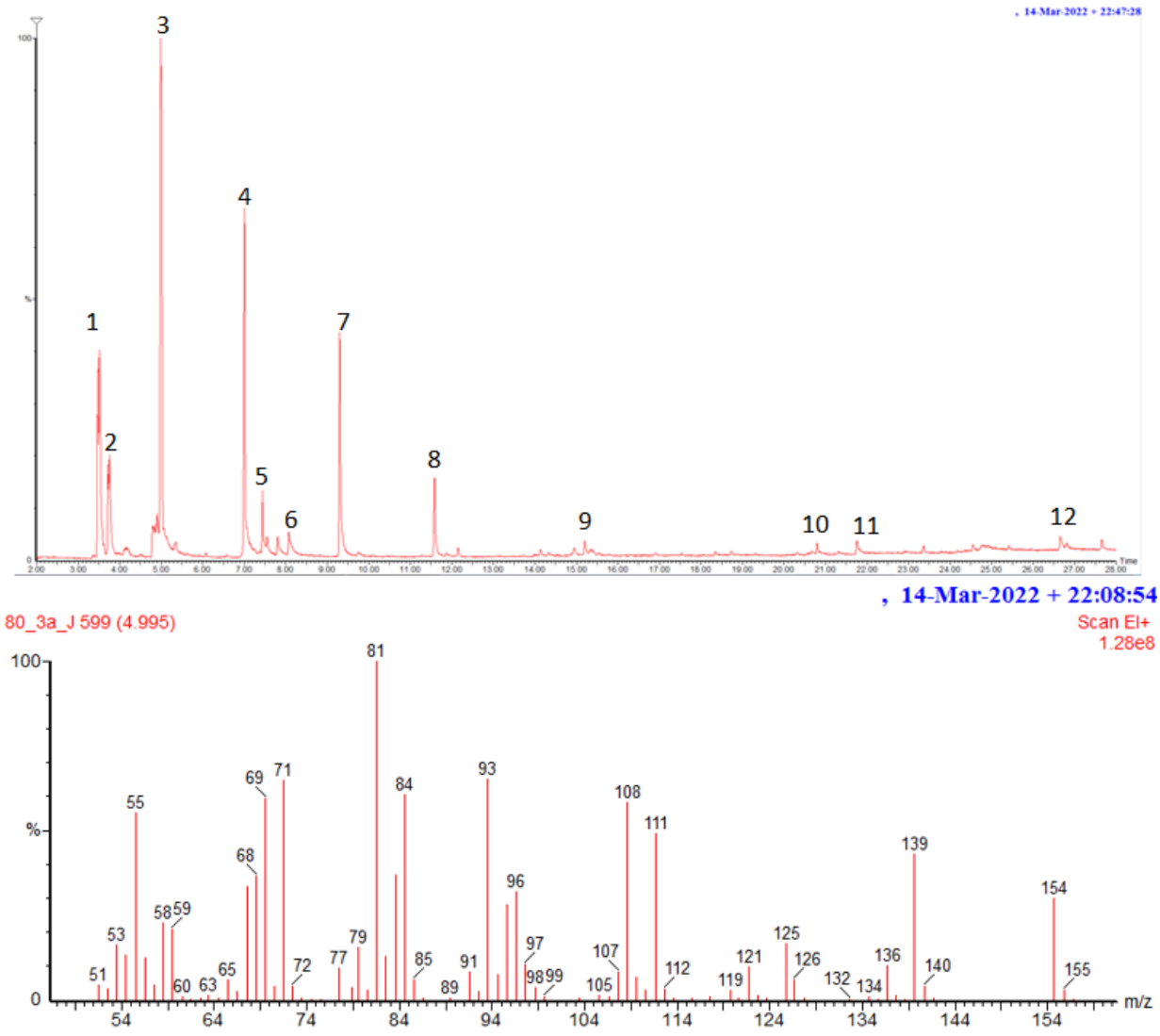


Figure 4. 3: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% at room temp. of rosemary leaves in the Umm Lasfah village region sample. The peak numbers of the components are listed in **Table (4.3)**.

Table 4. 3: Compounds detected in methanolic extracts 80% at room temp. of rosemary leaves in the Umm Lasfah village sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.479	3-Carene	136	$C_{10}H_{16}$
2	3.754	Camphene	136	$C_{10}H_{16}$
3	4.995	Eucalyptol	154	$C_{10}H_{18}O$
4	7.005	Camphor	152	$C_{10}H_{16}O$
5	7.436	Borneol	154	$C_{10}H_{18}O$
6	8.076	D-verbenone	150	$C_{10}H_{14}O$
7	9.301	Isoborneol acetate	196	$C_{12}H_{20}O_2$
8	11.582	Caryophyllene	204	$C_{15}H_{24}$
9	15.199	Humulen-(V1)	204	$C_{15}H_{24}$
10	20.791	Menthol	156	$C_{10}H_{20}O$
11	21.751	Isoparvifuran	254	$C_{16}H_{14}O_3$
12	26.653	2(1h)-phenanthrenone, 3, 4, 4A, 10, 10A-hexahydro-6-methoxy-1, 1, 4A-	314	$C_{21}H_{30}O_2$

Table (4.4) and **Figure (4.4)** show the 13 compounds found in rosemary leaves in 80% methanol at room temperature in the Hebron region. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.4)**.

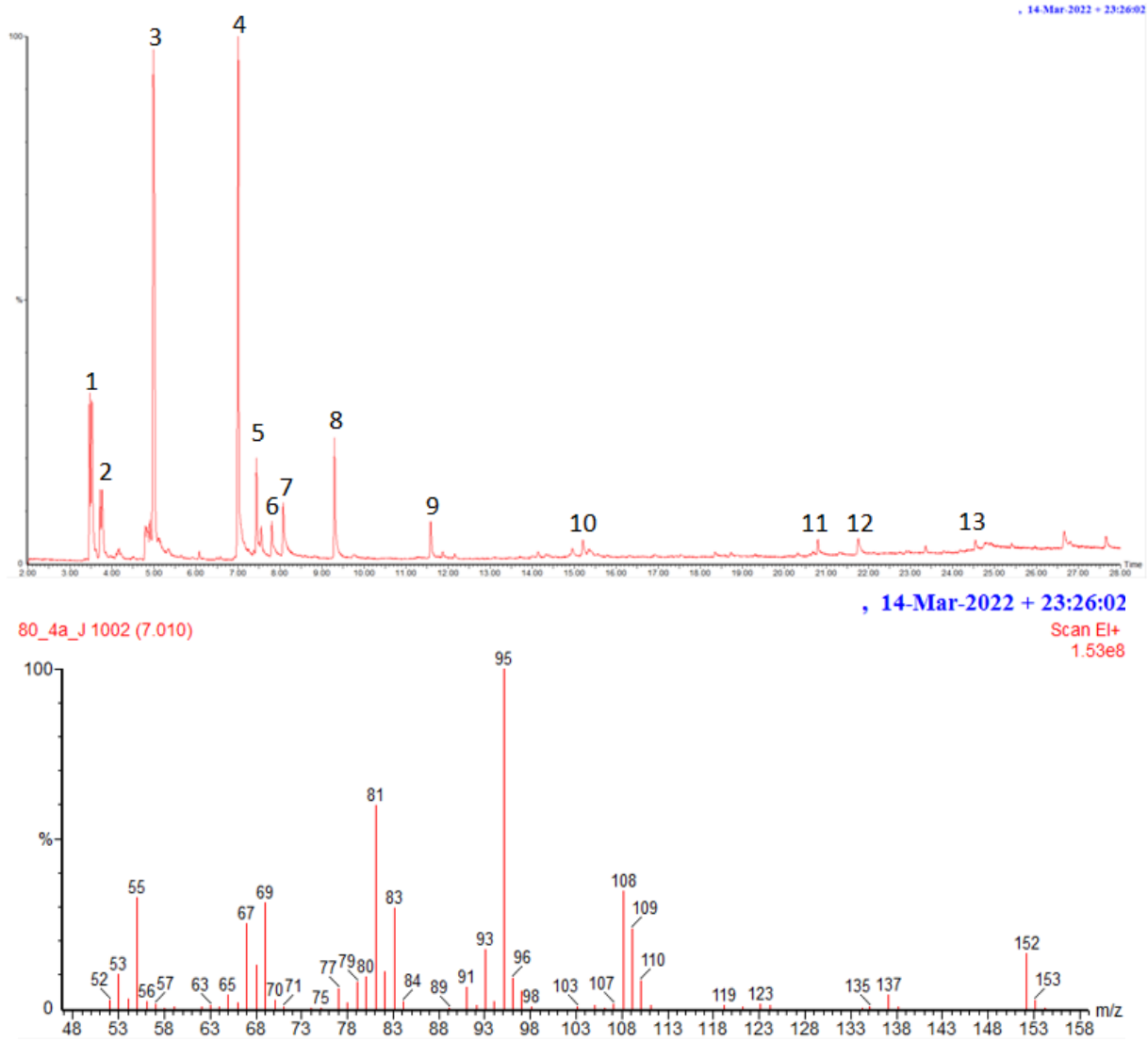


Figure 4. 4: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% at room temp. of rosemary leaves in the Hebron region sample. The peak numbers of the components are listed in **Table (4.4)**.

Table 4. 4: Compounds detected in methanolic extracts 80% at room temp. of rosemary leaves in the Hebron sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	Rt	Compound	MW	Molecular Formula
1	3.474	3-Carene	136	$C_{10}H_{16}$
2	3.719	Camphene	136	$C_{10}H_{16}$
3	5.005	Eucalyptol	154	$C_{10}H_{18}O$
4	7.005	Camphor	152	$C_{10}H_{16}O$
5	7.446	Borneol	154	$C_{10}H_{18}O$
6	7.806	Bornyl Chloride	172	$C_{10}H_{17}Cl$
7	8.076	D-verbenone	150	$C_{10}H_{14}O$
8	9.301	Bornyl acetate	196	$C_{10}H_{20}O_2$
9	11.587	Caryophyllene	204	$C_{15}H_{24}$
10	15.209	Beta-humulen	204	$C_{15}H_{24}$
11	20.801	Menthol	156	$C_{10}H_{20}O$
12	21.766	Isoparvifuran	254	$C_{16}H_{14}O_3$
13	24.552	2-Bromo-5,8-dimethoxy-3-methyl-1-naphthol	296	$C_{13}H_{13}O_3Br$

Table (4.5) and **Figure (4.5)** show 13 compounds found in rosemary leaves in 80% methanol at room temperature in the Bani Naim region. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.5)**.

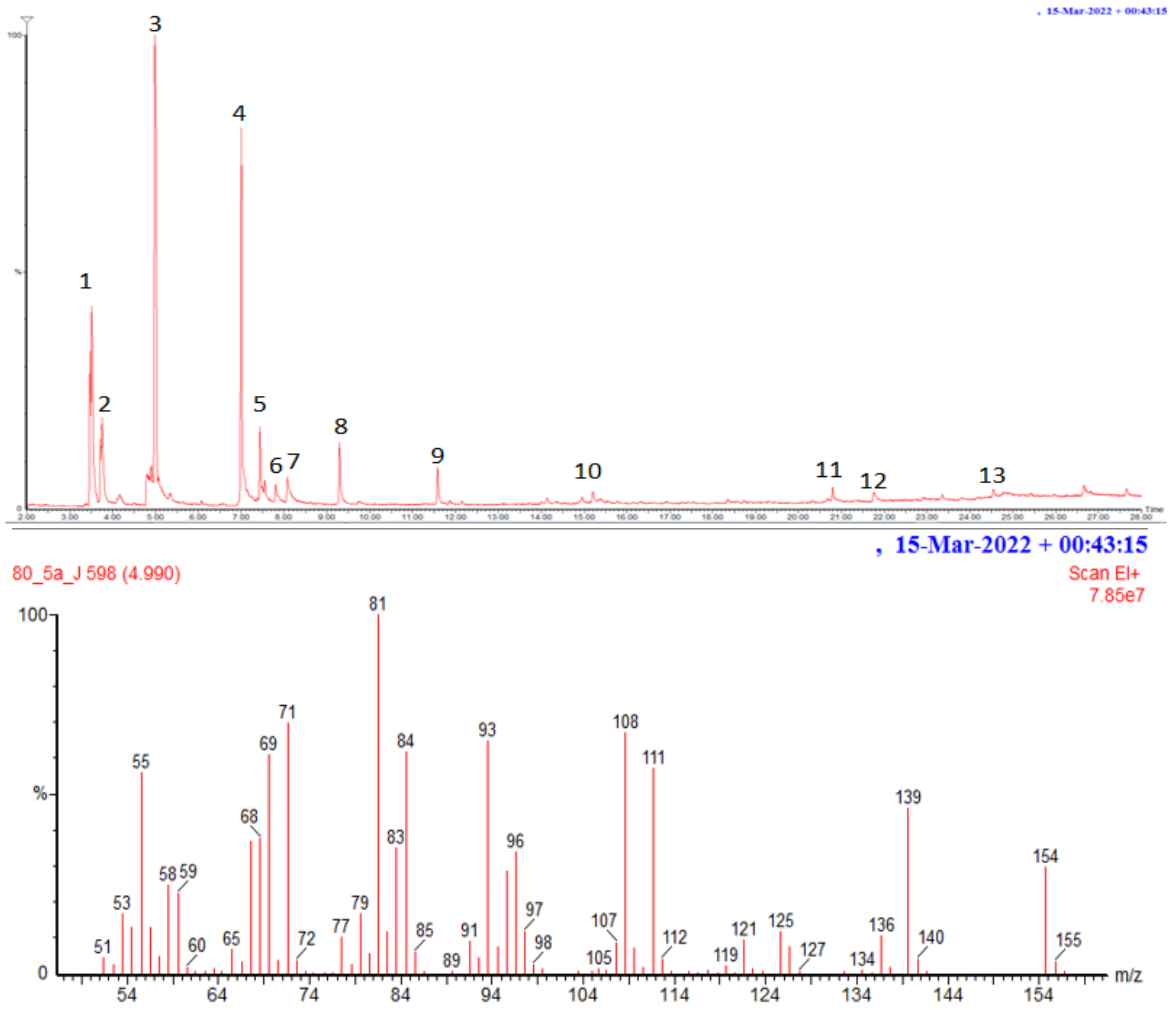


Figure 4. 5: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% at room temp. of rosemary leaves in the Bani Naim region sample. The peak numbers of the components are listed in **Table (4.5)**.

Table 4. 5: Compounds detected in methanolic extracts 80% at room temp. of rosemary leaves in the Bani Naim sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.474	3-Carene	136	$C_{10}H_{16}$
2	3.759	Camphene	136	$C_{10}H_{16}$
3	4.990	Eucalyptol	154	$C_{10}H_{18}O$
4	7.005	Camphor	152	$C_{10}H_{16}O$
5	7.446	Borneol	154	$C_{10}H_{18}O$
6	7.806	Bornyl Chloride	172	$C_{10}H_{17}Cl$
7	8.081	D-verbenone	150	$C_{10}H_{14}O$
8	9.301	Isoborneol acetate	196	$C_{12}H_{20}O_2$
9	11.582	Caryophyllene	204	$C_{15}H_{24}$
10	15.204	Beta-humulen	204	$C_{15}H_{24}$
11	20.796	Menthol	156	$C_{10}H_{20}O$
12	21.766	Isoparvifuran	254	$C_{16}H_{14}O_3$
13	24.552	2-Bromo-5,8-dimethoxy-3-methyl-1-naphthol	296	$C_{13}H_{13}O_3Br$

Table (4.6) and **Figure (4.6)** the data indicate that 11 components found in the methanolic extracts 80% Reflux method of rosemary leaves in the Raqaa region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Borneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.6)**.

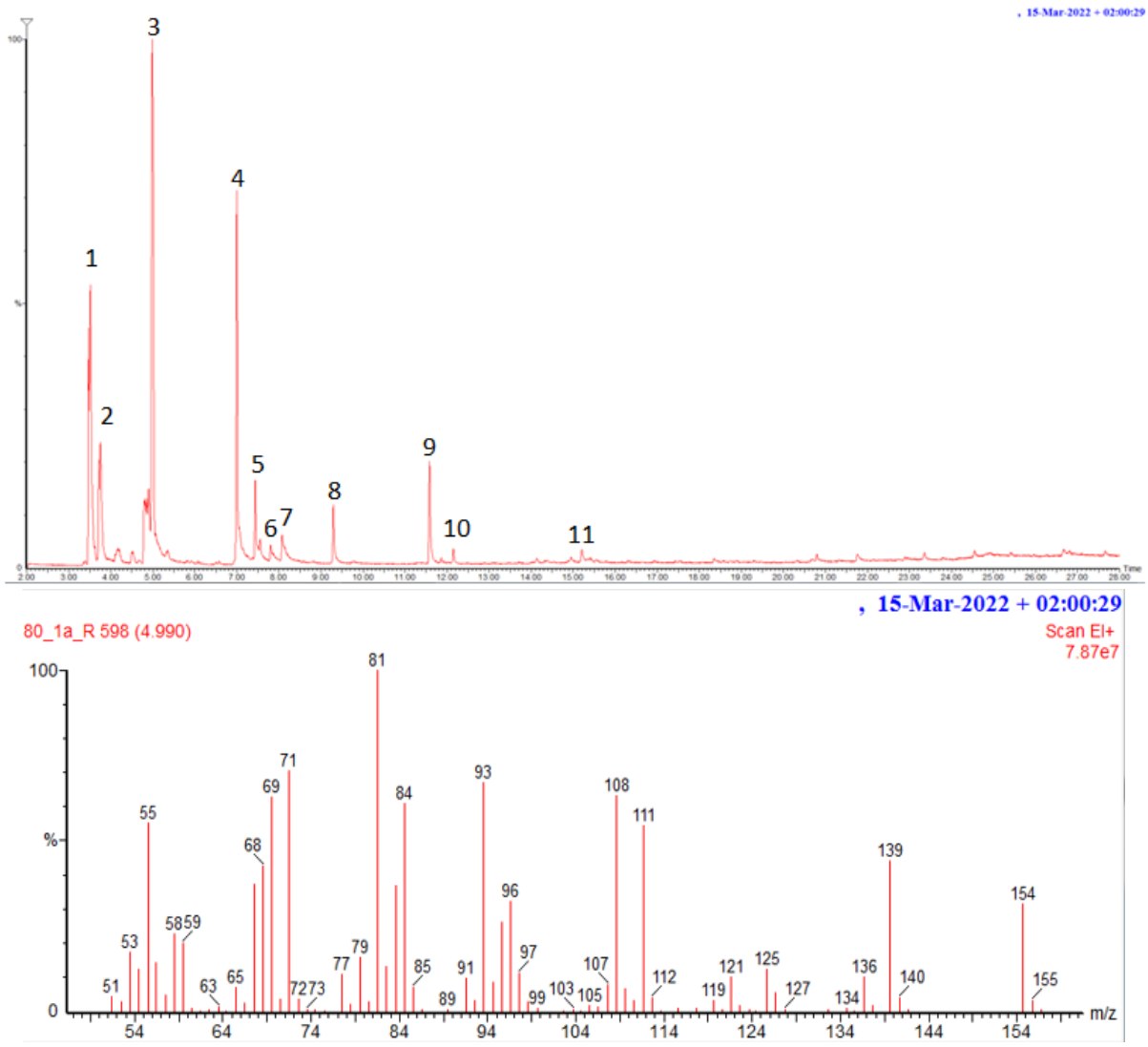


Figure 4. 6: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% Reflux method of rosemary leaves in the Raqaa region sample. The peak numbers of the components are listed in **Table (4.6)**.

Table 4. 6: Compounds detected in methanolic extracts 80% reflux method of rosemary leaves in the Raqaa sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.479	3-Carene	136	$C_{10}H_{16}$
2	3.754	Camphene	136	$C_{10}H_{16}$
3	4.990	Eucalyptol	154	$C_{10}H_{18}O$
4	7.000	Camphor	152	$C_{10}H_{16}O$
5	7.441	Borneol	154	$C_{10}H_{18}$
6	7.806	Bornyl Chloride	172	$C_{10}H_{17}Cl$
7	8.076	D-verbenone	150	$C_{10}H_{14}O$
8	9.296	Borneol acetate	196	$C_{12}H_{20}O_2$
9	11.582	Caryophyllene	204	$C_{15}H_{24}$
10	12.152	Alpha- caryophyllene	204	$C_{15}H_{24}$
11	15.209	Humulen-(V1)	204	$C_{15}H_{24}$

Table (4.7) and **Figure (4.7)** present the 12 compounds extracted from rosemary leaves in the methanolic extracts 80% Reflux method in the Khilt Al-Adrah region sample. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.7)**.

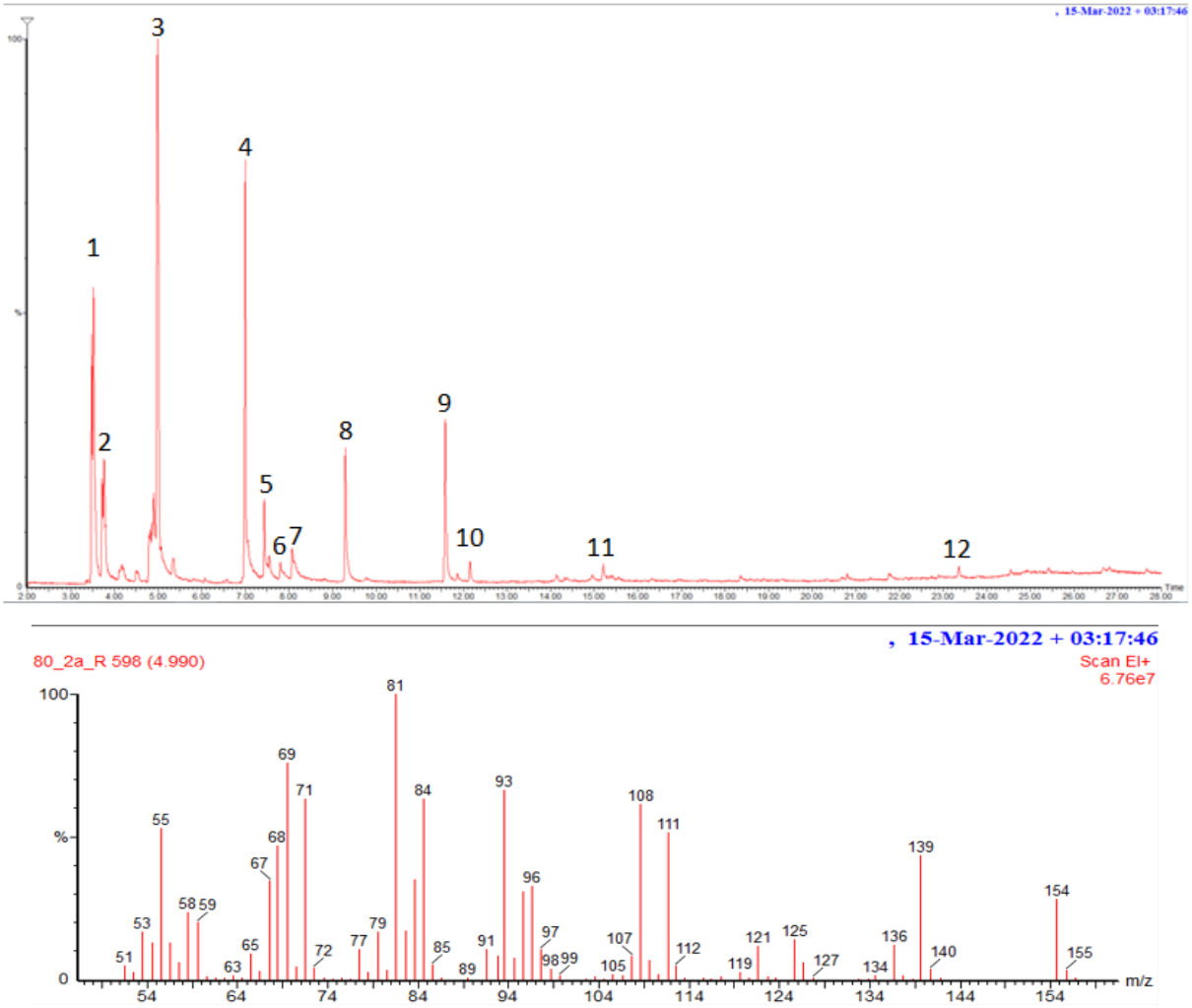


Figure 4. 7: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% Reflux method of rosemary leaves in the Khilt Al-Adrah region sample. The peak numbers of the components are listed in **Table (4.7)**.

Table 4. 7: Compounds detected in methanolic extracts 80% reflux method of rosemary leaves in the Khilt Al-Adrah sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.529	3-Carene	136	$C_{10}H_{16}$
2	3.764	Camphene	136	$C_{10}H_{16}$
3	4.995	Eucalyptol	154	$C_{10}H_{18}O$
4	7.000	Camphor	152	$C_{10}H_{16}O$
5	7.441	Borneol	154	$C_{10}H_{18}O$
6	7.806	Bornyl Chloride	172	$C_{10}H_{17}O_2$
7	8.076	D-verbenone	150	$C_{10}H_{14}O$
8	9.301	Borneol acetate	196	$C_{12}H_{20}O_2$
9	11.587	Caryophyllene	204	$C_{15}H_{24}$
10	12.152	Alph- caryophyllene	204	$C_{15}H_{24}$
11	15.209	Humulen-(V1)	204	$C_{15}H_{24}$
12	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table (4.8) and **Figure (4.8)** show the 11 compounds found in the methanolic extracts 80% Reflux method of rosemary leaves in the Umm Lasfah village region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.8).**

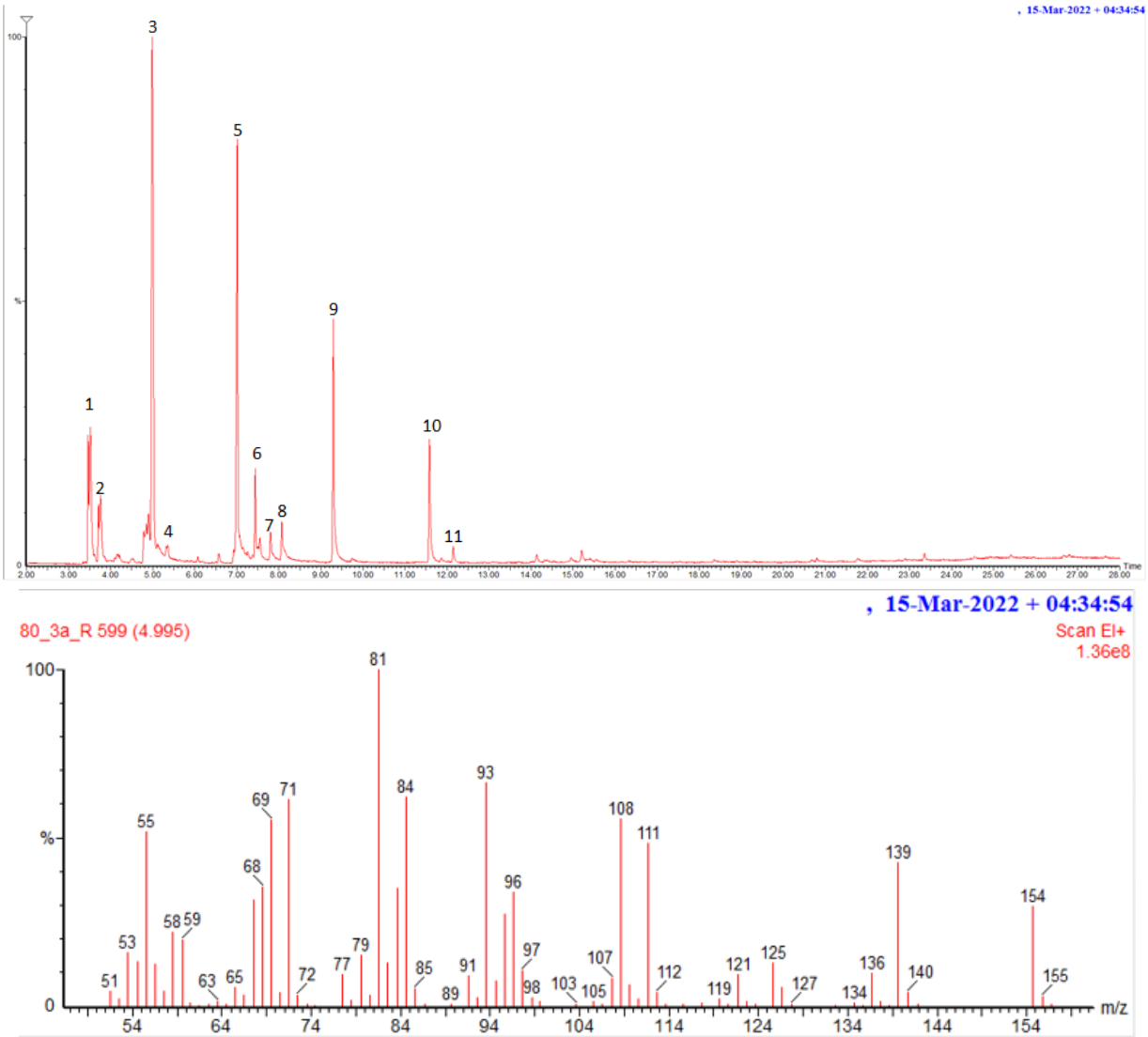


Figure 4. 8: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% Reflux method of rosemary leaves in the Umm Lasfah village sample. The peak numbers of the components are listed in **Table (4.8)**.

Table 4. 8: Compounds detected in methanolic extracts 80% reflux method of rosemary leaves in the Umm Lasfah village sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.524	3-Carene	136	$C_{10}H_{16}$
2	3.764	Camphene	136	$C_{10}H_{16}$
3	4.990	Eucalyptol	154	$C_{10}H_{18}O$
4	5.365	Alpha- pinene	136	$C_{10}H_{16}$
5	7.015	Camphor	152	$C_{10}H_{16}O$
6	7.441	Borneol	154	$C_{10}H_{18}O$
7	7.801	Bornyl Chloride	172	$C_{10}H_{17}O_2$
8	8.071	D-verbenone	150	$C_{10}H_{14}O$
9	9.301	Isoborneol acetate	196	$C_{12}H_{20}O_2$
10	11.587	Caryophyllene	204	$C_{15}H_{24}$
11	12.147	Alph- caryophyllene	204	$C_{15}H_{24}$

Table (4.9) and **Figure (4.9)** the data indicate that 11 components found in the methanolic extracts 80% Reflux method of rosemary leaves in the Hebron region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Borneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.9)**.

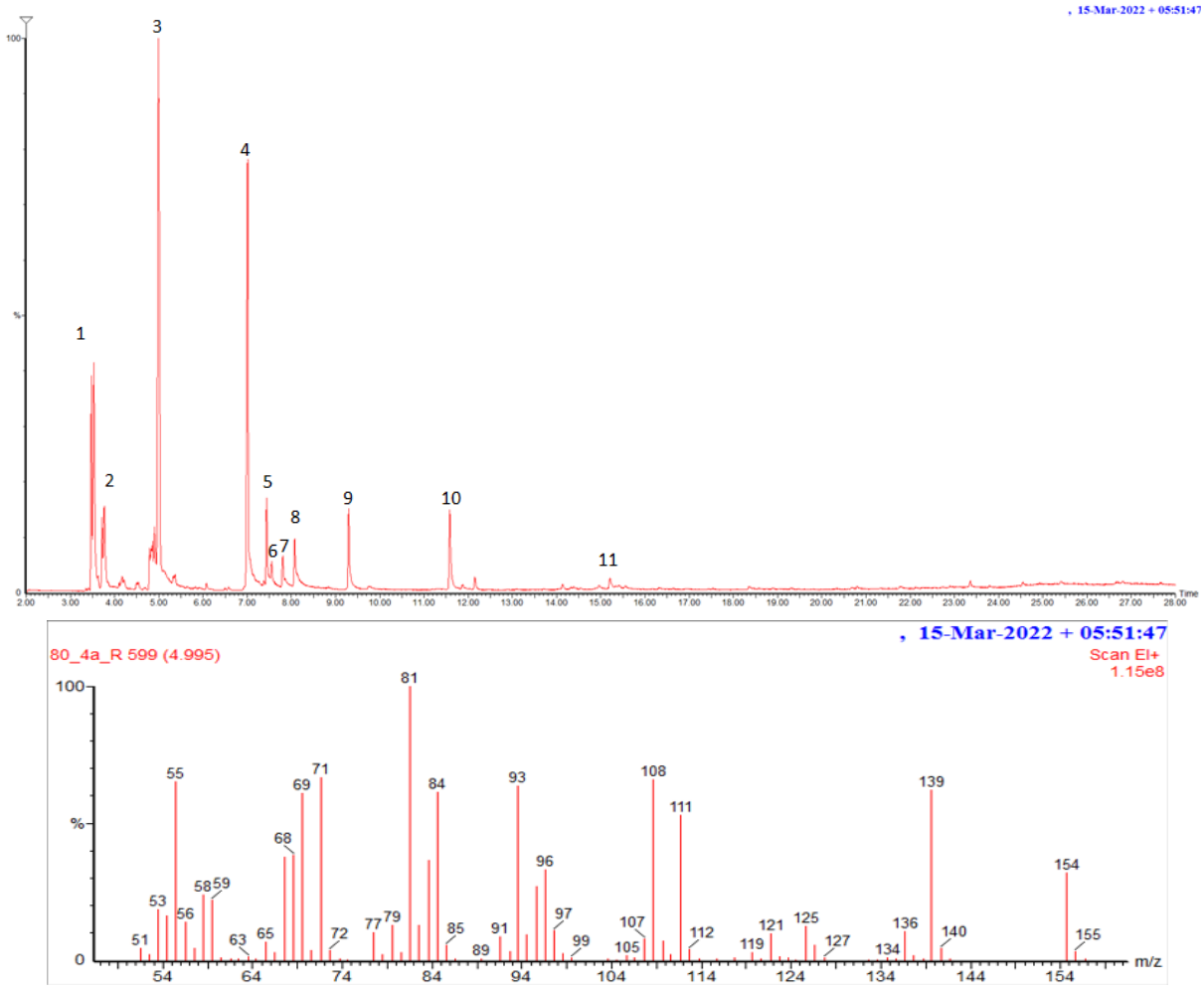


Figure 4. 9: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% Reflux method of rosemary leaves in the Hebron sample. The peak numbers of the components are listed in **Table (4.9)**.

Table 4. 9: Compounds detected in methanolic extracts 80% reflux method of rosemary leaves in the Hebron sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.534	3-Carene	136	$C_{10}H_{16}$
2	3.769	Camphene	136	$C_{10}H_{16}$
3	4.995	Eucalyptol	154	$C_{10}H_{18}O$
4	7.005	Camphor	152	$C_{10}H_{16}O$
5	7.441	Borneol	154	$C_{10}H_{18}O$
6	7.556	Bornyl Chloride	172	$C_{10}H_{17}O_2$
7	7.806	P-Menth-1-en-8-ol	154	$C_{10}H_{18}O$
8	8.076	D-verbenone	150	$C_{10}H_{14}O_2$
9	9.296	Bornyl acetate	196	$C_{12}H_{20}O_2$
10	11.587	Caryophyllene	204	$C_{15}H_{24}$
11	15.214	Humulen-(V1)	204	$C_{15}H_{24}$

Table (4.10) and **Figure (4.10)** present the 11 compounds extracted from rosemary leaves in the methanolic extracts 80% Reflux method in the Bani Naim region sample. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.10)**.

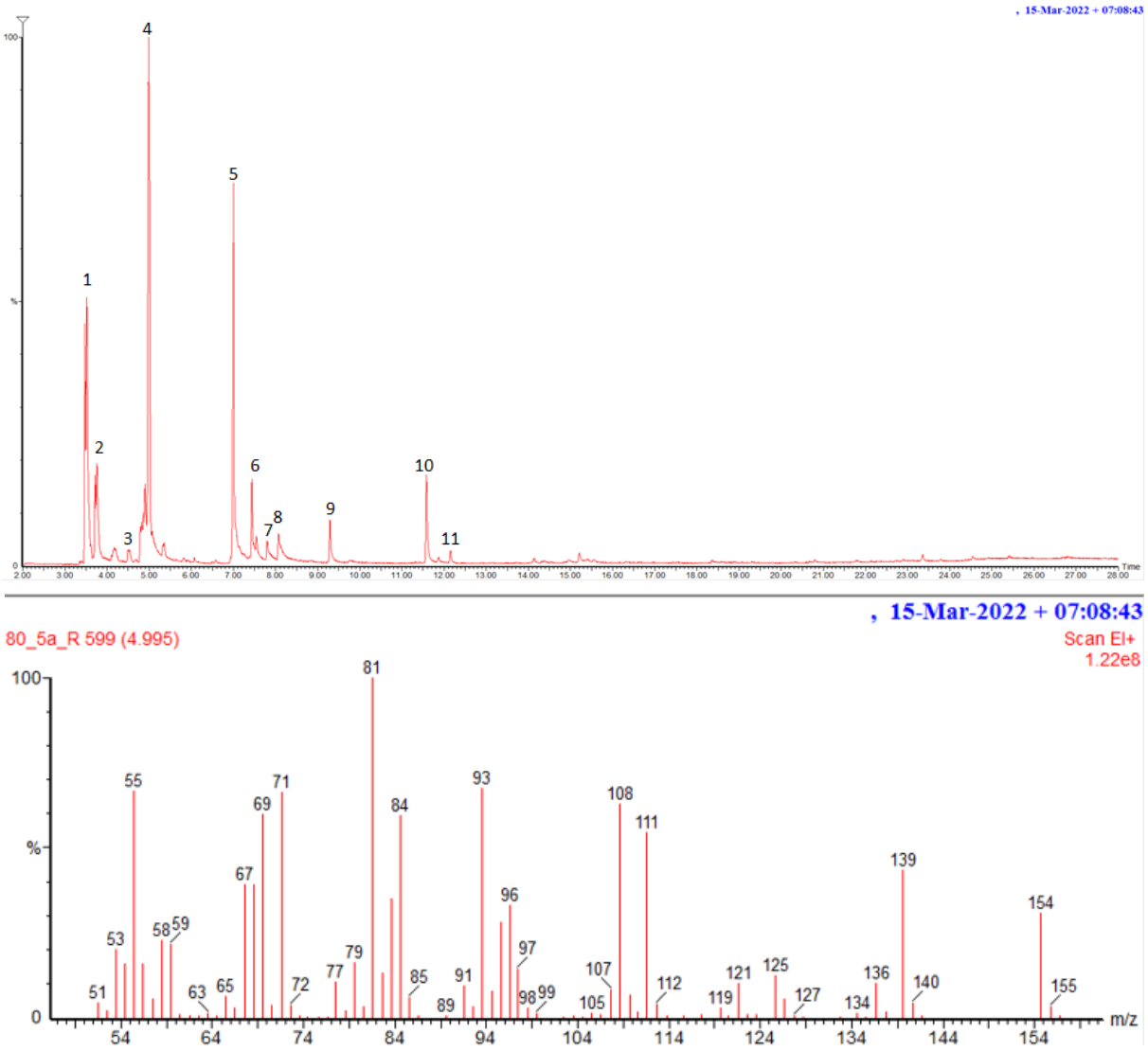


Figure 4. 10: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 80% Reflux method of rosemary leaves in the Bani Naim sample. The peak numbers of the components are listed in **Table (4.10)**.

Table 4. 10: Compounds detected in methanolic extracts 80% reflux method of rosemary leaves in the Bani Naim sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.524	3-Carene	136	$C_{10}H_{16}$
2	3.764	Camphene	136	$C_{10}H_{16}$
3	4.529	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.005	Eucalyptol	154	$C_{10}H_{18}O$
5	7.000	Camphor	152	$C_{10}H_{16}O$
6	7.446	Borneol	154	$C_{10}H_{18}O$
7	7.801	Bornyl Chloride	172	$C_{10}H_{17}O_2$
8	8.081	D-verbenone	150	$C_{10}H_{14}O_2$
9	9.321	Bornyl acetate	196	$C_{12}H_{20}O_2$
10	11.592	Caryophyllene	204	$C_{15}H_{24}$
11	12.177	Alph- caryophyllene	136	$C_{10}H_{16}$

Table (4.11) and **Figure (4.11)** show 9 compounds found in rosemary leaves in 90% methanol at room temperature in the Raqaa region. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.11)**.

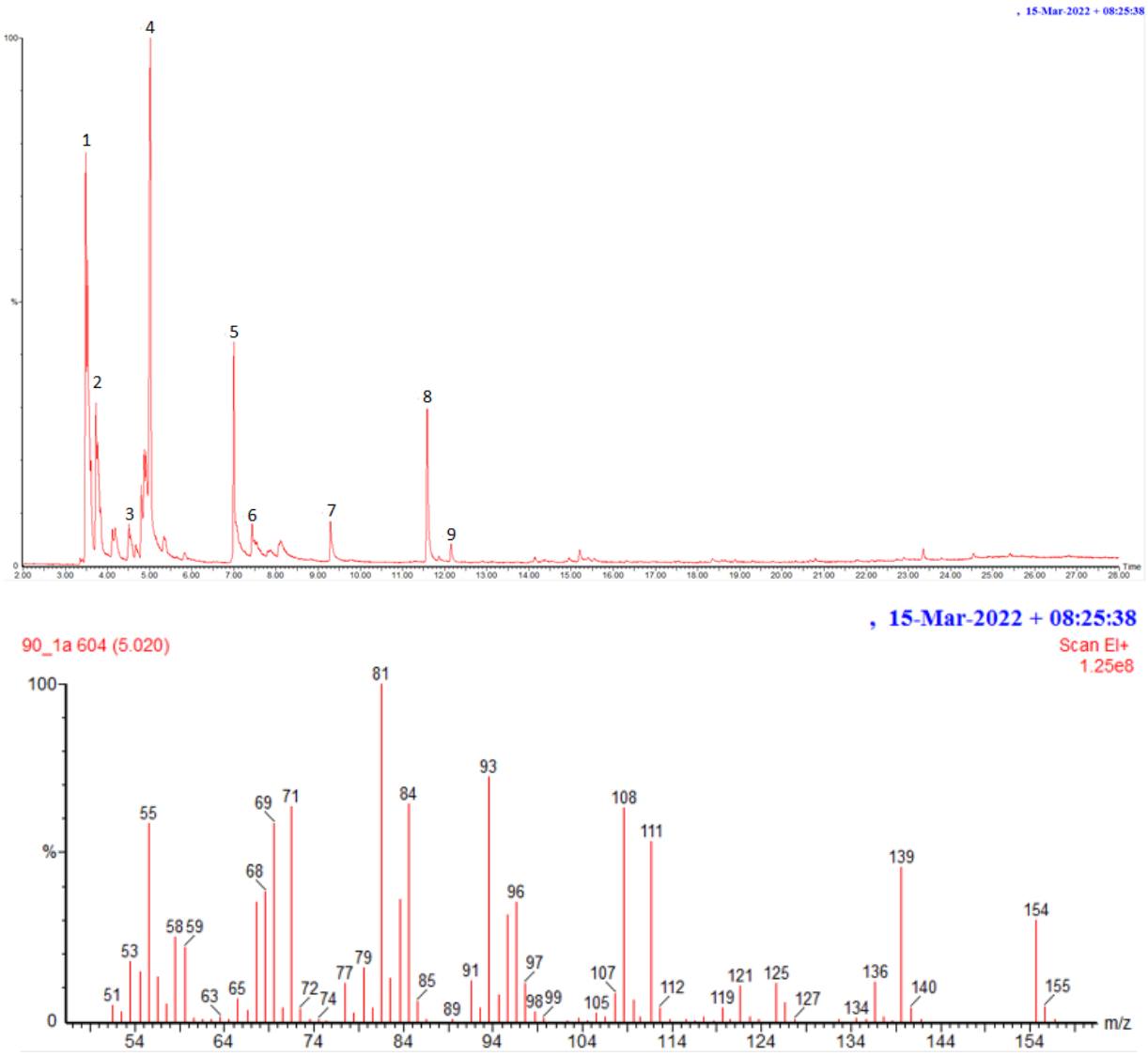


Figure 4. 11: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% at room temp. of rosemary leaves in Raqaa region sample. The peak numbers of the components are listed in **Table (4.11)**.

Table 4. 11: Compounds detected in methanolic extracts 90% at room temp. of rosemary leaves in the Raqaa sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.489	3-Carene	136	$C_{10}H_{16}$
2	3.729	Camphene	136	$C_{10}H_{16}$
3	4.509	Alph- caryophyllene	136	$C_{10}H_{16}$
4	5.020	Eucalyptol	154	$C_{10}H_{18}O$
5	7.000	Camphor	152	$C_{10}H_{16}O$
6	7.436	Borneol	154	$C_{10}H_{18}O$
7	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
8	11.592	Caryophyllene	204	$C_{15}H_{24}$
9	12.152	Alpha- Caryophyllene	204	$C_{15}H_{24}$

Table (4.12) and **Figure (4.12)** present the 15 compounds extracted from rosemary leaves in 90% methanol at room temperature in the Khilt Al-Adrah region sample. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.12).**

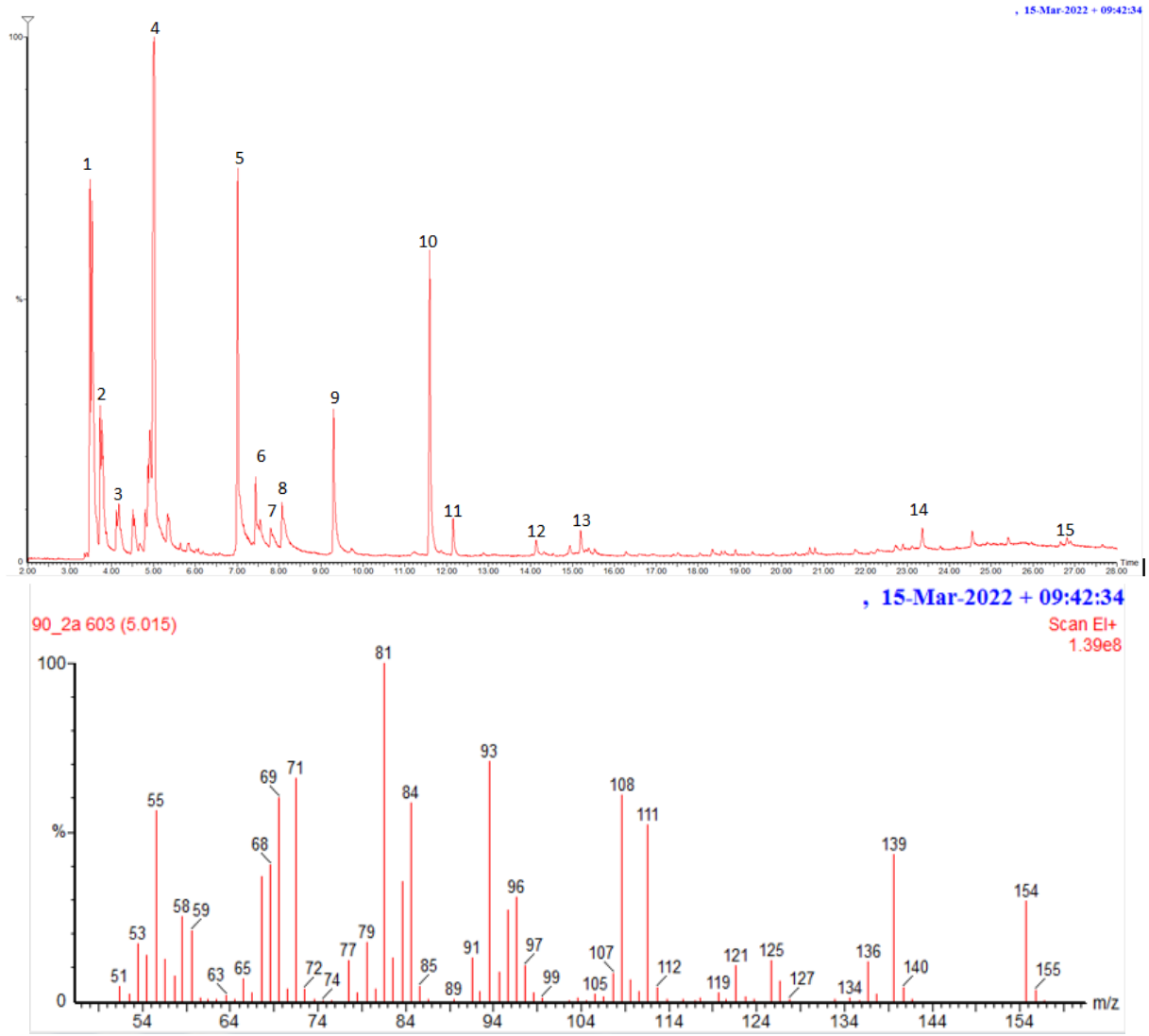


Figure 4. 12: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% at room temp. of rosemary leaves in Khilt Al-Adrah region sample. The peak numbers of the components are listed in **Table (4.12)**.

Table 4. 12: Compounds detected in methanolic extracts 90% at room temp. of rosemary leaves in the Khilt Al-Adrah sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.534	3-Carene	136	$C_{10}H_{16}$
2	3.729	Camphene	136	$C_{10}H_{16}$
3	4.509	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.015	Eucalyptol	154	$C_{10}H_{18}O$
5	7.020	Camphor	152	$C_{10}H_{16}O$
6	7.446	Borneol	154	$C_{10}H_{18}O$
7	7.811	Bornyl chloride	172	$C_{10}H_{17}Cl$
8	8.076	D-verbenone	150	$C_{10}H_{14}O$
9	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.602	Caryophyllene	204	$C_{15}H_{24}$
11	12.157	Alpha- caryophyllene	204	$C_{15}H_{24}$
12	14.143	Vitamine A aldehyde	284	$C_{20}H_{28}O$
13	15.204	Methyl steviol	204	$C_{21}H_{32}O_3$
14	23.367	Ferruginol	286	$C_{20}H_{30}O$
15	26.833	Alpha- amyrin	426	$C_{30}H_{50}O$

Table (4.13) and **Figure (4.13)** show the 13 compounds found in rosemary leaves in 90% methanol at room temperature in the Umm Lasfah village region. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.13)**.

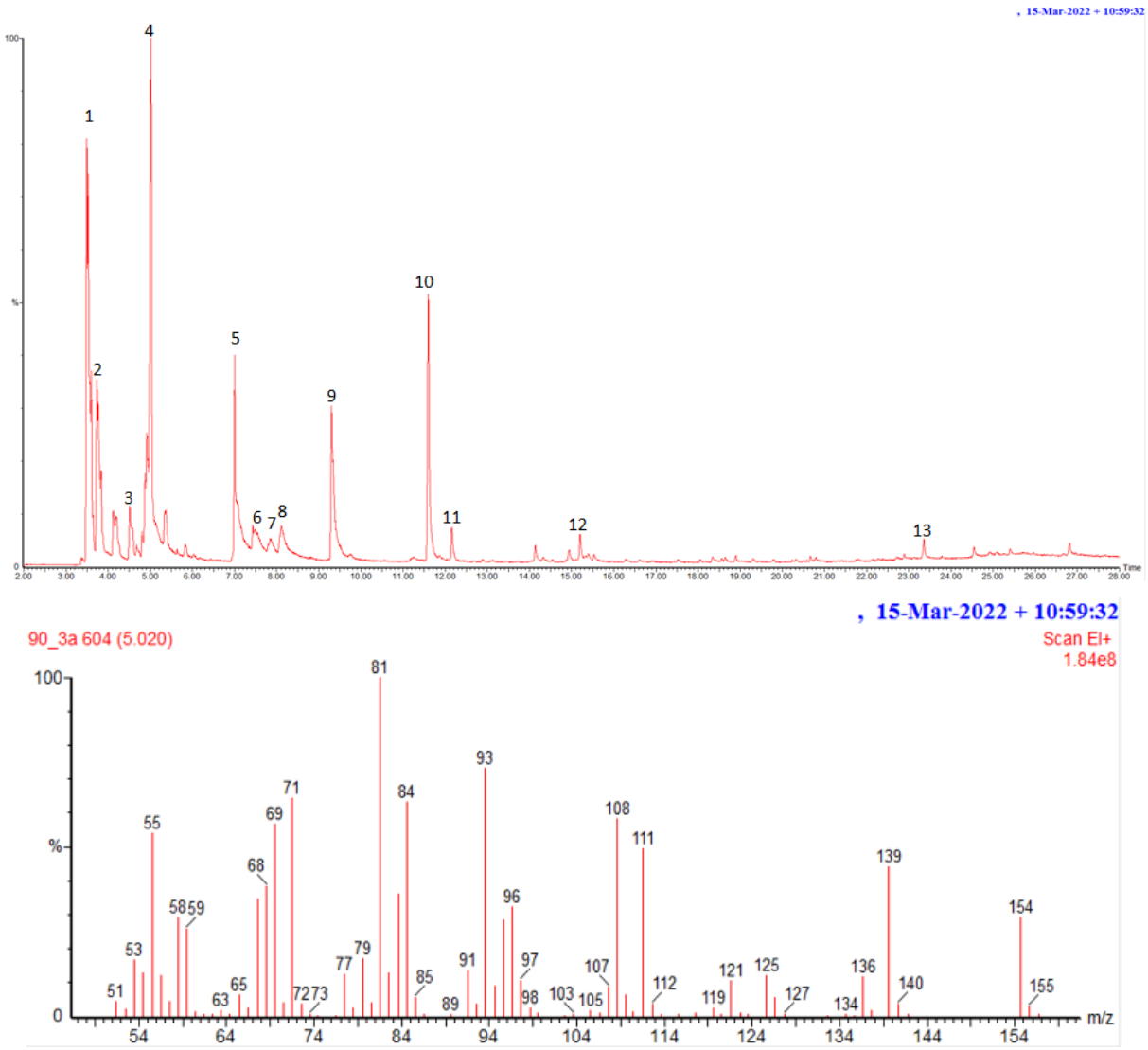


Figure 4. 13: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% at room temp. of rosemary leaves in Umm Lasfah village sample. The peak numbers of the components are listed in **Table (4.13)**.

Table 4. 13: Compounds detected in methanolic extracts 90% at room temp. of rosemary leaves in the Umm Lasfah village sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	Rt	Compound ID	MW	Molecular Formula
1	3.434	3-Carene	136	$C_{10}H_{16}$
2	3.729	Camphene	136	$C_{10}H_{16}$
3	4.509	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.005	Eucalyptol	154	$C_{10}H_{18}O$
5	7.010	Camphor	152	$C_{10}H_{16}O$
6	7.441	Borneol	154	$C_{10}H_{18}O$
7	7.801	Bornyl chloride	172	$C_{10}H_{17}Cl$
8	8.066	D-verbenone	150	$C_{10}H_{14}O$
9	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.597	Caryophyllene	204	$C_{15}H_{24}$
11	12.152	Alpha- caryophyllene	204	$C_{15}H_{24}$
12	15.194	Methyl steviol	204	$C_{21}H_{32}O_3$
13	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table (4.14) and **Figure (4.14)** show 9 compounds found in rosemary leaves in 90% methanol at room temperature in the Hebron region. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.14)**.

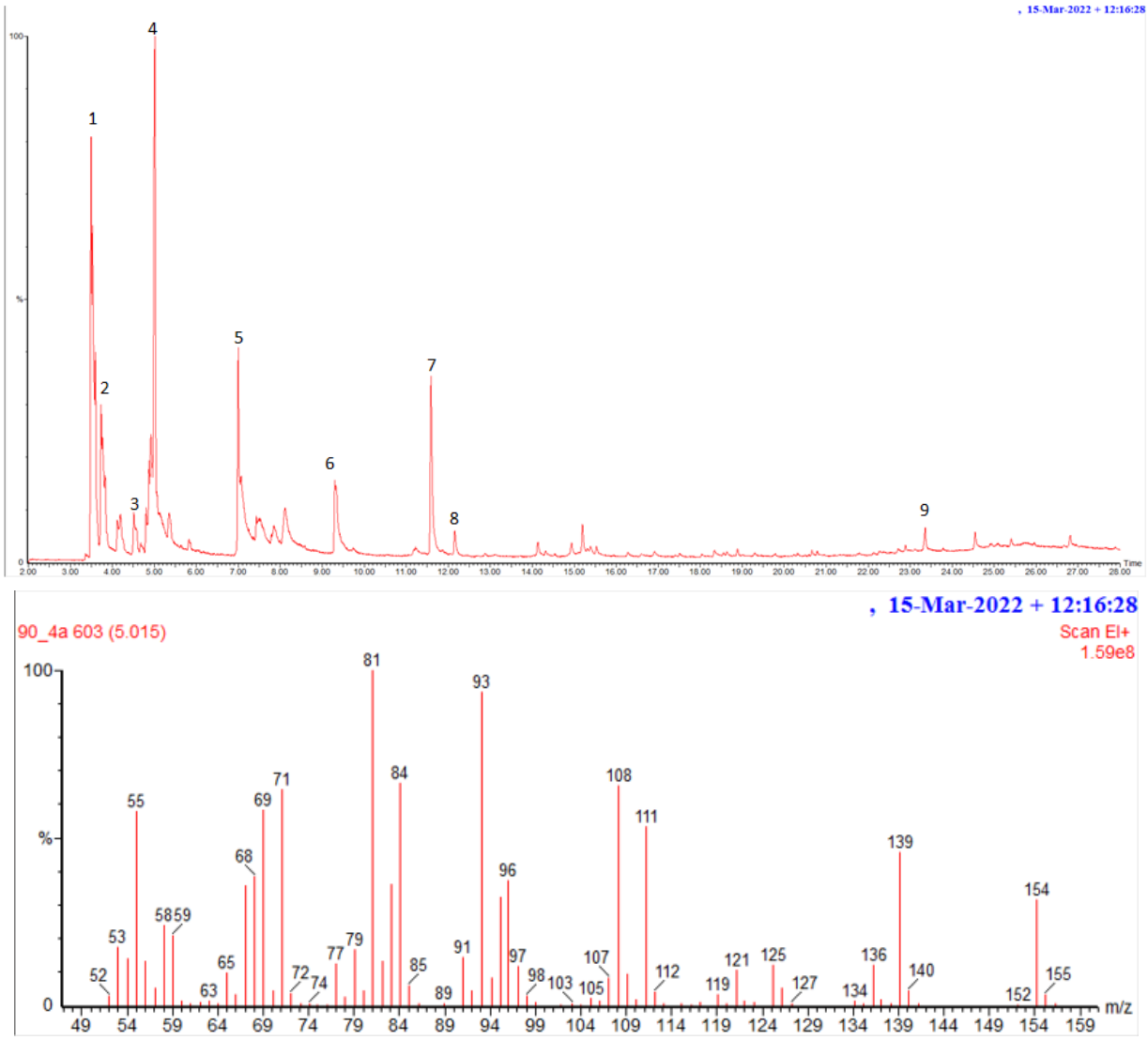


Figure 4. 14: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% at room temp. of rosemary leaves in Hebron sample. The peak numbers of the components are listed in **Table (4.14).**

Table 4. 14: Compounds detected in methanolic extracts 90% at room temp. of rosemary leaves in the Hebron sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.489	3-Carene	136	$C_{10}H_{16}$
2	3.734	Camphene	136	$C_{10}H_{16}$
3	4.509	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.025	Eucalyptol	154	$C_{10}H_{18}O$
5	7.015	Camphor	152	$C_{10}H_{16}O$
6	9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$
7	11.597	Caryophyllene	204	$C_{15}H_{24}$
8	12.157	Alpha- caryophyllene	204	$C_{15}H_{24}$
9	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table (4.15) and **Figure (4.15)** present the 12 compounds extracted from rosemary leaves in 90% methanol at room temperature in the Bani Naim region sample. The major compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.15)**.

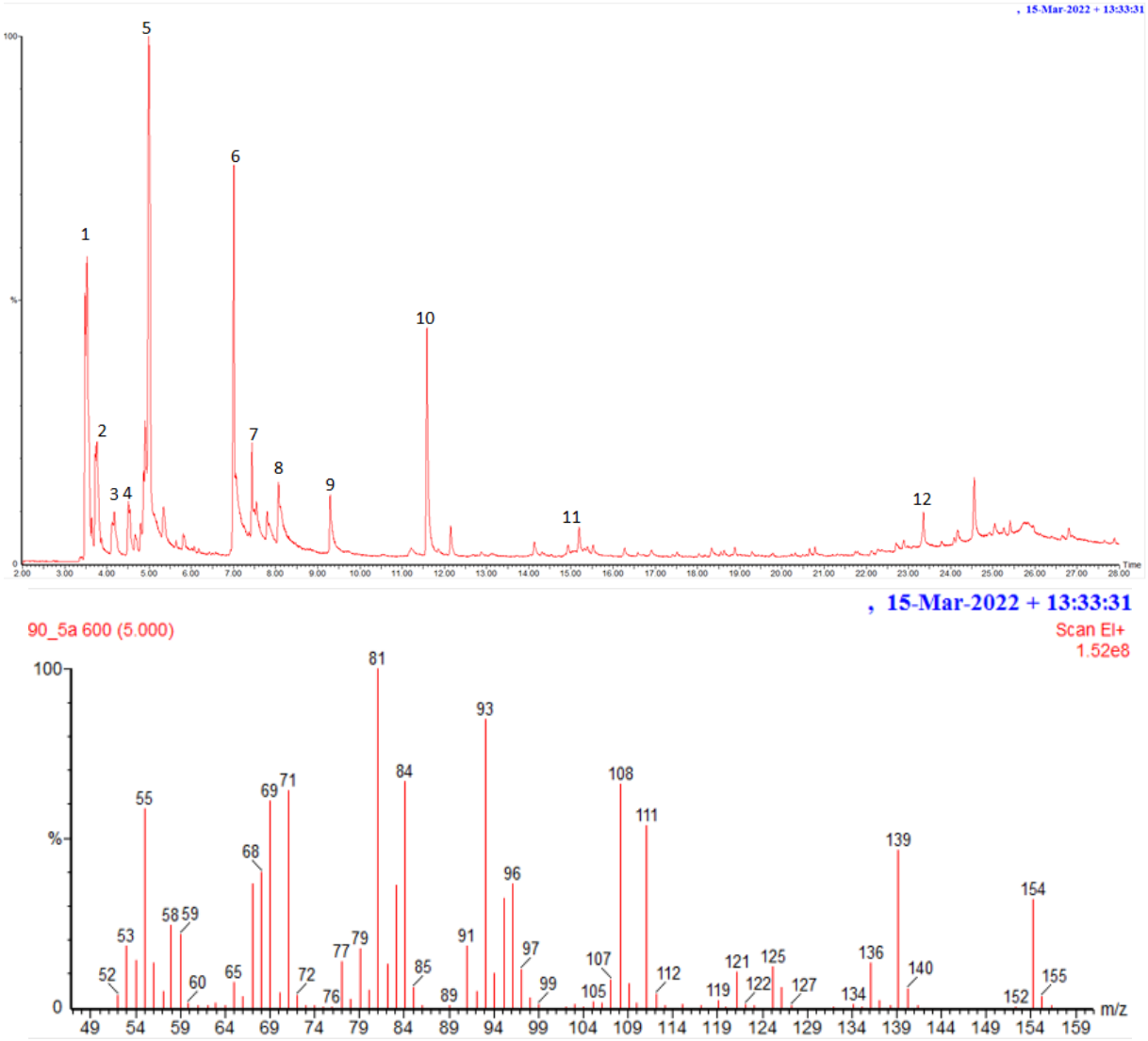


Figure 4. 15: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% at room temp. of rosemary leaves in Bani Naim sample. The peak numbers of the components are listed in **Table (4.15)**.

Table 4. 15: Compounds detected in methanolic extracts 90% at room temp. of rosemary leaves in the Bani Naim sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.494	3-Carene	136	$C_{10}H_{16}$
2	3.734	Camphene	136	$C_{10}H_{16}$
3	4.179	Alpha- pinene	136	$C_{10}H_{16}$
4	4.514	Alpha- phellandrene	136	$C_{10}H_{16}$
5	5.015	Eucalyptol	154	$C_{10}H_{18}O$
6	7.015	Camphor	152	$C_{10}H_{16}O$
7	7.446	Borneol	154	$C_{10}H_{18}O$
8	8.116	D-verbenone	150	$C_{10}H_{14}O$
9	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.597	Caryophyllene	204	$C_{15}H_{24}$
11	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
12	23.367	Ferruginol	286	$C_{20}H_{30}O$

Table (4.16) and **Figure (4.16)** the data indicate that 13 components found in the methanolic extracts 90% Reflux method of rosemary leaves in the Raqaa region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.16)**.

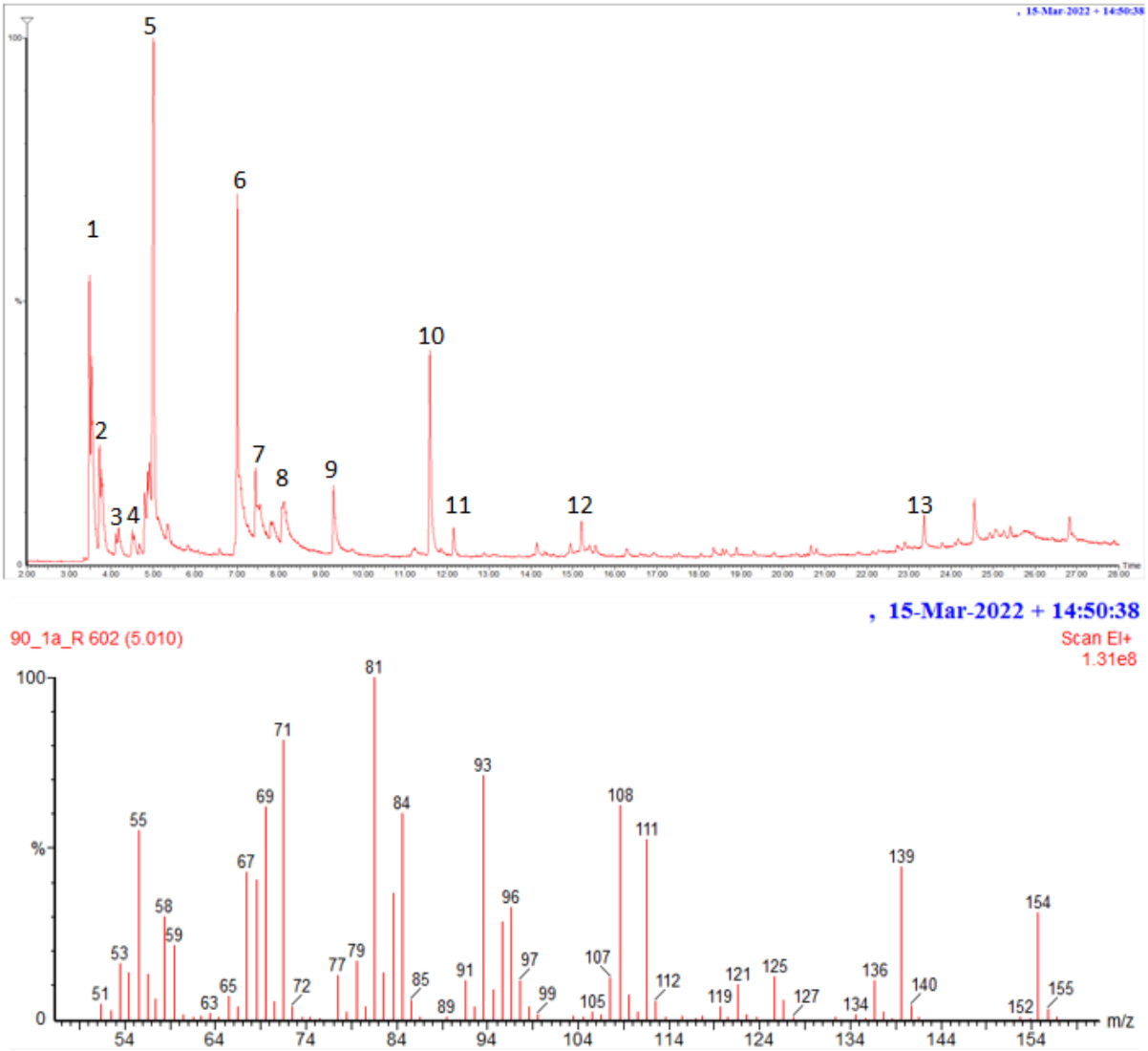


Figure 4. 16: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% Reflux method of rosemary leaves in the Raqaa sample. The peak numbers of the components are listed in **Table (4.16)**.

Table 4. 16: Compounds detected in methanolic extracts 90% reflux method of rosemary leaves in the Raqaa sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound ID	MW	Molecular Formula
1	3.489	3-Carene	136	$C_{10}H_{16}$
2	3.734	Camphene	136	$C_{10}H_{16}$
3	4.179	Alpha- pinene	136	$C_{10}H_{16}$
4	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
5	5.020	Eucalyptol	154	$C_{10}H_{18}O$
6	7.015	Camphor	152	$C_{10}H_{16}O$
7	7.451	Borneol	154	$C_{10}H_{18}O$
8	8.076	D-verbenone	150	$C_{10}H_{14}O$
9	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.597	Caryophyllene	204	$C_{15}H_{24}$
11	12.152	Alpha- Caryophyllene	204	$C_{15}H_{24}$
12	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
13	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table (4.17) and **Figure (4.17)** present the 12 compounds extracted from rosemary leaves in the methanolic extracts 90% Reflux method in the Khilt Al-Adrah region sample. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.17)**.

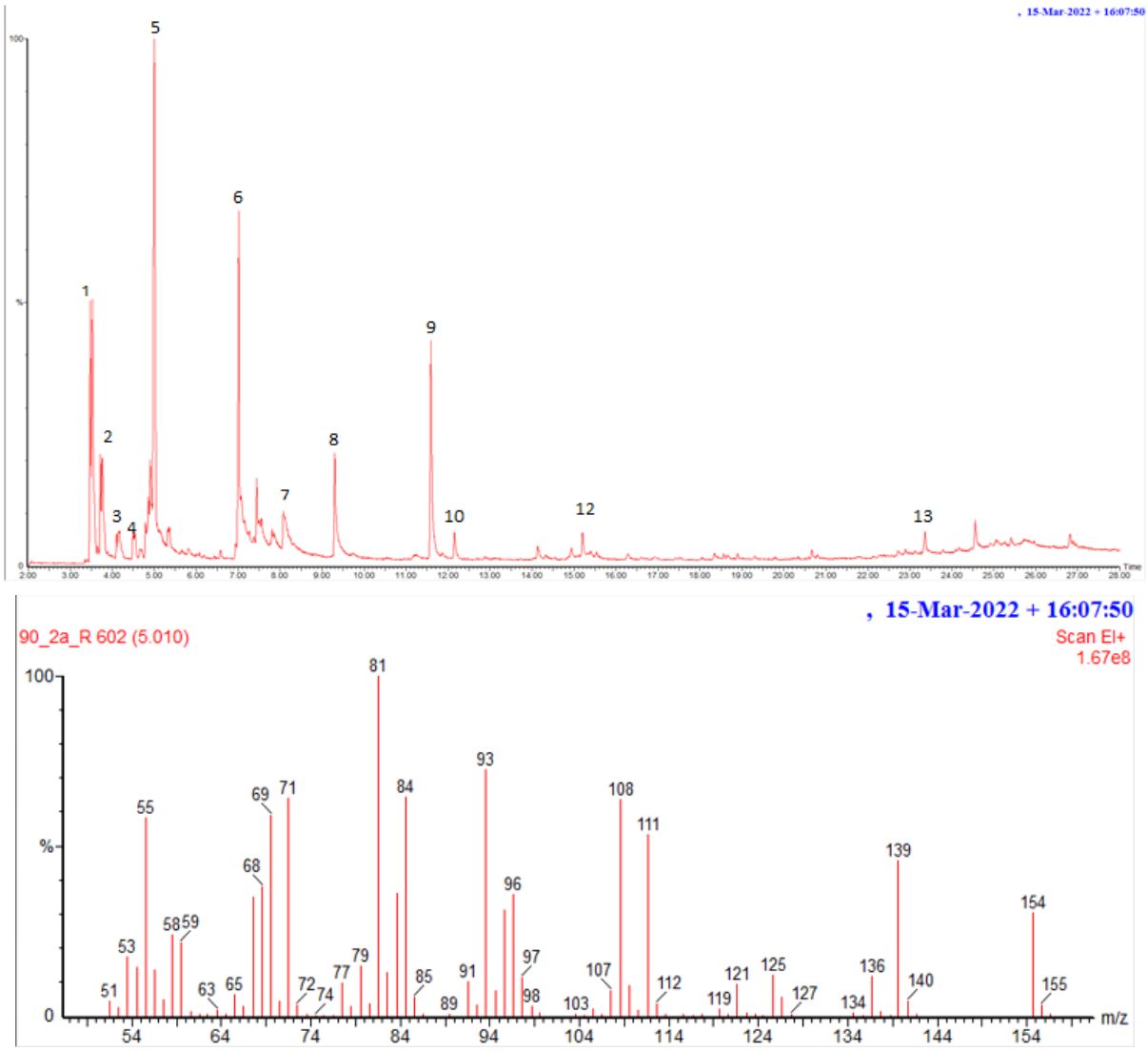


Figure 4. 17: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% Reflux method of rosemary leaves in the Khilt Al-Adrah sample. The peak numbers of the components are listed in **Table (4.17)**.

Table 4. 17: Compounds detected in methanolic extracts 90% reflux method of rosemary leaves in the Khilt Al-Adrah sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.479	3-Carene	136	$C_{10}H_{16}$
2	3.764	Camphene	136	$C_{10}H_{16}$
3	4.179	Alpha- pinene	136	$C_{10}H_{16}$
4	4.499	Alpha- phellandrene	136	$C_{10}H_{16}$
5	5.005	Eucalyptol	154	$C_{10}H_{18}O$
6	7.015	Camphor	152	$C_{10}H_{16}O$
7	8.076	D-verbenone	150	$C_{10}H_{14}O$
8	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
9	11.597	Caryophyllene	204	$C_{15}H_{24}$
10	12.152	Alpha- Caryophyllene	204	$C_{15}H_{24}$
11	15.199	Vitamin A aldehyde	284	$C_{20}H_{28}O$
12	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table (4.18) and **Figure (4.18)** show the 13 compounds found in the methanolic extracts 90% Reflux method of rosemary leaves in the Umm Lasfah village region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.18)**.

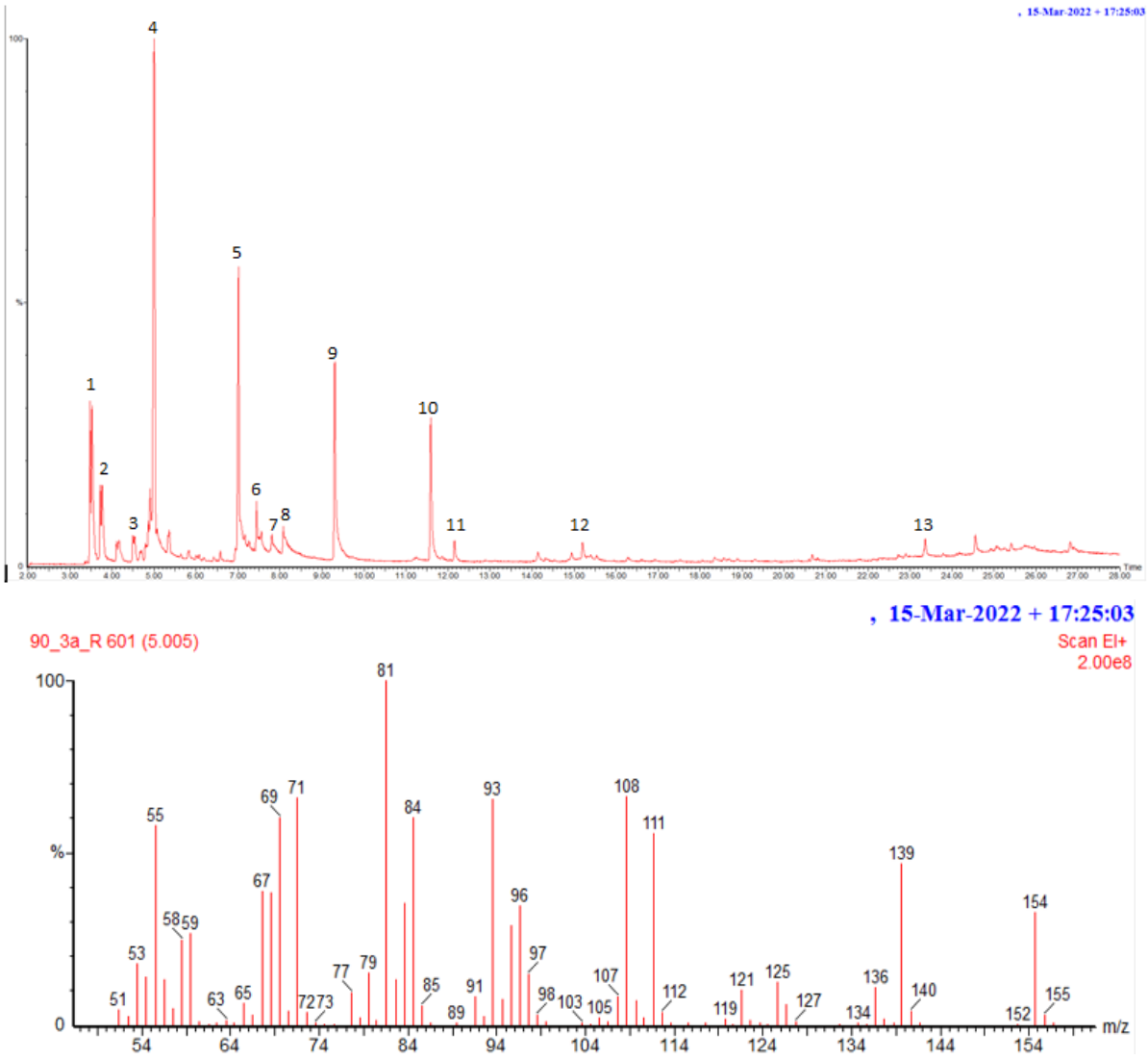


Figure 4. 18: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% Reflux method of rosemary leaves in the Umm Lasfah village sample. The peak numbers of the components are listed in **Table (4.18)**.

Table 4. 18: Compounds detected in methanolic extracts 90% reflux method of rosemary leaves in the Umm Lasfah village sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.479	3-Carene	136	$C_{10}H_{16}$
2	3.769	Camphene	136	$C_{10}H_{16}$
3	4.544	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.000	Eucalyptol	154	$C_{10}H_{18}O$
5	7.010	Camphor	152	C10H16O
6	7.446	Borneol	154	$C_{10}H_{18}O$
7	7.806	Bornyl chloride	172	$C_{10}H_{17}Cl$
8	8.076	D-verbenone	150	$C_{10}H_{14}O$
9	9.311	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.592	Caryophyllene	204	$C_{15}H_{24}$
11	12.152	Alpha- Caryophyllene	204	$C_{15}H_{24}$
12	15.209	Humulen-(V1)	204	$C_{15}H_{24}$
13	23.367	Ferruginol	286	$C_{20}H_{30}O$

Table (4.19) and **Figure (4.19)** the data indicate that 12 components found in the methanolic extracts 90% Reflux method of rosemary leaves in the Hebron region. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.19)**.

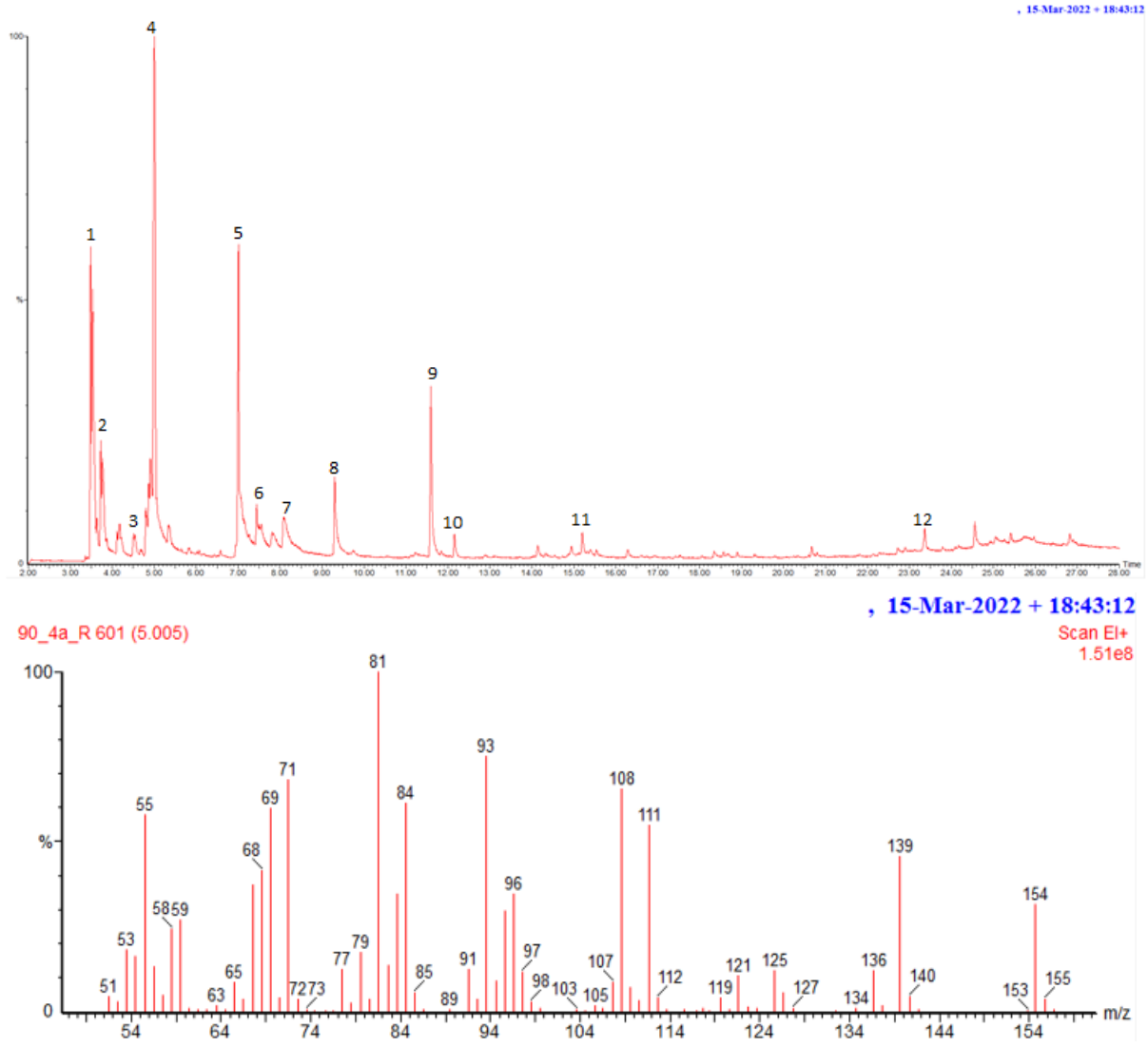


Figure 4. 19: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% Reflux method of rosemary leaves in the Hebron sample. The peak numbers of the components are listed in **Table (4.19)**.

Table 4. 19: Compounds detected in methanolic extracts 90% reflux method of rosemary leaves in the Hebron sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.494	3-Carene	136	$C_{10}H_{16}$
2	3.734	Camphene	136	$C_{10}H_{16}$
3	4.509	Alpha- phellandrene	136	$C_{10}H_{16}$
4	5.010	Eucalyptol	154	$C_{10}H_{18}O$
5	7.005	Camphor	152	$C_{10}H_{16}O$
6	7.446	Borneol, heptafluorobutyrate (ester)	350	$C_{14}H_{17}O_2F_7$
7	8.081	D-verbenone	150	$C_{10}H_{14}O$
8	9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$
9	11.592	Caryophyllene	204	$C_{15}H_{24}$
10	12.162	Alpha- Caryophyllene	204	$C_{15}H_{24}$
11	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
12	23.367	Ferruginol	286	$C_{20}H_{30}O$

Table (4.20) and **Figure (4.20)** present the 12 compounds extracted from rosemary leaves in the methanolic extracts 90% Reflux method in the Bani Naim region sample. The major volatile compounds identified were 3-Carene, Camphene, Eucalyptol, Camphor, Borneol, Isoborneol acetate, and Caryophyllene. The other minor volatile compounds identified were shown in **Tables (4.20)**.

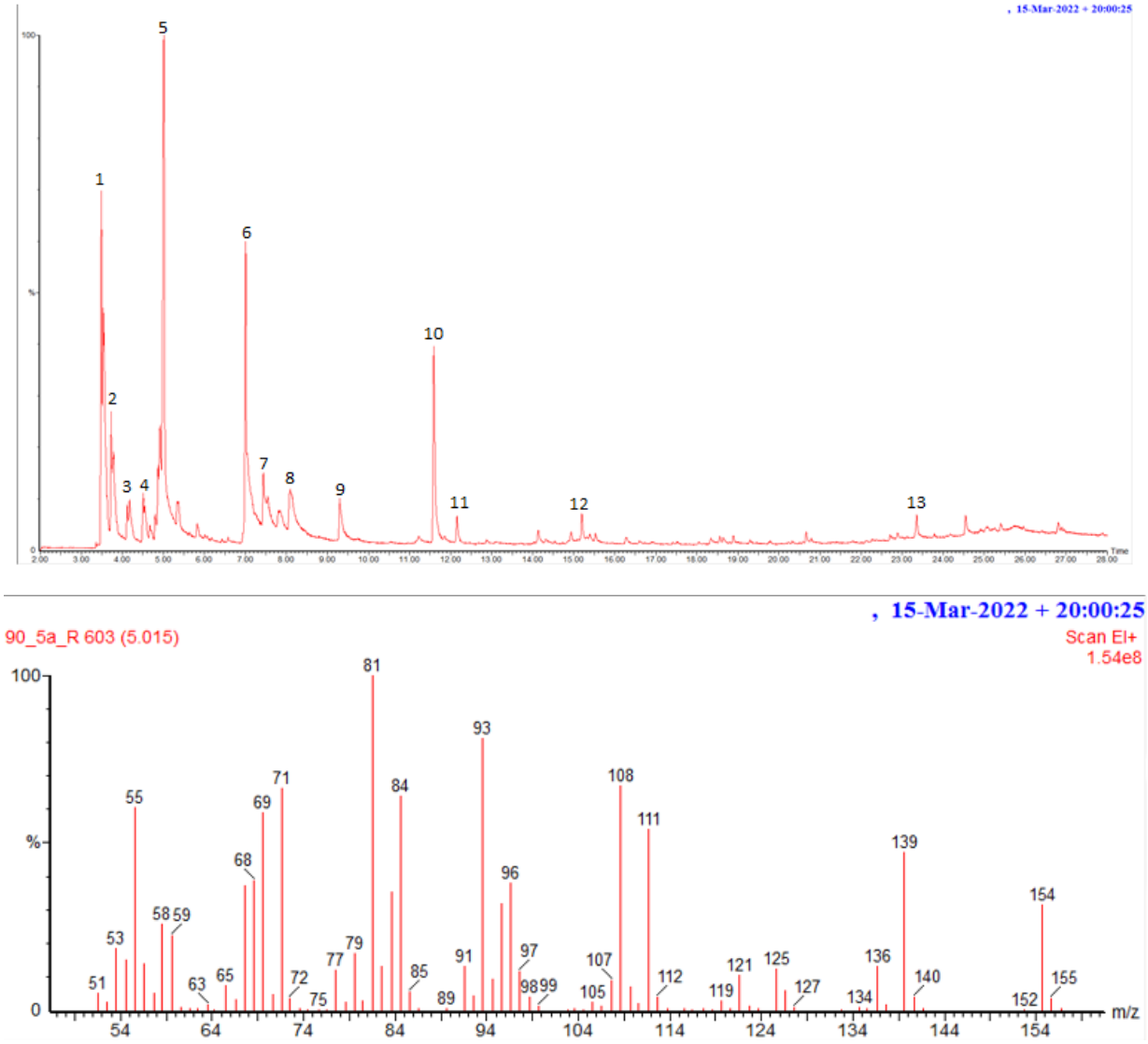


Figure 4. 20: Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts 90% Reflux method of rosemary leaves in the Bani Naim sample. The peak numbers of the components are listed in **Table (4.20)**.

Table 4. 20: Compounds detected in methanolic extracts 90% reflux method of rosemary leaves in the Bani Naim sample with their retention time (*Rt*), molecular weight (MW), molecular weight, and the molecular formula (MF).

Peak	*Rt*	Compound	MW	Molecular Formula
1	3.494	3-Carene	136	$C_{10}H_{16}$
2	3.739	Camphene	136	$C_{10}H_{16}$
3	4.129	Alph-pinene	136	$C_{10}H_{16}$
4	4.514	Alpha- phellandrene	136	$C_{10}H_{16}$
5	5.020	Eucalyptol	154	$C_{10}H_{18}O$
6	7.005	Camphor	152	$C_{10}H_{16}O$
7	7.476	Borneol, heptafluorobutyrate (ester)	350	$C_{14}H_{17}O_2F_7$
8	8.126	D-verbenone	150	$C_{10}H_{14}O$
9	9.316	Isobornyl acetate	196	$C_{12}H_{20}O_2$
10	11.597	Caryophyllene	204	$C_{15}H_{24}$
11	12.162	Alpha- Caryophyllene	204	$C_{15}H_{24}$
12	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
13	23.367	Ferruginol	286	$C_{20}H_{30}O$

4.2. Qualitative phytochemical screening

The results of the rosemary leave qualitative phytochemical screening tests were carried out and showed that the methanolic extract of the samples contains a wide range of phytochemical groups such as cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins, and terpenoids in all regions samples. Interestingly, alkaloids were found only in the leaves of the regions Raqaa and Bani Naim samples. However, Other groups such as anthocyanins, anthraquinone, Flavonoids, Glycosides and Phlobatnnins were not present. Qualitative results of the phytochemical compounds of rosemary samples are expressed as (+) for the presence and (-) for the absence, presented in **Table (4.21)**.

Table 4. 21: Phytochemical screening for the methanolic extracts from rosemary leaves samples.

Rosemary sample location	Parts	Phytochemical screening tests													
		Cardiac glycosides	Phenolic groups	Alkaloids	Anthocyanin	Coumarin	Saponins	Anthraquinone	Quinones	Steroids	Tannins	Terpenoids	Flavonoids	Glycosides	Phlobatnnins
Raqaa	**Leaves**	+	+	+	-	+	+	-	-	+	+	+	-	-	-
Khilt Al-Adrah		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Umm Lasfah village		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Hebron		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Bani Naim		+	+	+	-	+	+	-	-	+	+	+	-	-	-

4.3. Antioxidant activity

4.3.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging capacity

The antioxidant activity of rosemary leaves was examined by the DPPH method. The diluted methanolic extract (1:10) of the different rosemary samples showed antioxidant activities and the average percentage of scavenging capacity is shown in **Figure (4.21).** Leaves extracts of Bani Naim (76.36%) and Umm Lasfah village (75.99%) samples exhibited the highest levels of antioxidant capacity followed by Raqaa (75.86%) and Khilt Al-Adrah (74.58%) samples. The Hebron sample has the lowest level of antioxidant capacity (73.25%).

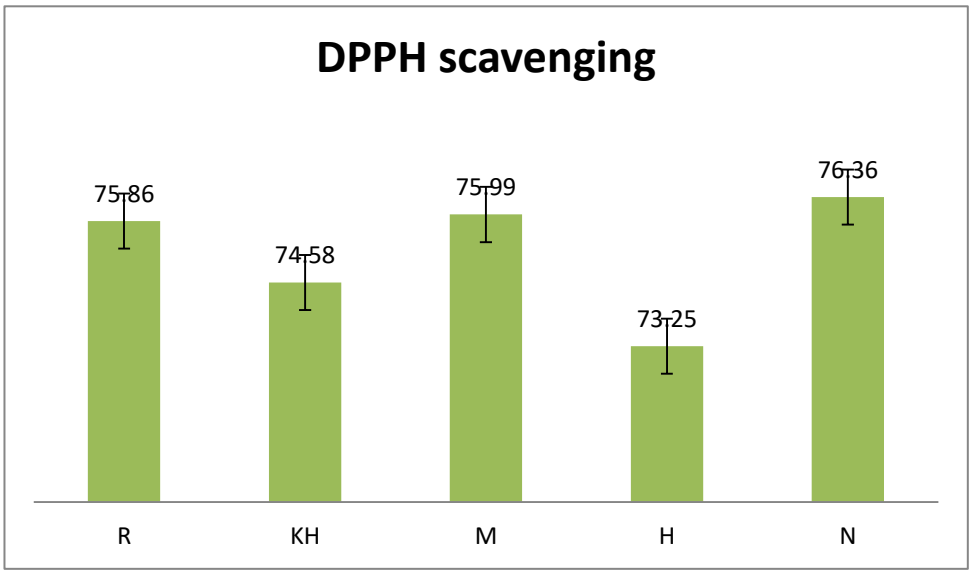


Figure 4. 21: Antioxidant capacity (%) of the methanolic extracts of five rosemary samples, assayed by the DPPH free radical scavenging assay, n=3. **(R):** Raqaa, **(KH):** Khilt Al-Adrah, **(M):** Umm Lasfah village, **(H):** Hebron, **(N):** Bani Naim samples.

4.3.2. 2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging capacity

The antioxidant activity of rosemary leaves was examined by the ABTS method. The diluted methanolic extracts (1:10) of the different rosemary samples showed antioxidant activities and the average percentage of scavenging capacity is shown in **Figure (4.22).** The percentage of scavenging of diluted methanolic extracts of leaves of Umm Lasfah village (88.82%) sample exhibited the highest levels of antioxidant capacity followed by Bani Naim (79.67%), Raqaa (75.89%), Khilt Al-Adrah (75.58%), and Hebron (73.91%) samples.

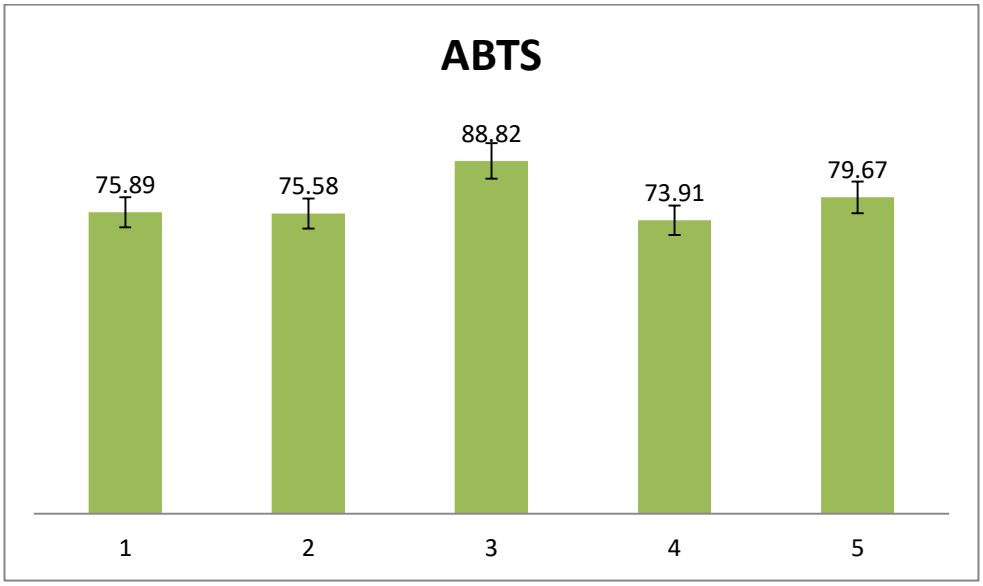


Figure 4. 22: Antioxidant capacity (%) of the methanolic extracts of five rosemary samples, assayed by the ABTS free radical scavenging assay, n=3. **(R):** Raqaa, **(KH):** Khilt Al-Adrah, **(M):** Umm Lasfah village, **(H):** Hebron, **(N):** Bani Naim samples**.**

4.3.3. Total phenols

The total phenols in the methanolic extract of different rosemary samples were quantitatively estimated as mg of Gallic acid equivalent (GAE mg /l) (n=3) as shown in Figure (4.22). The diluted (1:10) in methanol showed pronounced phenols as assessed by the Folin-Ciocalteu reagent. The total phenolic contents of diluted methanolic extracts of leaves of Umm Lasfah village (876.7 mg GAE/g), Khilt Al-Adrah (728.3 mg GAE/g), Raqaa (693.3mg GAE/g), Hebron (652.8 mg GAE/g), and Bani Naim (616.7 mg GAE/g), samples respectively (Figure 4.24).

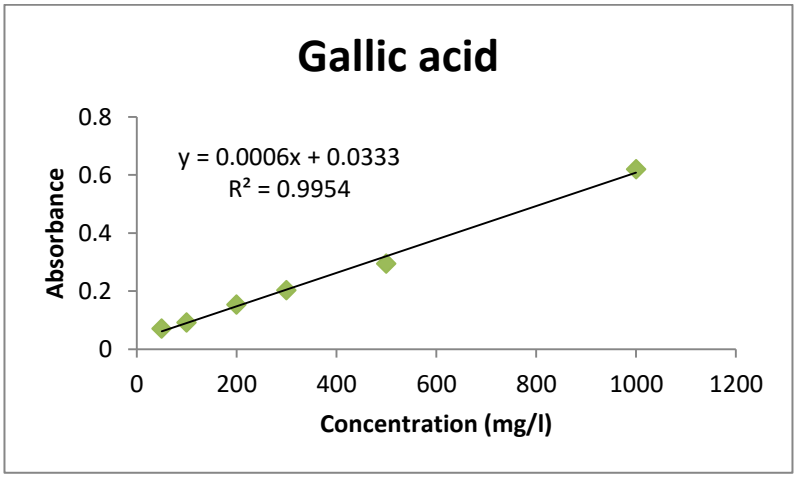


Figure 4. 23: Calibration curve of gallic acid. Each point represents the mean of triplicates

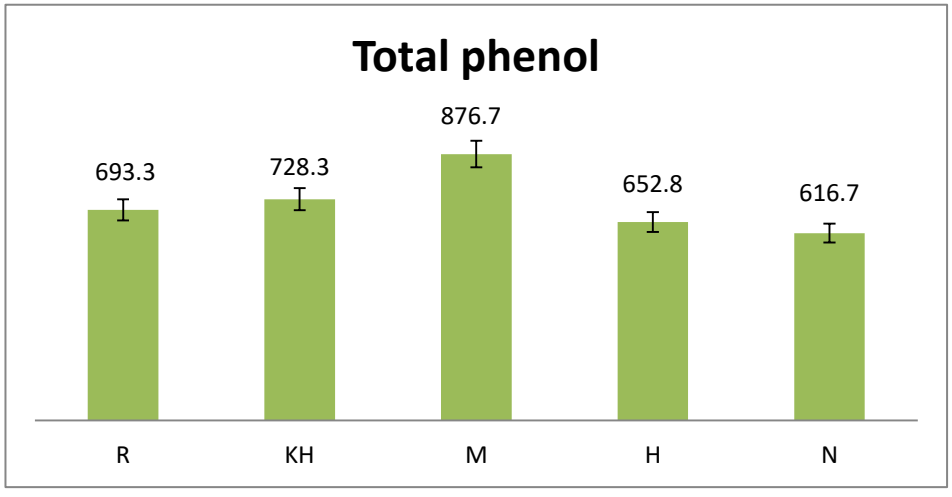


Figure 4. 24: Total phenols contents of five rosemary samples methanolic extracts produced by Folin -Ciocalteu method, n=3. **(R):** Raqaa, **(KH):** Khilt Al-Adrah, **(M):** Umm Lasfah village, **(H):** Hebron, **(N):** Bani Nai samples.

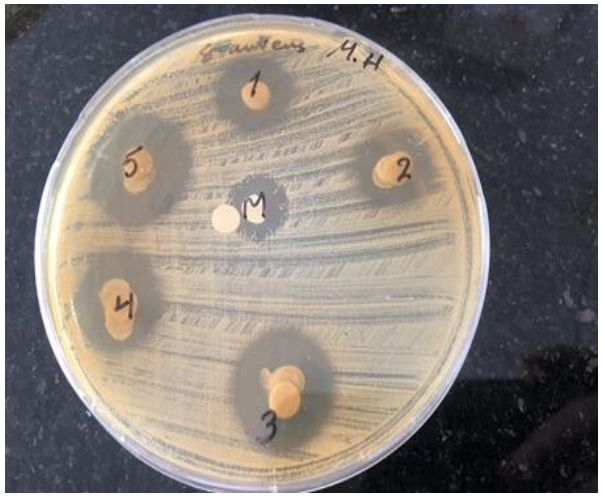
4.4. Antibacterial activity

The initial screening of the different concentrations of methanolic extracts of *R. officinalis* against different types of organisms was performed. The antibacterial activity of 50 µl of *R. officinalis* leaves extracts was examined on gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by using the disc diffusion method. The zones of inhibition measured were shown in **Figure (4.25)** and the results of the zones of inhibition were summarized in **Table (4.22).**

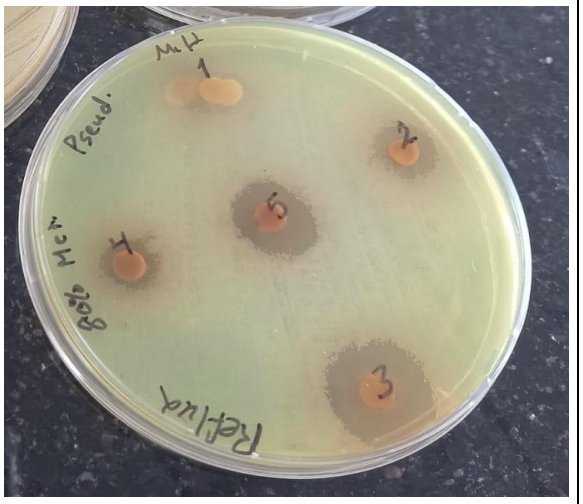
It is clear that 50 µl of *R. Officinalis* exhibits notable antibacterial activities against gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*). For the 80% methanolic extracts at room temperature and reflux methods of extractions, the observed activities in gram-positive bacteria were higher than in gram-negative bacteria. Conversely, no activity on the *P. aeruginosa* strain was shown by the Raqaa sample, and both Hebron and Umm Lasfah extracts (methanolic extractions of 80% and reflux method) were inactive against the *E. coli* strain. It was also observed that the antibacterial activity of 90% methanol extract at room temperature was high against the *P. aeruginosa* strain. Conversely, no activity was shown by Hebron, Bani Naim, Raqaa, and Umm Lasfah extracts samples on *E. coli* in both methanolic extractions methods (90% at room temperature and reflux).

These findings demonstrated that these plants have antibacterial effects and the activities depended upon the presence of the phytochemicals which are affected by the method of extraction. These findings demonstrate that the phytochemicals present in certain ethnobotanical species including rosemary have antibacterial properties, this is the main reason for their use as a traditional remedy in Palestinian folk medicine.

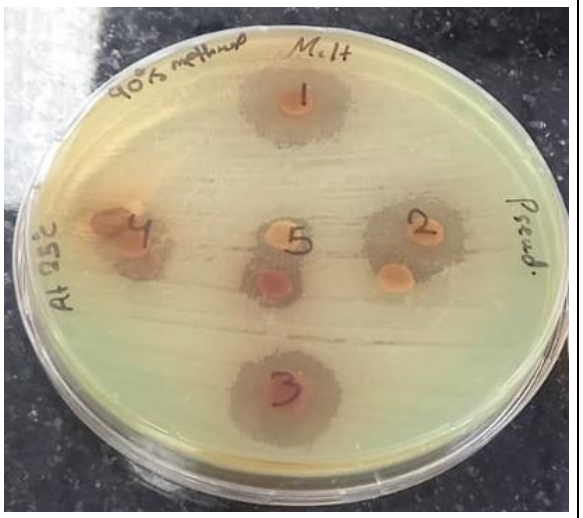
A

Rosemary Extract	***Staphylococcus Aureus***	***E. coli***	***Pseudomonas***
At room temperature (80% Methanol)			

B

Reflux (80% Methanol)			

C

At room temperature (90% Methanol)			

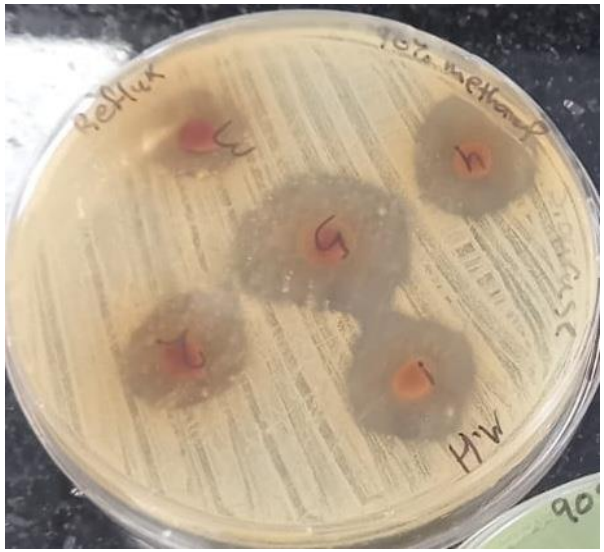
Reflux (90% Methanol)			

Figure 4. 25: Zone of inhibition of *R. officinalis* leaves Extracts for A (80% Methanol, RT.), B (80% Methanol, Reflux), and C (90% Methanol; RT. & Reflux): **(1):** Raqaa, **(2):** Khilt Al-Adrah, **(3):** Umm Lasfah village, **(4):** Hebron, **(5):** Bani Naim samples.

Table 4. 22: The antibacterial activity results of *R. officinalis* leaves Extracts for A (80% Methanol, RT.), B (80% Methanol, Reflux), and C (90% Methanol; RT. & Reflux): (R): Raqaa, (KH): Khilt Al-Adrah, (M): Umm Lasfah village, (H): Hebron, (N): Bani Naim samples

Average zone of inhibition (cm)						
	At room temperature (80% Methanol)					
Sample	**R**	**KH**	**M**	**H**	**N**	**Control**
S. aureus	1.7	1.6	1.8	1.7	2.2	**No effect**
E. coli	0.9	1	1.1	1.2	1.2	**No effect**
P. aeruginosa	1.2	1.4	1.6	1.6	1.6	**No effect**
Average zone of inhibition (cm)						
	Reflux (80% Methanol)					
Sample	**R**	**KH**	**M**	**H**	**N**	**Control**
S. aureus	1.4	1.3	1.2	1.2	1.5	**No effect**
E. coli	1.4	1.4	**No effect**	**No effect**	0.6	**No effect**
P. aeruginosa	**No effect**	1.2	1.8	0.8	1.5	**No effect**

Average zone of inhibition (cm)						
	At room temperature (90% Methanol)					
Sample	**R**	**KH**	**M**	**H**	**N**	**Control**
S. aureus	**0.5**	**0.4**	**0.4**	**0.1**	**0.1**	**1.1**
E. coli	**1.2**	**2**	**1.5**	**No effect**	**No effect**	**No effect**
P. aeruginosa	**1.4**	**1.6**	**1.2**	**1.6**	**1.2**	**0.8**
	Average zone of inhibition (cm)					
	Reflux (90% Methanol)					
Sample	**R**	**KH**	**M**	**H**	**N**	**Control**
S. aureus	**0.9**	**0.5**	**0.4**	**0.7**	**1.4**	**1.1**
E. coli	**No effect**	**1.4**	**No effect**	**1**	**1.2**	**No effect**
P. aeruginosa	**1.2**	**1**	**0.4**	**0.8**	**0.8**	**0.8**

4.5. Proximate composition and mineral contents

The values of the proximate composition of the dried rosemary samples used in the experiment are presented in **(Table 4.23).** All the samples collected from different regions showed similar values of dry matter content (93.3 %-94.5%) for dried rosemary leaf powders. The fiber content of the samples showed that the Hebron region sample has the highest percentage (28.7%), while the lowest percentage was recorded in the Raqaa sample (23.5 %). The fat content in the samples was found to be approximately similar in all samples; Khallet Al-Adra, Hebron, and Bani Naim (14.01%, 14.85%, and 14.54%). As for the regions of Al-Raqqa and Umm Lasfafa samples, the percentages of fat were lower (11.95%, and 12.87%). The ash values of the samples showed that the sample of Raqaa'a region has the highest percentage (9.31%), then Khalil Al-Adra (8.2%), Bani Naim (8.2%), and Hebron (8.1%) samples, while the lowest percentage was recorded in the sample of Umm Lasfa village (7.3%).

Mineral analysis

Several studies on the mineral content of medicinal plants have been published in many developed countries. To the best of our knowledge, there is no single report in Palestine on the mineral composition of these herbs. Our present work was carried out to screen the availability of minerals in *R. officinalis* leaves by using inductively coupled plasma optical emission spectrometry (ICP-OES). In our samples, seven minerals were screened namely; calcium (Ca), potassium(K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), and Phosphorus (P). Based on the results of the analysis, a list of the detected minerals was compiled by their percentage content in the ash residue of leaves of rosemary: Ca>K>Mg>Na>Fe>Mn>P **Figure (4.26)**, **Table (4.23)**. The calcium content was higher in the regions of Raqaa and Bani Naim, while the potassium content was higher in the regions of Raqaa and Umm Lasfah village.

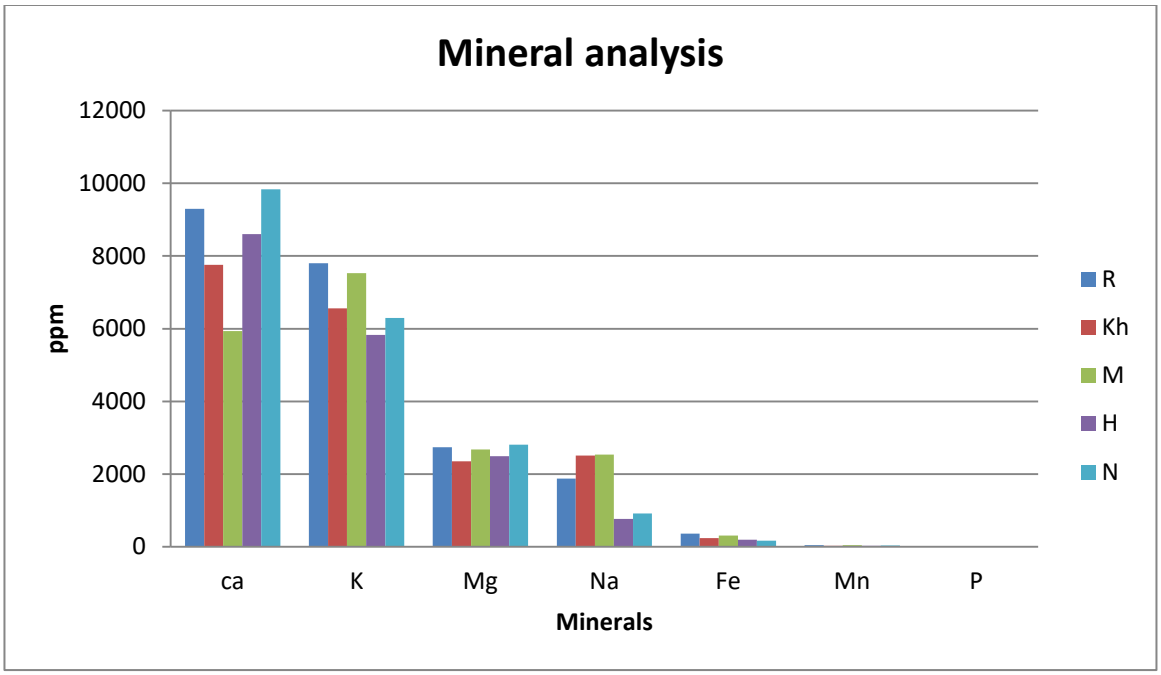


Figure 4. 26: Minerals in rosemary leaves. (R): Raqaa, (KH): Khilt Al-Adrah, (M): Umm Lasfah village, (H): Hebron, (N): Bani Naim samples

Table 4. 23: Proximate composition and mineral contents of rosemary leaf

Samples	Dry Matter %	Moisture %	Fiber %	Fat %	Ash %	Ca (*ppm*)	K (*ppm*)	Mg (*ppm*)	Na (*ppm*)	Fe (*ppm*)	Mn (*ppm*)	P (*ppm*)
Raqaa	94.4	5.6	23.5	11.95	9.3	9302.2	7803.2	2734.1	1874.5	361.0	42.7	6.8
Khilt Al-Adrah	93.3	6.7	27.2	14.01	8.2	7758.2	6556.1	2348.5	2504.6	234.3	25.7	6.6
Umm Lasfah village	94.0	6.0	26.7	12.87	7.3	5932.0	7526.7	2672.4	2532.2	311.2	40.2	5.2
Hebron	94.5	5.5	28.7	14.84	8.1	8604.2	5831.9	2492.0	761.4	190.3	23.0	0.5
Bani Naim	93.8	6.2	28.2	14.54	8.2	9835.1	6297.2	2809.0	912.4	169.9	37.3	0.9

Chapter Five

Discussion

5. Discussion

The main goals of this research were to identify the main phenolic and volatile compounds found in a rosemary plant in Palestine, estimate the antioxidant effect using ABTS and DPPH assays, study the impact of the extracts of this plant if they have activity against specific strains of pathogenic bacteria, show some phytochemical screening, and analysis of the proximate composition and mineral contents. According to our study results, rosemary has high antioxidant activity and polyphenols content. Rosemary showed high antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli.* Rosemary plant samples from all regions tested for the phytochemicals were found to have cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins, and terpenoids.

5.1. GC-MS analysis

The volatile components of (80% and 90% Methanol; at room temp and reflux) for rosemary leaf extracts were detected using GC-MS with the Electron Impact (EI) mode and compared to the National Institute of Standards and Technology (NIST) database. The main components of the rosemary plant samples are Eucalyptol, Camphor, 3-Carene, Camphene, Borneol, Isoborneol acetate, Bornyl acetate, and Caryophyllene. These results are also compatible with the results obtained by (Verma *et al.*, 2012; Masumoto *et al.*, 2018). Borneol, Bornyl acetate, Camphor, 1,8-Cineole (Eucalyptol), and Verbenone are the main volatile components that contribute to the distinctive flavor and aroma of Rosemary extracts (Senanayake, 2018). Rosemary contains several phytochemicals which could be used to treat diseases or/ and disorders. It was found that the major chemotype for Mediterranean-grown rosemary such as Eucalyptol and Camphor (Jordán *et al.*, 2013). Eucalyptol has several drug properties that are gaining medical attention, as well as evidence of its anti-inflammatory and antioxidant mode of action (Juergens, 2014). Eucalyptol also is a powerful cytokine inhibitor with a significant improvement in anti-inflammatory action, according to research by (Juergens *et al.*, 2017). Volatile components like Eucalyptol and α-pinene have an anti-hyperglycemic impact because they lower plasma glucose levels, raise insulin levels, and aid in glucose utilization by cells (Selmi *et al.*, 2017). Camphor is also widely used on the skin for its antipruritic, analgesic, and anti-irritant properties (Burkhart and Burkhart, 2003).

5.2. Qualitative phytochemical screening

Phytochemicals can vary greatly depending on plant parts (stems, leaves, flowers, and root) (Gurbuz *et al.*, 2016), extraction circumstances (time, solvents, and extraction method) (Altemimi *et al.*, 2017), processing procedures, and environmental conditions in which plants grow (Zeroual *et al.*, 2021). In the present study, the results concluded that the studied plants contained an appreciable number of cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins, and terpenoids **(Table 4.21).** According to a study by Tabassum and others, the leaves of rosemary plants present tannins, flavonoids, terpenoids, alkaloids, cardiac glycosides, phenols, and saponins (Tabassum *et al.*, 2021). In addition, Johar *et al.* showed that rosemary has terpenoids, flavonoids, and saponins, but not tannins (Johar *et al.*, 2015).

The presence of phenolic compounds may help in preventing several chronic illnesses, including diabetes, cancer, cardiovascular disease, and infections caused by bacteria and parasites. The rosemary leaf extracts tested in this current study show that rosemary also contains Tannins, which have antioxidant properties, enhance wound healing, and are beneficial against peptic ulcers. The presence of terpenoids also in the leaves extracts of rosemary may give cardioprotection and antioxidant properties (Mumtaz *et al.*, 2014). Another secondary metabolite detected in rosemary leaf extracts was steroids, which aid to reduce cholesterol and improve airway inflammation in asthma (Maharaj *et al.*, 2022). Saponins have historically been used as natural detergents too. Their physicochemical and biological properties are exploited in food, cosmetics, and medicine (Kregiel *et al.*, 2017; Mohan *et al.*, 2021). It was reported that alkaloids have pharmacological activities like antimicrobial, analgesic, antioxidant activity, and anti-inflammatory activity (Muhamad *et al.*, 2022). Rosemary has been demonstrated to reduce iron absorption and utilization. Thus, it should be used with caution in patients at risk of iron deficiency; this is because the extract is rich in phenolic according to (Samman *et al.*, 2001).

5.3. Antioxidant activity

Our bodies produce free radicals (ROS) and reactive nitrogen species (RNS) as a result of various endogenous systems, environmental exposure, or pathological situations. When free radicals outnumber the body's ability to manage them, the condition is known as oxidative stress. Oxidative stress plays a central role in the pathogenesis of diverse chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer (Mohammed *et al.*, 2015; Sharifi-Rad *et al.*, 2020). The application of an external source of antioxidants can assist in coping with this oxidative stress. Herbs are a good source of naturally occurring antioxidants, particularly phenolic compounds that have hydroxyl groups that may readily donate an atom of hydrogen to a free radical. The herb rosemary is a rich source of lipid-soluble antioxidants, particularly Carnosic acid, which has been proven to be a powerful antioxidant. Actually, it is more efficient than synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Reische *et al.*, 2008). Saito *et al.* in a study found that the volatile constituents of rosemary, particularly 1,8-Cineole, exert an antioxidant effect (Saito *et al.*, 2004). Rosemary extracts are used as a natural antioxidant, extending the shelf life of perishable foods. In fact, rosemary extract (E392) has received approval from the European Union (EU) as a safe and reliable natural antioxidant for food preservation (Younes *et al.*, 2018).

Investigation of the diluted 1:10 methanolic extracts of the rosemary extract showed a remarkable scavenging capacity for both DPPH and ABTS free radical scavenging assays. The two types of radical scavenging activity assays showed an approximately close percentage for each of the samples. Some studies have shown that rosemary extract has powerful antioxidant effects (Moreno *et al.*, 2006; Özcan, 2003). Our result rosemary leaves of our samples showed antioxidant activity, with a range percentage of scavenging activity from 73.25% to 75.99% for DPPH. As stated by Aktaş and Malayoğlu the antioxidant activity (DPPH radical scavenging activity) of rosemary extract 94 % was a higher value than our result (Aktas and Malayoglu, 2019). In another study (Kasparavičienė *et al.*, 2013), antioxidant activity was high in all tested rosemary (72–85% inactivation of DPPH), these ratios are consistent with our study.

According to (Martínez *et al.*, 2019), the capacity of rosemary extract to scavenge the DPPH free radical was recorded at 81.8 %. Additionally, the ABTS assays were higher than 80%; this result is consistency with our study, especially in Umm Lasfah village (88.82).

This investigation aimed to evaluate the total Phenols of leaves of rosemary plant, as milligrams of Gallic acid equivalent per gram of dried plant (mg GAE⁄g). As shown in **Figure (4.24**), the total phenolic contents of the methanolic extract of rosemary leaves recorded the highest value of 876.7 mg GAE/g and the lowest value of 616.7 mg GAE/g. These values are higher than the value for the total phenolic content of the rosemary extract which is 318 mg GAE/g, according to (Abramovič *et al.*, 2012). Another study showed that dry rosemary leaves found the concentration of total phenol in water extracts to be 185 mg Gallic acid equivalents/g of extract, which is lower than the results of the present study. (Dorman *et al.*, 2003).

It has been noted that the solvent employed in extraction can have an impact on the antioxidant activity of the extract, depending on the phenolic content. Liu *et al.* discovered that methanol extracts of rosemary have more phenolic and flavonoid contents than hexane extracts. The importance of the antioxidant capacity of methanol extracts of rosemary is probably due to their richness in phenolic compounds (Liu *et al.*, 2007). The compounds associated with the antioxidant activity of rosemary are the phenolic diterpenes (Carnosol, Rosmanol, 7-methyl-epirosmanol, Isorosmanol, and Carnosic acid), and the phenolic acids (Rosmarinic and Caffeic acids) (Cetin *et al.*, 2017). The qualitative phytochemical analysis shown in **Table (4.21)** indicated that rosemary leaves contain terpenoids and phenolic acid in all the samples tested, which are responsible for their antioxidant activity. The total phenol contents in plants depend on plant species, plant tissue, developmental stage, and environmental factors such as temperature, water stress, and light conditions (Chaves *et al.*, 2020; Zeroual *et al.*, 2021). These results suggested that the rosemary plant could be of great industrial importance and can support the development of natural additives with the potential for application in food technology. Plant-based antioxidants are becoming increasingly important in nutrition (food preservation and stability) as well as preventive medicine (Andrade *et al.*, 2018).

5.4. Anti-bacterial activity

The worldwide prevalence of bacterial infections is a major public health concern (Khan *et al.*, 2013). Today's widespread use of synthetic antibiotics has increased the prevalence of resistant strains and side effects (Kloy *et al.*, 2020). The antimicrobial activities of medicinal plants are based on their bioactive phytochemicals known as secondary metabolites, which include terpenoids, alkaloids, flavonoids, tannins, and glycosides (Sisay *et al.*, 2019). The phenolic compounds damage the cell membrane and block the cell's functional characteristics, eventually leaking the inner materials of the cell (Bajpai *et al.*, 2012). The present study investigated the antibacterial activities of the rosemary plant. However, different concentrations of methanolic extracts were investigated for their antibacterial activities against three bacterial strains, gram-positive (*Staphylococcus aureus*), and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) using the disc diffusion method for determining the inhibitory zone diameters. Methanol extracts showed good antimicrobial activities against all microorganisms tested (Moreno *et al.*, 2006). Our results indicated that the rosemary extracts showed antibacterial activities, these results are consistence with other studies (Weckesser *et al.*, 2007), that showed an effect against Gram-positive bacteria (*S. aureus*). Additionally, the extracts had an impact on Gram-negative bacteria *(E. coli* and *P. aeruginosa*). Rosemary extracts contain bioactive chemicals such as phenolic compounds that inhibit the growth of both gram-negative and gram-positive bacteria (Johar *et al.*, 2015). The major compounds that are responsible for the antibacterial actions include 1,8-Cineole, Camphor, Bornyl acetate, and α-Pinene (Genena A. K. *et al.*, 2008). The presence of α-pinene as a major component improves antimicrobial activity when compared to *S. aureus*, as stated by another study (Zaouali *et al.*, 2010). Some of our samples are inactive against some strains of bacteria as shown in **Table (4.22)**. The lack of an inhibition zone does not always imply that compounds are inactive. For instance, non-polar compounds might not diffuse into the culture medium (Moreno *et al.*, 2006).

It was suggested that the antibacterial activities of rosemary leaves extracts were probably due to their constituents (Eucalyptol, Camphor, Camphene, and Caryophyllene). Our results on rosemary leaves extracts showed that this plant can have the potential to be a source of secondary compounds having antimicrobial activities against Gram-positive and Gram-

negative bacteria. Indeed, more studies are required to identify which exact compound is responsible for the Gram-negative bacteria and Gram-positive bacteria inhibition in this plant.

5.5. Proximate composition and mineral contents

The fiber contents of rosemary leaves in this study were found to be higher than those reported by another study (Sharma and Dhuria, 2021), which demonstrated about 4.52% fiber content in Rosemary leaves. On the other hand, Polat *et al.* demonstrated the crude fiber in rosemary leaves is 25.24% values, (Polat *et al.*, 2011), which is close to our results (23.5%-28.7%). The highest fat content was recorded in our Hebron sample (14.84%), which was slightly higher than that of Bani Naim (14.54%), and Khilt Al-Adrah (14.01%) samples. While the samples of Raqaa'a and Umm Lasfa regions recorded the lowest percentages (12.87%, and 11.95%). The value of 8.76% achieved by Genena *et al.* was a bit lower than our finding (Genena A. *et al.*, 2008). According to Mwithiga *et al.* method the fat content was measured using the Soxhlet extraction method of fresh rosemary herb (Mwithiga *et al.*, 2022), and the highest fat content was recorded as 13.36% when used cow manure, but fat content without any treatments was recorded as 11.48%. The proximate composition of herbs can vary depending on the agro-climatic conditions, maturity, and harvesting period (Kutbay and Tolga, 2001).

The ash values in our samples for the studied rosemary leaves were nearly similar compared to one study (Polat *et al.*, 2011). The rosemary samples achieved higher ash values; as the ash values of the samples studied were found to be for Raqaa'a (9.31%), Khilt Al-Adrah (8.2%), Bani Naim (8.2%), and Hebron (8.1%) samples respectively. Our results are consistent with other studies for one sample, the value of ash was close to the sample of the village of Umm Lasfa, which is 7.3% (Kassahun and Feleke, 2019; Sharma and Dhuria, 2021). Rosemary leaves may be considered an excellent source of key minerals as well as some trace elements essential to human body physiology (Zeroual *et al.*, 2021). The earlier findings in the leaf samples of the rosemary plant indicated that the plant has higher contents of Ca and K, moderate levels of Mg and Na, and lower contents of Fe, Mn, and P. The highest accumulation of minerals has been identified in rosemary leaves are calcium and potassium. In addition, these leaves were rich in Magnesium which is consistence with other studies (Kiczorowska *et al.*, 2015; Nikitina *et al.*, 2017). The main abundant mineral in the leaves was calcium, which

is very important to a healthy body. It plays an important role in building stronger, denser bones and keeping bones strong and healthy. Most research on the long-term effects of insufficient calcium consumption is focused on bone health, particularly rickets in children fractures, osteopenia, and osteoporosis in older adults (Shlisky *et al.*, 2022). Some people have dairy allergies, while others (vegetarians) avoid dairy products for health reasons, however, plants can be the main source of calcium in these groups to obtain the required daily intake of calcium (1000 mg for adults) including rosemary. As mentioned previously, rosemary reduces iron absorption; this might be due to the presence of calcium which reduces iron absorption as known. Rosemary leaves in all samples recorded a high accumulation value of potassium. Dietary potassium can lower blood pressure by reducing the harmful effects of sodium. A potassium-rich dietary plan may also lower the incidence of kidney stones and bone loss. Thus, rosemary could be a good source of potassium to get potassium Adequate Intake (AI) which is 4,700 mg per day for adults (Davis *et al.*, 2010). Sodium and potassium are essential for maintaining the human body's osmotic pressure equilibrium (Hasan *et al.*, 2014). ATP is used by the ion pump (Na+/K+ATPase) to move sodium ions out of the cell and potassium ions into the cell (Pirahanchi *et al.*, 2022). Mg is essential for energy metabolism, the excitability of muscles and nerves, bone formation, and enzymatic activity (Huskisson *et al.*, 2007). In conclusion, small amounts of herbs in a diet can supply a good source of supplementing mineral deficiencies (Zengin *et al.*, 2008). The World Health Organization (WHO) recommended that medicinal plants should be also tested for the presence of certain metals that may harm general health (Organization, 2007).

Chapter Six

Conclusions

6. Conclusions

Based on the results obtained in this study, it was revealed that the methanolic extract of the leaves rosemary commonly grown in Palestine from different locations (Bani Naim, Hebron, and Yatta city) have the following valuable effects:

The methanolic extracts were analyzed by GC-MS technology. The GC-MS technique was utilized and found to be accurate and reliable in the separation and identification of the components of *Rosmarinus officinali*s L. complex volatile mixtures. Several volatile components were detected and identified in rosemary leaves with two extraction methods (at room temperature and reflux apparatus). The major volatile compounds detected in the methanolic extracts (80%, 90%) of rosemary samples were characterized by high-level peaks of Eucalyptol and Camphor in all regions (Bani Naim, Hebron, and Yatta city). The usage of rosemary leaves is recommended for medicinal use. In other words, it is advisable to use rosemary leaves in skin preparations to utilize the high concentration of camphor in this plant.

The results showed that the methanolic extract of the rosemary leaves in three regions is a good and valuable source of natural antioxidants that can be used in the food and medical sectors. The important antioxidant capacity of methanolic extracts of rosemary is probably due to their richness in phenolic compounds. They are well known to positively impact human health and can be considered for future uses as antioxidant components in agro-food industries.

Rosmarinus officinalis L. leaf extracts could also be considered to be preliminary components having potential applications in antibacterial medication development.

The rosemary leaves are rich in calcium (Ca), potassium(K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), and Phosphorus (P) in all regions, as well as contain fiber and fat percentages. The screening of minerals of rosemary leaves in other locations all over Palestine is highly recommended to draw general decisive conclusions. Although there are several works that have been reported on the mineral contents of medicinal plants in many developed countries, however, this work was the first to be conducted in Palestine.

We have estimated that these data will be useful for studies to improve the nutritional and medicinal content of rosemary. This work is also essential to assess the *Rosmarinus officinalis* L. use by Palestinians.

More research is needed to identify active compounds and study their effects against pathogen mechanisms. Besides, more research is necessary to isolate the secondary metabolites in order to conduct clinical studies for human benefit.

References

1. Abramovič, H., Terpinc, P., Generalić, I., Skroza, D., Klančnik, A., Katalinić, V., & Smole-Možina, S. (2012). Antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosmarinus officinalis*) and vine (*Vitis vinifera*) leaves. *Croatian Journal of Food Science and Technology*, 4(1), 1-8.
2. Aburjai, T., & Natsheh, F. M. (2003). Plants used in cosmetics. *Phytotherapy Research*, 17(9), 987-1000.
3. Aguilar, F., Autrup, H., Barlow, S., Castle, L., Crebelli, R., Dekant, W., Engel, K., Gontard, N., Gott, D., & Grilli, S. (2008). Use of rosemary extracts as a food additive-Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food. *EFSA Journal*, 721, 1-29.
4. Akhtar, N., & Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry*, 11(8), 1223-1235.
5. Aktaş, B., & Malayoğlu, H. B. (2019). Comparison of phenolic compounds and antioxidant activities of the extracts of grape seed, rosemary, green tea and olive leaf. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 56(1), 81-90.
6. Al-Maharik, N., Jaradat, N., Hawash, M., Al-Lahham, S., Qadi, M., Shoman, I., & Issa, L. (2022). Chemical Composition, Antioxidant, Antimicrobial and Anti-Proliferative Activities of Essential Oils of *Rosmarinus officinalis* from five Different Sites in Palestine. *Separations*, 9(11), 339.
7. Al-Sheikh, B., & Mahassnehb, M. (2017). Flora of Wadi Al-Quff Protected Area, Hebron Governorate, Palestine. *Jordan Journal of Natural History*, 47-57.
8. Al-Sheikh, B., & Qumsiyeh, M. B. (2021). Imperiled ecosystems in Palestine: Rare plants as Indicators. *Earth Systems and Environmental Sciences*, 1-7.
9. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42.
10. Amin, T., Sharma, N., & Bhat, S. V. (2012). A general overview on *Rosemarinus officinalis* L.(Rosemary) as a medicinal plant. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 4(3), 177-181.
11. Anadón, A., Martinez-Larranaga, M. R., Martínez, M. A., Ares, I., García-Risco, M. R., Senorans, F. J., & Reglero, G. (2008). Acute oral safety study of rosemary extracts in rats. *Journal of Food Protection*, 71(4), 790-795.
12. Andrade, J., Faustino, C., Garcia, C., Ladeiras, D., Reis, C., & Rijo, P. (2018). *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. *Future Science*, 4(4), 283.
13. Arslan, D., & Özcan, M. M. (2008). Evaluation of drying methods with respect to drying kinetics, mineral content and colour characteristics of rosemary leaves. *Energy Conversion and Management*, 49(5), 1258-1264.
14. Aziz, E., Batool, R., Akhtar, W., Shahzad, T., Malik, A., Shah, M., Iqbal, S., Rauf, A., Zengin, G., & Bouyahya, A. (2022). Rosemary species: A review of phytochemicals, bioactivities and industrial applications. *South African Journal of Botany*, 151, 3-18.
15. Bailly, F., & Cotelle, P. (2005). Anti-HIV activities of natural antioxidant caffeic acid derivatives: toward an antiviral supplementation diet. *Current Medicinal Chemistry*, 12(15), 1811-1818.

16. Bajpai, V. K., Baek, K.-H., & Kang, S. C. (2012). Control of Salmonella in foods by using essential oils: A review. *Food Research International*, 45(2), 722-734.
17. Bakırel, T., Bakırel, U., Keleş, O. Ü., Ülgen, S. G., & Yardibi, H. (2008). In vivo assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. *Journal of Ethnopharmacology*, 116(1), 64-73.
18. Balaram, V. (2019). Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. *Geoscience Frontiers*, 10(4), 1285-130.
19. Bartlett, H. E., & Eperjesi, F. (2007). Effect of lutein and antioxidant dietary supplementation on contrast sensitivity in age-related macular disease: a randomized controlled trial. *European Journal of Clinical Nutrition*, 61(9), 1121-1127.
20. Batool, H., Hussain, M., Hameed, M., & Ahmad, R. (2020). A review on Calotropis procera its phytochemistry and traditional uses. *Big Data Agriculture*, 2(11), 29-31.
21. Begum, A., Sandhya, S., Vinod, K. R., Reddy, S., & Banji, D. (2013). An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Scientiarum Polonorum Technologia Alimentaria*, 12(1), 61-74.
22. Benincá, J. P., Dalmarco, J. B., Pizzolatti, M. G., & Fröde, T. S. (2011). Analysis of the anti-inflammatory properties of *Rosmarinus officinalis* L. in mice. *Food Chemistry*, 124(2), 468-475.
23. Berdahl, D. R., & McKeague, J. (2015). Rosemary and sage extracts as antioxidants for food preservation. *Handbook of Antioxidants for Food Preservation*, 177-217.
24. Birtić, S., Dussort, P., Pierre, F. X., Bily, A. C., & Roller, M. (2015). Carnosic acid. *Phytochemistry*, 115, 9-19.
25. Borges, R. S., Ortiz, B. L. S., Pereira, A. C. M., Keita, H., & Carvalho, J. C. T. (2019). *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. *Journal of Ethnopharmacology*, 229, 29-45.
26. Bozin, B., Mimica-Dukic, N., Samojlik, I., & Jovin, E. (2007). Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. *Journal of Agricultural and Food Chemistry*, 55(19), 7879-7885.
27. Brückner, K., Božić, D., Manzano, D., Papaefthimiou, D., Pateraki, I., Scheler, U., Ferrer, A., de Vos, R.C., Kanellis, A.K. & Tissier, A. (2014). Characterization of two genes for the biosynthesis of abietane-type diterpenes in rosemary (Rosmarinus officinalis) glandular trichomes. *Phytochemistry*, 101, 52-64.
28. Burkhart, C. G., & Burkhart, H. R. (2003). Contact irritant dermatitis and anti-pruritic agents: the need to address the itch. *Journal of Drugs in Dermatology*, 2(2), 143-146.
29. Cajka, T. (2013). Gas chromatography–time-of-flight mass spectrometry in food and environmental analysis. *Comprehensive Analytical Chemistry*, 61, 271-302.
30. Cetin, I., Yesilbag, D., Cengiz, S. S., & Belenli, D. (2017). Effects of supplementation with rosemary (*Rosmarinus officinalis* L.) volatile oil on growth performance, meat MDA level and selected plasma antioxidant parameters in quail diets. *Kafkas Univ Vet Fak Derg*, 23(2), 283-288.
31. Charles, D. J. (2012). Antioxidant properties of spices, herbs and other sources. *Springer Science & Business Media*, 495-507.

32. Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants*, 9(1), 76.
33. Christaki, E., Bonos, E., Giannenas, I., & Florou-Paneri, P. (2012). Aromatic plants as a source of bioactive compounds. *Agriculture*, 2(3), 228-243.
34. Cohen, M. L. (2000). Changing patterns of infectious disease. *Nature*, 406(6797), 762-767.
35. Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and medicinal uses of terpenes. *Medicinal Plants*, 333-359.
36. Daher, C. F. & Kashour, B. M. (2008). Rosmarinus officinalis leaves water extract: A possible anti-inflammatory and anti-ulcerogenic remedy. *Planta Medica*, 74 (9), 1-56.
37. Dai, P., & Liu, H. (2021). Research on the biological activity of rosemary extracts and its application in food. *E3S Web of Conferences*, 251(02034), 1-7.
38. Dar, R. A., Shahnawaz, M., & Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 6(6), 349-351.
39. Datiles, M. J., & Acevedo-Rodríguez, P. (2014). Rosmarinus officinalis (rosemary). *CABI Compendium*, 47678.
40. Davis, C., Mcmurry, K., Britten, P., Trumbo, P., & Rodgers, A. (2010). US Department of Agriculture and US Department of Health and Human Services. *Dietary Guidelines for Americans*.
41. De Macedo, L. M., Santos, É. M. d., Militão, L., Tundisi, L. L., Ataide, J. A., Souto, E. B., & Mazzola, P. G. (2020). Rosemary (*Rosmarinus officinalis* L., syn Salvia rosmarinus Spenn.) and its topical applications: a review. *Plants*, 9(5), 651.
42. De Oliveira, J. R., Camargo, S. E. A., & De Oliveira, L. D. (2019). Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent. *Journal of Biomedical Science*, 26(1), 1-22.
43. de Sousa, L. L., de Andrade, S. C., Athayde, A. J., de Oliveira, C. E., de Sales, C. V., Madruga, M. S., & de Souza, E. L. (2013). Efficacy of Origanum vulgare L. and Rosmarinus officinalis L. essential oils in combination to control postharvest pathogenic aspergilli and autochthonous mycoflora in Vitis labrusca L. (table grapes*). International Journal of Food Microbiology*, 165(3), 312–318.
44. Desbois, A. P., & Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, 85, 1629-1642.
45. Dorman, H., Peltoketo, A., Hiltunen, R., & Tikkanen, M. (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. *Food Chemistry*, 83(2), 255-262.
46. Dowek, S., Fallah, S., Basheer-Salimia, R., Jazzar, M., & Qawasmeh, A. (2020). Antibacterial, antioxidant and phytochemical screening of palestinian mallow, Malva sylvestris L. *International Journal of Pharmacy and Pharmaceutical Sciences*, 12(10), 12-16.
47. Dzoyem, J. P., Tshikalange, E., & Kuete, V. (2013). Medicinal plants market and industry in Africa. *Medicinal Plant Research in Africa*, 859-890.
48. El-Sayed, S. M., & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*, 5(6), 1-7.

49. Estefan, G., Sommer, R., & Ryan, J. (2013). Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region. *International Center for Agricultural Research in the Dry Areas*, 65-119.
50. Garcia, A., & Barbas, C. (2011). Gas chromatography-mass spectrometry (GC-MS)-based metabolomics. *Metabolic Profiling*, 191-204.
51. Genena, A. K., Hense, H., Smânia Junior, A., & Souza, S. M. d. (2008). Rosemary (*Rosmarinus officinalis*): a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. *Food Science and Technology*, 28, 463-469.
52. Ghosh, S., Prasanna, V. L., Sowjanya, B., Srivani, P., Alagaraja, M., & Banji, D. (2013). Inductively coupled plasma–optical emission spectroscopy: a review. *Asian Journal of Pharmaceutical Analysis*, 3(1), 24-33.
53. Ghule, H. N., & Ghule, Y. L. (2020). Review on herbal cosmetic for hair and skin. *World Journal of Pharmaceutical Research*, 9(7), 470-481.
54. Ginsburg, S. R., & Maleky, F. (2020). Extraction of lipid-soluble antioxidants from rosemary leaves using vegetable oils. *International Journal of Food Science & Technology*, 55(9), 3135-3144.
55. Gird, C. E., Nencu, I., Popescu, M. L., Costea, T., Duţu, L. E., Balaci, T. D., & Olaru, O. T. (2017). Chemical, antioxidant and toxicity evaluation of rosemary leaves and its dry extract. *Farmacia*, 65(6), 978-983.
56. Golshani, Z., & Sharifzadeh, A. (2014). Evaluation of antibacterial activity of alcoholic extract of rosemary leaves against pathogenic strains. *Zahedan Journal of Research in Medical Sciences*,16 (3), 12-15.
57. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9(1639), 1-9.
58. Gurbuz, B., Bagdat, R. B., Uyanik, M., & Rezaeieh, K. A. P. (2016). Rosemary (Rosmarinus officinalis L.) cultivation studies under Ankara ecological conditions. *Industrial Crops and Products*, 88, 12-16.
59. Habte, G., Hwang, I. M., Kim, J. S., Hong, J. H., Hong, Y. S., Choi, J. Y., & Kim, K. S. (2016). Elemental profiling and geographical differentiation of Ethiopian coffee samples through inductively coupled plasma-optical emission spectroscopy (ICP-OES), ICP-mass spectrometry (ICP-MS) and direct mercury analyzer (DMA). *Food Chemistry*, 212, 512-520.
60. Habtemariam, S. (2016). The therapeutic potential of rosemary (*Rosmarinus officinalis*) diterpenes for Alzheimer's disease. *Evidence-Based Complementary and Alternative Medicine*, 1-14.
61. Hameed, I. H., & Mohammed, G. J. (2017). Phytochemistry, antioxidant, antibacterial activity, and medicinal uses of aromatic (medicinal plant *Rosmarinus officinalis*). *Aromatic and Medicinal Plants*, 175-189.
62. Hamidpour, R., Hamidpour, S., & Elias, G. (2017). *Rosmarinus officinalis* (Rosemary): a novel therapeutic agent for antioxidant, antimicrobial, anticancer, antidiabetic, antidepressant, neuroprotective, anti-inflammatory, and anti-obesity treatment. *Biomedical Journal of Scientific & Technical Research*, 1(4), 1-6.
63. Hammer, M., & Junghanns, W. (2020). *Rosmarinus officinalis* L.: Rosemary. *Medicinal, Aromatic and Stimulant Plants*, 501-521.

64. Hanson, J. R. (2016). Rosemary, the beneficial chemistry of a garden herb. *Science Progress*, 99(1), 83-91.
65. Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. *Springer Science & Business Media*. p. 302.
66. Hasan, H. M. I., El-Mehdawe, M. F., & Saad, E. K. (2014). Heavy metals and mineral elements contents of leaves and stems for some herbal plants at AL-Gabal AL-Akhder region. *Chemical Science Review and Letters*, 3(12), 980-986.
67. Hassanpour, S., MaheriSis, N., & Eshratkhah, B. (2011). Plants and secondary metabolites (Tannins): A Review. *International Journal of Forest, Soil and Erosion*, 1(1), 47-53.
68. Heinrich, M., Kufer, J., Leonti, M., & Pardo-de-Santayana, M. (2006). Ethnobotany and ethnopharmacology—Interdisciplinary links with the historical sciences. *Journal of Ethnopharmacology*, 107(2), 157-160.
69. Hejaz, H. A., Sabbobeh, R., Halayqa, H., Jahajha, A., & Abu-Laf, S. (2022). Minerals analysis of *Salvia palaestina* leaves: Quantification and detection. *Journal of Plant Sciences*, 17, 53-61.
70. Hernández, M. D., Sotomayor, J. A., Hernández, Á., & Jordán, M. J. (2016). Rosemary (*Rosmarinus officinalis* L.) oils. *Essential Oils in Food Preservation, Flavor and Safety*, 677-688.
71. Herrero, M., Ibáñez, E., Cifuentes, A., & Bernal, J. 2009. Multidimensional chromatography in food analysis. *Journal of Chromatography A*, 1216(43), 7110-7129
72. Ho, C. T., Wang, M., Wei, G. J., Huang, T. C., & Huang, M. T. (2000). Chemistry and antioxidative factors in rosemary and sage. *Biofactors*, 13(1-4), 161-166.
73. Huskisson, E., Maggini, S., & Ruf, M. (2007). The role of vitamins and minerals in energy metabolism and well-being. *Journal of International Medical Research*, 35(3), 277-289.
74. Ighbareyeh, J., Suliemieh, A., Ayash, A., Sheqwara, M., Ortiz, A., & Carmona, E. (2021). Biodiversity and Phytosociological Analysis of Plants in Wadi Al-Quf Nursery Reserve North-Western of Hebron City in Palestine. *Journal of Plant Sciences*, 9(1), 13-24.
75. Jahiman, N., Pa'ee, F., Manan, N., & Salleh, N. M. (2021). Development of herbal bag for herbal bath during postnatal care from Temuan traditional knowledge. *IOP Conference Series: Earth and Environmental Science*, 736, 1755-1315.
76. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, 7(1), 1-7.
77. Jaradat, N. (2005). Ethnopharmacological survey of natural products in palestine. *An-Najah University Journal for Research - A (Natural Sciences)*, 19, 13-67.
78. Jaradat, N. A., Zaid, A. N., Al-Ramahi, R., Alqub, M. A., Hussein, F., Hamdan, Z., Mustafa, M., Qneibi, M., & Ali, I. (2017). Ethnopharmacological survey of medicinal plants practiced by traditional healers and herbalists for treatment of some urological diseases in the West Bank/Palestine. *BMC Complementary and Alternative Medicine*, 17(1), 1-18.
79. Jarrar, N., Abu-Hijleh, A., & Adwan, K. (2010). Antibacterial activity of Rosmarinus officinalis L. alone and in combination with cefuroxime against methicillin–resistant Staphylococcus aureus. *Asian Pacific Journal of Tropical Medicine*, 3(2), 121-123.
80. Jeevalatha, A., Kalaimathi, R., Basha, A. N., Kandeepan, C., Ramya, S., Loganathan, T., & Jayakumararaj, R. (2022). Profile of bioactive compounds in *Rosmarinus officinalis*. *Journal of Drug Delivery and Therapeutics*, 12(1), 114-122.

81. Jiang, Y., Wu, N., Fu, Y., Wang, W., Luo, M., Zhao, J., & Liu, X. L. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental Toxicology and Pharmacology*, 32(1), 63-68.
82. Johar, S., Irfan, S., Ahmed, S. S., & Jabeen, R. (2015). Phytochemical screening and antibacterial activity of *Rosmarinus officinalis* L. against *Escherichia coli*. local isolates. *International Journal of Basic and Applied Sciences*, 4(4), 413.
83. Johnson, J. J. (2011). Carnosol: a promising anti-cancer and anti-inflammatory agent. *Cancer Letters*, 305(1), 1-7.
84. Jordán, M. J., Lax, V., Rota, M. C., Lorán, S., & Sotomayor, J. A. (2013). Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control*, 30(2), 463-468.
85. Juergens, L. J., Racké, K., Tuleta, I., Stoeber, M., & Juergens, U. R. (2017). Anti-inflammatory effects of 1, 8-cineole (eucalyptol) improve glucocorticoid effects in vitro: a novel approach of steroid-sparing add-on therapy for COPD and asthma?. *Synergy*, 5, 1-8.
86. Juergens, U. (2014). Anti-inflammatory properties of the monoterpene 1.8-cineole: current evidence for co-medication in inflammatory airway diseases. *Drug Research*, 64(12), 638-646.
87. Kasparavičienė, G., Ramanauskienė, K., Savickas, A., Velžienė, S., Kalvėnienė, Z., Kazlauskienė, D., Ragažinskienė, O., & Ivanauskas, K. (2013). Evaluation of total phenolic content and antioxidant activity of different *Rosmarinus officinalis* L. ethanolic extracts. *Biologija*, 59(1), 39-44.
88. Kassahun, A., & Feleke, G. (2019). Proximate analysis, physicochemical properties and chemical constituent's characterization of *Rosemarinus officinalis* oil. *Natural Volatiles and Essential Oils*, 6(2), 20-24.
89. Khan, S. R., Sharma, B., Chawla, P. A., & Bhatia, R. (2022). Inductively coupled plasma optical emission spectrometry (ICP-OES): a powerful analytical technique for elemental analysis. *Food Analytical Methods*, 1-23.
90. Khan, U. A., Rahman, H., Niaz, Z., Qasim, M., Khan, J., Tayyaba, & Rehman, B. (2013). Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology and Immunology*, 3(4), 272-274.
91. Kheiria, H., Mounir, A., María, Q., José, J. M., & Bouzid, S. (2021). Total Phenolic Content and Polyphenolic Profile of Tunisian Rosemary (*Rosmarinus officinalis* L.). *Residues Natural Drugs from Plants*, 137-149.
92. Kiczorowska, B., Klebaniuk, R., Bakowski, M., & Al-Yasiry, A. R. M. H. (2015). Culinary herbs-the nutritive value and content of minerals. *Journal of Elementology*, *20*(3), 599-608.
93. Kloy, A., Ahmad, J., Yusuf, U., & Muhammad, M. (2020). Antibacterial properties of rosemary (*Rosmarinus officinalis*). *South Asian Research Journal of Pharmaceutical Sciences*, 2(1), 4-7.
94. Kondeti, R. R., Mulpuri, K. S., & Meruga, B. (2014). Advancements in column chromatography: A review. *World Journal of Pharmaceutical Sciences*, 2(9), 1375-1383.
95. Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., Babic, L., & Zhang, B. (2017). Saponin-based, biological-active surfactants from plants. *Application and Characterization of Surfactants*, 6(1), 184-205.
96. Ksouri, S., Djebir, S., Bentorki, A. A., Gouri, A., Hadef, Y., & Benakhla, A. (2017). Antifungal activity of essential oils extract from Origanum floribundum Munby,

Rosmarinus officinalis L. and Thymus ciliatus Desf. against Candida albicans isolated from bovine clinical mastitis. *Journal De Mycologie Médicale*, 27(2), 245-249.
97. Kutbay, H., & Tolga, O. (2001). Foliar N and P resorption and nutrient levels along an elevational gradient in Zelkova carpinifolia (Pall.) C. Koch subsp. yomraensis Ansin & Gercek. *Annals of Agri Bio Research*, 6(1), 1-8.
98. Labban, L., Mustafa, U. E.-S., & Ibrahim, Y. M. (2014). The effects of rosemary (*Rosmarinus officinalis*) leaves powder on glucose level, lipid profile and lipid perodoxation. *International Journal of Clinical Medicine*, 5(06), 297-304.
99. Lazarova-Zdravkova, N., Terzieva, T., Peshev, D., & Georgieva, N. (2020). Antibacterial activity of microencapsulated via spouted-bed hydro-alcoholic rosemary extracts. *Journal of Chemical Technology and Metallurgy*, 63(1), 40-44.
100. Lešnik, S., Furlan, V., & Bren, U. (2021). Rosemary (*Rosmarinus officinalis* L.): extraction techniques, analytical methods and health-promoting biological effects. *Phytochemistry Reviews*, 20(6), 1273-1328.
101. Lin, W. Y., Yu, Y. J., & Jinn, T. R. (2019). Evaluation of the virucidal effects of rosmarinic acid against enterovirus 71 infection via in vitro and in vivo study. *Virology Journal*, 16, 1-9.
102. Liu, Q., Meng, X., Li, Y., Zhao, C.-N., Yi-Tang, G., & Li, H. (2017). Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*, 18(6), 1283.
103. Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X., & Yan, G. (2007). Antioxidant activity and phenolics of an endophytic Xylaria sp. from Ginkgo biloba. *Food Chemistry*, 105(2), 548-554.
104. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8), 118.
105. Maharaj, A., Naidoo, Y., Dewir, Y. H., & Rihan, H. (2022). Phytochemical Screening, and Antibacterial and Antioxidant Activities of Mangifera indica L. Leaves. *Horticulturae*, 8(10), 909.
106. Martínez, L., Castillo, J., Ros, G., & Nieto, G. (2019). Antioxidant and antimicrobial activity of rosemary, pomegranate and olive extracts in fish patties. *Antioxidants*, 8(4), 86.
107. Masabni, J., & King, S. (2014). Easy Gardening. *Texas A&M Agrilife Extension: Collage Station*, 1-3.
108. Masumoto, N., Nishizaki, Y., Sugimoto, N., & Sato, K. (2018). Phytochemical profiling of rosemary extract products distributed as food additives in the Japanese market. *Japanese Journal of Food Chemistry and Safety*, 25(2), 105-113.
109. Minaiyan, M., Ghannadi, A., Afsharipour, M., & Mahzouni, P. (2011). Effects of extract and essential oil of *Rosmarinus officinalis* L. on TNBS-induced colitis in rats. *Research in Pharmaceutical Sciences*, 6(1), 13.
110. Miraj, S. (2016). An evidence-based review on herbal remedies of *Rosmarinus officinalis*. *Der Pharmacia Lettre*, 8(19), 426-436.
111. Miroddi, M., Calapai, G., Isola, S., Minciullo, P., & Gangemi, S. (2014). *Rosmarinus officinalis* L. as cause of contact dermatitis. *Allergologia et Immunopathologia*, 42(6), 616-619.
112. Mohammad-Saeed, B., Mohammad-Ali, A., & Mahmoud, R. K. (2013). Effect of diabetan on blood glucose, glycosylated hemoglobin, lipid profile, liver and kidney function tests of diabetic patients: A clinical, double blind, randomized trial. African *Journal of. Pharmacy and Pharmacology*, 7, 50-53.

113. Mohammed, M. T., Kadhim, S. M., Jassimand, A. N., & Abbas, S. (2015). Free radicals and human health. *International Journal of Innovation Sciences and Research*, 4(6), 218-223.
114. Mohan, E., Suriya, S., Shanmugam, S., & Rajendran, K. (2021). Qualitative phytochemical screening of selected medicinal plants. *Journal of Drug Delivery and Therapeutics*, 11(2), 141-144.
115. Moore, J., Yousef, M., & Tsiani, E. (2016). Anticancer effects of rosemary (Rosmarinus officinalis L.) extract and rosemary extract polyphenols. *Nutrients*, 8(11), 731.
116. Moreno, S., Scheyer, T., Romano, C. S., & Vojnov, A. A. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research*, 40(2), 223-231.
117. Mosleh, Y. Y., Mofeed, J., Almaghrabi, O. A., Kadasa, N. M., El-Alzahrani, H., & Fuller, M. (2014). Residues of heavy metals, PCDDs, PCDFs, and DL-PCBs some medicinal plants collected randomly from the Jeddah, central market. *Life Science Journal*, 11(7), 1-8.
118. Muhamad, M., Ai Sze, W., Zulkifli, N. S., & Ab-Rahim, S. (2022). Qualitative Analysis on the Phytochemical Compounds and Total Phenolic Content of Cissus hastata (Semperai) Leaf Extract. *International Journal of Plant Biology*, 14(1), 53-62.
119. Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed Research International*, 2014, 1-11.
120. Mukadam, M., Shaikh, M. F. S., Anam, M. S., Ahmed, K., & Mandlekar, M. B. F. (2021). Gas chromatography of Volatile oil. *International Journal of Pharmaceutical Research and Applications*, 6(5), 399-406.
121. Mumtaz, F., Raza, S. M., Ahmad, Z., Iftikhar, A., & Hussain, M. (2014). Qualitative phytochemical analysis of some selected medicinal plants occurring in local area of Faisalabad, Pakistan. *Journal of Pharmacy and Alternative Medicine*, 3(3), 17-21.
122. Mwithiga, G., Maina, S., Gitari, J., & Muturi, P. (2022). Rosemary (*Rosmarinus officinalis* L.) growth rate, oil yield and oil quality under differing soil amendments. *Heliyon*, 8(4), 1-7.
123. Nagegowda, D. A., & Gupta, P. (2020). Advances in biosynthesis, regulation, and metabolic engineering of plant specialized terpenoids. *Plant Science*, 294, 1-45.
124. Naimi, M., Vlavcheski, F., Shamshoum, H., & Tsiani, E. (2017). Rosemary extract as a potential anti-hyperglycemic agent: Current evidence and future perspectives. *Nutrients*, 9(9), 1-19.
125. Nasr-Eldin, M. A., Abdelhamid, A., & Baraka, D. (2017). Antibiofilm and antiviral potential of leaf extracts from Moringa oleifera and rosemary (*Rosmarinus officinalis* Lam.). *Egyptian Journal of Microbiology*, 52(1), 129-139.
126. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451-1474.
127. Neves, J. A., Neves, J. A., & Oliveira, R. d. C. M. (2018). Pharmacological and biotechnological advances with *Rosmarinus officinalis* L. *Expert Opinion on Therapeutic Patents*, 28(5), 399-413.
128. Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): A review. *Medicines*, 5(3), 1-13.

129. Nikitina, A., Tohsirova, Z., & Popova, O. (2017). Elemental composition of rosemary shoots (*Rosmarinus officinalis* L.), introduced in the botanical garden of pyatigorsk medical-pharmaceutical institiute. *Pharmacy & Pharmacology*, 5(6), 581-588.
130. Nisar, A. (2022). Medicinal plants and phenolic compounds. Phenolic Compounds: Chemistry, Synthesis, Diversity, Non-Conventional Industrial, *Pharmaceutical and Therapeutic Applications*, 131-141.
131. Noriega, P., Gortaire, G., & Osorio, E. (2021). Mass Spectrometry and Its Importance for the Analysis and Discovery of Active Molecules in Natural Products. *Natural Drugs from Plants*, 33-43.
132. Official, A. (2000). Methods of analysis of AOAC International. *AOAC International, Maryland, USA*, 2000.
133. Oga, F., Sekine, S., Shitara, Y., & Horie, T. (2016). Pharmacokinetic herb-drug interactions: insight into mechanisms and consequences. *European Journal of Drug Metabolism and Pharmacokinetics*, 41, 93-108.
134. Özcan, M. (2003). Antioxidant activities of rosemary, sage, and sumac extracts and their combinations on stability of natural peanut oil. *Journal of Medicinal Food*, 6(3), 267-270.
135. Pal, S. K., & Shukla, Y. (2003). Herbal medicine: current status and the future. *Asian Pacific Journal of Cancer Prevention*, 4(4), 281-288.
136. Pawłowska, K., Janda, K., & Jakubczyk, K. (2020). Properties and use of rosemary (*Rosmarinus officinalis* L.). *Pomeranian Journal of Life Sciences*, 66(3), 76-82.
137. Pérez, M. B., Calderon, N. L., & Croci, C. A. (2007). Radiation-induced enhancement of antioxidant activity in extracts of rosemary (*Rosmarinus officinalis* L.). *Food Chemistry*, 104(2), 585-592.
138. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, 6(11), 1-5.
139. Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11-26.
140. Pirahanchi, Y., Jessu, R., & Aeddula, N. R. (2022). Physiology, sodium potassium pump. *StatPearls [Internet]*.
141. Polat, U., Yesilbag, D., & Mustafa, E. (2011). Serum biochemical profile of broiler chickens fed diets containing rosemary and rosemary volatile oil. *Journal of Biological and Environmental Sciences*, 5(13), 23-30.
142. Prasad, R., Garg, A. P., & Varma, A. (2005). Interaction of medicinal plants with plant growth promoting rhizobacteria and symbiotic fungi. *Basic Research and Applications of Mycorrhizae*, 1, 363-407.
143. Pratyusha, S. (2022). Phenolic compounds in the plant development and defense: an overview. *Plant Stress Physiology-Perspectives in Agriculture*, 125-140.
144. Pravallika, S. (2016). Gas chromatography: a mini review. *Research and Reviews Journal of Pharmaceutical Analysis*, 5(2), 55-62.
145. Qawasmeh, A., Bourke, C., Lee, S., Gray, M., Wheatley, W., Sucher, N. J., & Raman, A. (2011). GC-MS analysis of volatile secondary metabolites in "Mediterranean" and "Continental" Festuca arundinacea (Poaceae) infected with the fungal endophyte Neotyphodium coenophialum strain AR542. *Acta Chromatographica*, 23(4), 621-628.
146. Qawasmeh, A., Obied, H. K., Raman, A., & Wheatley, W. (2012). Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction

between Lolium perenne and different strains of Neotyphodium lolii. *Journal of Agricultural and Food Chemistry*, 60(13), 3381-3388.
147. Rafieian-Kopaei, M., Baradaran, A., & Rafieian, M. (2013). Plants antioxidants: From laboratory to clinic. *Journal of Nephropathology*, 2(2), 152-153.
148. Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging*, 2(2), 219.
149. Rani, K. (2017). Role of antioxidants in prevention of diseases. *Journal of Applied Biotechnology & Bioengineering*, 4(1), 1-2.
150. Ray, P. D., Huang, B. W., & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular Signalling*, 24(5), 981-990.
151. Reische, D., Lillard, D., Eitenmiller, R., Akoh, C., & Min, D. (2008). Food lipids: chemistry, nutrition, and biotechnology. *Antoxidants*, 409.
152. Ribeiro-Santos, R., Carvalho-Costa, D., Cavaleiro, C., Costa, H. S., Albuquerque, T. G., Castilho, M. C., Ramos, F., Melo, N. R., & Sanches-Silva, A. (2015). A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). *Trends in Food Science & Technology*, 45(2), 355-368.
153. Roomiani, L., Soltani, M., Akhondzadeh Basti, A., Mahmoodi, A., Taheri, A., Taheri Mirghaed, A., & Yadollahi, F. (2013). Evaluation of the chemical composition and in vitro antimicrobial activity of Rosmarinus officinalis, Zataria multiflora, Anethum graveolens and Eucalyptus globulus against Streptococcus iniae the cause of zoonotic disease in farmed fish. *Iranian Journal of Fisheries Sciences*, 12, 702–716.
154. Sabbobeh, R., Hejaz, H., Jahajha, A., Al-Akhras, S., Al-Jaas, H., & Abu-Lafi, S. (2016). Antioxidant, Antimicrobial Activities and Phytochemical Profiling of the Leaf Extract of Salvia palaestina. *Journal of Applied Pharmaceutical Science*, 6(1), 76-82.
155. Saibabu, V., Fatima, Z., Khan, L. A., & Hameed, S. (2015). Therapeutic potential of dietary phenolic acids. *Advances in Pharmacological Sciences*, 2015, 1-10.
156. Saito, Y., Shiga, A., Yoshida, Y., Furuhashi, T., Fujita, Y., & Niki, E. (2004). Effects of a novel gaseous antioxidative system containing a rosemary extract on the oxidation induced by nitrogen dioxide and ultraviolet radiation. *Bioscience, Biotechnology, and Biochemistry*, 68(4), 781-786.
157. Samman, S., Sandström, B., Toft, M. B., Bukhave, K., Jensen, M., Sørensen, S. S., & Hansen, M. (2001). Green tea or rosemary extract added to foods reduces nonheme-iron absorption. *The American Journal of Clinical Nutrition*, 73(3), 607-612.
158. Santos, F., & Galceran, M. (2003). Modern developments in gas chromatography–mass spectrometry-based environmental analysis. *Journal of Chromatography A*, 1000(1-2), 125-151.
159. Sasikumar, B. (2012). Rosemary. *Handbook of Herbs and Spices*, 452-468.
160. Satyal, P., Jones, T. H., Lopez, E. M., McFeeters, R. L., Ali, N. A. A., Mansi, I., Al-Kaf, A. G., & Setzer, W. N. (2017). Chemotypic characterization and biological activity of *Rosmarinus officinalis*. *Foods*, 6(3), 20.
161. Selmi, S., Rtibi, K., Grami, D., Sebai, H., & Marzouki, L. (2017). Rosemary (*Rosmarinus officinalis*) essential oil components exhibit anti-hyperglycemic, anti-hyperlipidemic and antioxidant effects in experimental diabetes. *Pathophysiology*, 24(4), 297-303.
162. Selmi, S., Rtibi, K., Grami, D., Sebai, H., & Marzouki, L. (2017). Rosemary (*Rosmarinus officinalis*) essential oil components exhibit anti-hyperglycemic, anti-hyperlipidemic and antioxidant effects in experimental diabetes. *Pathophysiology*, 24(4), 297-303.

163. Selvaraj, K. V., Sivakumar, P., Shalom, S. V., Bharathi, A., Velayutham, A., & Prabha, T. (2022). A review on the in vitro regeneration of the timeless panacean medicinal and aromatic herb: *Rosmarinus officinalis* (L.). *Annals of Phytomedicine*, 11(1), 247-252.
164. Senanayake, S. N. (2018). Rosemary extract as a natural source of bioactive compounds. *Journal of Food Bioactives*, 2, 51–57.
165. Sharangi, A. B., & Guha, S. (2013). Wonders of leafy spices: Medicinal properties ensuring Human Health. *Science International*, 1(9), 312-317.
166. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., & Peluso, I. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694.
167. Sharma, A., & Dhuria, R. (2021). A nutritional evaluation of rosemary leaf powder and black cumin seed powder as phytogenic feed additives. *The Pharma Innovation Journal*, 10(10), 983-985.
168. Shirzad, H., Shahrani, M., & Rafieian-Kopaei, M. (2009). Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes in vivo. *International Immunopharmacology*, 9(7-8), 968-970.
169. Shlisky, J., Mandlik, R., Askari, S., Abrams, S., Belizan, J. M., Bourassa, M. W., Cormick, G., Driller-Colangelo, A., Gomes, F., & Khadilkar, A. (2022). Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes: Wiley Online Library. *Annals of the New York Academy of Sciences*, 1512(1), 10-28.
170. Singh, R. P., Sharad, S., & Kapur, S. (2004). Free radicals and oxidative stress in neurodegenerative diseases: relevance of dietary antioxidants. *Journal Indian Association of Clinical Medicine*, 5(3), 218-225.
171. Singh, R., Kaushik, R., & Jaglan, V. (2018). Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review. *Pharma Innovation Journal*, 7(6), 568-573.
172. Sisay, M., Bussa, N., Gashaw, T., & Mengistu, G. (2019). Investigating in vitro antibacterial activities of medicinal plants having folkloric repute in Ethiopian traditional medicine. *Journal of Evidence-Based Integrative Medicine*, 24, 1-9.
173. Stefanaki, A., & van Andel, T. (2021). Mediterranean aromatic herbs and their culinary use. *Aromatic Herbs in Food*, 93-121.
174. Tabassum, T., Shlini, P., & Johnson, J. T. (2021). Isolation and Purification of bioactive component from *Rosmarinus officinalis*. *Journal of Pharmaceutical Sciences and Research*, 13(5), 279-283.
175. Teonata, N., Wijaya, V. A., Vithaloka, V. S., & Thariq, M. (2021). An Introduction to Different Types of Gas Chromatography. *Jurnal Sains Dan Terapan Kimia*, 15(1), 8-17.
176. Tigist, G., Mekonnen, M., Mengesha, B., & Philiphos, M. (2016). Rosemary Production and Utilization. *Ethiopian Institute of Agricultural Research*,1-26.
177. Tilburt, J. C., & Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. Bulletin of the World Health Organization, 86, 594-599.
178. Tsukamoto, Y., Ikeda, S., Uwai, K., Taguchi, R., Chayama, K., Sakaguchi, T., & Fujita, T. (2018). Rosmarinic acid is a novel inhibitor for Hepatitis B virus replication targeting viral epsilon RNA-polymerase interaction. *PLoS One*, 13(5), 1-16.

179. Uritu, C. M., Mihai, C. T., Stanciu, G.-D., Dodi, G., Alexa-Stratulat, T., Luca, A., Leon-Constantin, M.-M., Stefanescu, R., Bild, V., & Melnic, S. (2018). Medicinal plants of the family Lamiaceae in pain therapy: A review. *Pain Research and Management, 2018*. 1-44.
180. Valenzuela, A., Sanhueza, J., & Nieto, S. (2003). Natural antioxidants in functional foods: from food safety to health benefits. *Grasas y Aceites*, 54(3), 295-303.
181. Veenstra, J. P., & Johnson, J. J. (2021). Rosemary (*Salvia rosmarinus*): Health-promoting benefits and food preservative properties. *International Journal of Nutrition*, 6(4), 1-10.
182. Verma, R., Rahman, L., Mishra, S., Verma, R., Singh, A., Chauhan, A., & Yadav, A. (2012). Volatile terpenoid composition of *Rosmarinus officinalis*, "CIM-Hariyali": Variability in north India during annual growth. *Journal of the Chilean Chemical Society*, 57(2), 1066-1068.
183. Wansutha, S., Yuenyaow, L., Jantama, K., & Jantama, S. S. (2018). Antioxidant activities of almond milk fermented with lactic acid bacteria. *Thai Journal of Pharmaceutical Sciences*, 42, 115-119.
184. Weckesser, S., Engel, K., Simon-Haarhaus, B., Wittmer, A., Pelz, K., & Schempp, C. á. (2007). Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine*, 14(7-8), 508-516.
185. Wong, P. Y., & Kitts, D. D. (2006). Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. *Food Chemistry*, 97(3), 505-515.
186. World Health Organization. (2007). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. *World Health Organization*,1-118.
187. Xie, J., VanAlstyne, P., Uhlir, A., & Yang, X. (2017). A review on rosemary as a natural antioxidation solution. *European Journal of Lipid Science and Technology*, 119(6), 1-18.
188. Yahaya, M. F., Kubmarawa, D., Yelwa, J. M., & Runde, M. (2018). Antioxidant and antimicrobial activity of essential oils extracted from aromatic plants. *World Scientific News*, (111), 13-25.
189. Yıldız, P. O. (2016). Effect of thyme and rosemary essential oils on the shelf life of marinated rainbow trout. *Journal of Animal and Plant Sciences*, 26(3), 665-673.
190. Yılmazer, M., Karagöz, S. G., Ozkan, G., & Karacabey, E. (2016). Aroma transition from rosemary leaves during aromatization of olive oil. *Journal of Food and Drug Analysis*, 24(2), 299-304.
191. Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipič, M., Frutos, M. J., Galtier, P., & Gott, D. (2018). Refined exposure assessment of extracts of rosemary (E 392) from its use as food additive. *EFSA Journal*, 16(8), 1-25.
192. Zaouali, Y., Bouzaine, T., & Boussaid, M. (2010). Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. *Food and Chemical Toxicology*, 48(11), 3144-3152.
193. Zengin, M., Özcan, M. M., Çetin, Ü., & Gezgin, S. (2008). Mineral contents of some aromatic plants, their growth soils and infusions. *Journal of the Science of Food and Agriculture*, 88(4), 581-589.
194. Zeroual, A., Sakar, E., Ibourki, M., Bijla, L., Ainane, A., Mahjoubi, F., Chaouch, M., Gharby, S., Chaqroune, A., & Ainane, T. (2021). Phytochemical screening and mineral profiling of wild and cultivated rosemary (*Rosmarinus officinalis* L.) from Taounate region (northern Morocco). *Pharmacology Online*, 2, 576-582.

195. Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Natural Product Communications*, 17(1), 1-14.

Abstract in Arabic

الأنشطة البيولوجية والتركيب الغذائي للزيت العطري من أوراق نبات إكليل الجبل

الملخص

تحظى طب الأعشاب في فلسطين بشعبية كبيرة. أحد أكثر الأعشاب استخدامًا هو إكليل الجبل. ومع ذلك، فإن استخدامه مرتبط بالتقاليد الموروثة أكثر من اعتماده على البحث العلمي. أصبح من المعروف أن إكليل الجبل على وجه الخصوص يحتوي على مستقلبات ثانوية لها استخدامات واسعة في الطب الشعبي وفي صناعة الأغذية كمنكهات ومواد حافظة. في هذا البحث العلمي، تم تقييم المستخلصات الميثانولية لأوراق نبات إكليل الجبل من مواقع مختلفة للمركبات المتطايرة باستخدام تحليل GC-MS وتم تقدير الأنشطة المضادة للأكسدة باستخدام طرق DPPH و ABTS ، وتم فحص الأنشطة المضادة للبكتيريا بطريقة نشر القرص وتحديد المحتوى المعدني للأوراق الجافة، باستخدام جهاز ICP-OES. تم تحديد عدد من المركبات المتطايرة في مستخلص أوراق إكليل الجبل (80٪ و90٪ في room temperature and reflux conditions). المكونات الرئيسية في جميع العينات المدروسة من إكليل الجبل هي Eucalyptol ثم Camphor. أظهر التحليل الكيميائي النباتي لأوراق إكليل الجبل وجود مركبات كيميائية نباتية مثل cardiac glycosides, phenolic groups, coumarin, saponins, steroids, tannins, and terpenoids في جميع العينات. أظهرت المستخلصات الميثانولية لأوراق إكليل الجبل للعينات المدروسة والتي تم جمعها من جميع المناطق قدرة كسح تتراوح بين 73.25٪ -76.36٪ باستخدام مقايسة DPPH وباستخدام مقايسة ABTS كانت قيم النطاق 73.91٪ -88.82٪. علاوة على ذلك ، فإن المحتوى الفينولي الكلي (TPC) الذي قدموا فيه قيمًا أعلى لأوراق إكليل الجبل في قرية أم لصفة (876.7 ملم GAE / غم) يحتوي على أعلى نسبة من إجمالي الفينولات، يليه خلة العدرة (728.3 ملم GAE / غم)، رقعة (693.3 ملم GAE / غم)، الخليل (652.8 ملم GAE / غم)، وعينات بني نعيم (616.7 مجم GAE / جم). أظهرت الدراسات المضادة للبكتيريا باستخدام طريقة الانتشار القرصي أن المستخلصات الميثانولية (80٪ و90٪ في room temperature and reflux conditions) من إكليل الجبل لها منطقة تثبيط ضد بكتيريا *S. aureus* , *E. coli*, *K. pneumonia*. كانت النتيجة الأكثر إثارة للاهتمام هي 80٪ من مستخلص الميثانول عند درجة حرارة الغرفة في جميع العينات مقارنة ب methanol control. تم اكتشاف أن أوراق إكليل الجبل التي تم جمعها غنية بالمعادن خاصة الكالسيوم والبوتاسيوم في جميع المناطق التي تم اختبارها. تشير الأنشطة المضادة للأكسدة والبكتيريا في إكليل الجبل إلى أن هذا النبات يمكن أن يكون مضادًا واعدًا للأكسدة وعلاجًا محتملًا مضادًا للبكتيريا. وفقًا لمعرفتنا، كانت هذه الدراسة الأولى من نوعها لفحص وتقييم المركبات الكيميائية النباتية لنبات إكليل الجبل الفلسطيني لأنشطتها المضادة للبكتيريا ومضادات الأكسدة.