An-Najah National University
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Variations of the Chemical Constituents and Pharmacological Activities of *Pelargonium Graveolens* Essential Oil from Three Regions in Palestine

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Dedication

To my father spirit, your kindness and support are still with me forever

To my mother, thanks for your support and kindness

To my sisters and brothers, thanks for your support

To my doctors at Aleppo University

To my doctors at An-Najah National University

To all my loyal friends

I dedicate this work

Acknowledgment

First of all, my immeasurable thanks to my God, who has enabled me to accomplish this work.

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الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

تباينات المكونات الكيميائية والانشطة الدوائية لزيت العطري لنبتة العطريه من ثلاث مناطق في فلسطين

Variations of the Chemical Constituents and Pharmacological Activities of *Pelargonium Graveolens* Essential Oil from Three Regions in Palestine

اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

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

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Signature:	.....	التوقيع:
Date:	**12/06/2021**	التاريخ

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List of abbreviation

Amu	Atomic Mass Unit
ATCC	American Type Culture Collection
BC	Before Christ
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toluene
C.A.M.	Complementary and Alternative Medicine
CI	Clinical Isolate
C.N.S.	Central Nervous System
DMSO	Dimethyl Sulfoxide
D.N.A.	Deoxyribo Nucleic Acid
DNSA	3, 5-Dinitrosalicylic Acid
DPN	Diabetic Peripheral Neuropathy
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
GC-MS	Gas Chromatography/Mass Spectrometry
H.C.T.	Human Colon Tumor cell
I%	Percentage Inhibition
IC_{50}	Inhibition Concentration of 50%
M.A.C.	MacConkey Agar
M.A.P.	Medicinal Aromatic Plants
MCF7	Mammary Carcinoma F7
M.H.B.	Mueller Hinton Broth
M.I.C.	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant Staphylococcus Aureus
M.S.A.	Mannitol Salt Agar
NA	Nutrient Agar
DN	Diabetic Nephropathy
NIST	The National Institute of Standards And Technology
NSAID	Non-Steroidal Anti-Inflammatory Drug
P.D.A.	Potato Dextrose Agar
PL	Pancreatic Lipase
PNPB	P-Nitro Phenyl Butyrate
R.O.S.	Reactive Oxygen Species
STDV	Standard Deviation
Ss	Stock Solution
Subsp.	Subspecies
Syn.	Synonym
UV-Vis	Ultraviolet-Visible
E.O.	Essential Oil
W/W%	Weight by weight%
W.B.	West Bank
WHO	World Health Organization

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Abstract

Pelargonium graveolens or *Pelargonium roseum* is a species that originated in Southern Africa and is related to the *Geraniaceae* family. It grows in the Mediterranean part of Europe starting in the eighteenth century.

Pelargonium graveolens essential oils (E.O.) of the three samples were extracted using microwave ultrasonic apparatus. The chemical constituents of *P.graveolens* E.O samples were analyzed using Gas Chromatography – Mass Spectroscopy.

To detect the antioxidant activity of *P.graveolens* Essential oil samples we have used the method of Inhibition of 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical to screen the three samples E.O. antioxidant activity. We studied the antilipase activity of *P.graveolens* E.O samples by using porcine pancreatic lipase and the p-nitro phenylbutyrate (PNPH) method. To detect the α-amylase activity we used the 3,5-dinitrosalicylic acid (DNSA) procedure.

The broth microdilution test was used to detect the biological activity of *Pelargonium graveolens* E.O towards six types of bacteria and against *Candida albicans*.

The essential oil product yield range was (0.5 to 1%) (w/w%). The Gas Chromatography-Mass Spectroscopy test presented the high percentages of oxygenated components in the range of (86.2-88.5%), and non-oxygenated components in the range of (11.5%-13.8%). Twenty-six components were observed, *R-Citronellol* was the most abundant component in the three samples in the range of (74.4-86%), *Citronellyl Formate* was the second abundant component with the range of (3.2-14.4%). The sample from Nablus was the potent antioxidant and antiamylase agent with IC_{50} 146.14 and IC_{50} 199.6 µg/ml, respectively. Ramallah's sample (middle region) was the potent antilipase agent with IC_{50} 584.112 µg/mL. The three samples exhibited broad antimicrobial activity; and showed potent antifungal activity with minimum inhibitory concentration (M.I.C.) of 0.3125µg/mL. The sample from Hebron (Southern region) showed the highest potency against *"Methicillin" Resistant Staphylococcus Areus* (MRSA) with the lowest reported M.I.C. value (0.3125µg/mL) meanwhile the M.I.C. value from Ramallah reached 12.5µg/mL.

The study appeared that *Pelargonium graveolens* E.O. samples from different governorates in Palestine contained different percentages of chemical constituents, which explained the different potential biological activities such as antioxidant, antiobesity, antidiabetic, and antimicrobial activities due to their chemical components. These variations of chemical components are

percentages due to the differences in the climate and rainfall average also type of the soil, the old of the plant, and the cultivation season in the different governorates. In Hebron which is sited in the south part of Palestine, the climate is cold in winter and there is significant rainfall throughout the year also it is a height above sea level from 860m-1008m while Ramallah governorate which sited in the middle of Palestine also is weather so cold in winter and there is heavy rainfall during winter but the rate is less than rainfall in Hebron and it is the height above the sea level is 760m-885m. Nablus governorate which is sited in the north part of Palestine also its climate is cold in winter and its rainfall average is moderate its height level above the sea is 430 m -931 m.

More in vivo studies are recommended to check out the E.O.'s clinical efficacy and determine the oil's proper clinical uses. It is also recommended to separate the oil's main active ingredients and check out the toxicity of active ingredients, in addition, studying *P.graveolens* E.O as a food and a medicine *flavoring agent, and a preservative.*

Chapter One

Introduction

Chapter One

Introduction

1.1 History of medicinal herbs

Medicinal plants have been utilized in practical medicine practices starting from ancient. Plants were discovered in the personal effects of Otzi the Iceman. The Ancient Egyptians wrote the Ebbers Papyrus in 1500 B.C., which contains over 850 plants.

The proof is that plants had different medicinal applications and purposes for more than 60000 years ago. There was a burial site of a Neanderthal man which was uncovered in 1960. This appeared in some stories expressed by eight species of plants buried with him, and most of them have recent applications these days [1].

Many traditional drugs originate from plant sources; as some of the most effective drugs are plant sources like Aspirin, derived from foxglove, and Quinine, which is usually derived and prepared from Cinchona's bark, and Morphine was synthesized from the Opium poppy.

The old Sumerians and old Egyptians used willow bark as a practical medicine for pain relief for more than 3500 years.

Some medicinal plants have disinfectant characters which are used for destroying diseases that are causing germs. At the same time

inhibits growing of pathogenic microbes, which causes communicable diseases. The herbal medicine applications recommend calmative herbs that lead and provide a soothing effect on the body. They are often used as a sedative [2]

1.2 Current use of Medicinal Plants

Large numbers of populations have used traditional medicinal plants from the senior population till now to meet their health care requirements; according to World Health Organization (WHO), about 80% of people worldwide use medicinal plants in their health care system, such as plant extracts or their active principles [3]. In developing countries, herbal medicines have been used as an alternative source of medication to solve health problems and the high costs of medicines [4]. In the developed countries, the drugs extracted from plants take an important place in their health care system [3]. In North America, the vast attention on nutraceuticals and medicinal herbs products were augmented in the last years, in which phytochemical products can enhance medicinal qualities and long-term health. Aromatherapy is a type of alternative medicine that uses essential oils to improve a person's health or mood [5]. The percentage of herbal medicine use in the U.S.A. is 25% of the total drug market [6]. The international herbal medicine market is expected to increase from 61 billion U.S. $ to 5 trillion U.S. $ in 2025 [7]. Pharmaceutical industries in China and Japan are interested in investigating plants as sources of new medicine [7].

According to the "WHO" statical analysis, about 62 of verified different therapeutic drug categories used worldwide are produced from 119 chemicals isolated from 91 plant species [1]. It is different from the traditional single drug. Plant extracts or raw plant has several phytochemicals and bioactive ingredients that lead to synergistic effects that allow for several effects in curing diseases [7]. The medicinal plants and their claimed conventional applications are considered practical approaches in producing a new drug from natural sources [8].

Plants produce primary and secondary metabolites essential for plant growth, protection, and beneficial for the human body [2]. Secondary metabolites produced by plants like (alkaloids, phenols, saponins, tannins, and E.O.) which are necessary for healing diseases and may be responsible for the therapeutic effect of plants like terpenoids and E.O., which have anti-inflammatory, anthelmintic, antiviral, antibacterial, and cholesterol inhibition and insecticide [9, 10,11].

Different drugs used in recent times were derived from medicinal plants such as Artemisinin, Atropine, and Quinin.

1.3 Essential oils

Essential oils or "ethereal oil" are extracted from different parts of plants by several methods of extraction depending on the plant origin and the volatility of the oils; some of these methods as simple pressing, which used in the extraction of orange and lemon oils, and

fermentation method which followed by distillation used in the extraction of mustard and bitter almond oils. The most common commercial method is steam distillations [12]. E.O.'s are hydrophobic and usually very soluble in alcohol and non-polar or weakly polar solvents as waxes and oil. They are without color or with pale yellow liquid with a lower density than water. They are easily oxidized by air, heat, and light and have different biological activities depending on their chemical composition [13]. The most common use of E.O., especially in the European Union, is cosmetics such as perfumes, aftershaves, and fragrances. It is also used as a flavoring and preservative food agent and alternative medicine pharmacy for its therapeutic effects. The investigation of those plants and their E.O. that have been used in traditional medicine is vital to improving healthcare quality [14]. The chemical compounds of E.O. can be classified into two types: oxygenated (ketones, alcohols, phenols, etc.) and the other is hydrocarbons (limonene, pinene, etc.) or phenylpropanoids and terpenoids [15]. In this case, the active compounds may be divided into three groups that have to do with their chemical structure: *terpenes, terpenoids, phenylpropenes* [16].

Monoterpenes are considered a type of terpenes consisting of two isoprene units with molecular formula C_{10} H_{16} and *monoterpenes* mostly linear (acyclic) and contain rings (monocyclic and bicyclic).

Modified *terpenes* like those containing oxygen functionality or sometimes missing a methyl group are called *monoterpenoids.*

Monoterpenes are found in many plants' essential oils, such as limonene, which have been shown to prevent mammary, liver, lung, and other cancers.

Sesquiterpenes: These aggregates are known for their calming properties, and they can also be useful to the immune system in protecting humans from hurtful microbes, acting as antioxidants, and assisting in cellular repair.

When inhaling E.O., it takes twenty-two seconds to reach the brain, the only way to reach the limbic system is through smell, so this is the main reason why oils are such an excellent option for those with depression, anxiety, and panic attacks.

Terpenes are considered hydrocarbons produced from the binding of several *isoprene* units (C_5H_8) (Figure 1). The primary terpenes are *monoterpenes* ($C_{10}H_{16}$) and *sesquiterpene* ($C_{15}H_{24}$), *diterpenes* ($C_{20}H_{32}$), and *triterpenes* ($C_{30}H_{48}$), examples of *terpenes* (limonene, pinene).

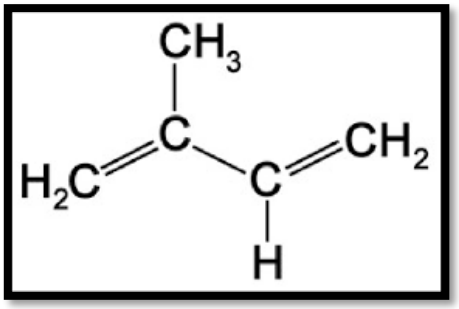

Figure (1): Isoprene.

Terpenoids are terpenes that are produced by adding oxygen molecules and moving or removing methyl groups under the influence of enzymatic biochemical modifications; terpenoids, also called "isoprenoids," organize a large number of natural components obtained from isoprene units C_5 linked head to tail form (Figure 1) [16]. Terpenoids can be sub-divided into aldehydes, alcohols, ethers, esters, epoxides, phenols, and ketones [16].

Phenylpropanoids are presented in E.O. in few amounts and derived from Shikimic acid (Figure 2) [17]. *Phenylpropanoids* - contain a sizeable organic product produced by plants to protect themselves from wounds, infections, herbivores, and ultraviolet irradiation [18]. Examples of *Phenylpropanoid* are *eugenol and vanillin* [19].

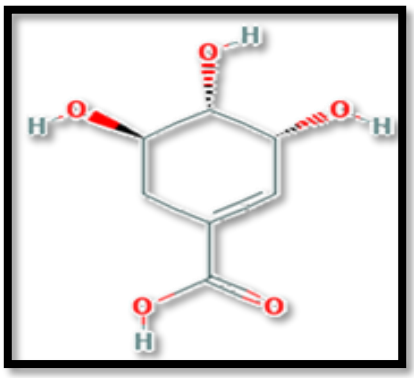

Figure (2): Shikimic Acid.

Other Essential oil constituents: several types of degraded products and formulas that are originating from terpenes, unsaturated fatty acids, glycosides, lactones, and sometimes sulfur- and nitrogen-containing compounds (allyl isothiocyanate (AITC) and allicin) [16].

The chemical structures of E.O. usually give an idea about their therapeutic activities; such as antiviral, antimicrobial, antiseptic, vasodilators, hypotensive, calming, sedative, spasmolytic, antipyretic, analgesic, anti-inflammatory, expectorant, stimulant, tonic, antitumor, anesthetic, cell-regenerating, and digestive [13].

1.4 Background

1.4.1 *Pelargonium graveolens*

Pelargonium is one of the types of flowering plants containing about 280 species of *perennials succulents* and is usually known as *geraniums* and *pelargoniums storksbills*. *Geranium* is considered a separate genus that is related to plants and is known as *cranesbills*. All *genera* are classified into the family *Ericaceae*. Carl Linnaeus included most of these species in one genus are named *Geranium* and later classified into two genera by Charles Louis Heritier de Brunelle in 1789.

1.4.1.1 *P. graveolens* subspecies and traditional names

The plant list has 250 accepted species names (including two primary hybrids) and nine subspecies or varieties for the genus Pelargonium.

The family *Pelargonium* obtains the name from the fruit related to the stork's beak. The specific epithet reinformed, kidney-shaped in Latin, are referred to the leaf shape and room portion; in the typical

African, that name is usually referred to as the stem color. The genus *Pelargonium* is classified in the family *Ericaceae*, a massive family of 11 genera and tropical worlds. More than 270 species of *Pelargonium* exist in all parts of the world. Two hundred nineteen species of several types are still incorrectly referred to as *geraniums*. The genus *Geranium* species have regular flowers that have shapes and are divided into equal halves in any plane. In contrast, the flowers of *pelargoniums* can be divided into equal halves in only one plane.

1.4.1.2 Description of *Pelargonium graveolens*

Pelargonium graveolens consist of woody and straight stems with branches. Their leaves are usually palmately lobed or pinnate. They often have long legs that occasionally contain light or dark patterns covered with short and brutal hairs that give the plant a robust and attractive rose-like scent. The upstanding stems hold five-petals flowers in umbel-like clusters; the flower has a single harmony plane (zygomorphic) that differs from the *Geranium* flower with radial symmetry (actinomorphic). Their height can reach up to 1.3 m and spread to 1 m. with hairy stems, and they are herbaceous when they are young and later becoming woody with age.

Figure (3): *Pelargonium graveolens.*

1.4.1.3 Folk uses

In current years, *Pelargonium* species have been used to treat intestinal illness, wounds, and respiratory problems. Respiratory and cold medications usually contain *Pelargonium* oil and have been used in Europe and the United States. In several studies, *Pelargonium* oil was used to help in hormonal balance, liver and kidney dysfunctions, and remove toxins from the liver that may affect the body's overall health; besides, it has other advantages like supporting the digestive and nervous system. *Pelargonium graveolens* have been used in conventional medicine to relieve hemorrhoids, dysentery, inflammation, cancer, and the perfumery, cosmetic, and aromatherapy industries worldwide.

Palestine:

As archived in the Palestinian Museum Gardens leaflet, the leaves and flowers of *P. graveolens* are used as flavoring agents in desserts, jams, salads, and tea; it is also used in making perfumes, aromatherapy, and massages.

Africa:

In Southern Africa, people using it as medicine for many diseases, such as wounds, fevers, colds, inflammation, and more.

Essential oil is extracted from the plant, which has an orange to rose fragrance. This type of plant is the source of *geranium* oil that is usually used extensively in aromatherapy, skincare, and food flavoring. Another application of this oil has been used as a substitute for the attar of roses in the perfume trade. Finally, the leaves are used in potpourri.

1.4.1.4 Chemical composition

The chemical composition and the percentages of the constituents of the E.O. of *Pelargonium graveolens* species may differ depending on the differences in several parameters like growing season and from younger and older branches and the leaf pairs in the same plant. The major constituents of E.O. and aqueous extract of the plant were *monoterpenes* (*R-Citronellol, Cis-Menthon, α-pinene, Geranyl isobutyrate, Beta Myrcene, Trans Menthone,*

Geranyl bromide, P-Menthone-3-one, Ocimene, 8-P- Methane, L-β-Pinene, Cis -Crane, Limonene, Rose Oxide) and fatty alcohol esters (*Citronellol Format, Neryl propionate, Geranyl taiglate)* and then sesquiterpenes (Gamma Eudesmol, Copaene, Caryophyllene, Beta Bourbone, (+)Leden, Delta Cadinene, Alpha -Cubebin) and Bicycle [8] octane; 8-methylene.

1.4.2 Evidence-based uses

Pelargonium graveolens antibacterial activity gave promise results and could be used as an antibacterial drug source. Recently many chemical components of *Pelargonium graveolens* have powerful antibiotic, antimutagenic, analgesic anti-inflammatory, and sedative effects such as the monoterpenes Beta Myrcene. Other components, such as Copaene, have anti-proliferative, antioxidant, anti-genotoxic, and cytotoxic properties. Moreover, Caryophyllene, which is natural bicyclic sesquiterpenes due to its ability to bind with CB2 receptors, has potent anti-inflammatory, antimicrobial, Antibacterial, anxiety, pain relief, reduce cholesterol, osteoporosis, and seizures. α- Pinene is a component of *P. graveolens* which is bicyclic monoterpenes; it functions by inhibiting or slowing acetylcholine esterase activity in the brain helps people retain memories more effectively. Other benefits like euphoria, increased alertness, reduced oil production in oily skin, and finally, anticancer properties. α-pinene has been given orally combined with several

other terpenes to treat gallstones without adverse effects in the drug with the trade name Rowachol. Another component, Citronoellyl Formate, which belongs to fatty alcohol esters, is available as a liquid with an intensely fruity, rose-like odor suitable for fresh, top notes in rose and lily of the valley fragrances. Also, trans – Menthone is a monoterpene used as a flavoring agent in perfumes and cosmetics for its aromatic characteristic and minty odor. Geranyl bromide available in *P. graveolens* is monoterpenes used as a perfume component for creams and soaps and as a flavoring agent. Ocimene, which is monoterpenes, is used in perfumery for its sweet herbal scent, antifungal properties. Like the related acyclic terpene Myrcene, Ocimene is unstable in the air.

Ocimene is used as a decongestant and expectorant. Anecdotal evidence indicates that Ocimene helps clear airways and improve respiration when smoked. Neryl propionate, also known as feme 2777, belongs to the class called fatty alcohol esters is used as an antioxidant; human TRPA1 receptors are activated by Geranyl propionate and closely acetate because of its physiological advantages of TRPA1 agonists such as enhancement of energy metabolism. Delta Cadinene is a kind of sesquiterpenes and is considered a member of the lyases family with carbon-oxygen lyases acting on phosphate groups. Usually, this enzyme type's common systematic name is 2-trans, 6-trans -farnesyl - diphosphate -lyase (cyclizing, (+) -delta -cadinene -forminyl. This enzyme

usually participates in terpenoid biosynthesis. It uses one cofactor of magnesium. Beta bourbon, known as sesquiterpenes that has antitumor, anti-inflammation, and antifungal effects. P-methane-3-one is oxygenated monoterpenes and has been used as insect repellents.

1.4.2.1 Uses in the food industry

P.graveolens have several applications in the food industry. Several studies for essential oil show practical advantages against bacteria and fungi. This antimicrobial action led to the testing of the oil against food spoilage pathogens. Simultaneously, it shows good results, making it a good candidate for the food industry and used as a preservative agent.

1.5 Problem statement

Nowadays, many dangerous global health problems affect the global health sector badly.

One of this problem is the oxidative stress which is the result of bad distributing between the oxidants and the antioxidants, and this imbalance distribution affects the cell activity, viability which leads to cell damage and then affects the organ jobs which lead to more health problems such as diabetes, hypertension, atherosclerosis, asthma, pulmonary fibrosis, ischemia, neurological dysfunction, immune system diseases, cancer and promote aging.

Natural antioxidants from plants are recently considered the first choice of a cell defense system for protection against oxidants or Reactive Oxygen Species (R.O.S.) by preventing or delaying the oxidation process. These natural antioxidants can act as radical scavengers[12].

Obesity is a global problem that threatens more than 52% of European adult people. This problem affects the personal life of the obese person and his health[15].

As details mentioned by the world health organization (WHO) in 2016, the number of adults obese was 650 million, represented 13% of adults.

Obesity is considered one of the most causes of death in the United States of America. Different natural components have been investigated to have suitable activities for preventing or treating obesity and related metabolic and cardiovascular disease. Orlistat is still the drug of choice used to treat obesity, but it is still not enough for treating obesity in many cases.

Diabetes is another global health problem caused when the body does not generate enough insulin or cannot be used effectively. A diabetic person who does not take suitable treatment may suffer other health problems such as cardiovascular disease, retinopathy, kidney dysfunction, and uncontrolled sugar level to neuropathy.

Hence, the studies for antidiabetic agents from plant extract have increased to discover new valuable drugs for restraining the disease.

Another global health problem is antimicrobial resistance, which occurs when microscopic organisms like bacteria, viruses, parasites, and fungus are modified when exposed to antimicrobial medicine like antibiotics and antivirals, anthelmintics, antimalarials, and antifungal[11].

Over time, antimicrobial resistance develops naturally out of changes in genetic deformation.

According to the problem mentioned earlier, oxidative stress, obesity, diabetic diseases, and antimicrobial resistance and their consequences of developing severe diseases in human beings result in elevated mortality worldwide. The global trend toward natural products. It was encouraging to study the national and traditional herbs that were historically used to treat different diseases. Thus, the study aimed to define the chemical compositions and their characteristics of E.O. of *Pelargonium graveolens[10]*.

1.6 Objectives of the Study

1.6.1 General objective

The study's main aim was to compare the chemical composition of *Pelargonium graveolens* E.O collected from three different governorates in Palestine; the geographical impact was considered

in choosing the regions (i.e., northern, middle, and southern areas) and determine the potential biological activities and pharmacological properties of E.O. of *Pelargonium graveolens*.

1.6.2 Specific objectives

In this study, screening the potential antimicrobial, antioxidant, and enzymatic activity (antilipase and antiamylase) of the E.O. was examined and compared. However, the specific objectives of the current thesis were:

a. To analyses, the chemical composition of *Pelargonium graveolens* E.O. using GC/MS.

b. To test the antioxidant activities of *Pelargonium graveolens* E.O.

c. To assess the enzymatic properties of *Pelargonium graveolens* E.O. such as antilipase and antiamylase.

d. To investigate the antibacterial, antifungal activities of *Pelargonium graveolens* E.O.

e. To conduct a comparative study of the tests' findings mentioned above among three geographical regions in Palestine.

1.7 Significance of the study

Previous studies declare that *Pelargonium graveolens* E.O. can act as antioxidants, antibacterial, anti-fungal, and other biological properties. However, as we have known, there have been no studies that explore the potential changes in these activities regarding the differences in geographical conditions and, subsequently, the chemical constituents. This is the first study conducted on the chemical compositions and biological activities of *Pelargonium graveolens* E.O. relative to different geographical conditions in three regions in Palestine. Consequently, the study may be a valuable tool to:

a. Explore the chemical constituents of *Pelargonium graveolens* E.O.

b. Illustrate if there are differences in the chemical constituents of *Pelargonium graveolens* E.O. in the different regions of Palestine.

c. Investigate the biological activities, the antioxidant, and the enzymatic properties of *Pelargonium graveolens* E.O. in the different regions of Palestine.

Chapter Two

Materials and Methodologies

Chapter Two

Materials and Methodologies

2.1 Materials

The substances used in the research were of analytical grade and utilized without more purification. DMSO 100% was used in antimicrobial Testing, and the dilution of 10% was used in the enzymatic Testing.

2.1.1 Materials used in producing E.O.

Calcium Chloride, used for drying E.O.

2.1.2 Materials used for antioxidant Testing

DPPH (2,2-Diphenyl-1-picrylhydrazyl), methanol, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acids).

2.1.3 Materials used for antilipase Testing

Porcine pancreatic lipase, Tris-HCl, and PNPB (p-nitrophenyl butyrate), Orlistat, and Acetonitrile.

2.1.4 Materials used for antiamylase Testing

The Amylase type four-B, more or equal 10 unit/mg, Acarbose, and the antiamylase acid (DNSA). The sodium-potassium tartrate tetrahydrate. Other chemicals like sodium hydroxide (NaOH) and

disodium hydro phosphate/hydroxonium phosphate (Na_2HPO_4/ NaH_2PO_4).

2.1.5 Materials used for antimicrobial Testing

2.1.5.1 Antibacterial

Methanol, Nutrient Agar 28.0 g/L, Mannitol 111 g/L, MacConkey Agar 49.53g/L, and Mueller Hinton Broth 21.9 g/L.

2.1.5.2 Antifungal

Ethyl alcohol 99.9% and Sodium Hydroxide 1 M, Sodium Chloride and Sodium potassium tartrate, Sabouraud Dextrose Agar 65g/L w, 3-[N-morpholino] propane sulfonic acid (MOPS) buffer 34.53 g/L and RPMI-1640-medium (with L-glutamine, without sodium bicarbonate) 1.165 mol/L (was improved at Roswell Park Memorial Institute), and 0.05% Tween 80.

2.2 Equipment

2.2.1 Essential Oil Extraction and chemical testing

Miller (Molineux model) was used to cut the dehydrated herbs. Balances were used to weigh the plant material; microwave-ultrasonic (CW-2000). Other devices were used for the preparation of Essential extraction oil. The GC-MS (QP-5000 Shimadzu GC-MS, Japan) was used for the chemical testing of E.O.

2.2.2 Antioxidant and enzymatic Testing

Weight scale device maximum capacity 4.5 kg, UV-Vis (Ultraviolet-Visible) Spectrophotometer was used for assessment of the antioxidant and enzymatic activities of E.O. Water bath, water bath sonicator, Heater, Refrigerator, Single micropipette 100-5000 µL, single micropipette 100 -1000 µL and 20-200, large glass test tubes, volumetric flasks 10, 50, 100 mL, and plastic cuvettes.

2.2.3 Antimicrobial screening

Weight scale device max 3kg, d=0.001g, Heater, Autoclave used for sterilization media, water, and disposed materials, Bunsen Burner and Hood were used for working under aseptic conditions. Refrigerator, Water bath, and Incubator, the pH of RPMI solution which was adjusted by using pH meter, Multichannel micropipette 30-300µL, Multichannel micropipette 1-10 µL, Single micropipette 100-1000 µL, Single micropipette 20-200 µL, tips white, yellow and blue were used for measuring the minute volumes of essential plant oil, nutrient broth, and bacterial solutions. Microplates 96 Well Cell Culture. Aluminum foil is used for enveloping the material for autoclaving. Disposable sterile syringe 5, 10 mL, sterile syringe filter 0.25 µm, in addition to other types of equipment were used like large and small glass test tubes, large and small plates, Loops, disposable sterile pipette volumes 1,5,10 mL and Eppendorf tubes, autoclave sterilization tapes, and parafilm M.

2.3 Methodologies

2.3.1 Plant materials gathering and preparation

The aerial parts of *Pelargonium graveolens* were collected in April 2020 from three governorates in Palestine: Nablus, Ramallah, and Hebron. After that, all samples were botanically characterized and coded by Dr. Nidal Jaradat, the Pharmacognosist at An-Najah National University (ANNU). The obtaining of E.O. is followed the mechanism which was supposed by Dr. Nidal Jaradat in reference [22]. The new aerial parts of *Pelargonium graveolens* were detached carefully, flushed two times using distilled water, and dried for about two weeks in the shade and room temperature. After drying, the dried specimens were fractured and stored in plastic bags.

2.3.2 Essential Oil Extraction

The three samples of the *Pelargonium graveolens* plant's essential oil were extracted utilizing microwave and ultrasonic methods and tested locally. The supernatant of plant fragmentation was directed toward the ultrasonic waves, enhancing the extraction process [23]. The extraction instrumentation is shown in (Figure 4). A 100 g from the dried parts of each plant sample were placed in a 1 L round bottom flask then 300 mL of distilled water was added. Then the flask was moved to the oven and then connected with the Clevenger

apparatus. The extraction process was carried for 10 min at 100 ˚C. Three times of these procedures were repeated for each sample. The extracted E.O. was collected into a separate clean small glass bottle and then chemically dried over calcium chloride while the dried oil was stored in a small closed dark glass bottle. All the bottles were labeled by the name and date of each plant. After that, all samples were wrapped with aluminum foil and stored in the refrigerator at 2-8˚C for further use [24].

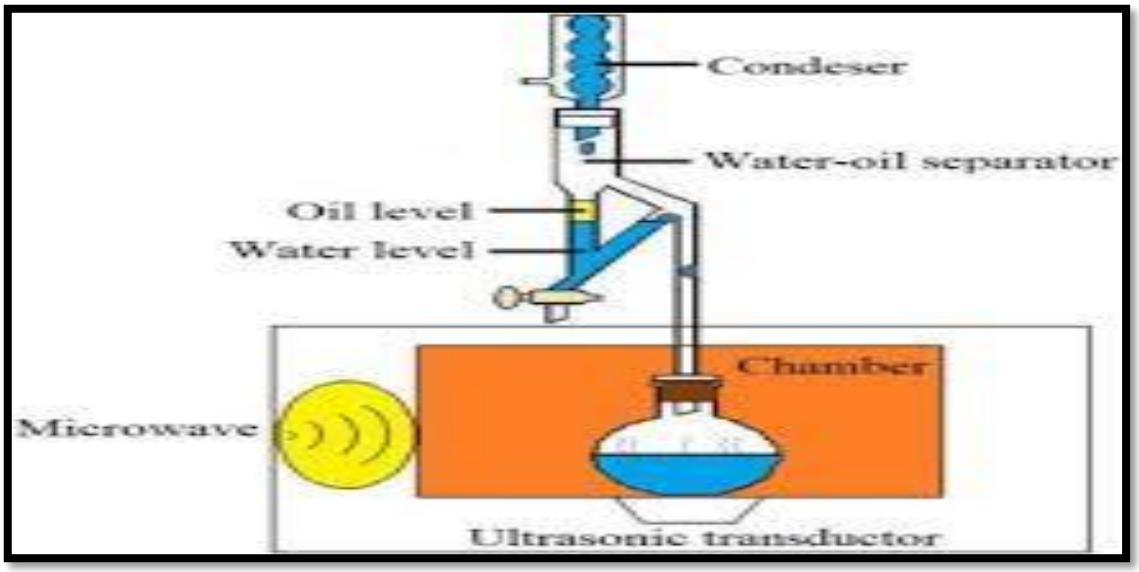

Figure (4): Microwave - ultrasonic apparatus.

2.3.3 Gas Chromatography/Mass Spectrometry (GC-MS)

GC-MS technique was used to identify and measure the chemical composition of the three samples of *Pelargonium graveolens* E.O. The method used was described by Al-Hamwi et al. [25] and Jaradat et al. [24] with some modifications. The analysis was carried using GC-MS chromatograms (Shimadzu QP-5000 GC-

MS), a column Rtx-5ms (0.250 mm interior diameter, 0.25μm thickness, and 30m long). The purge gas was helium at a flow rate of 1 mL/min. The injector's temperature was set at 220°C while the oven's temperature was set from 50°C (1min hold) at 5 °C/min to 130°C, then at 10 °C/min to 250°C and kept at a constant temperature for 15 min. The temperature of the transfer line was 290°C. The detection type was carried using the electron ionization method. The scan speed of 1000 amu/sec and the scan rate of 0.5 s were used in a range of 38-450 M/Z [25]. A NIST library was used to identify all essential ingredients besides the Kovats index in the literature to compare their retention times. The quantitative data were obtained electronically from integrated peaks, area percentages without using a correction factor [25].

2.3.4 Antioxidant activity DPPH assay

The scavenging activity of *Pelargonium graveolens* E.O. of the three samples from three regions of the West Bank in Palestine was evaluated using Sonboli and Jaradat et al. [45]. Stock solutions of E.O. of the three samples and Trolox (the reference product) were prepared in methanol at a concentration of 0.1 mg/mL (10 mg of sample in 100 mL methanol). Operation solutions with the following concentrations: 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 and 100 μg/mL was set by taking volumes of (0.1, 0.2, 0.3, 0.5, 0.7, 1, 2, 3, 4, 5, 8 and 10 mL) respectively of stock solution and bringing

them up to 10 mL with methanol, using 10 mL volumetric flask (VF). The DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared freshly at a concentration (0.002% w/v), 2 mg of DPPH was dissolved in 100 mL of methanol using 100 mL V.F. A mixture of DPPH, Methanol, and E.O. of each of the above-mentioned working solutions was prepared at a 1:1:1 ratio. A blank solution was prepared by mixing the DPPH solution with methanol at a 1:1 ratio. After that, all of those solutions were incubated at room temperature in the dark for 30 minutes. The absorbance of those solutions was measured after the incubation period by UV-Vis spectrophotometer at 517 nm wavelength, and methanol was used to zero the spectrophotometer. The antioxidant activities of *Pelargonium graveolens* E.O. and Trolox were evaluated by their capability to give a hydrogen atom or electron and were distinguished from turning the deep violet color of a methanol solution of DPPH to colorless or soft yellow as shown in figure 5, for that the inhibition% of DPPH activity was used to define the antioxidant activity of *Pelargonium graveolens* E.O. and Trolox using the following equation:

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

Equation (1): INHIBITION% OF ANTI-OXIDANT ACTIVITY.

A $_{blank}$ stated the absorption of the control reaction (all reagents without the sample), and A $_{sample}$ stated the absorbance of the sample.

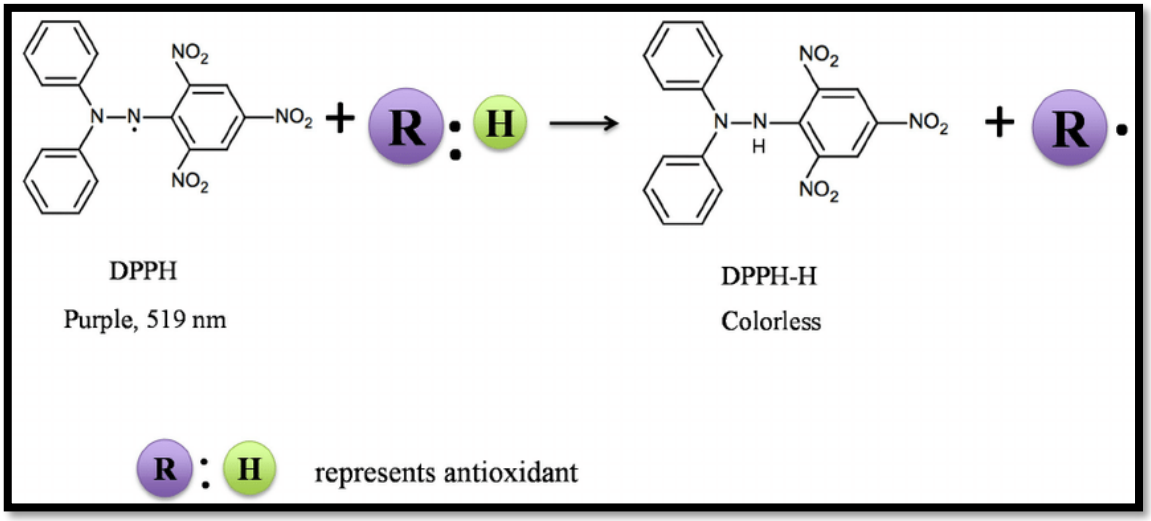

Figure (5): Principle of Dpph Radical Scavenging Capacity Assay.

The IC_{50}, the antioxidant half_ maximal inhibitory concentration (providing 50% inhibition) for each studied *P.graveolens* E.O, Trolox standard solution, and their standard deviations were calculated from the graph that plotted the inhibition percentage against extract concentration. Microsoft Office Excel 2017 was used for presenting the plots.

2.3.5 Pancreatic Lipase (P.L.) Inhibition

The Porcine Pancreatic Lipase (PPL) inhibitory test was performing as the methods mentioned by Jaradat et al. [17], Bustanji et al. [20], and Siew-Ling et al. [21] with some adjustment.

2.3.5.1 Preparation stock and working solutions

- Essential oil and Orlistat stock and working solutions

Five different concentrations of samples were prepared (200, 400, 600, 800, and 1000 µg/mL) from E.O.'s stock solution of 1mg/mL that has been prepared in 10% Dimethyl sulfoxide (DMSO) and then diluted with 10%DMSO.

The reference for pancreatic lipase inhibition assay was Orlistat. The same procedure prepared it as a plant extract.

- Pancreatic lipase enzyme

Pancreatic lipase enzyme stock solution was made before use by suspending 10% DMSO at a concentration of 1 mg/mL. By weighing 25 mg of lipase suspending in a small amount of 10% DMSO, bringing up the volume up to 25 mL in V.F. (25 mL), putting in water bath sonicator at 37 ºC for 15 minutes.

- p-nitrophenyl butyrate (PNPB) lipase substrate

The stock solution of p-nitrophenyl butyrate (PNPB) was made according to manufacture directions (20.9 mg of PNPB in 2 mL of Acetonitrile) by dissolving 104.5 mg of PNPB in Acetonitrile, bringing up the volume to 10 mL in V.F (10 mL).

2.3.5.2 Assay of Pancreatic Lipase (P.L.) Inhibition

The Pancreatic Lipase (P.L.) Inhibition assay was performed using the directions in the references [19-23] with some adjustment. Every working solution of E.O. prepared above, 200 µL plant extract was taken in a separate test tube, 100 µL of porcine pancreatic lipase (1 mg/mL) was added. The preparing mixture was then continued up to 1000 µL by adding 700 µL of (Tris– HCl) solution and was incubated at 37 °C in a water bath for about 15 min. After the incubation, a 100 µL of PNPB (p-nitrophenyl butyrate) solution was added to each test tube. The mixture was again placed in a water bath for 30 min at 37 °C. A negative control solution was prepared without E.O. by mixing 100 µL of porcine pancreatic lipase (1 mg/mL) solution with Tris– HCl solution with 1mL by adding 900 µL. The same procedure was followed for Orlistat, and that was used as a positive control. Tris-HCl buffer was involved in resetting UV-Vis spectrophotometer at 405 nm. Pancreatic lipase activity was measured by determining the hydrolysis of p-nitrophenolate to p-nitrophenol (figure 6) at 405 nm using a UV-Vis spectrophotometer. The lipase inhibition activity of *Pelargonium graveolens* E.O. or Orlistat as a reference was identified by detecting and determining the effect on the enzyme reaction rate after involving extracts and then compared with the control. I% was calculated by using equation 2 [24].

$$I\% = [(Absorbance_{BLANK} - Absorbance_{sample})/Absorbance_{BLANK}*100$$

Equation (2): PANCREATIC LIPASE INHIBITION [24].

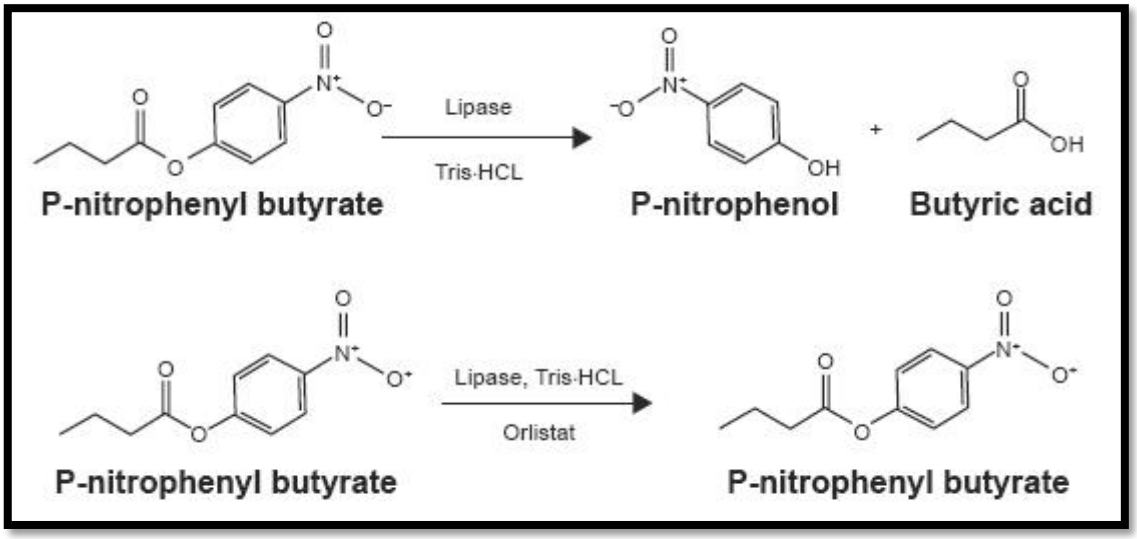

Figure (6): The Mechanism of P-Nitro Phenyl Butyrate (PNPB) Hydrolysis With and Without Orlistat.

2.3.6 α-amylase inhibitory screening

The α-amylase inhibition assay was carried out depending on the procedure by Wickramaratne et al. [32] with some changes. The assay was conducted using the 3,5-dinitrosalicylic acid (DNSA) procedure.

2.3.6.1 Preparation of stock and working solutions

- Sodium phosphate buffer (20 mM) with sodium chloride (6.7mM), pH 6.9.

Solutions of 20 mM sodium phosphate monobasic and sodium phosphate dibasic buffer including 6.7 mM sodium chloride (NaH_2PO_4 and Na_2HPO_4, both containing 6.7 mM NaCl, pH 6.9),

sodium chloride (NaCl) was utilized for ionic strength and osmolarity purposes). The solutions were prepared by partially filling a beaker with the NaH_2PO_4 and NaCl solution. The mixture was subjected to a magnetic stirrer while adjusting the pH by inserting a calibrated pH electrode in the solution. Then gradually adding the Na_2HPO_4 and NaCl solution until the pH reached 6.9. A weight of 5.36 g of 20 mM $Na_2HPO_4.\ 7H_2O$ and 0.39 g of 6.7 mM NaCl were dissolved in distilled water to make 1 liter and a weight of 2.76 g of NaH_2PO_4. H_2O and 0.39 g of NaCl were dissolved in distilled water to make 1 liter.

- Essential oil stock and working solutions

The stock solution (S.S) of *Pelargonium graveolens* EOs of 1 mg/mL concentration, was made in the least amount of 10% DMSO (1:100 dilution) and was further softened in buffer (Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9). Functioning solution of concentrations (10, 50, 100, 500 and 1000 µg/mL) were prepared by mixing (0.1 mL, 0.5 mL, 1 mL, 5mL, 10 mL) respectively of *Pelargonium graveolens* EOs S.S and further diluting with buffer (Na_2HPO_4/NaH_2PO_4 (0.02 M)), NaCl (0.006 M) at pH 6.9) and bring up to 10 mL using VF (10 mL).

- Acarbose stock solutions

The stock and functioning solutions of Acarbose were made using the same procedure of *Pelargonium graveolens* E.O.s. Acarbose was performed as a reference.

- α-amylase solution

α-amylase solution (2 unit/mL) was made by dissolving 12.5 mg of amylase enzyme in the smallest amount of DMSO10 %, then bringing up to 100 mL with buffer (Na_2HPO_4/NaH_2PO_4 (0.02 M)), NaCl (0.006 M) at pH 6.9) in V.F. (100 mL).

- Starch stock solution

The starch solution was prepared in a concentration of 1% (w/v) by suspending 1000mg of starch in 100 mL distilled water using V.F (100 mL) and kept in a water bath at 37ºC until use with slightly mixing to prevent starch precipitation.

- 3.5-dinitrosalicylic acid stock solution (DNSA)

DNSA was a reactive reagent that reduced sugars to get 3-amino-5-nitro salicylic acid (Figure 7), which mightily absorb light at 540 nm. The reagent was prepared by making a solution of 12 g of sodium potassium tartrate tetrahydrate dissolved in 8.0 mL of 2 M NaOH (8 g in 100 mL distilled water) and then diluted in 20 mL of 96 mM of 3.5-dinitrosalicylic acid solution.

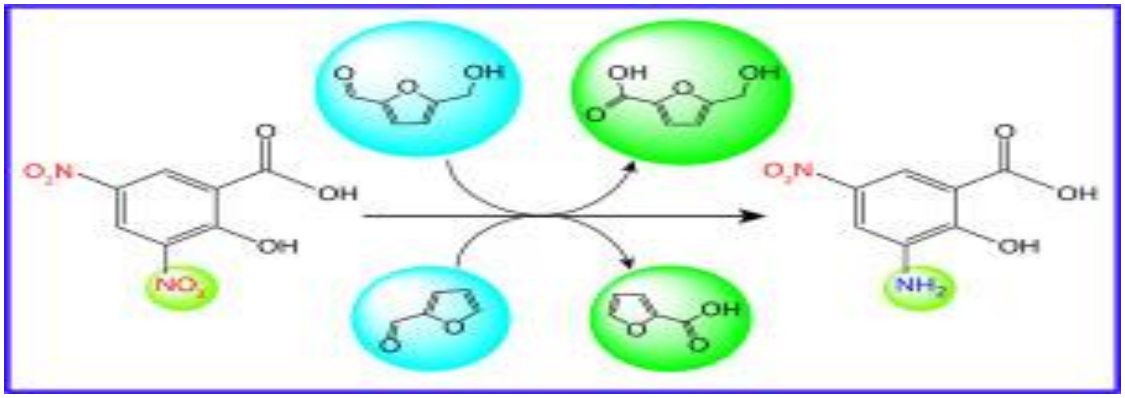

Figure (7): DNSA (3,5-Dinitro Salicylic acid reaction with reducing sugars to form 3-Animo-5-Nitro salicylic in Alkali medium and at temperature 100° C [31].

2.3.6.2 Assay of α-amylase inhibition

Wickramaratne 2016 described the procedure as "volume of 200 µL of α-amylase solution (2 unit/mL) was mixed with 200 µL of each E.O. working solutions and incubated for 10 min at 37°C. Then all of the starch solutions were added to each tube and incubated for 3 mins at 37°C. Adding 200µL DNSA reagent and boiling it for 10 min in a water bath at 85-90°C will terminate the reaction.

After cooling, the mixture to room temperature, 5 mL of distilled water has been added, and the absorption was measured at 540 nm using a UV-Vis Spectrophotometer. A 100% activity blank was prepared by replacing the plant extract prepared before with 200 µL of the buffer. A control sample of Acarbose was used. The α-amylase is used to determine the inhibitor percent using equation 3. The percent of α-amylase inhibition was then plotted against the extract concentration for the determination of IC_{50} values were obtained from the graph" [39].

(3)% α-amylase inhibition = Abs $100\%_{Blank}$ - $Abs_{Sample/Abs}100\%_{Blank}$

Equation (3): % A-AMYLASE INHIBITION [30].

2.3.7 Antimicrobial screening

2.3.7.1 Microorganisms and condition for cultivation

- Bacterial strains

The E.O. samples of *Pelargonium graveolens* were studied for their antimicrobial activities. The antibacterial activities of *Pelargonium graveolens* E.O. were studied against the growth of six references bacterial strains that have been obtained from the *American Type Culture Collection (ATCC*): *Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 13883*, U.K.), *Pseudomonas aeruginosa (ATCC 27853*, U.S.A.), *Staphylococcus aureus (ATCC 25923*,U.S.A.). Moreover, diagnostically confirmed clinical isolates *Proteus mirabilis and MRSA*.

Fungal strains

The American Type Culture Collection (ATCC) Candida albicans (ATCC 90028, U.S.A.) were examined to determine the antifungal activity of essential oils tested against fungal strains' growth.

2.3.7.2 Preparation of growth media

- Bacterial growth media

Nutrient Agar (N.A.) was made by dissolving 5.6 g of N.A. powder in 200 mL autoclaved distilled water. The mixture was heated to a boiling point with stirring with a magnetic stirrer, then autoclaved for 15 min at 121ºC; the sterilization process was confirmed using sterilization indicator tapes. After autoclaving and under aseptic condition, the sterilized solution of N.A. was poured into small and large plates (around 15 mL and 20 mL, respectively), allowed the media to solidify and condensate to dry. The plates were labeled, incubated in an invert position to prevent condensation of vapor on agar, reduce contamination, enveloped with a plastic bag, labeled, and kept in the refrigerator at 4-8 ºC until use.

Mueller Hinton Broth (M.H.B.) was made by dissolving 8.4 g of M.H.B. powder in 400 mL autoclaved distilled water. The solution was heated to boiling point with stirring with a magnetic stirrer. After reaching a touchable temperature, the solution was poured into ten small test tubes with 5 mL in each, and into another ten small test tubes with 4 mL in each, and finally, the rest amount of M.H.B. was poured in 10 mL in giant test tubes. All the tubes were autoclaved for 15 min at 121ºC, and the sterilization was confirmed

using sterilization indicator tape. Then the tubes were kept in the refrigerator at 4-8 ºC, until use.

2.3.7.3 Preparation of microorganism strains

- Bacterial strains

Freshly prepared bacterial strains were used; the strains were cultured before 24 hours of use. Initially, the bacterial strains were cultured on its specific growth medium; *Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis, and Pseudomonas aeruginosa* on MacConkey Agar, *Staphylococcus aureus,* and *MRSA* on Mannitol Salt Agar. The stock solution of bacterial strain suspensions was prepared in 5 mL M.H.B. by gently swabbing the colony surface of overnight bacterial stains subculture onto specific agar with a sterile swab, adjusting the turbidity to 0.5 McFarland solution with a concentration of $1.5*10^8$ CFU/mL. Working solutions were prepared by mixing 2 mL of stock solutions with 4 mL M.H.B. in a large plate to produce a concentration of 0.5 $*10^8$ CFU ($5*10^7$), which was further used in the experiment of microdilution method, the concentration achieved in the wells was $2.5*10^5$ CFU.

- Fungal strains

The fungal strains were cultured freshly using Potato Dextrose Agar and Sabouraud Dextrose Agar; *Candida albicans* before 24 hours of using:

Candida albicans stock solution in sterile M.H.B. was prepared by gently swabbing the colony surface of overnight *Candida albicans* subcultured onto S.D.A. with a sterile swab, adjusting the turbidity to 0.5 McFarland solution with a concentration of $1*10^6$ to $5*10^6$ CFU/mL. The working solution was made to be used for inoculation in microdilution method with the final concentration of $1*10^3$ to $5*10^3$ CFU/mL by 1: 1000 dilution in two times; first dilution was 1:50 in M.H.B. (100 µL to 4900), the second dilution was 1:20 in RPMI µL (500 µL to 9500 µL), and finally 100 µL was used in microdilution method [32], the final concentration in wells was $0.333*10^3$ to $1.666*10^3$ CFU/mL.

2.3.7.4 Preparation of plant E.O.s solutions

The initial concentrations of *Pelargonium graveolens* E.O. for bacterial and *Candida albicans* assays were 50 mg/mL. The concentration of 50 mg /mL was obtained by initially mixing 50 mg of E.O. with 0.5 mL of DMSO 100%, then bringing up the volume to 1 ml with DMSO 100% using Eppendorf tubes size 2 mL. The same procedure was used to prepare the concentration of 25 mg /mL, except the weight of E.O. was 25 mg.

2.3.7.5 Preparation of antimicrobial agents

- Antibacterial agents

The antibacterial agents used for susceptibility tests on the six bacterial strains that were used with *Pelargonium graveolens* E.O. were dissolved in a particular volume of appropriate solvents as stated by the solubility test to obtain stock solution:

Azithromycin was dissolved in Ethanol 95% to get stock solution of concentration (4.5 mg/mL), two dilutions of (1:10) were made to get concentration (45µg/mL) carried out on all bacterial strains.

Levofloxacin was dissolved in sterile distilled water to acquire the stock solution of concentration (3.28 mg/mL), three dilutions of (1:10) were made to get a concentration (3.28 µg/mL) was performed on *MRSA* and *Klebsiella pneumoniae*. A fourth dilution was made of (1:10) to get a concentration (0.328 µg/mL) was performed on *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*.

Cefuroxime was dissolved in Dimethyl sulfoxide (DMSO) 100% to obtain a stock solution of concentration (3.77 mg/mL), two dilutions of (1:10) were made to get concentration (37.7 µg/mL), was tested on all bacterial strains.

Doxycycline was dissolved in DMSO 100% to get a stock solution of concentration (6.20 mg/mL), three dilutions were made of (1:10)

to get concentration (6.20 µg/mL) was carried out on all bacterial strains [33-36].

- Antifungal agents

Stock solutions were obtained by using antifungal agents for sensitivity tests on *Candida Albicans*, which used with *Pelargonium graveolens* E.O. oils, were dissolved in a specific volume of relevant solvents according to the solubility test

Terbinafine was dissolved in an equal portion of sterile distilled water and methanol (1:1) to get a stock solution of concentration (50 mg/mL), two dilutions were made of (1:10) to obtain concentration (500 µg/mL).

Tinidazole was dissolved in sterile distilled water to get a stock solution of concentration (50 mg/mL), two dilutions were made of (1:10) to obtain concentration (500 µg/mL) [37].

2.3.7.6 Antimicrobial assays

The susceptibility tests carried out on microorganisms were determined using the broth microdilution method as defined by the procedure in the following references with some modifications [38-39].

- Antibacterial assay

The broth microdilution method was used to detect the Minimum Inhibitory Concentration (M.I.C.) values to estimate the antibacterial activity. Overnight cultures of two Gram-positive bacteria, i.e., *Staphylococcus aureus* and the *methicillin-resistant Staphylococcus aureus (MRSA)* and four Gram-negative strains; *Proteus mirabilis, Pseudomonas aeruginosa, Escherichia coli*, and *Klebsiella pneumoniae*, were adjusted to 0.5 McFarland standard turbidity. Sterile Mueller Hinton broth (100) for bacterial Investigation was added to all microplate wells using a multichannel pipette (30-300 µL). The stock solutions of samples (100 µL) were added to microplate wells in duplicate; at row A and B for the first sample, C and D for the second sample, E and F for the third sample, G and H for DMSO 100 % to detect the presence of antibacterial activity for DMSO in broth microdilution method conditions. Then, using multichannel pipettes (30 -300 µL) 100 µL of the mixture (the samples or DMSO 100% and the sterile broth) from wells number one was transferred to wells number two to obtain (2-fold) serial dilution and so on till row wells number 11, wells number 12 served as positive control without E.O.s or DMSO 100%. The inoculate (1µL) from bacterial suspension was added to each well using multichannel pipettes (1-10 µL). The entire process was conducted under aseptic conditions. The final concentrations of bacteria were 2.5 $*10^5$ CFU/mL. The concentrations of

Pelargonium graveolens E.O. were ranged from 0.049 to 25 mg/mL and for DMSO 100% from 0.098% to 50%. Wells numbers 11 were served as negative (free of inoculating) controls. Plates were incubated for 16-20 hours at 35°C. Bacterial growth was indicated by the presence of turbidity in the wells. The lowest concentrations where did not show any bacterial growth were estimated as the M.I.C. value. To control the tested microorganisms' sensitivity, the M.I.C. of antibacterial agents, Azithromycin, Levofloxacillin, Doxycycline, Cefuroxime, were also evaluated in parallel experiments.

- Antifungal assay

The broth microdilution method was used to evaluate the minimum inhibitory concentration (M.I.C.) values to estimate the activity against *C. Albicans*. The same procedure of bacterial strains was used for *C. Albicans* with some modifications. Sterile RPMI 1640 medium (100 μL) was used for *C. Albicans* investigation, and the inoculate of *C. Albicans* was (100 μL) was added to each well using multichannel pipettes (30-300 μL). Thus, the final concentration of *C. Albicans* was in the range of ($0.333*10^3$-$1.666*10^3$ CFU/mL); the concentrations of *P. graveolens* E.O.s were ranged from $8.467*10^{-4}$ to 16.666 mg/mL and for DMSO 100% from $1.69*10^{-3}$ to 33.33%. Plates were incubated for 48 hours at 35°C. The growth of *C. Albicans* was indicated by the presence of pellets at the

bottom of the wells. The lowest concentrations which did not show any *Candida* growth were estimated as the M.I.C. value. To control the tested *C. Albicans'* sensitivity, the M.I.C. of antifungal agents, Terbinafine, and Tinidazole were also evaluated in a parallel experiment.

Chapter Three

Results

Chapter Three

Results

Our study's main goal was to screen the chemical constituent. The potential pharmacological activities of three samples of *Pelargonium graveolens* essential oils representing three areas in Palestine and finally compare the findings.

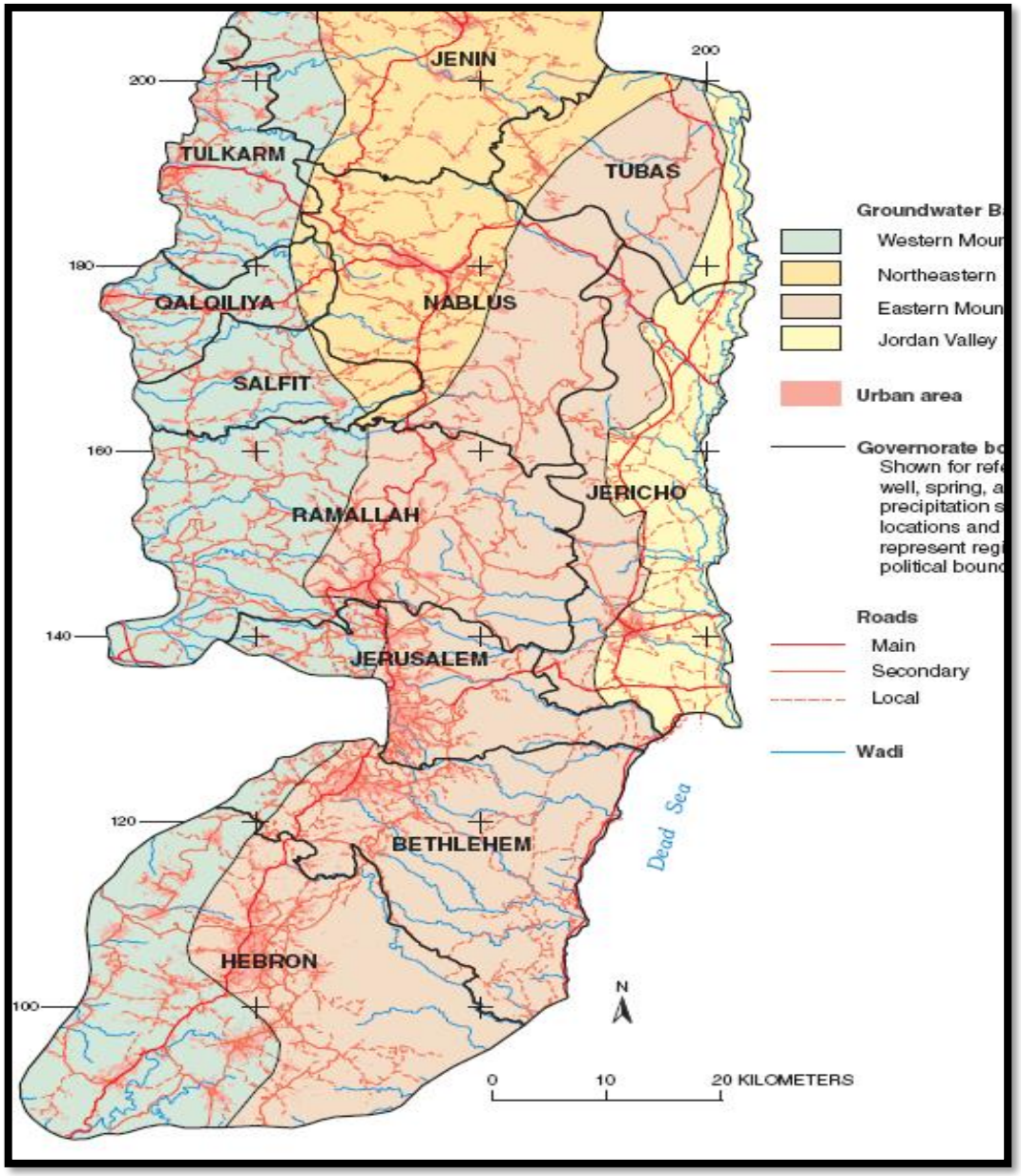

Figure (8): Palestinian map resembles Palestinian governorates.

3.1 Essential oils analysis

The Microwave-Ultrasonic Apparatus method was used to extract the essential oils of the three samples of *Pelargonium graveolens*. The generated oils were fragrant, sticky, yellowish to green in color, and had a lovely scent of rose and citrus. Studying the E.O.s yields of the aerial parts of *Pelargonium graveolens* samples indicated variations between the three samples: 0.12% ± 0.5%, 0.88± 0.15%, and 0.99± 0.19% (w/w%) from Nablus, Ramallah, and Hebron, respectively (Table 1). The chemical analysis was obtained using GC-MS and characterized the E.O.s using 26 components that have been classified into oxygenated ingredients like ketones. The other is non-oxygenated ingredients hydrocarbons for the three samples (Table 1and Figure 9). The most available components in all three samples were R-citronellol, citronellyl formate, cis-menthone, gamma-eudesmol, L-β-Pinene, geranyl isobutyrate, phenyl ethyl tiglate, geranyl bromide, beta myrcene, neryl propionate, 8-P-menthene, and geranyl tiglate. Table 1 and Figure 9 represent detailed results. Other mutual compounds specified in all three samples with a total percentage less than 11.31% in Hebron, and less than12.29 % in Ramallah, and less than 10.62 % in Nablus were: P-menthan-3-one, beta-bourbonene, (+)-Ledene, citronelly propionoate,alpha-cubebene, Caryophyllene, Caryophyllene,

Bicyclo[5.1.7] octane; 8methylene, Delta cadinene, limonene,

copaene, trans menthone, ocimene, and cis-carane (Table 1). Referring to the Appendix components 1, the basic chemical structure chromatograms of *Pelargonium graveolens* E.O. from Hebron, Ramallah, and Nablus were shown chromatograms. The chemical analysis of *Pelargonium graveolens* E.O. from three governorates in Palestine was illustrated in (Figure 9).

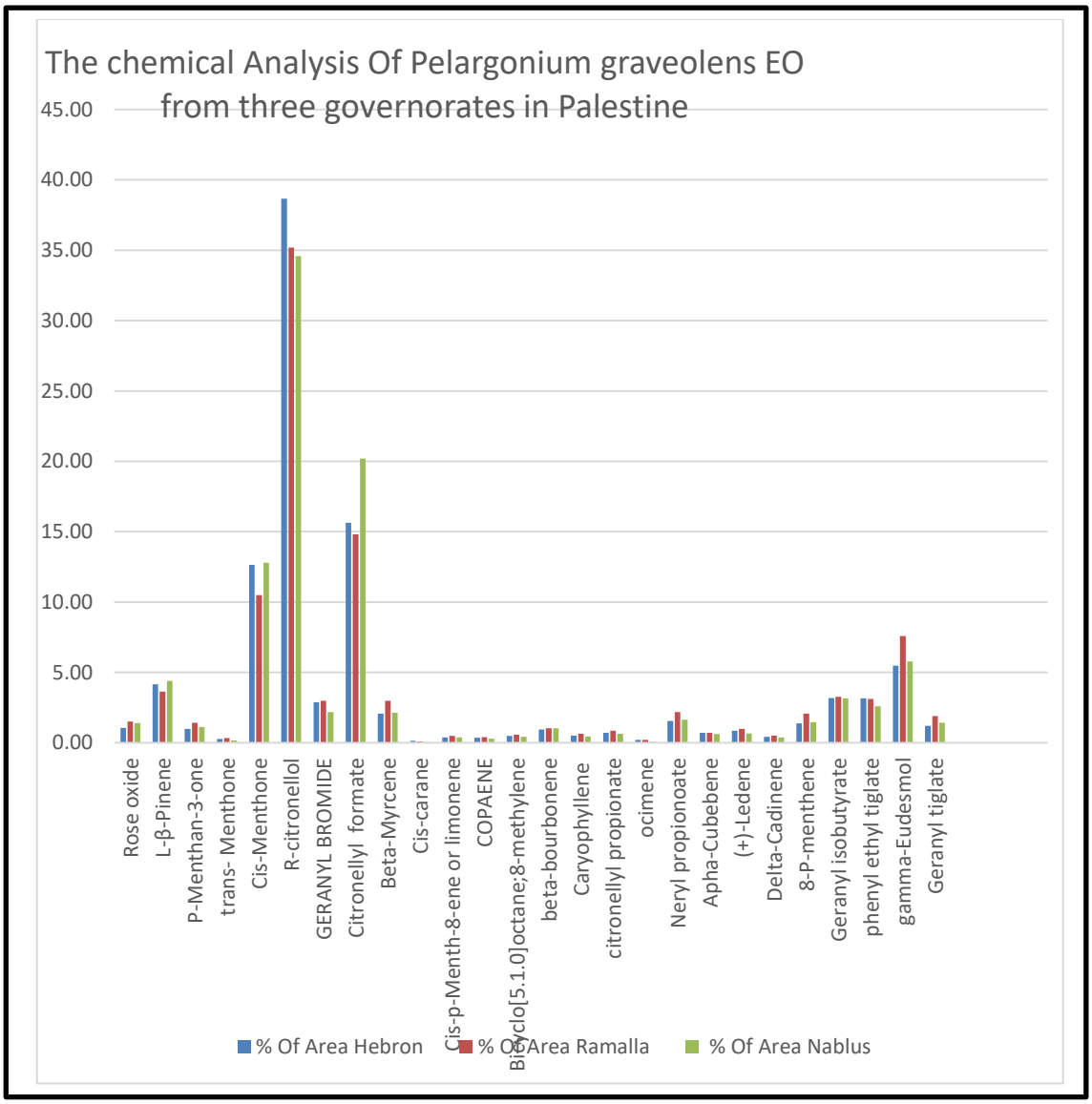

Figure (9): Chemical analysis of three samples P. graveolens E.O.s.

Table (1): Summarizes the total percentage of yields, chemical compounds with their groups of three samples of *Pelargonium graveolens* E.O.s

(w/w) % yield	% total EO Hebron	% total E.O. Ramallah	% total E.O. Nablus
Rose oxide	1.04	1.50	1.39
L-β-Pinene	4.15	3.62	4.39
P-Menthan-3-one	0.99	1.41	1.12
trans- Menthone	0.27	0.34	0.17
Cis-Menthone	12.64	10.50	12.79
R-citronellol	38.67	35.20	34.58
GERANYL BROMIDE	2.87	2.99	2.19
Citronellyl formate	15.64	14.81	20.18
Beta-Myrcene	2.06	2.98	2.14
Cis-carane	0.14	0.09	0.04
Cis-p-Menth-8-ene or limonene	0.38	0.50	0.38
COPAENE	0.36	0.40	0.29
Bicyclo [5.1.0] octane;8-ethylene	0.49	0.56	0.43
beta-bourbonene	0.94	1.02	1.03
Caryophyllene	0.50	0.65	0.44
citronellyl propionate	0.71	0.87	0.64
Ocimene	0.20	0.21	0.08
Neryl propionoate	1.56	2.18	1.64
Apha-Cubebene	0.71	0.71	0.62
(+)-Ledene	0.85	0.99	0.66
Delta-Cadinene	0.43	0.51	0.39
8-P-menthene	1.38	2.08	1.46
Geranyl isobutyrate	3.19	3.27	3.16
phenyl ethyl tiglate	3.16	3.11	2.59
gamma-Eudesmol	5.48	7.58	5.78
Geranyl tiglate	1.20	1.90	1.42
Total non-oxygenated constituents	11.31	12.29	10.62
Total oxygenated constituents	88.69	87.71	89.38

3.2 Antioxidant

The possible antioxidant activity of the *Pelargonium graveolens* E.O. from different governorates in Palestine was screened, and the free radical-scavenging activity was determined by DPPH assay, which was used as in vitro approach. IC_{50} values were used to assess the examined samples' ability to inhibit DPPH. They identified the number of antioxidants required to inhibit the radical (DPPH) concentration by 50%, and they inversely linked to their antioxidant activities. The assay revealed that the three samples of E.O exhibited DPPH free radical IC_{50} for the Nablus E.O sample that showed the highest scavenging effect (146.14 µg ⁄ ml). The E.O samples from Ramallah IC_{50} are 172.17 µg ⁄ ml, and Hebron showed the lowest potency and scavenging effect with IC_{50} is 189.46 µg ⁄ ml. Trolox owned scavenging effect (87.9%) and potency at IC_{50} value (36.135 µg/mL). The results of IC_{50} and the DPPH radical scavenging effect of the three samples and Trolox were shown in (Table 2 and Figure 10). The lowest antioxidant potency of the three E.O. samples exhibited compared to Trolox. There were significant differences in antioxidant potency and efficacy of E.O samples compared to Trolox.

Table (2): The DPPH radical scavenging activity and I.C. $_{50}$ of EO samples OF *P. graveolens* from different regions of Palestine and Trolox

	Trolox	Nablus	Ramallah	Hebron
IC_{50} µg/Ml	36.135	146.15	172.17	189.46
% DPPH radical scavenging activity	87.9%	21.37 %	19.35%	17.33%

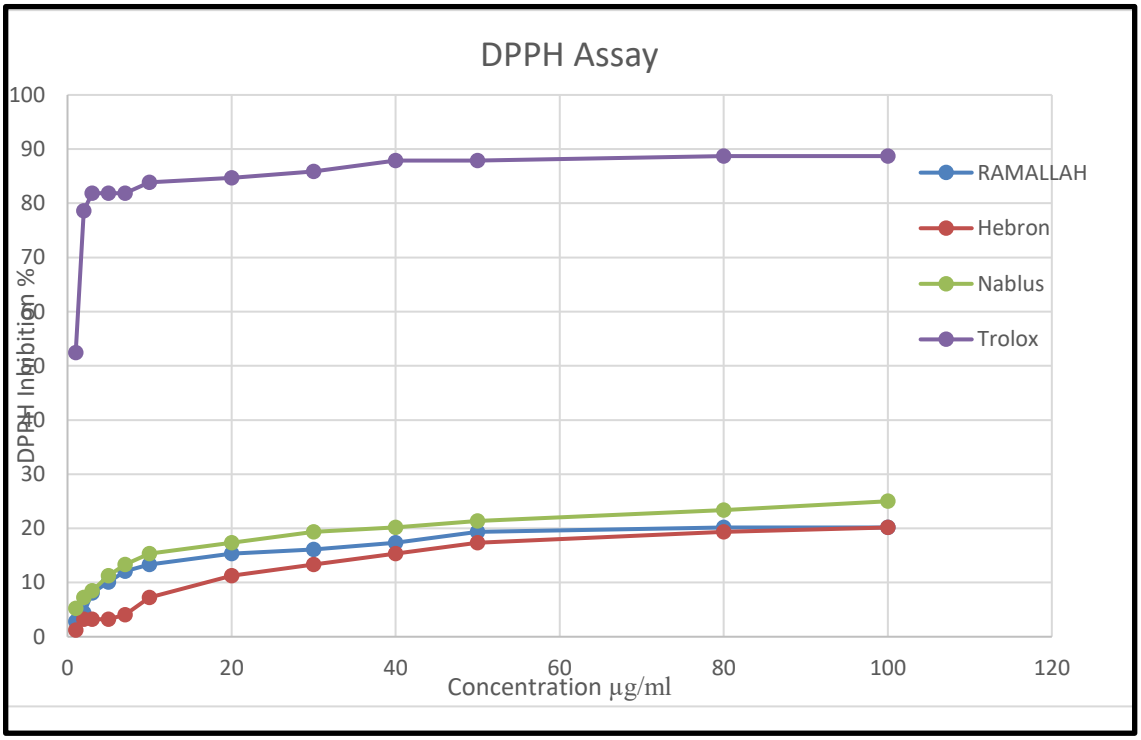

Figure (10): DPPH radical scavenging activity IC_{50} OF the three samples *Pelargonium graveolens* E.O. and Trolox.

3.3 Lipase inhibition assay

The influence of *Pelargonium graveolens* E.O. of the three samples on Pancreatic Lipase Enzyme was measured using the hydrolysis of p-nitrophenyl butyrate to p-nitrophenol. Orlistat comparing to a potent lipase inhibitory agent used to detect the essay, the three E.O. samples showed nearly the same activity. However, the E.O. sample from Ramallah showed the highest potency with an IC_{50} value of (584.112 µg/mL), while the E.O sample obtained from

Nablus showed the IC_{50}% value (626.566%), while Hebron IC_{50} % value is (634.517%). However, Orlistat has potency at IC_{50} of 91.00 µg/mL with antilipase inhibition (99.13%). The IC_{50} values with their antilipase activity of the three samples and Orlistat were presented (Table 3 and Figure 11). Comparative statistical analysis of the results of the three samples of E.O. showed significant differences in antilipase potency and efficacy of E.O. compared to Orlistat. Also, there were insignificant differences in antilipase potency and efficacy of E.O compared to each other.

Table (3): Lipase inhibition assay of the three samples of *P. graveolens* E.O. and Orlistat

	Orlistat	**Nablus**	**Ramallah**	**Hebron**
IC_{50} µg/mL	91.00	626.5664	584.112	634.5177
Antilipase activity	100%	55%	60%	58%

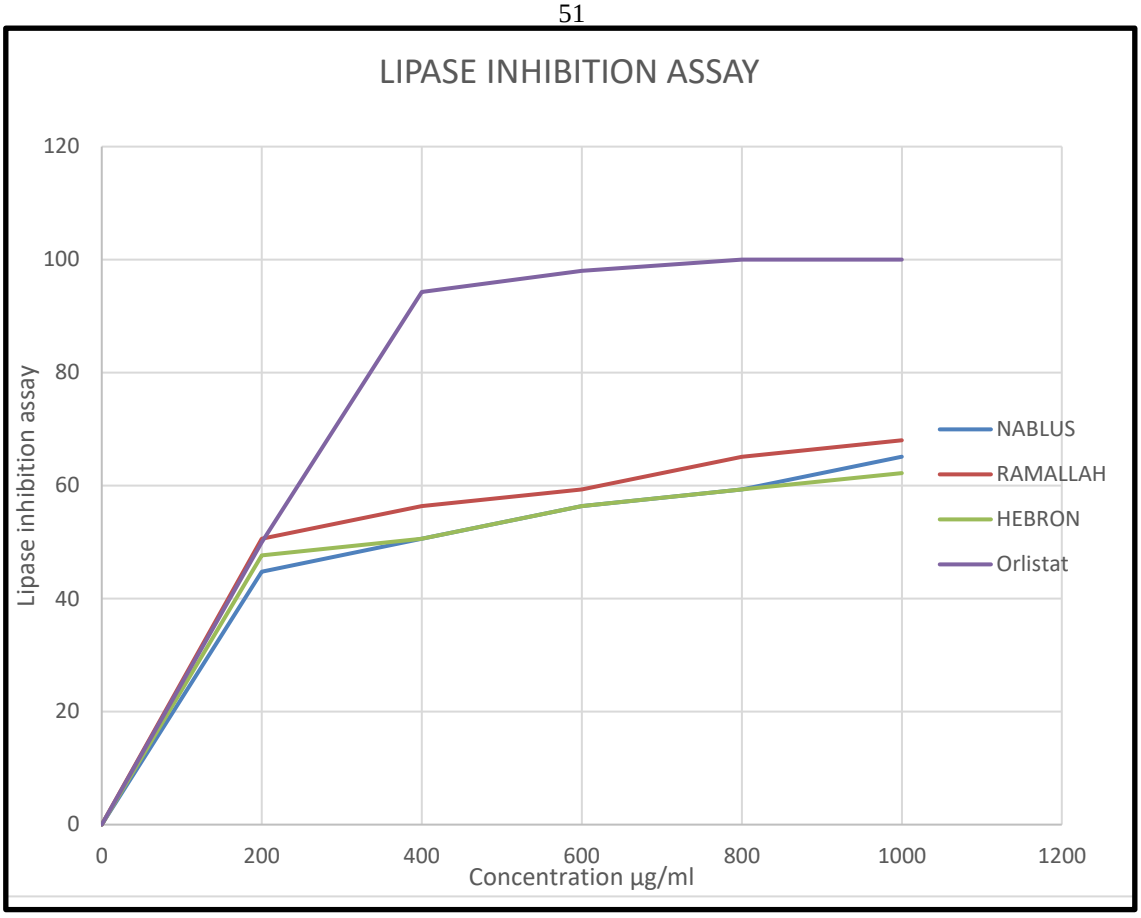

Figure (11): Assay of α- Amylase Inhibition (IC_{50}) for the three samples of *P.graveolens* EO from three regions of Palestine.

3.4 α-Amylase inhibition assay

In vitro assay of alpha-amylase inhibitory activities using starch as a substrate and Acarbose as a positive control was carried out on *P. graveolens* E.O of three samples. The results appeared that the three samples of E.O. showed different degrees of inhibition. Nablus EO sample showed the highest potency with an IC_{50} value of (199.6 µg/mL) and the highest antiamylase activity (98%), the E.O.s samples from Hebron have an IC_{50} value of 211.8640 µg ⁄ ml and Ramallah with IC_{50} 232.12µg ⁄ ml. In comparison, Acarbose showed potency at IC_{50} value (9.00 µg/mL) and I% effect (91.39%). The IC_{50} values and the three samples' antiamylase activity and

Acarbose were demonstrated in (Table 4 and Figure 12). The three E.O. samples have shown higher potency in α-Amylase inhibition that was compared to Acarbose. There were insignificant differences in antiamylase potency and efficacy of E.O. compared to Acarbose and compared to each other.

Table (4): α-Amylase inhibition assay of the three samples of *P. graveolens* E.O.s and Acarbose

	Acarbose	Nablus	Ramallah	Hebron
IC_{50} µg/ml	9.00	199.6	232.12	211.864
antiamylase activity	91.39%	98%	82%	91%

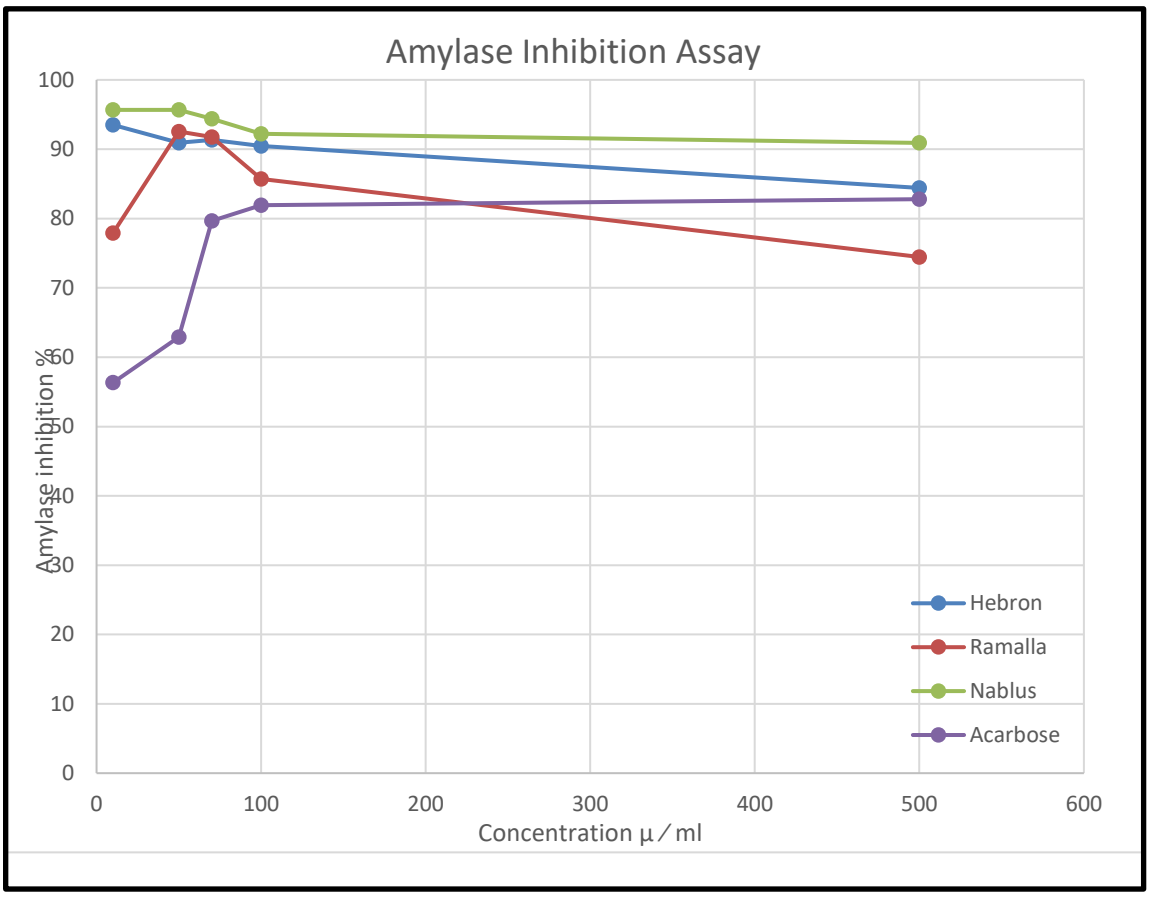

Figure (12): Amylase inhibition assay of *P.graveolens* E.O from different regions of Palestine.

3.5 Antimicrobial activity

3.5.1 Antibacterial activity

The Minimum Inhibitory Concentrations (M.I.C.) of *Pelargonium graveolens* E.O from different Palestine regions were presented in Table 5. The majority of Gram (+) and Gram (-) bacterial strains were sensitive to *Pelargonium graveolens* E.O at M.I.C. of 3.125µg/mL. There were statistically significant differences in activity against six microbial strains between *Pelargonium graveolens* E.O from the three governorates in Palestine. *P.graveolens* E.O has a significant effect on *Staphylococcus aureus (ATCC6538)* with M.I.C. (6.25µg⁄ml) from Hebron and Ramallah samples. While Nablus sample the M.I.C. was (12.5µg⁄ml). There were significant differences of Ramallah E.O. sample compared to Nablus and Hebron E.O samples against the *American Type Culture Collection MRSA (ATCC 25931),* the E.O sample from Ramallah had the highest potency at M.I.C. value of 50 µg/mL while M.I.C. value for Hebron and Nablus were (25µg⁄ml). The E.O. samples from the three regions showed low antibacterial activity towards *E.coli (ATCC25922),* and *Pseudomonas argionosa (ATCC27853)* with a M.I.C. value was (50µg⁄ml). While the E.O. antibacterial activity towards *Proteus* was high for the three regions samples with M.I.C. value was (12.5µg⁄ml). However, the antibacterial activity of E.O. samples showed intermediate activity towards *Klebsiella* with a M.I.C. value (25µg⁄ml). Four antibacterial drugs

were used to evaluate bacterial strains' sensitivity: Azithromycin 250 mg, Levofloxacin 500 mg, Doxycycline 100 mg, Cefuroxime 250 mg. The drugs' MICs values were in the range ($01.28125*10^{-6}$ mg/ml mg/mL); table 6 listed the MICs for drugs.

3.5.2 Antifungal activity

To study the sensitivity of *Pelargonium aeruginosa* E.O against fungus, only the yeast the American Type Culture Collection *C. Albicans (ATCC90028)* studied. *The American Type Culture Collection C. Albicans (ATCC 90028)* yeast was the most sensitive to *P. graveolens* E.O samples at M.I.C. value (3.125µg⁄ml). Evaluation of fungal strains' sensitivity, two antifungal drugs have been used: Terbinafine 250 mg and Tinidazole 500 mg, and the M.I.C. value of antifungal drugs was (18.52 µg/mL) Table 5.

Table (5): Antimicrobial activity (M.I.C. in µg/mL) of E.O obtained from different regions of Palestine using broth microdilution method and agar dilution method

	M.I.C. P.g.N.	**M.I.C. P.g.R.**	**MIC P.g.H**	**DMSO 100%**
Yeast				
C. Albicans (ATCC 90028)	3.125	3.125	3.125	30%
Bacterial strains (Gram-Positive)				
***Staphylococcus aureus* (ATCC 25923)**	12.5	6.25	6.25	30%
MRSA (CI)	25	50	25	30%
Bacterial strains (Gram Negative)				
Escherichia coli (ATCC 25922)	50	50	50	30%
Pseudomonas aeruginosa* (*ATCC 27853)	50	50	50	30%
Proteus mirabilis (CI)	12.5	12.5	12.5	30%
Klebsiella pneumoniae* (*ATCC 13883)	25	25	25	30%

Table (6): MICs (µg/mL) of Antimicrobial drugs

	Azithromycin	Levofloxacin	Doxycycline	Cefuroxime
Bacterial strains				
***Staphylococcus aureus* (ATCC 25923)**	0.352	$5.125*10^{-3}$	0.012	2.356
Staphylococcal enterotoxin B (SEB) CI	0.352	$6.4*10^{-3}$	0.097	4.713
MRSA CI	0.176	$6.4*10^{-3}$	0.097	4.713
Proteus mirabilis CI	5.625	$1.28*10^{-3}$	0.387	4.713
Pseudomonas aeruginosa (ATCC 27853)	0.703	$1.28*10^{-3}$	0.387	2.356
Escherichia coli (ATCC 25922)	0.703	$1.28*10^{-3}$	0.012	2.356
***Klebsiella pneumoniae (ATCC 13883*)**	1.406	0.012	0.387	4.713
Shigella Sonnie (ATCC 25931)	0.703	-	0.387	2.356
	Terbenafine	Tinidazole		
Candida albicans (ATCC 90028)	18.5185	-		

Chapter Four

Discussion

Chapter Four

Discussion

4.1 Chemical analysis

The components of *Pelargonium graveolens* essential oils in this study were lower than the findings of a study performed in Tunisia by Imen Ben El Hadj Ali et al., [43], which reported that they identified forty-two components of *P.graveolens* EO by GC-FID and GC-MS analysis. In this Tunisian study, the main components were Citronellol (24.3% -26.98%), Geraniol (20.65%-21.81%), and 10-epi-gamma-eudesmol (5.13%-13.006%) were the main constituents for leaves and flowers oils.

The GC-MS analysis in our study identified twenty-six compounds listed in Table1. *Pelargonium graveolens* E.O. chromatographic profiles of the three samples. The analysis of the results was dominated by oxygenated ingredients presented by ketones with the range of (86.2107–88.5%) and hydrocarbons ranging from (11.499-13.7892%). Among the oxygenated compounds, R-Citronellol was the most available component with a range of (34.6 – 38.7%), and citronellyl formate was detected to be the second most available component with a range of (14.8–20.2%). The non-oxygenated components detected were minor than 11.5%-13.8%: L-β- pinene,

Geranyl bromide, β- Myrcene, limonene, Caryophyllene, (+)-Ledene and Bicyclo [5.1.0] octane-8-methylene (Appendix 2).

Studies conducted previously on *Pelargonium graveolens* E.O. growing in Iran by Ghannadi et al. reported that E.O acquired after hydro distillation of the aerial parts of *P. graveolens* presented an average yield of 0.8% and 1% (w ⁄ v) based on the dry weight. Analysis of the oil's components found that more than twenty compounds were identified in each E.O. were β- Citronellol (36.4%), citronellyl formate (12.1%), caryophyllene Oxide (24.4%), n-hexadecane (12.5%), and α-terphenyl acetate (11.6%) were the main constituents of the E.O.

There are differences in the composition of these E.O. compared to the E.O. oils of the other countries. These variations seem to depend on distillation and the condition of distillation and climate type and type of soil in which the *P.graveolens* were planted.

In Palestine, no previous studies about the chemical composition of *P. graveolens*, so that this work will be a core for further studies in the future.

Table (7): Main components and their structures of *P. graveolens* E.O. from different origin

Origin	Sample period	Extraction method	Compound and concentration	Reference
Tunis	the flowering period during April	hydro distillation	Citronellol (24.3%-26.98%) Geraniol (20.65%-21.81%) 10-epi-γ-eudesmol (5.13%-13.06%)	Iman BenEl Hadj Ali et al., (2020) [43]
Iran	whole flowering period in June	hydro distillation	B- citronellol (36.4%) Citronellyl formate (12.5%) Caryophyllene Oxide (24.9%)	Ghannadi et al., (2012) [4]

4.2 Antioxidant

Recently most studies are interested in finding new natural antioxidants use as medications to reduce the impacts of free radicals and oxidative stress. *P. graveolens* species were investigated to be a natural source of antioxidant agents [42]. DPPH assay was used as an in vitro approach to evaluate the free radical-scavenging function and investigate the plant E.O. three samples' potential antioxidant activity. While results were reported by Imen Ben ElHadj Ali et al. in Tunis in which antioxidant activity of the E.O. of *P.graveolens* was observed with IC_{50} value 711-1280µg/mL that appears antioxidants activity was relatively high. [21]. The study showed that E.O.s available with oxygenated monoterpenes like Citronellol (24.3%- 26.98%) and Geraniol (20.65%-21.81%) have antioxidant activity [43-44]. However, this result may be supported by the antioxidant potency of the Hebron sample E.O has owned the maximum amount of total oxygenated compounds as R-

citronellol (38.67%) and citronellyl formate (15.64%), respectively, among the three samples of E.O.

4.3 Lipase inhibition assay

Lipase is an enzyme its function in the body to impart fats in the food so it could be able to absorb the fats in the intestines, and this enzyme performs essential roles in digestion, transport, and processing of dietary lipids (e.g., triglycerides, fats, oils). Lipases catalyze the breakdown of fats and oils into fatty acids and glycerol in the small intestine.

Obesity and overweight are a significant universal problem because it causes other health problems such as diabetes, cardiovascular diseases, self-esteem problems [45]. Traditional medicinal plants are widely investigated for their phytochemicals to produce new lipase inhibitory agents for preventing or curing chronic diseases [27]. The anti-lipase properties of *P.graveolens* EO samples from different areas of Palestine were investigated by inhibiting pancreatic lipase (P.L.). Presumably, no prior studies were conducted to explore *P. graveolens* E.O. against the P.L. enzyme in Palestine. The current study results showed that the E.O. sample from Hebron owned the highest potency with an IC_{50} value of 634.5177% µg/ml and an inhibition effect of 58% (Table 3, Figure 10). However, the antilipase activity for *P.graveolens* and other plants is related to their component of monoterpene and

sesquiterpene (α-pinene, β-pinene, D-limonene, and Caryophyllene) and were responsible for antilipase activity of the oil. The phytochemical findings of the three samples of *P.graveolens* EO support monoterpenes and sesquiterpenes in all three samples of E.O. Hebron sample owned the highest percentages of monoterpenes and sesquiterpenes. In contrast, the Nablus sample owned the highest percentage of (Cis-menthone), which support the highest antilipase potency and efficacy of Hebron and Nablus samples, respectively (634.517µg/ml and 626.566 µg/ml.

4.4 Amylase inhibition assay

α-amylase is an essential enzyme, its vital role in metabolizing and absorption of starch. The reduction process of the enzyme can actively control blood glucose levels in diabetes mellitus patients. Plant extracts that showing inhibition of α- amylase enzyme properties help control diabetes.

Diabetes mellitus is a metabolic disease that causes high blood sugar. The critical management for diabetes is to control high blood glucose levels. Many pharmaceutical drugs are now available to treat and manage the glucose levels in type 2 diabetes patients, but it has many adverse effects such as gastrointestinal problems. By inhibiting α- amylase enzyme, we can inhibit the carbohydrate hydrolyzing, so this strategy lowers postprandial blood glucose levels [46].

The inhibition of α-amylase activity investigated *P. graveolens* E.O. samples' antidiabetic properties from different regions in Palestine. Many plants have the activity of inhibition α-amylase, such as Sideritis galatica Born, which is growing in Turkey. This plant was studied by Zengn et al. (2016). Its activity was related to the abundance of monoterpene hydrocarbons ingredients, mainly α-pinene and β-pinene. In the study of the α-amylase inhibitory activity of J. phoenicea essential oil growing in Tunisia, the results showed powerful α-amylase inhibition properties due to terpenes' presence like α-pinene [29]. The *P. graveolens* E.O. sample from Nablus owned a high amount of β-pinene components (4.39 %), compared with Hebron samples and Ramallah, which may explain its highest potency against the α-amylase enzyme.

4.5 Antimicrobial activity

Extracts of essential oils have been examined as new potential antimicrobial agents, bio-preservation products, and suitable antiseptic enhancers for topical uses [47]. *Pelargonium graveolens* species E.O. were considered broad-spectrum solid antimicrobial activity [48]. The antimicrobial properties of *P.graveolens* EO of the three samples from different regions of Palestine were examined against six bacterial strains (2Gram (+) and 4 Gram (-)) and one yeast in a recent study. They were quantitatively determined by M.I.C. values using the Broth microdilution method. The results shown in Table 5 presented that the E.O. of the three samples

promoted considerable antifungal potency with a few antibacterial potencies. The results of antimicrobial activity of the E.O. of three samples showed that this activity was specific against *Staphylococcus aureus* and *Proteus mirabilis* and nonspecific against the rest of microbial organisms. Screening for the potential antimicrobial activity of *P.graveolens* E.O and methanolic extract growing in Tunisia, which was studied by Ben ElHadj Ali et al. (2020) [21], showed that the E.O. gave a broader spectrum and solid antibacterial activity depending on the determination of M.I.C. according to oils and extracts was shown against eleven bacterial strains. The antibacterial activity was tested against three gram-positive bacteria and nine gram-negative bacteria. All samples were more active against gram-positive than gram-negative bacteria. The M.I.C. values for strains sensitive to the *P.graveolens* essential oils ranged from 9.5-21mm and 0.5-20ml⁄ml for leaf oils and 12.5-22.5mm and 0.5-5µl⁄ml for flower oils respectively. They demonstrated that the potent Antibacterial of Tunisian *P.graveolens* essential oils could be due to a high amount of Citronellol (25.64%), Geraniol (21.23%), citronellyl formate (8.15%), and 10-epi-γ-eudesmol (9.1%).

A study conducted by Ghannadi et al. showed the antibacterial activity against *P.graveolens* E.O against six bacterial species, included *Listeria monocytogenes(PTCC1297*), *Salmonella enteritis* (PTCC1091), *Pseudomonas aeruginosa* (*PTCC1074*), *Escherichia coli (PTCC1330), Staphylococcus aureus (PTCC1112),* and

Bacillus subtilis(PTCC1023). In their study, all bacterial strains were susceptible to *P.graveolens* essential oils except *L.monocytogenes*.The most sensitive strain was *S.aureus*, and this essential oil showed an excellent inhibitory effect on its growth. The oils' activity would be expected to relate to the plant's composition of essential oils and possible synergistic interaction between components. The antibacterial activity for β-citronellol and Caryophyllene were studied and showed high percentages [4]. Studying the chemical ingredients with the antibacterial and antifungal activity of the essential oils for four plants that have Mentha spicata [49], and the crude menthone (the dominant component) for the antimicrobial activity that has been reported that E.O. showed powerful antimicrobial properties against *Staphylococcus aureus*, all of *Shigella species*, *Escherichia coli*, *Klebsiella sp*, *Pseudomonas aeruginosa*, *Proteus sp*, *Candida albicans*, and other strains and concluded that menthone (iso menthone) exhibited strong antibacterial properties with MIC 1.5-3.5 µg/mL. These results could be related to our results obtained from Hebron. The E.O. s of *P.graveolens* with the highest quantity of R-Citronellol (Table 1) led us to believe in the effectiveness against *Staphylococcus aureus*. On the other hand, Citronellol represented the lowest number of constituents in Nablus (Table 1) and gave the lowest potency against *Staphylococcus aureus and MRSA*.

Chapter Five

Conclusion and Recommendations

Chapter Five

Conclusion and Recommendations

5.1 Conclusion

Pelargonium graveolens E.O. oil from three various areas in Palestine represented by three governorates showed various antioxidant, anilipase, antiamylae and antimicrobial activities depending on the phytochemical constituents' essential oils. *P.graveolens* EO of three governorates have the same chemical components but in different percentages. The sample from southern Palestine (Hebron) showed the highest total percentages of yield, identified compounds, and oxygenated compounds, producing antioxidant potency. The middle Palestine sample (Ramallah) exhibited antlipase potency due to higher amounts of Caryophyllene and hydrocarbons. The highest amount of α-pinene and β-pinene in Ramallah's sample exhibited the highest anti-lipase activity. The E.O. exhibited intense antifungal activities and lower antibacterial activities. The middle region sample showed higher potency against *MRSA*, while the sample of the northern region showed higher potency against *Staphylococcus aureus*. The study enables *P.graveolens* E.O to be an excellent natural source in treatment or preventing hypercholesterolemia, diabetes, weighting loss agent, skin dermatitis, and good food preservative agent.

5.2 Recommendations

Future work may include:

1. In addition to in vivo studies are required to evaluate the antiamylase, antilipase, antioxidant and antibacterial activities.

2. In addition to more studies are needed to separate the essential components responsible for potential pharmacological activities.

3. Further studies are required to evaluate the safety and toxicity of plant extract.

4. Further studies are required to evaluate the proper dose of E.O. to achieve the highest efficacy of the drug.

5. More studies are required to estimate the suitable amount of E.O. to be used as a food preservative and flavoring agent in the food and drug industry.

6. We have noticed that the pharmacology effect of *P.graveolens* E.O. depends on the time we have extracted the oil and the time we have experimented. To have a high efficacy of *P.graveolens* E.O., it must be fresh extraction.

7. More studies are required to detect the ability to use *P.graveolens* E.O. as a herbal medicine in treating exocrine pancreatic insufficiency patients.

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Appendices

Appendix (1)

Chemical Structure of Constituents

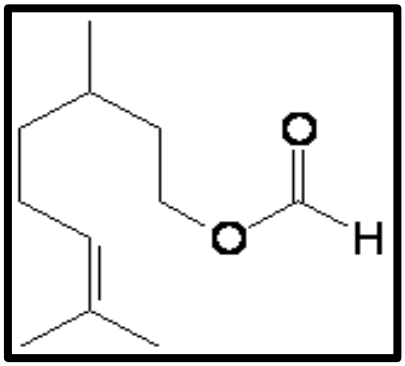


FIGURE 1 CITRONELLYL FORMATE

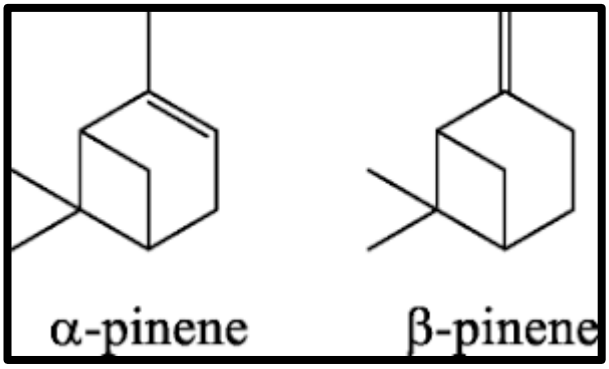


FIGURE 2 L–B–PINENE

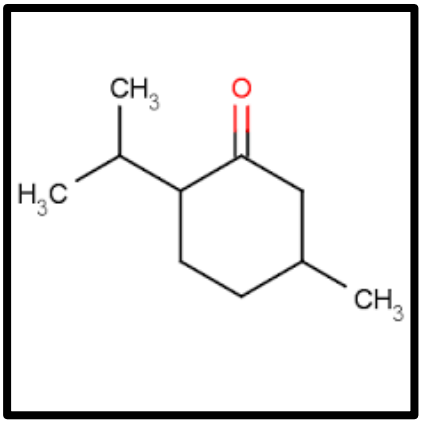


FIGURE 3 P–MENTHAN–3–ONE

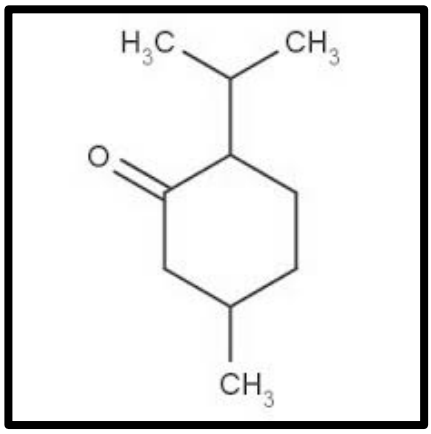


FIGURE 4 TRANS– MENTHONE

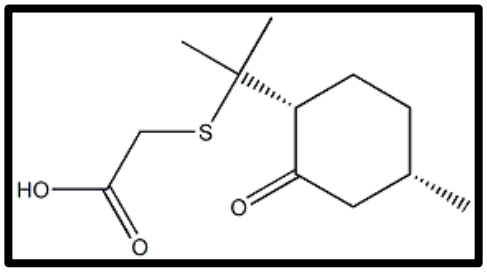


FIGURE 5 CIS–MENTHONE

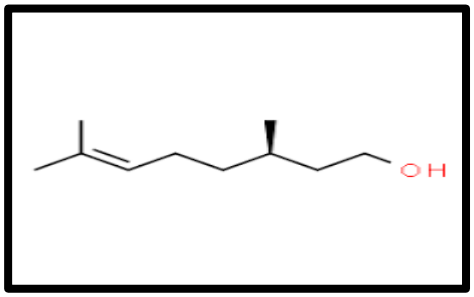


FIGURE 6 R–CITRONELLOL

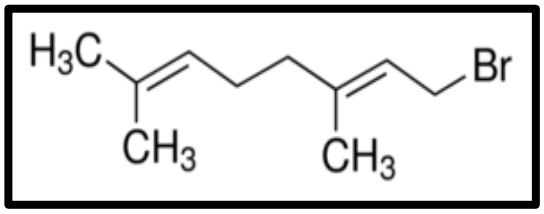


FIGURE 7 GERANYL BROMIDE

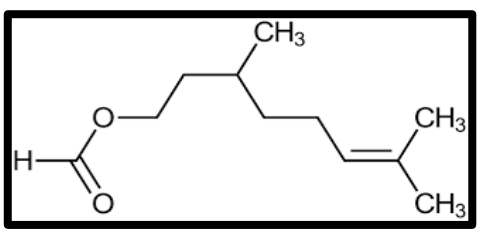


FIGURE 8 CITRONELLYL FORMAT

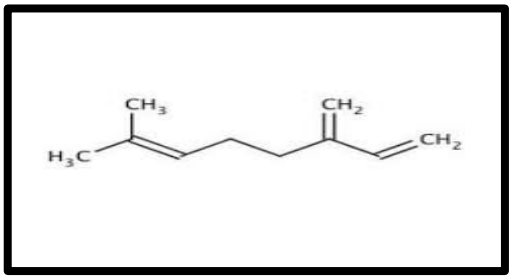


FIGURE 8 BETA-MYRCENE

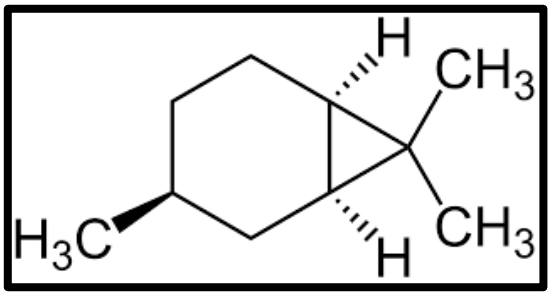


FIGURE 9 CIS-CARANE

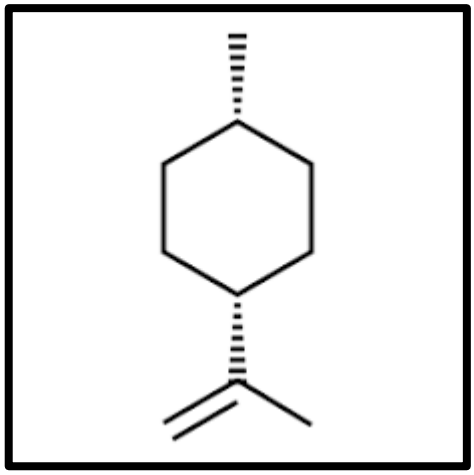


FIGURE 10 CIS-P-MENTH-8-ENE

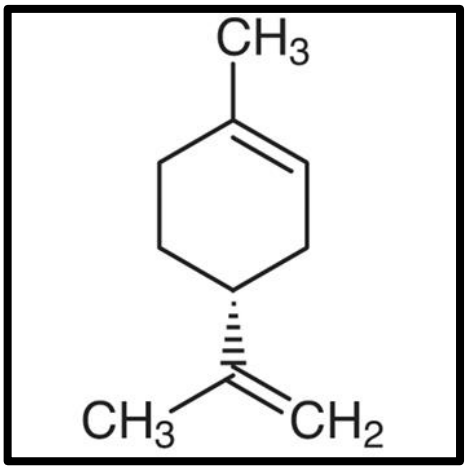


FIGURE 11 LIMONENE

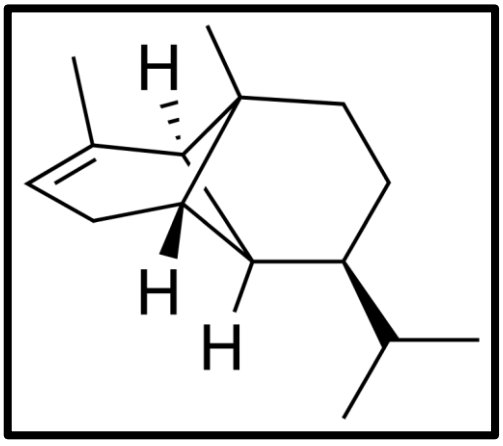


FIGURE 12 COPAENE

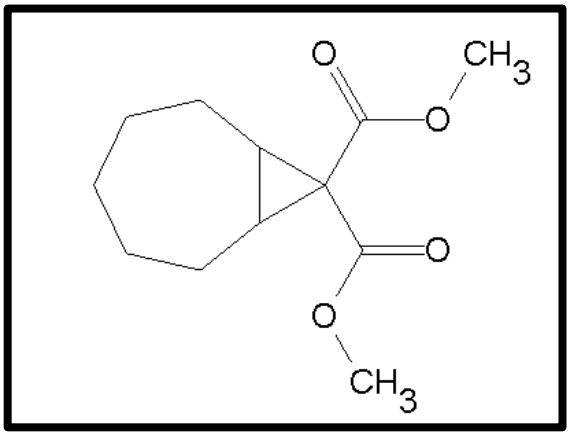


FIGURE 13 BICYCLO [5.1.0] OCTANE;8-ETHYLENE

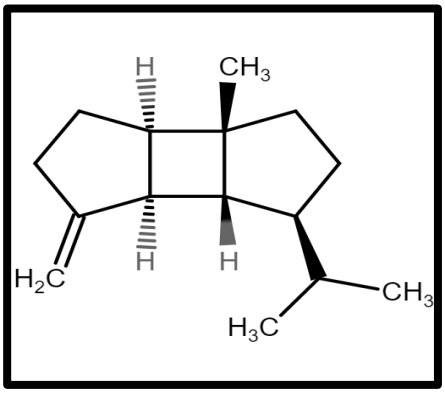


FIGURE 14 BETA–BOURBONENE

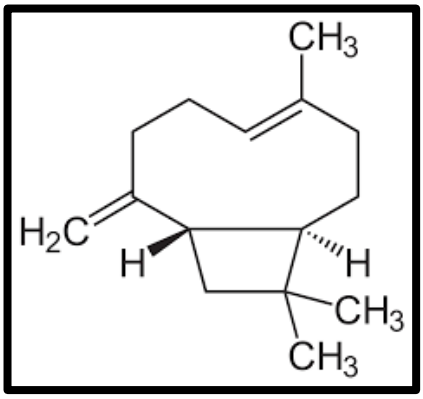


FIGURE 15 CARYOPHYLLENE

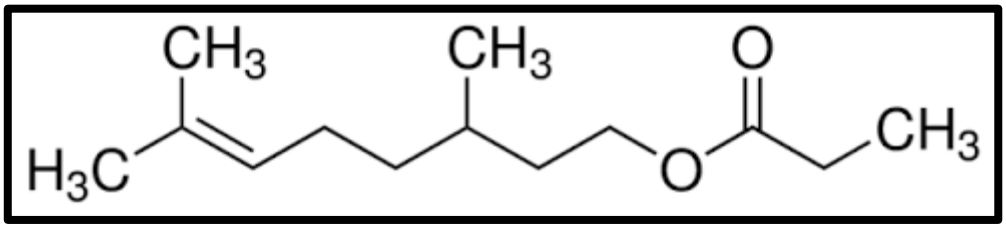


FIGURE 16 CITRONELLYL PROPIONATE

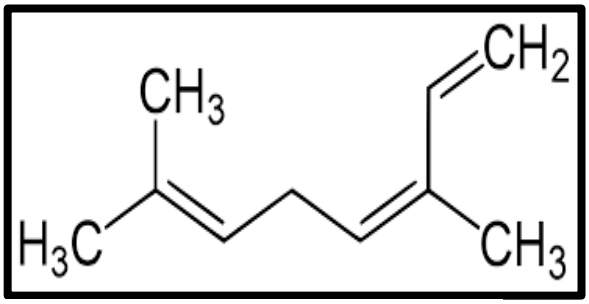


FIGURE 17 OCIMENE

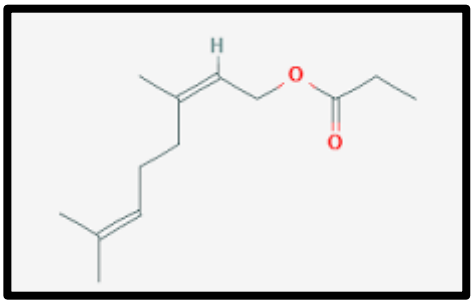


FIGURE 18 NERYL PROPIONATE

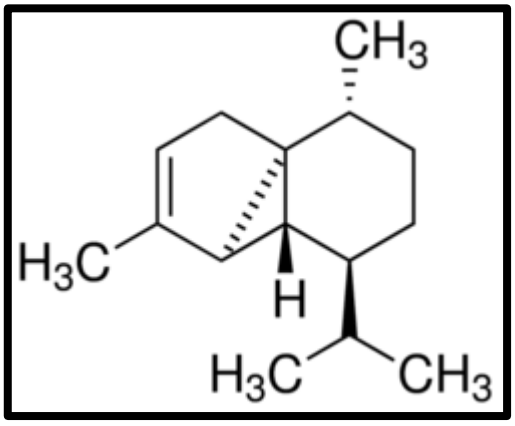


FIGURE 19 APHA–CUBEBENE

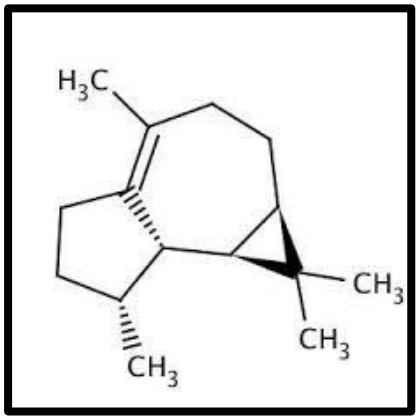


FIGURE 20 (+)–LEDENE

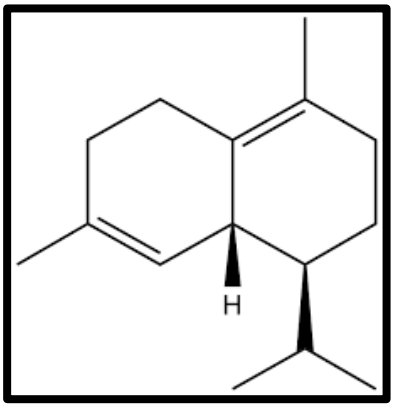


FIGURE 21 DELTA–CADINENE

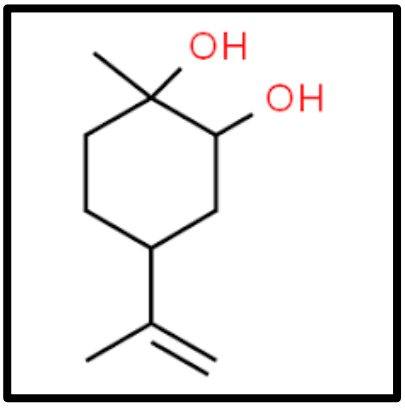


FIGURE 22 8–P–MENTHENE

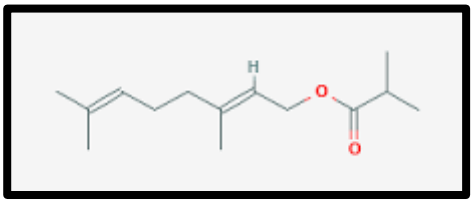


FIGURE 23 GERANYL ISOBUTYRATE

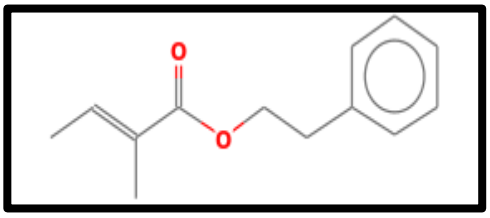


FIGURE 24 PHENYL ETHYL TIGLATE

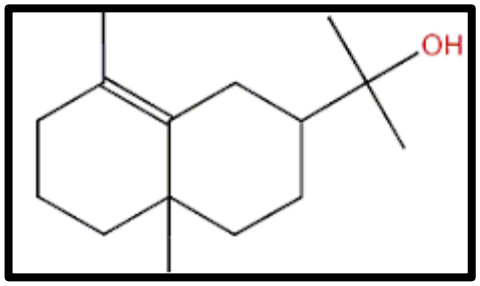


FIGURE 25 GAMMA–EUDESMOL

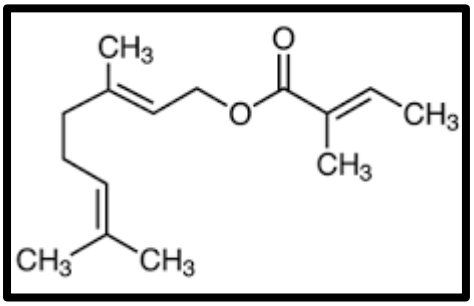
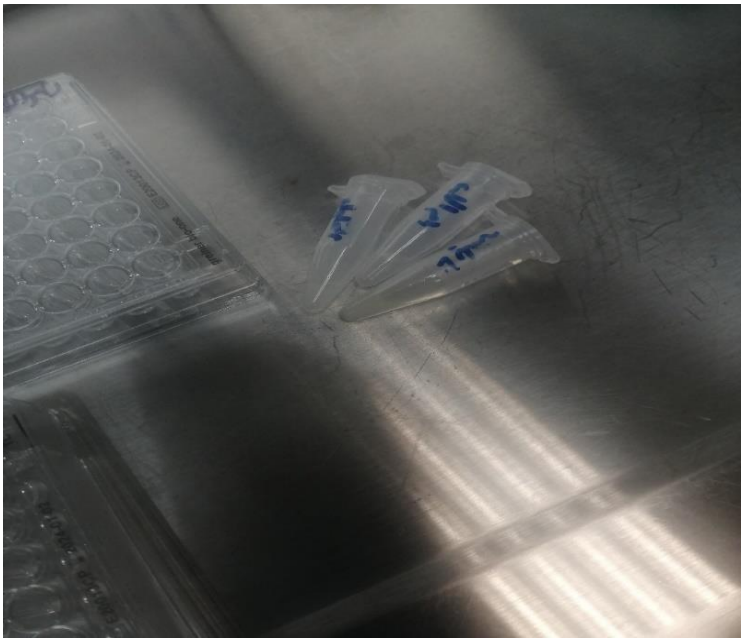


FIGURE 26 GERANYL TIGLATE

Appendix (2)

Pelargonium graveolens Preparations



essential oil *of P.graveolens* samples

Arial parts of *P.graveolens* dried at the shadow

P.graveolens gathered from Palestinian regions.

جامعة النجاح الوطنية
كلية الدراسات العليا

تباينات المكونات الكيميائية والأنشطة الدوائية لزيت العطري لنبتة العطريه من ثلاث مناطق في فلسطين

اعداد
نوار حسني ولد علي

اشراف
أ. د. شحده جوده
د. نضال جرادات

قدمت هذه الأطروحه استكمالا لمتطلبات درجة الماجستير في الكيمياء بكلية الدراسات العليا، جامعة النجاح الوطنية، نابلس، فلسطين.
2021

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اعداد

نوار حسني ولد علي

اشراف

أ. د. شحده جوده

د. نضال جرادات

الملخص

العطريه (*Pelargonium graveolens*) أو (*Pelargonium roseum*) هي نبتة تنتمي الى الفصيلة الغرنوقيه (*Geraniaceae*). توجد في المناطق الجنوبيه من أفريقيا وتنمو في مناطق البحر المتوسط من أوروبا حيث انتشرت في هذه المناطق منذ القرن الثامن عشر الميلادي.

تم استخراج الزيوت الطياره لنبتة العطريه من ثلاث عينات تم احضارها من مناطق من فلسطين تمثل شمال ووسط وجنوب فلسطين وهي نابلس ورام الله والخليل على التوالي .وقد تمت طريقه الاستخلاص باستخدام جهاز (Microwave ultrasonic). وتم تحليل المكونات الكيميائية لعينات الزيوت الطيارة باستخدام (Gas Chromatography -Mass Spectroscopy). تم فحص فاعلية الزيوت الطيارة من العينات الثلاثة كمضاد للأكسده 2,2 −diphenyl picryl hydrazyl) ولفحص الفعالية كمثبط لانزيم (lipase) فقد تم استخدام (porcine pancreatic lipase PL) وأيضا p-nitrophenyl butyrate (PNPB). تم تقييم الفعالية كمضاد لانزيم (amylase) باستخدام (p-nitrophenyl butyrate (DPPH) (dinitrosalicylic-3.5, DNSA). ولفحص الفعالية المضادة للميكروبات فقد تم استخدام طريقة (Broth microdiltution) بشكل منفصل كمضاد للبكتيريا وايضا كمضاد للفطر. تم اختبار ستة سلالات بكتيريه وخميره واحده هي فطريات المبيضات البيض (Candida albicans).

كان معدل الناتج من الزيوت الطيارة في المدى(0.5-1%) (%w/w). وأظهر تحليل GC-MS النسب المئوية من المكونات المؤكسدة كانت في المدى (86.2−88.5%)، والمكونات غير

المؤكسدة في نطاق (11.499–13.78%). وقد تم التعرف على ست وعشرين مركب، وكان (R-Citronellol) من المكونات الأكثر وفرة في العينات الثلاث في نطاق (74.43–86.04%)، وكان المكون (Citronellyl formate) في الدرجة الثانية وكان في نطاق (3.16–14.41%). العينة من نابلس(المنطقة الشماليه) كانت اكثر فعالية كمضاد للأكسدة ومضاده لانزيم الأميليز حيث بلغت قيمة IC_{50} (146.14 و 199.6)µg⁄ml على التوالي، واظهرت العينة من الخليل (المنطقة الجنوبية) اكثر فعالية كمثبط لانزيم الليبيز بقيمة 634.517 IC_{50} µg/mg كما واظهرت العينة من رام الله (المنطقة الوسطى) اكثر فعالية كمثبط لانزيم الليبيز بقيمة µg/mL 584.112 IC_{50}. واظهرت العينات الثلاث فعاليه جيدة كمضاد للميكروبات وبخاصه كما أظهرت العينات الثلاث نشاط عال كمضاد للفطريات في الحد الأدنى من التركيز المثبط (M..IC) في نطاق (0.312 µg/mL).

كما أن عينة الزيت العطري الطيار من الخليل أبدت فعالية عاليه اتجاه بكتيريا المكورات العنقوديه الذهبية المقاومه للميثيسلين (MRSA) بحيث كانت قيمة الحد الادنى من التركيز المثبط M.I.C بلغت 12.5 µg⁄ml.

أظهرت الدراسة أن عينات الزيوت الطياره للنبات الطبي (*Pelargonium graveolens*) من ثلاث محافظات مختلفة من فلسطين تحتوي على نفس المكونات الفعالة ولكن بنسب متفاوته وهذا يفسر الاختلافات في الانشطه البيولوجيه لعينات الزيت العطري من محافظات مختلفه مثل: مضادات الأكسدة، ومضادات السمنة، ومضادات السكري، وأنشطة مضادات الميكروبات.يعود هذا الاختلاف الى الاختلافات في الطقس ومعدل هطول الامطار وطبيعة التربة وعمر النبات وموسم الحصاد بين المحافظات المختلفه.

في محافظه الخليل والتي تقع في جنوب فلسطين يكون الطقس بارد في الشتاء ومعدل هطول الامطار مرتفع ويبلغ معدل ارتفاع الخليل عن مستوى سطح البحر 860–1008 م. في حين ان محافظه رام الله والتي تقع في وسط فلسطين يكون الطقس بارد في الشتاء وتتساقط الامطار بشكل مرتفع ولكن بنسب اقل من الخليل ويبلغ ارتفاعها عن سطح البحر 760–885 م. في حين ان

محافظه نابلس والتي تقع في شمال فلسطين يكون ايضا طقسها بارد شتاء" ومعدل هطول الامطار متوسط ويبلغ ارتفاعها عن سطح البحر 430–931م.

انه من الضروري إجراء دراسات في الجسم الحي لتقييم الأنشطة البيولوجية المحتملة وايضا تقييم الامان والسمية للمستخلص النباتي. وانه من الضروري إجراء مزيد من التجارب والدراسات لفصل المكونات الرئيسية المسؤولة عن الأنشطة البيولوجية وتحديدها وتوصيفها.