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Pharmacognosy & Medicinal Plants

Biochemical Studies on Selected Palestinian Wild Plants

By

Enas Adnan Abu ‘Eid

Supervisor

Dr. Mohannad Jazzar

Co-Supervisor

Dr. Abdel Qader Qawasmeh

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Biochemical, GC-MS Analysis, antioxidant, and Phytochemical Screening of some Wild Palestinian Plants

Prepared by:

Enas Adnan Abu 'Eid

This thesis was successfully defended on 19/5/2024 and approved by:

Dr. Mohannad Jazzar	**Supervisor** .....
Dr. Abdel Qader Qawasmeh	**Co-Supervisor** .....
Dr. Iyad Al-Ali	**External Examiner** .....
Dr. Alaa khraiwesh	**Internal Examiner**.....

Declaration

I certify that this thesis submitted for the degree of the master is the result of my research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institute.

Signed…..……

Enas Adnan Abu ‘Eid

…19…/…1… /2024

Dedication

This thesis is dedicated:

To my father and mother, a special feeling of gratitude to my loving parents, whose words of encouragement and push for the tenacity to finish this thesis. To my brothers and sisters who have never left my side and are very special. To my dearest husband: for his endless support and encouragement. My beloved kids: Hasan, Ahmad, Basel lighten my life up and give me the power to keep on. To my friends and work-family who have supported me throughout the process and finishing this thesis.

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Contents

List of Abbreviations

WPP	Wild Palestinian Plant
WHO	World Health Organization
TAPHM	Traditional Arabic Palestinian Herbal Medicine
$DPPH^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl hydrate
$ABTS^{\bullet+}$	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
FRAP	Ferric reducing antioxidant power
CUPRAC	Cupric reducing antioxidant power assays
HPLC/PDA/MS	High-liquid performance chromatography–photodiode array Detection- mass spectrometry
GC-MS	Gas chromatography-mass spectrometry
IC_{50}	half-maximal inhibitory concentration
mg RU/g	1 (milligram RU) / gram = 44.45 microns
mg GA/g	Milligram Gallic acid per gram
R. graveolens	*Ruta gravenoles*
H .aureus	*Hyoscusem auerues*
C. iphionoides	*Chiliadenus iphionoides*
S. pneumoniae	*Streptococcus pneumonia*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
P. aeruginosa	*Pseudomonas aeruginosa*
K. pneumonia	*Klebsiella pneumonia*
S. cerevisiae	*Saccharomyces cerevisiae*
A. niger	*Aspergillus niger*
P. vulgaris	*Proteus vulgaris*
ATCC	American Type Culture Collection
Salmonella sp	Scientific Name of Salmonella
M. longifolia	*Mentha longifolia*
M. officinalis	*Melissa officinalis*
R. damascene	*Rosa damascene*
M. luteus	*Micrococcus luteus*
GC-FID	gas chromatography equipped with flame ionization detector
MIC	Microdilution
ROS	reactive oxygen species
CAM	Complementary and alternate medicine
MRSA	methicillin-resistant *Staphylococcus aureus*
v/v	Volume/Volume
Rpm	Round per minute

w⁄v	Weight by volume
TM	traditional medicine
µL	Microliter
UV/Visible spectrophotometer	Ultraviolet-visible spectrophotometer
Nm	Nanometer
µg	Microgram
°C	Celsius
ADR	Antimicrobial Drug Resistance
CDC	Centers for Disease Control and Prevention
CFUs/Ml	(no. of colonies x dilution factor) / volume of culture plate
Me OH	Methanol
Vos	Volatile oils
N	Normal
NIST05	MS Library
REF	Reference
EOS	essential oils
TOSC	total oxidant scavenging capacity

Biochemical, GC-MS Analysis, antioxidant, and Phytochemical Screening of some Wild Palestinian Plants

Abstract:

Palestinian traditional medicine makes extinctive use of Wild plants. This investigation focused on the study of *Chiliadenus iphionoides, Hyoscyamus aureus* and *Ruta graveolens* usually grow in the mountains of Palestine. The purpose of this research was to study biochemical analysis, anti- bacterial, antioxidants phytochemical screening and GC-MS analysis using gas spectrometer of those plants. The anti-bacterial activity of the methanolic extract (80%) of plant leaves was tested in a well diffusion method, antioxidant activity was conducted using both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ tests, whereas, GC-MS gas spectroscopy was carried out following standard protocols. The investigation of the 80% methanolic extract demonstrated that plants have antibacterial properties. *Chiliadenus iphionoides*, showed activity against strains of the Gram-negative bacteria *Proteus mirabilis* and *Pseudomonas aeruginosa,* where the activity reached 96 % ±7.3and 70.4%±2.8, respectively using well diffusion method. Where for Gram-positive Bacteria *Staphylococcus aureus*, its activity against the positive control was 50.1%±1.5. *Hyoscusem aureus* plant exhibited activity against Gram-positive *Staphylococcus aureus* and strains of Gram-negative bacteria *Proteus mirabilis* and *Pseudomonas aeruginosa,* with activity levels of 68.6 %±3.5 and 80.1%±4, respectively. *Ruta graveolens* plant demonstrated activity against Gram-negative bacteria strains *Proteus mirabilis* and *Pseudomonas aeruginosa*, where its activity reached 68. %± 3 and 60 %± 3, respectively, and against positive *Staphylococcus aureus,* whose activity reached 89.9% ± 2 by well diffusion method compared with the positive control. Gram-positive Staphylococcus *aureus*, whose well diffusion method activity was 80.1%±4. By using the well diffusion method, its activity was 89.9 %±2 higher than the positive control. *Ruta graveolens* plant exhibited activity against the Gram-positive *Staphylococcus aureus* and the Gram-negative bacteria strains *Proteus mirabilis* and *Pseudomonas aeruginosa*, with activity levels reaching 68.6±3 and 60±3, respectively. Its activity was 80.1%± 4 higher than the positive control using the well diffusion method. Additionally, the methanolic extract from these plants showed some capabilities. employing the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ tests. *Ruta gravenoles* had a 61% antioxidant capacity, *Chiliadenus iphionoides* 43%, and *Hyoscusem aureus* had 55.5% activity for ABTS●+ and DPPH●. *Ruta gravenoles* had a strong antioxidant capacity of 59.24%, *Hyoscusem aureus* had 44.96%, and *Chiliadenus iphionoides* had 33.78% activity.

Additionally, the study demonstrated the presence of a broad range of phytochemical

compounds like: cardiac glycosides, alkaloids, coumarins, phenolic groups, flavonoids, steroids, and phlobatannins in those plants through phytochemical and GC-MS analyses of plant leaves. Further investigation needed to investigate the pharmacological importance and find out the potential health benefits.

Keywords: GC-MS analysis, antibacterial biologically active compounds, *Chiliadenus iphionoides, Hyoscusem aureus* and *Ruta graveolens,* $ABTS^{\bullet+}$ and $DPPH^{\bullet}$

الملخص:

التحليل الكيميائيّ والحيوي بواسطة المطياف الغازي GC-MS، ومضادات الأكسدة، والفحص الكيميائي النباتي لبعض النباتات الفلسطينية البرية

النباتات البرية تنمو عادةً، في جبال فلسطينَ. تركز هذه الدراسة على دراسة النباتات البرية الفيجن والبنج الذهبي اشتيلا، والتي تستخدمُ على نطاق واسع في الطب الفلسطيني التقليدي. ويعود الهدف من هذا البحث هو دراسةُ التحليل الكيميائي الحيوي ومضادات الأكسدة والبكتيريا، والتحليل بواسطة المطياف الغازي GC-MS لتلك النباتات. حيث تم فحص النشاط المضاد للبكتيريا للمستخلص الميثانولي (80٪) لأوراق النباتات بطريقة الانتشار البئري، وتم إجراء نشاط مضادات الأكسدة باستخدام كل من فحوصات $ABTS^{\bullet+}$ و $DPPH^{\bullet}$ وتمّ إجراء الفحص الكيميائي النباتي والتحليل الطيفي الكتلي للغاز (GC-MS) باتباع البروتوكولات القياسية. حيث أظهرت دراسةُ المستخلص الميثانولي (80٪) لتلك النباتات نشاطًا واضحًا مضادًا للبكتيريا فمثلا نبات الفيجن اظهر نشاطا وضحا ضد سُلالات البكتيريا سالبة الجرام *Proteus mirabilis* و *Pseudomonas aeruginosa* حيث بلغ نشاطه3.7 ± 96 و2.8±70.4 على التوالي، وضد المكورات العنقودية الذهبية إيجابية الجرام *Staphylococcus aureus*، حيثُ بلغ نشاطه 1.5 ± % 50.1 وذلك بطريقة الانتشار البئري مقارنة مع السيطرة الإيجابية, كما أظهر نبات البنج الذهبي ضد سُلالات البكتيريا سالبة الجرام *Proteus mirabilis* و *Pseudomonas aeruginosa* حيث بلغ نشاطه 3.5 ± % 68.6 و4 ± .801على التوالي، وضد المكورات العنقودية الذهبية إيجابية الجرام *Staphylococcus aureus*، حيثُ بلغ نشاطه 2 ± 89.9 وذلك بطريقة الانتشار البئري مقارنة مع السيطرة الإيجابية , ونبات اشتيلا اظهر ضد سُلالات البكتيريا سالبة الجرام *Proteus mirabilis* و *Pseudomonas aeruginosa* حيث بلغ نشاطه.3 ±68.6 و3± 60 على التوالي، وضد المكورات العنقودية الذهبية إيجابية الجرام *Staphylococcus aureus*، حيثُ بلغ نشاطه 4 ± % 80.1 وذلك بطريقة الانتشار البئري مقارنة مع السيطرة الإيجابية, أيضا أظهر المستخلصُ الميثانولي لتلك النباتات قدرات واضحة فمثلا لنبات الفيجن مضادة للاكسدة تبلغ 61% و 55% للبنج الذهبي و لنبات ال اشتيلا 43% من النشاط باستخدام فحوصات $ABTS^{\bullet+}$ بينما سجلت مضادات الاكسدة ان $DPPH^{\bullet}$ لكسح الجذور الحرة لنبات الفيجن 59.2% وفي البنج الذهبي 44.96% ايضا نبات ال اشتيلا وصلت 33.78% . كما بيّنت الدراسةُ بواسطة الفحص الكيميائيّ النباتيّ وفحوصات GC-MS لأوراق النباتات عن وجود مجموعة واسعة من المركبات الكيميائية النباتية مثل جليكوسيدات القلب، الالكلويدات، الكومارين، المجموعات الفينولية، الفلافونويدات، المنشطات، والفلوباتانين في تلك النباتات، الأمر الذي يمكن أنْ يفتحَ المزيدَ من الأبحاث القيّمة للتّحقيق في فوائدها الدوائية.

الكلمات المِفتاحية: فيجن، شتيلا والبنج الذهبي $ABTS^{\bullet+}$ و$DPPH^{\bullet}$, المركبات النّشطة بيولوجيا المُضادة للبكتيريا، تحليل GC-MS.

Chapter 1: introduction

1.1 Introduction:

Human beings have been utilizing medicinal plants for basic preventive and curative healthcare for various kinds of illnesses all over the world since time immemorial. Recent estimates suggest that over 9000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Hemmami et al., 2023; Owusu et al., 2021).

Plant physiology and Biochemistry, according to WHO, more than 90% of the total population on the earth rely on herbal therapies as a principal means of preventing and curing illness, where, several traditional medicinal and folkloric methods are based on the use of medicinal plants. There are several benefits for using medicinal plants such as: the plants are readily available, easy to transport, and do not disintegrate quickly. In addition, remedies based on these plants often have minimal side effects, and the relatively high cost of synthetic therapeutic medicine in developing countries often makes traditional herbal medicine an affordable choice for the poor in these lands,
(Kilari et al,.2015). Medicinal and aromatic plants are important products found naturally in the plains and mountains of Palestine and worldwide (Molassiotis et al,.2005). The therapeutic and healing power of plants is well demonstrated scientifically. Medicinal plants and their extracts are used as abortifacients, astringents, antiphonic, cardio tonic, emetics, expectorants, purgatives, expectorants, and many other conditions (Al Husseini, 2010; Bachheti et al., 2022; Bamola, Verma, & Negi, 2018; Kumar et al., 2017). Evidenced based alternative and complementary medicine, BMC complementary alternative medicine (Nidal Jaradat et al., 2017).

Medicinal plants are used at the household status by women taking care of their families, at the local level by folkloric medicinal practitioners using folkloric classical traditional method of healing such as Ayurveda, oriental medicine, traditional Arabic plant healing, and many other alternative medicines. It is estimated over 80% of the world's folk relies upon such traditional plant-based systems of medicine to provide them with primary healthcare (Payyappallimana, 2010).

Moreover, it is well documented that modern pharmacopeia includes at least 25% drugs derived from plants, many are synthetic similarities built on prototype compounds isolated from plants. Demand for medicinal plants is increasing in together developing and developed nations due to growing recognition of natural products, being non-toxic, having no side effects, and simply available at reasonable values (Sen et al., 2011).

Many Allopathic and therapeutic drugs are derived and indebted a tremendous debt to medicinal plants: 1 in 4 prescriptions filled in a country like USA are either a manufactured form of or resultant from plant materials (Frohock, 1995; Sharma, Kumar, & Singh, 2019). In Palestine, Medicinal plants have been traditionally available and used in the socio cultural, spiritual and medicinal arena (Harlow et al., 2023). Palestinian households use medicinal plants in a self-help mode for preventive, promotive and curative applications. (Hemmami et al., 2023). The following table (1-1) lists some of these plants that are readily available in the Palestinian culture.

Table (1.1): Some wild Palestinian plants their secondary compounds and biological activities used in Palestinian Folkloric Medicine.

Latin name	Family	Part used	Secondary compound	Biological Activity	Study Country	REF
Malva sylvestris	*Malvaceae*	Arial parts	Malvone, Malvidin, Malvin	Antiseptic, Antimicrobial, Antifungal	Iran	(Mousavi et al., 2021; Pirbalouti & Koohpyeh, 2011; Razavi, Zarrini, Molavi, & Ghasemi, 2011)
Ephedra alata	*Ephedraceae*	Aerial parts	Polyphenolic, Alkaloids	Against human breast cancer Antioxidant activity antibacterial cocci and, Candida	Tunisia	(Danciu et al., 2019)
Arum palestinum	*Araceae*	Aerial parts	Piperazirum, Isoorientin	Anticancer, urinary disorders	Egypt palestine	(farid, hussein, & saker, 2016; Zaid, rayan et al., 2010; zaid et al., 2012)
Asparagus officinalis	*Liliaceae*	Aboveground part	Polysaccharides, polyphenols, anthocyanins, and saponins	Anti-cancer, anti-tumor, antioxidant, immunomodulatory, hypoglycemic, antihypertensive, anti-epileptic.	China	(Guo et al., 2020)
Urtica urens	*Urticaceae*	Aerial part	Flavonoids, phenolics, alkaloids, tannins, phlobatannins, terpenoids, saponins, steroids, coumarins anthocyanins	Cancer, stomach, intestine pain and inflammation, liver diseases, and circulatory system	Southern Africa, Palestine	(Abou Auda et al., 2011; Paul & Pillai, 2021)
Micromeria fruticose	*Lamiaceae*	Aerial part	Phenolic acids, flavonoids, Coumarin, and tannins	Stomach, intestine pain and respiratory system ,inflammation, fever and asthma.	Turkey Palestine	(Said et al ., 2002; Taskin et al., 2021)

The various cultures like: (Egypt, Syria, China, India and other civilizations), were treated by plants since long a time. This is due to the fact that, medicinal plants are the only inexpensive and reachable source of primary health care for them, particularly in the lack of access to contemporary medical facilities. Recent study reveal that there are more conventional medicine providers than allopathic providers, particularly in rural areas (Porwal et al., 2019). Recently, materials derived from plants have gained important attention because of their versatile applications such as: The richest bio reserve of pharmaceuticals with conformist drug structures, food supplements, traditional medicines, modern medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs is found in remedial plants (Porwal et al., 2019). Medicinal plants form a huge group of inexpensively major plants that provide the essential raw materials for indigenous pharmaceuticals. Plant products still remain the most important source of pharmaceutical agents used in conventional medicine (Gunjan et al., 2015). According to the WHO the primary step for recognition and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies that are necessary for any phytopharmaceutical products used for standard formulation (Abdelhalim et al, .2020). Preliminary phytochemical studies are obliging in finding out chemical constituents in the plant material that may fine lead to their quantitative estimation (Huma et al., 2012).

Lately, much concentration has directed towards extracts and biologically active compounds isolated from accepted plant species. In the present age of drug development and discovery of newer drug molecules, a lot of plant products are assessed on the basis of their conventional uses. The healing properties of medicinal plants are mostly due to the occurrence of various multifaceted chemical substances of dissimilar compositions which happen as secondary metabolites (Najmi et al., 2022). The important of these bioactive constituents of plants are steroids, tannins, alkaloids, flavonoids and phenolic compounds. Therefore, it is enviable to know the phytochemical composition of the plant material before testing its effectiveness for medicinal purpose (Jemal et al., 2022). Plants are also main natural sources of medicinal compounds in present pharmacopoeias. Indian Materia Medici comprises about 2000 drugs of natural origin and most of them are resulting from different conventional system and myths practices. However, there are large numbers of plants, which have not been mentioned in these reports, in malice of their usage in the conventional and

folk medicinal systems. S. Mariana common name is milk thistle; it is an edible plant belongs to the Asteraceae family. It is an annual or biennial native to the Mediterranean regions of Europe, North Africa and the Middle East and in some parts of USA (Máthé & Khan, 2022). In India, it is commonly found in Jammu and Kashmir (Mir et al., 2021).

Milk thistle plant grows to a height of three to ten feet with an erect stem that bears large, alternating, prickly edged leaves. Throughout history, man used various natural materials as a remedy for various diseases (Porwal et al., 2019). In the past few decades, most natural products were replaced with synthetic drugs that were based on modern chemistry and biotechnology. However, we are recently witnessing a vastly growing and renewed interest in natural medicines in western countries. In particular, the herbal medicine market has exploded and became prosperous in pharmacies and many drug stores. For example, there was a seven-fold increase in the number of people using herbal medicines between 1990 and 1997 in the U.S. A (Eisenberg et al., 1998). With this increasing interest in natural medicine, more individuals will explore the possibility of using natural medicines to complement conventional therapy, as is already the case in certain minority cultures. Furthermore, natural products are still a major source of new drug discoveries, for example: 65% of the drugs that were approved for marketing between the years 1983 and 1994 were based on natural sources. Ethno pharmacological research is considered crucial in the development and discovery of new drugs from natural sources. In Palestine, there are numerous medicinal plants described for treatment of many diseases (Molassiotis et al., 2005). Herbal medicine is considered an integral part of the Palestinian culture and plays a pivotal and indispensable role in the current public healthcare. The hills and mountains of Palestine are covered with more than 2600 plant species of which more than 700 are noted for their uses as medicinal herbs or as botanical pesticides (Amenu et al., 2014). An extensive ethnobotanical study was previously carried out in West Bank/Palestine to evaluate the relative efficacy of medicinal plants for the treatment of skin and prostate disorders (Jaradat et al., 2005). Another recent ethno pharmacological survey study was conducted in some parts of Palestine among the most well-known Arabic indigenous herbal practitioners in order to evaluate the potential of local plants used in treating different diseases and illnesses (Jaradat et al., 2016). The study has shown that one hundred and twenty-nine (129) plant species are still in use in Arabic traditional medicine: forty (40) plant species are

used for skin diseases; twenty-seven (27) species for treating kidney and urinary tract; twenty-six (26) species for treating diabetes; twenty-three (23) species for treating digestive system diseases; twenty-two (22) species for treating liver diseases; sixteen (16) species for treating respiratory and coughing; thirteen (13) species for cancer and nine species for treating weight loss and cholesterol reduction (10). Other similar studies were carried out, found that eighty per cent (80%) of patients suffering from diabetes mellitus or cardiac diseases use medicinal plants because they believe that they are cheaper, more efficient and better than modern medicine (Jaradat et al., 2005).

1.2 Wild Palestinian plant contain a rich medicinal value

Many recent studies about the medicinal value of WPP have been emerged over the past decade to provide evidence proving or declining the traditional uses of WPP (Azaizeh, Saad, Cooper, & Said, 2010). According to these studies WPP have been tested for their argued antimicrobial (Abu-Shanab et al.,2007), antioxidant (Husein AI et al., 2014), and anticancer effects *in vitro* (Jaradat& Shawahna, 2016).

Palestine's climate is ideal for growing a wide variety of plants, for example: *Rosmarinus officinalis* a traditional plant in Palestine used traditionally to treat disorders like renal colic (as antispasmodic), rheumatoid arthritis (as ant rheumatic), as well as general analgesic, diuretic, and expectorant (Al-Sereiti et al., 1999). In addition, this medicinal plant registered to have antimicrobial and antioxidant activities (W. Wang et al., 2012). Another medicinal plant which is called as *Teucrium polium (T. polium)* that has been shown to have anti-inflammatory, anti-diabetic, analgesic, antioxidant, antispasmodic properties, and antimicrobial properties against several clinical pathogens (Darabpour et al., 2010; Abbas et al., 2019).

1.2.1 Wild Palestinian plant act as an antioxidant

Many Palestinian researchers have mentioned about the antioxidant activity of different WPP, (Jaradat et al., 2017; Jaradat, Damiri, & Abualhasan, 2016; Salameh et al., 2020), these studies clearly shown that WPP have a pronounced effect of antioxidants activity (Abu-Qaoud et al., 2018; F Al-Rimawi et al., 2017; Hawash et al., 2020). To study the antioxidant capacity of plants, it refers mainly to the ability of methanolic extracts to scavenge free radicals. This is done using the *in vitro* testing on plants, which involve the antioxidant capacity of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and

2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals (Jamuna & Swarup, 2012).

A study carried by Al-Rimawi and his colleagues (Al-Rimawi et al., 2017), on *Ephedra alata* wild plant using the phenolic content, flavonoid content, antioxidant activity were tested by applying $DPPH^{\bullet}$, $ABTS^{\bullet+}$, Ferric reducing antioxidant power, and Cupric reducing antioxidant power assays, and phenolic and flavonoid content of *Ephedra alata*. In addition, this study applied three different solvents were used to extract the *Ephedra alata*؛ 100% water, 80% ethanol, and 100% ethanol, by HPLC/PDA/MS. The extract results showed that this wild Palestinian plant *Ephedra alata* has got a profound effect of antioxidant activity due to the presence of phenolic, and flavonoid groups. According to this study the antioxidant activity of ethanol (80 %) extract was the highest one, Ferric reducing antioxidant power (FRAP) was 21.3 ±0.4, Cupric reducing antioxidant power assays (CUPRAC) was 6442 ± 52, $DPPH^{\bullet}$ was
482.5 ±1.7, and $ABTS^{\bullet+}$ was 66±1.5. There was a direct correlation between antioxidant activity and total phenolic content but not with total flavonoids content, each of the four antioxidant activity tests was highly and consistently effective expressively correlated with each other.

In an another study carried out by Jaradat (Jaradat et al., 2016), on different wild Palestinian plants like: *Urtica urens, Rumex cyprius, and Borago officinalis*, the results of free radical scavenging activity evaluated by DPPH• showed that the overall antioxidant activity of *Rumex cyprius* was the greatest among the plants, followed by *Urtica urens*, and *Borago officinalis*; correspondingly and the half-maximal inhibitory concentration (IC50) values of the methanolic extracts were 29.70±0.60µg/ml, 5.07±0.49µg/ml, 39.92±0.52µg/ml for them respectively. On the other hand, a study carried by Jahajha on the *Calamintha genus*, this study has shown the presence of flavonoids, terpenoids, tannins, and phenolic compounds, these compounds obviously indicated the important pharmacological and antioxidant activities present in these plants (Mukherji et al.,2017).

(Hasan et al.,2018) carried another study on *Borage. officinalis* which was designed to evaluate plant antioxidant activity using $DPPH^{\bullet}$ assay, and to examine the presence of phytochemical compounds, and to determine total flavonoid using rutin reference standard method, total phenols contents using the Folin Ciocalteu method of wild and

cultivated *Borago officinalis*, Hasan investigation on this wild plant leaves extract showed that the IC_{50} was reported to be 6.3 µg /mL, while the cultivated leaves extract was found to have a higher IC_{50} value of 8.7 µg /ml, this means lower antioxidant activity compared to wild-growing plants, the total flavonoid content of the methanolic extract of wild *Borage. officinalis* was 22.4 mg RU/g, this value was reduced to 13.1 mg RU/g for the cultured methanolic extract the total phenol content decreased from 5.21 mg GA/g to 2.37 mg GA/g.

1.2.2 The antibacterial effect of Wild Palestinian plant

plant Many studies mentioned about the microbial activity of wild Palestinian plants that has been carried last few years in Palestine (Jaradat et al., 2017) (Hamarsheh et al.,2021; Salameh et al., 2020; Saleh et al., 2013). In addition, several other studies have implicated WPP as antibacterial agents (Lubna et al., 2021; Rayan et al., 2020). Hamarsheh and colleagues (Hamarsheh et al., 2021) have evaluated the antibacterial activity of some Palestinian medicinal plants namely; *Achillea fragrantissima, Artemisia inculta Delile Coridothymus capitatus* (L.), *Rchb.f, and Malva sylvestris* (L). These studies clearly indicated that, the presence of inclusions was examined by microscopy after 48 hours of incubation with different extracts, only *Artemisia inculta* extracts have inhibited *Chlamydia trachomatis* infection *in vitro* in Hela 229 cells. Other species in this study have been reported to have no anti-chlamydial activity. Saad and his colleagues carried a study (Saad et al.,2005) ,on five wild Palestinian medicinal plants, these plants are: *Echinops adenocaulos, Parietaria judaica, Urtica urens, Verbascum fruticulosum,* and *Vitex agnus-castus* using extract of holly Mecca Zamzam water, all these plants extract showed strong antibacterial activity against a multidrug-resistant strain of *Streptococcus pneumoniae*, these findings strongly revealed that all Zamzam extracts from the five plant species investigated suppressed *S. pneumoniae* growth. As a result of these findings, it can be concluded that these plant species can be used in the medicinal and pharmaceutical industry to manufacture new drugs for the treatment of multidrug-resistant clinical isolates of S. *pneumoniae*. Another study carried out by (Abu-Shanab et al., 2007), and his colleagues using the ethanol extracts and water extract of four different plants namely: *Rosa damascene, Althaea officinalis, Mentha longifolia, Melissa officinalis*, and by using the disk diffusion method, 1 µg oxacillin disks (Antimicrobial Agents) were used, zone sizes were measured after incubation at 35°C for 24 hours.

Isolates with zones sizes ≤ 10, were considered methicillin-resistant, and just three species effectively demonstrated antibacterial action, *M. longifolia, M. officinalis,* and *R. damascene* with water extract, and ethanol extract. (Abu-Shanab et al., 2007). A study by Qabaha carried by using well diffusion method (Qabaha et al.,2013), where he found that plant extracts from *Rosmarinus Officinalis* and *Teucrium polium* showed broad antibacterial action against eight clinical pathogenic microbes, including *S. aureus, E. coli, M. luteus, Candida albicans, Bacillus subtilis, Aspergillus niger, P. aeruginosa,* and *K. pneumoniae*. The extracts have robust and effective natural fungistatic, fungicidal, bactericidal, bacteriostatic, and antioxidant activity.

Salameh and his colleagues have also examined the antimicrobial activity of *Micromeria fruticosa serpyllifoli*a from three different regions in Palestine (north, middle, and south) using the broth microdilution method, the three samples exhibited broad antimicrobial activity, most of the potency against *Shigella sonnei* was found in the southern region, and *Staphylococcus aureus* clinical isolates and "methicillin-resistant" *Staphylococcus aureus* from the northern region showed the least potency (Salameh et al., 2020). Another study carried out by Naseef and his colleagues (Naseef et al.,2020), in-vitro studies have been conducted on the medicinal properties of *Arum palaestinum Boiss*, several types of extracts were obtained from the leaves of this plant, their antibacterial and anticancer activities were tested, the study showed that extracts of the *Arum palaestinum* plant do not inhibit the growth of bacteria *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

1.3 Wild Palestinian medicinal plants as a rich source of pharmaceutical agents

Several studies showed that WPP have a profound range of secondary metabolites present in these plants including phenols and polyphenols, alkaloids, terpenoids, essential oils, lectins, and others. These secondary metabolites have been detected in different parts of. *Malva sylvestris europaea, Plumbago auriculata, Majorana syriaca*, *Ephedra alata*, and *Tragopogon porrifolius* plants (Abu- Lafi et al., 2008; Al-Rimawi et al., 2017; Fuad Al-Rimawi et al., 2016; Dowek et al., 2020; Jaradat et al.,2016), Amongst the most important flavonoids found were malvin and mavadine, myricetin (Mousavi et al., 2021), tannins are hydrolysable tannins (Abu- Reida et al., 2014), and total phenols are catechins were present (Abu-Lafi et al.,2020).

Palestine (the whole historic and occupied Palestinian territory) has a unique and rich natural biodiversity and flora, this is due to its unique geographic position at the crossroads of Africa, Asia, and Europe (Barros et al., 2011). In addition, Palestine is well known as one of the 25 countries in the world designated as biodiversity hotspots (Daoud et al., 2008). There is a great deal of biodiversity in Palestine due to its varying climatic, phytogeographical, and zoological areas in Palestine that creates such a huge biodiversity (Sadeghi et al., 2006). For these points the biodiversity in this region of the world is mostly rich (Qumsiyeh & Abusarhan, 2021; Salem, 2010; Soto-Berelov et al., 2015). In addition, Palestine land topology and geography contains mountains, hills, valleys, coastal plains, desert, Mediterranean Sea, and Dead Sea. The various climatic, phytogeographical, and zoological areas in Palestine create huge biodiversity (Vogiatzakis et al.,2006).

It has been estimated that in the hills and mountains of Palestinian territories, there around 2780 plant taxa were covered and recorded as local or naturalized (Awaja et al., 2015). From the native medicinal benefits (Ali-Shtayeh & Jamous, 2002; Dafni et al., 1984), The diverse topography of the Palestinian territories has allowed the survival of traditional knowledge regarding the plant resources that the local people use as food. However, there are only a few ethnobotanical studies have been conducted on medicinal plants in some parts of the country (Ali-Shtayeh et al., 2003; Said et al.,2002), with little or no emphasis on edible wild plants (Abu-Rabia et al.,2005).

This study is carried to investigate the medicinal values of some 'selected' herbs wildly grown in Palestinian regions and identify their secondary compounds based on the fact that the Palestinian lands are rich, with herbs that are often used in folkloric medicine (Abu-Shanab et al., 2007).

The ethnobotanical educations that has been focused on wild Palestinian medicinal plants in some parts of the country is growing (Ali-Shtayeh et al., 2000; Azaizeh et al., 2003; Said et al., 2002), with slight or no importance on edible wild plants (Abu-Rabia, 2005). Wild Palestinian plants (WPP) have played a major role in the Palestinian folkloric medicine and treating illnesses (Hadjichambis et al., 2008).

1.4 The wild Palestinian medicinal plants and their traditional use

In Palestinian mountains, hills, and valleys there are many wild edible Palestinian plants used and have always been mentioned in the folklore of the Palestinian culture (Ali-

Shtayeh et al., 2008). However, these wild Palestinian plants nutritional and medicinal uses have been some of the most relevant and consistent reasons for the popular administration of plants. Therefore, the ethno-oriented research is highly of importance to discover and develop new drugs and food resources (Heinrich & Gibbons, 2001; Khafagi & Dewedar, 2000). It became of high importance to obtain data on the common uses of edible wild plants before this knowledge disappears. In many Mediterranean countries, these traditions are in danger of disappearing, and therefore there is an urgent need to study these systems of knowledge and find innovative ways to inculcate them in future generations (Hadjichambis et al., 2008; Hadjichambis et al., 2008; Pieroni et al.,2005).

In past few years many studies have been published about the consumption and collection of edible plants in many Mediterranean countries such as Greece (Forbes et al., 1976), Spain, and Italy (Paoletti et al., 1995; Tardío et al., 2006), Turkey (Ertuğ), Cyprus (Della et al.,2006). Some other studies focused the therapeutic importance of the main compounds of some wild plants in Palestine and Jordan, these plants therapeutic benefits are well shown in those studies (Al-Aboudi & Afifi, 2011; Jaradat et al.,2005; Said et al., 2002).

The biological activity of some these plants include: *Achillea santolina* Leaves, it has been shown that the flowering branches of this plant is used for reduction of blood glucose level, cold, anti-colic, and to treat kidney stones (Ardestani & Yazdanparast, 2007), another plant is *Ajuga iva* which mainly used to stimulate nervous and cardiovascular systems and to treat female sterility (Youssef et al., et al., *Ambrosia maritima* plant registered to exhibit hypoglycemic activity, *Artemisia herba* exhibited to have a hypoliposis effect (Slighoua et al., 2020). The leaves of *Achillea millefolium* are used as antiseptic, astringent, diuretic, carminative to stop bleeding, *Alhagi mannifera* roots used to treat kidney pebbles and sands (Teixeira et al., 2003). *Althea officinalis* flowers used for cough, toothache (Kianitalaei& Feyzabadi, 2019) , *Arctium lappa* leaves used for anti-diarrhea, antiseptic (Saravani, et al., 2020), *Astragalus gummifera* gums for protection of the kidney (Noori et al.,2019), *Capsella bursapastori* entire plant used for kidney stone (Jaradat et al., 2005), *Convolvulus arvensis*, and entire organism used for leg corns, skin inflammation, anticough, sedative *Cynoglossuf officinalis* root's bark used as antispasmodic, anti-inflammatory, demulcent (Joshi et al.,2016), *Valeriana officinalis* roots and flower stems used as antispasmodic, antiseptic, relief stomach pains, sedative, and arthritis (Wang et al., 2010).

It has been estimated about 4,200,000 flowering plants present worldwide, whereas, the number of plants used for therapeutic purposes estimated to be about 50,000 plants (Govaerts et al., 2001). In the traditional Palestinian folkloric medicine, it has been estimated that there are about 396 different species of plants used usually to cure various ailments in Palestine. (Ali-Shtayeh et al.,2014).

In Palestine it is well known that these traditional Palestinian plants play an important role Palestinian culture and Customs. According to Shtayeh et al, there are about 96 species native to Palestine, out of these, 84 species introduced from Asian nations (Shtayeh et al.,2014). According to the traditional healers and local therapists the locally obtained medicinal plants are those plants grow in Palestinian environment naturally and collected by individuals or therapists. As mentioned by Jaradat, there are about 43 plant species utilized in Palestinian traditional food, (Jaradat et al.,2005). Whereas, it is reported that the local Palestinian traditional therapists use about 40 different plant species for skin diseases, 32 plant species used for kidney and urinary tract diseases, 21 plant species used for diabetes, 22 plant species used for liver disease, 16 plant species used for respiratory and cough treatment, 13 plant species used for cancer species, and 9 types used to treat weight loss and cholesterol-lowering treatments, 51 plant species used for digestive system problems, and 11 plant species used for hypertension. (Jaradat et al.,2005; Said et al., 2002).

There are many wild and traditional Palestinian plant reported to have medicinal value, they include: *Majorana syriaca, Foeniculum vulgare, Malva sylvestris, Salvia fruticosa, Cyclamen persicum, Micromeria fruticosa, Arum palaestinum, Trigonella foenum-graecum, Gundelia tournefortii,* and *Matricaria aurea* and many others reported in the literature (Ali-Shtayeh et al., 2008; Tardío & Pardo-de-Santayana, 2008).

In the rural parts of the Palestinian territories, the traditional remedies are widely used. This could be related to the country's political unrest and the high expense of traditional medications (Ali-Shtayeh et al., 2016; Jaradat et al.,2016). From these traditional wild plant shrubs, trees, and herbs many antipyretics are maintained, in addition to other pharmacological effects like: diuretics, analgesics, antimicrobials, laxatives, antidiarrheal, heart tonics, and antiemetics (Jaradat et al.,2005). These plants are easily available as they grow wild (Abu-Rabia et al., 2005; Alkowni & Sawalha, 2012).

1.5 The selected WPP used in this study and their Literature survey

In many eastern countries and including Palestine like: Iraq, Yemen, Jordan, Syria, and Lebanon, in these countries plant and herbal healers largely depend on plants to treat various diseases. In addition, and according to the WHO, it has been estimated that about 80% of the world's population in developing countries depends on traditional medicinal plants for healing illnesses and health care, (Abu-Rabia et al., 2005; Alzweiri et al., 2011; HM et al.,2016; Al-Fatimi et al.,2019; Jaradat et al., 2016; Licata et al., 2016). Many traditional medicinal plants are registered to be used in pharmaceutical preparations and industry, this is due to the fact that plants have a diverse field of active pharmaceutical ingredients that play an important role in the therapeutic administration of many viral infections like Coronavirus (Abu-Rabia ; 2005; Jaradat; 2005), cancer (Desai et al., 2008) , malaria (Cox; 2010) , cardiovascular (Bachheti et al., 2022) , neurological diseases (Abdou, Yousef ; 2016).The medicinal effects of these plants are due to the presence of a range of secondary compounds and metabolites (Bamola et al., 2018). These compounds and according to their biochemical structure belong to a variety of biochemical groups like: volatile oils, saponins, alkaloids, and also phenolic compounds like: flavonoids, tannins, coumarins, and anthraquinones. The biochemical composition and combinations of these secondary metabolites are formed in plants by two mechanisms namely: shikimic acid/aromatic amino acid, and mevalonic acid pathways (Jaradat et al., 2016).

The pharmacological and therapeutic effect of the medicinal plants as antibacterial, antioxidant, anti-diabetes, anti-ulcer, and antiviral activity are globally documented in Palestine and worldwide (Abu-Shanab et al., 2007; Meléndez & Capriles, 2006)
In our present study, the traditional wild Palestinian plants used are: (*Varthemia iphionoides, Hyoscyamus arues* L, and *Ruta graveolnes,*), these plants have been chosen because they have high medicinal reputation in Palestinian society. Moreover, these plants lack the data related to their secondary metabolite, active components, the rareness of biochemical and pharmacological activities that has motivated this study.

In addition, and in reference to 'recommended traditional local plant therapists and local reports, these plants were carefully and accurately selected with no poisoning or toxicity documentation (Ali-Shtayeh et al., 2008; Jaradat, 2005; Said et al., 2002), since they

are used in healing and treating certain known illnesses, or as food. Therefore, the selected plants for this study were considered to be safe (Hamarsheh et al., 2021).

1.6 The selected traditional wild Palestinian plants are described below

1.6.1 *Chiliadenus iphionoides*:

Chiliadenus iphionoides is a small shrub, in Arabic it is called as (Shtaila) شتيال commonly grows in Palestine, Jordan, Lebanon, Syria, and Sinai, and other Mediterranean countries (Sbieh et al.,2022; Shahrajabian et al., 2022; Umeno et al., 2017). This plant is a perennial plant whose height ranges from 30-80 cm. and belongs to the Asteraceae family. It also grows in the Arab desert environment, the Mediterranean region, and the Iranian-Turonian region. It has been used in the treatment of many diseases such as abdominal pain, cramps, heartburn, weight loss, diabetes, and cold, and many studies have shown that it has a toxic effect against cancerous stem cell lines (A. R. Abdelhalim & A. Al-Munawarah, 2020; Gorelick et al., 2011). Many recent studies demonstrated the effectiveness of this plant as antidiabetic, treating fever, influenza, depression, stomach ache, and nervousness. (Sbieh et al., 2022). In general, the plant extract showed to decrease blood glucose both in long term feedings, as well as in an oral starch acceptance test. In another, study the plant also used in lowering lipid activity. The connection between dyslipidemia and diabetes is clear mechanism in which glucose mobility from the blood to peripheral areas implied by increase in insulin secretion and glucose uptake, and in decrease intestinal glucose absorption. (Gorelick et al., 2011).

Recent studies on *Varthemia iphionoides* aqueous extract is commonly used to treat diseases like diabetes mellitus and gastrointestinal disorders (Abdelhalim &Al-Munawarah, 2020). The plant also exhibited an antispasmodic effect, antibacterial, antiplatelet activity, and lowers blood glucose level in hyperglycemic mice, urine retention, anticancer, antioxidant, and antiplatelet activity, whereas the radical-scavenging and the anti- oxidative

e effects have not been yet reported (Abdelhalim & Al-Munawarah, 2020) (Al-Dabbas et al.,2010) (Al-Bakheit et al., 2017).

1.6.2 *Hyoscyamus aurues* L.

Hyoscyamus aurues L. is an annual perennial or biennial plant belongs to Solanaceae family grows in Palestine, Iraq, Jordan, Syria, and also distributed in many Mediterranean countries. The Arabic name of this plant is known as Al bing Althahabi (البنج الذهبي). The *Solanaceae* family is considered to be as one of the largest family among the plant kingdoms with about 33 species. Many plants in the Solanaceae family are considered as medicinal plants (El–Dahmy et al., 2022; Porwal et al., 2019). The plant leaves of this family medicinally are used as sedative, antispasmodic, anti-asthmatic and as hallucinogenic (El-Dahmy, et al 2022). Many studies showed that the plant is effective in reducing blood lipid and sugar, whereas, other studies showed that the plant has many valuable phytochemicals like: alkaloids like scopolamine and hyoscyamine, flavonoids, tropane, tannins, saponins, carbohydrate, cardiac glycosides, and anthraquinones. In medicine, it is used as anticancer, antioxidant, antidiabetic, antidiarrheal, insecticidal, antiasthenic, anticonvulsant, anti-allergic, anti-secretary, ca+2 channel–blocking, hypotensive cardio protective hepato protective, anti-hyperuricemia, anti-parkinsonian, anticholinergic effects and anti-depressant effects (Ahmed et al., 2016; Al-Snafi et al., 2018). The Solanaceae plants are also used as hallucinogenic and poisonous agents due to the presence of tropane alkaloids. Same studies indicated the plants of this family contain various types of alkaloids as secondary metabolites like: purines. tropane alkaloids, pyridines, indole alkaloids, pyrazole, isoquinoline, and many others. Hyoscyamus is a highly diversified genus of plants in the Solanaceae family that comprises between twelve and fifteen species. Some of these alkaloids are highly toxic to humans if taken in high amount, these alkaloids include: hyoscyamine, scopolamine, and atropine. In addition, Alkaloids have many medicinal activities specially on the Autonomic Nervous System (ANS), ophthalmic properties, and actions on the Central Nervous System (CNS), Parkinson's disease, actions on the Respiratory system, and anti-inflammatory actions. (El–Dahmy et al., 2022) and (İnci et al., 2022).

1.6.3 *Ruta graveolens*

Ruta graveolens is a small shrub plant belongs to the Rutaceae family, the common name is rue or herb of grace, in English, and called as sudab, sadab, or) فيجن) in Arabic (Jaradat et al.,2016). This plant is growing in the hills and mountains of the Palestinian territories, and in many parts of the world like: Egypt, Jordan, Iraq, and southern Europe. This plant is growing on the waste stony ground places. The plant is rich in

essential oils and many important chemical compounds with important medicinal activities like: abortifacient, diuretic, emmenagogue stimulant, antiulcer, cramps, skin inflammation, headache, anti-diarrheic ,anti-rheumatism, vermicide, anti-diabetics, anti-pharmacological trials have theirs as anthelmintic, abortion, ant parasitic , healing, anti-inflammatory, anti-diarrheic, anti-rheumatic, antifebrile, antiulcer, vermicide repellent, anti-diabetics, anti-rheumatism, and antimicrobial properties (Coimbra et al., 2020; Noori et al., 2019).

In a recent study (Colucci-D'Amato & Cimaglia, 2020) carried out on zebrafish, a genetic model animal, it has been shown that *Ruta graveolens* administration results in a decline in eggs exhibition and fertilization due to a disorder of gonadal or thyroid hormones.

This study also showed that rutin the major *Ruta graveolens* essential oil component could be used as possible drug in treating certain neurological disorders like: neurodegeneration and glioblastomas. (Colucci-D'Amato & Cimaglia, 2020). Other studies showed the essential oil of rutin contains many secondary metabolites like: furacridone coumarins, isorutarin, rutacultin, suberenone, furanocoumarins, 5-methoxypsoralen (bergapten) and 8-methoxypsoralen. (Shahrajabian et al.,2022).

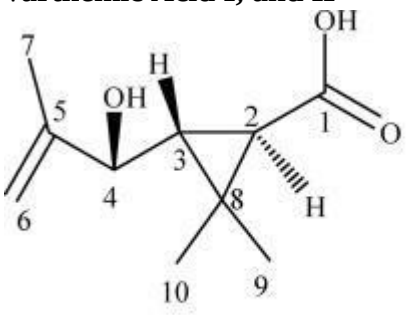
Table (1.2): Summaries of the selected WPP used in this study and some of their biological activities

Latin name	Family	Part used	Secondary Compound	Biological activity	Study country
Ruta graveolens	Rutaceae	Arial parts	Coumarins, alkaloids Volatile oils, flavonoids, lignanes and phenolic acids.	Rheumatism, dermatitis, eye problems, and pain relief	Palestine
Hyoscyamus aureus	Solanaceae	Arial parts	Torpan alkaloides	Treat pain, isomina and local ansethetic	Palestine
Varthemia iphionoides	Asteraseae	Arial parts	Essential oils, flavonoids and phenolic compouned.	Antiplatelet, Antidiabetic, Antioxidant, antispasmodic and antioxidant.	Palestine

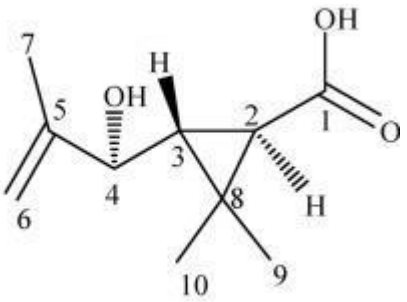
Figure 1.2 Some phytochemical structures of the selected WPP

1-*Varthemia or Chiliadenus*

varthemic Acid I, and II

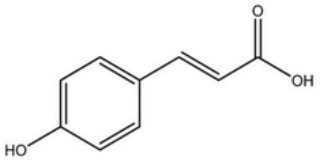


1

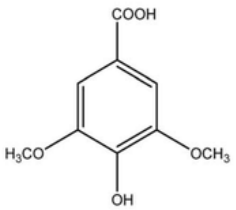


2

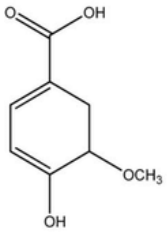
1- Ruta graveolens



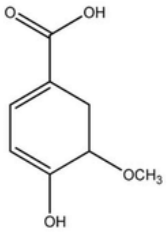
p-coumaric acid



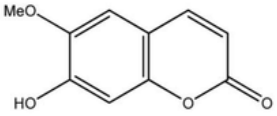
Syringic acid



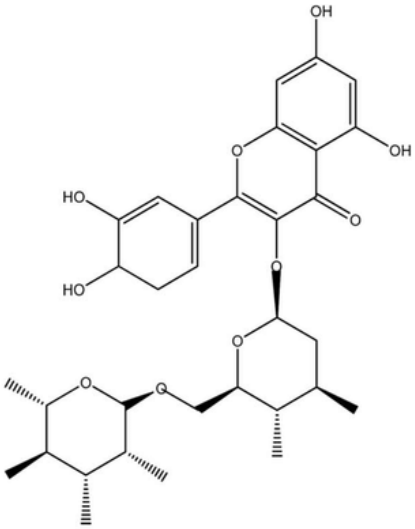
Vanillic acid



protocatechuic

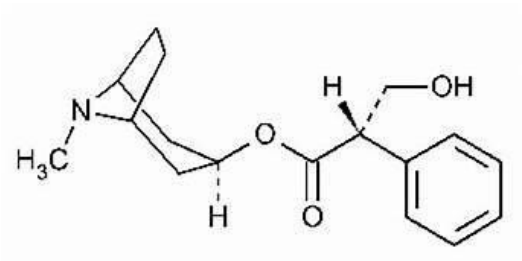


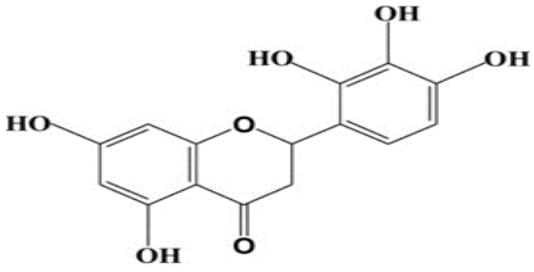
scopoletin



Rutin

2- Hyoscyamine pentahydroxyflavanone





1

1.7 Aim of this study

The main aim of this study was designed to investigate and document some biochemical, phytochemical, and pharmacological data on the selected WPP usually and traditionally used as folkloric medicinal herbs by Palestinian local therapists in many rural areas of the West Bank for treating of many diseases, using antioxidant, antibacterial, phytochemical screening by GC-MS biochemical methods. It is worthy to point out that and to the best of our knowledge, there are studies in the literature reporting about these wild plants as detail has been investigated in this analysis**.**

1.8 The objectives of this study

1. To evaluate the antioxidant activity of the volatile compounds of the above selected WPP leaves using $ABTS^{\bullet+}$ & $DPPH^{\bullet}$ assays.
2. To determine total phenols content using Folin Ciocalteu's
3. To determine whether the extracts above particular WPP leaves inhibited the growth of particular gram-negative bacterial strains, specifically (*Escherichia coli, Proteus Vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia),* and a gram-positive bacterium (*Staphylococcus aureus*) in comparison with positive controls.
4. To screen the presence of major phytochemical compounds and analyses these phytochemicals as plant secondary metabolites present in plant leaves extracts.
5. To identify the identity of the volatile compounds, present in the selected wild Palestinian plant leaves using GC-MS spectral analysis.

Chapter2
Materials and Methods

2.1 Study area

The Palestinian territories placed between the 31°21` to 32°33` latitude and between 34°52` to 35°32` longitude, due to this such location, it made this area highly affected by the Mediterranean climate. The climate in this area of the Mediterranean region is described by a long, hot, dry summer and a short, cool, rainy winter (Ighbareyeh et al., 2015). Rainfall is limited to the winter and spring months. It usually starts in the middle of October and continues up to the end of April. Snow and hail, although unfamiliar, may occur wherever in the area, especially to the west of and over the uplands (Aliewi et al, 2013). Palestine had a unique and wide range of ecosystem variety type due to its geographical place that lies between three landscapes: Africa, Asia, and Europe, this results in various climatic, zoogeographic, and phytogeography zones, and develops extensive natural biodiversity (Dowek et al., 2020). Tulkarm city is a district in the northern part of the west-bank, Palestine (Khader et al., 2017), the average temperature yearly almost ,23-31℃ in summer and from 8-18℃ in winter also, 60% average of the relative humidity and has limited rain full in winter nearly 586mm yearly (Al Abadla et al., 2020).

2.2 Sample Collection:

The selected WPP were cultivated from Tulkarm district of Palestine (32°18′37″ N, 135°01′43″ E), the plants harvested in July 2020, the leaves parts of these plants were used for research. These plants include: (*Hyoscumues auerues, Chiliadenus iphionoides* and *Ruta gravenoles* were visually identified at the laboratories of the college of agriculture, Hebron University. The plant leaves were separated, washed, and dried at room temperature until no change in weight was observed. The dried plant leaves of the above WPP were crushed into fine powders using a grinder (Morphy Richards, MR9100, British) and stored in clean dry tightly closed glassware at room temperature until analysis.

2.3 Antioxidant Activity

2.3.1 Extraction preparation

The extraction procedure of the selected WPP was mainly done following the protocol described by Qawasmeh et al 2012 and modified by Joseph et al 2020, in which one gram of each WPP sample was weighed, and then 10 ml of (80% v/v) methanol was added to a 25 ml beaker, kept in a shaking incubator, (Labtech, Model No. LSI-3016R, Daihan Labtech India Pvt. Ltd., Hyderabad, India) for 24 h at 80 rpm, at 25 °C. A 1.5 mL of the extract was transferred into Eppendorf tubes and spun down for 5 min at 4000 rpm) using Thermo scientific centrifuge (75002401, USA). The supernatants were transferred to another clean Eppendorf tube and stored at −20°C for $ABTS^{\bullet+}$ & $DPPH^{\bullet}$ assays (Joseph et al., 2020).

2.3.2 The 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) assay

$DPPH^{\bullet}$ or 2,2-diphenyl-1-picrylhydrazyl hydrate, is a chemical molecule contains a stable free radical used to test the scavenging capacity of substances (Sharma & Bhat, 2009). In our case, the extracts from the studied WPP were tested for their ability to scavenge free radicals using $DPPH^{\bullet}$. The scavenging capacity of extracted solutions of the studied plants was assayed based on the methods described by (Barros, et al., 2007) with slight modification carried in our laboratories, in which: $DPPH^{\bullet}$ stock solution was prepared by dissolving 2.3 mg of $DPPH^{\bullet}$ (Sigma Aldrich-STBD4146V) with 5.57 mL of (methanol (80% v/v). A 200 µL of $DPPH^{\bullet}$ stock solution was mixed with 2 mL 80% methanol and 20 µL of diluted plant extract (1:5, Sample) or 20 µL of methanol (80%, control) in plastic cuvettes. All the cuvettes were mixed by a vortex and incubated in a dark at room temperature for 1h. The absorbance's of plant extracts (A sample) and the methanol (A control) were measured at 734 nm using a Genway UV/Visible spectrophotometer at 517 nm (Dowek et al., 2020). The radical scavenging activity was calculated as a percentage of $DPPH^{\bullet}$ disc discoloration using the following equation:

$$\textbf{DPPH}^{\bullet}\ \textbf{Scavenging (\%)} = [(\textbf{A control} - \textbf{A sample})/\textbf{A control}] \times 100\%$$

2.3.3 The $ABTS^{\bullet+}$ Assay

In this assay, the $ABTS^{\bullet+}$ stock solution was mainly prepared according to the protocol described by (Qawasmeh et al., 2012), in which 18 mg of $ABTS^{\bullet+}$ (Sigma Aldrich,

Palestine) dissolved in 5 mL distilled water to get a final concentration of 7 mmol. An aliquot (88 µL) of potassium persulfate solution (2.45 mmol) was added to $ABTS^{\bullet+}$ solution. The mixture was incubated in the dark overnight before use. The working solution of $ABTS^{\bullet+}$ was prepared by diluting a stock solution of $ABTS^{\bullet+}$ with methanol (80% v/v) to final absorbance of 0.7000 ± 0.02 at 734 nm. A 30 µL of diluted plant extracts (1:5) solutions were mixed with 3 mL $ABTS^{\bullet+}$ working solution in micro cuvettes. For control, 30 µL methanol (80%) was mixed. All cuvettes were mixed by a vortex (Lab net international Inc. U.S.A) and incubated in a dark for 30 min at room temperature. The absorbances of plant extracts (A sample) and the methanol (A control) were measured at 734 nm using the Genway UV/Visible spectrophotometer (Joseph et al., 2020; Cao et al., 2019).

The percentage scavenging of $ABTS^{\bullet+}$ was calculated according to the following equation:

$ABTS^{\bullet+}$ Scavenging (%) = [(Acontrol − Asample)⁄Acontrol] × 100%

2.3.4 Determination of total phenols using Folin–Ciocalteu

Preparing Gallic acid as a stock solution

As Gallic acid (GA) is not water soluble, the GA was prepared by dissolving 250 mg of GA in 5 ml of (80%) methanol, following this the GA was diluted with distilled water up to 50 ml. Different concentrations of GA were prepared by adding six-different volumes of GA (50, 100, 200, 300, 500, and 1000 µl) in a cuvette, then adding (80%) methanol up to 10ml to use as a reference for total phenols determination in the *V. vinifera* samples. The extracts of *V. vinifera* samples prepared above were used to determine the total phenols as described in (Dowek et al., 2020).

Sodium carbonate (Na_2CO_3) solution preparation

The Na_2CO_3 was prepared by dissolving 5g of Na_2CO_3 in 20 ml D.W. in a beaker and then heating to boiling. The solution was cooled and filtered, and finally, adding D.W. up to 25 ml.

To determine the total phenols in *V. vinifera* samples, the spectrophotometer was used (λ max= 765 nm, and using D.W. as blank). In each cuvette, 20 µl of the extract, 1.58ml of

(D.W), and 150 µl of Folin–Ciocalteu reagent were added, after mixing the solution well about 300 µl of Na_2CO_3 solution was added to the solution in a cuvette. The resulting solution was kept for 1 hr. in dark before taking the reading. The absorbance of the resulting solution was measured at λ max of 760 nm. The blank used was water and the assay was done in triplicate, For the GA stock solution, 20 µl of GA was added (from each concentration in 10 cuvettes) instead of the extract. Data were expressed as milligrams of Gallic acid per gram of dried plant samples (mg GAE/g) (Dowek, Fallah, Basheer-Salimia, Jazzar, Qawasmeh, et al., 2020).

2.4 Phytochemical screening

The extract preparation mainly based on the protocol described by (Mujeeb et al., 2014) in which: 3g of each sample of *V. vinifera* were added to a beaker with 60 ml of (80%) methanol and kept for 24 h at 25 ^{o}C in a shaking incubator. The resulting extracts were filtered through a filter paper and used to screen the phytochemicals content in each sample as the following:

2.4.1 Test for Alkaloids

In a test tube, 1 mL of 1% HCl was added to 2 mL extract then a few drops of Meyer's reagent were added to the mixture. The presence of white precipitate indicates positive test for alkaloids.

2.4.2 Anthraquinones

In a test tube, 1 mL of 10% NH_3 solution was added to 2ml extract, which was mixed with benzene, the presence of red, pink, or violet color indicates positive test for Anthraquinones.

2.4.3 Anthocyanin

In a test tube, 1 mL of 1N NaOH was added to 1ml extract and heated for 5 min. The formation of bluish green color indicates a positive test for anthocyanin.

2.4.4 Cardiac glycoside

In a test tube, 2 mL of glacial acetic acid, 1 mL of conc. H_2SO_4 and a few drops of $FeCL_3$ were added to the 2 mL extract. The formation of brown ring indicates a positive test for Cardiac glycosides.

2.4.5 Coumarins

In a test tube, 1 mL of NaOH was added to 1 mL extract and kept in boiling water path for few minutes; the appearance of yellow color indicates positive test for Coumarins.

2.4.6 Flavonoids

In a test tube, a few drops of 1% NH_3 solution was mixed with 2 mL extract. The presence of yellow color indicates a positive test for Flavonoids.

2.4.7 Glycosides

In a test tube, 2 mL of 50% H_2SO_4 was added to the 2ml of extract. After 5 min of heating a mixture in a water bath, a few drops of Fehling's solution were added and boiled. The presence of red prick precipitate indicates positive test for Glycosides.

2.4.8 Phenolic group

In a test tube, 2 mL of distilled water and a few drops of 10% $FeCL_3$ were added to 1 mL extract. The formation of blue or black color indicates positive test for phenolic groups.

2.4.9 Phlobatannins

In a test tube, 1 mL of 10% NaOH was added to 2 mL extract. The formation of yellow color indicates a positive test for Phlobatannins.

2.4.10 Quinones

In a test tube, 1 mL of conc. H_2SO_4 was added to 1 mL of extract. The presence of red color indicates a positive test for quinines.

2.4.11 Saponins

In a test tube, 5 mL of distilled water was shaken with 2 mL of extract; the formation of foam indicates positive test for Saponins.

2.4.12 Steroids

In a test tube, 2 mL of $CHCl_3$, and 1 mL of H_2SO_4 were added to 1 mL extract, and the appearance of a reddish-brown ring indicates positive test for steroids.

2.4.13 Tannins

In a test tube, 1 mLof distilled water and 1-2 drops of $FeCL_3$, were added to 2 mL extract, the presence of green or blowback color indicates positive test for tannins.

2.4.14 Terpenoids

In a test tube, 2 mL of $CHCl_3$, and 3 mL conc. H_2SO_4 were mixed with 2 mL extract. The formation of a reddish-brown layer indicates a positive test for Terpenoids.

2.5 GC-MS analysis

Gas chromatography in addition to mass spectroscopy (GC–MS) is a method of analysis used to identify different substances within test sample, this method studies liquid, solid, and gas samples. In the present study our investigation basically carried according (Qawasmeh et al., 2011), with minor modification, in which volatile compounds present in leaves of the studied plants (1 g) were extracted 80% methanol (10 mL) overnight and analyzed using a GC–MS fitted with a BD-5 ms capillary column (30 m, 0.25 μm film thickness, 0.25 μm bore diameter) The injection volume was 1 μl. The oven temperature was maintained at 80 °C for 2 min and was programmed to rise to 280 °C at the rate of 30 °C/min. The temperatures of the injector and the detector were maintained at 250 °C and 260 °C, respectively. Helium was used as the carrier gas; the total-gas flow and velocity were maintained at 134.3 mL/min and 43.1 cm/s, respectively. MS scan speed was 1000 amu/s and the molecular masses (M/Z) of the compounds between 50 and 500 M/Z were acquired. The analysis for each sample was repeated. Compounds were uncertainly identified using the NIST05 mass spectral library, and when applicable, their mass spectra were compared with those published in the literature review (Mujeeb et al., 2014).

2.6 Antibacterial Activity

2.6.1 Extract preparation:

The method of extraction mainly carried out according to the protocol given by (Qawasmeh et al., 2012), in which twenty-five grams (25 g) of previously powdered plant samples of *(Chiliadenus iphionoides, Ruta graveolens and Hyoscyamus aurues*), seeds, and fruit-skins were weighed out using an analytical weighing scale. It was mixed with 100 milliliters of 80% v/v methanol and macerated overnight at 25°C in a shaking incubator. After 24 hours, the 80% methanol was decanted and filtered through

a Whatman No. 1 filter paper. Extracts were then concentrated in a beaker on a hotplate in a laminar flow cabinet with relatively lower heat at 30°C and agitated by magnetic stirring for 24 hours before being kept in a refrigerator at 4°C.

2.6.2 Media preparation

The media that used for bacterial culturing and identification was prepared as follows before pouring into Petri dishes:

- EMB: 35,96 g/ 1000 mL, for 250 mL, approximately 8,99g needed.
- Mannitol salt agar base (MSA): 111,2/1000ml, for 250ml, about 27,755g needed.
- MacConky agar: 51,53g/1000ml, for 250ml, about 12,883g needed.
- Nutrient agar: 28g/1000l, for 250ml, about 7g is needed.

Prepared small amounts of each type of media for identification of isolated bacteria and prepared three bottles of Muller Hinton (M.H) media to be used for culturing the bacteria and testing the sensitivity of the plant extract with positive and negative controls. Approximately 28.5 g of M.H powder was weighted in an autoclavable bottle, then 750 mL of distilled water was added. The bottles were then heated on a hot plate until they became clear (using a magnetic stirrer), wait 20 minutes, close the bottle and cover it with aluminum foil, and autoclave the media (EMB, MSA, MacConky, N.A, B.A, and M.H) for an hour and a half to become sterile. After an hour and a half of sterilization in the autoclave, the media was poured into the Petri dishes as follows, where 25 mL of M.H was poured into each Petri dishes and the quantity filled about 64 Petri dishes. In addition, differential media was poured into Petri dishes until the circle was covered. Finally, all the dishes were kept in the refrigerator at 4°C until needed.

2.6.3 Identification of Bacterial Isolates

This work was carried out at the Microbiology Department of the University of Hebron, bacterial isolates were obtained from the Microbiology laboratory. Bacterial isolates (*E. coli, Proteus, Pseudomonas, Staphylococcus aureus and Klebsiella*) were authenticated by secondary culture of the archived isolates. After thawing the isolates in 80% methanol, they were cultured on differential media plates (Owusu et al ., 2021). Differential media of EMB, Mannitol salt agar base (MSA), MacConky agar, and Nutrient agar aided in distinguishing between Gram-negative bacteria and Gram-positive bacteria. For Proteus: By using cotton swab, one touch of bacteria was put at the center of the petri dish.

Whereas follow the spread of other types of bacteria at the petri dish (primary, secondary, and tertiary). Then stayed in incubator at 37°C for 24 hours.

Isolates were characterized phenotypically using colonial morphology and Gram staining. A detailed description of the isolates' characterization follows:

a. *Escherichia coli (E. coli):* These isolates were characterized based on their appearance on using two differential media EMB and MacConky; a greenish metallic sheen forms colonies of *E. coli* on Eosin Methylene Blue agar (EMB), whereas a pink colony of *E. coli* fermenting lactose forms on MacConky agar.

b. *Proteus*: The strains were characterized by their appearance as swarming growth forms on Nutrient agar plates.

c. *Pseudomonas aeruginosa*: produce pigment Pyoverdine, which causes them to appear green on nutrient agar.

d. *Staphylococcus aureus*: appearance of golden-yellow colonies on Mannitol Salt Agar (MSA).

e. *Klebsiella*: These isolates were characterized based on their appearance on using two differential media MacConky and Eosin methylene blue agar (EMB); in both MacConky agar and EMB, Klebsiella pneumonia colonies were pink in color due to the lactose fermentation.

2.6.4 Antimicrobial Activity Evaluation of Plant Extracts by Agar Well Diffusion Method.

The antimicrobial activity was done according to the method of (Owusu et al., 2021; Balouiri et al., 2016). According to these methods, one ml distilled water was added to 4 test tubes, then using a cotton swap, a single touch of bacterial colony previously isolated and identified was taken and added to the distilled water and shacked. The density/turbidity of the inoculum and the absorbance should be about 0.07 Abs by using spectrophotometer with lamda max 450 nm, adjusted to 0.5 McFarland turbidity standard, resulting in a suspension of 1.5 × 108 CFU colony forming units.

Mueller Hinton agar plates were seeded with the test organisms and the plates left for five minutes to dry. After drying, five wells were made in the agar using sterile tips of micropipette measuring 9 mm in diameter, 3 for the extracts of each genotype, one for the

negative control and other for the positive control. Hundred microliters (10 µL) of the methanolic extract of samples were dispensed into the labelled wells, Meropenem disk was used as a Positive control of *E. coli, Proteus, Pseudomonas aeruginosa* and *Klebsiella*, while Vancomycin disk used for *Staphylococcus aureus*. In addition, 80% methanol was used as a negative control because it used to prepare the extract. The plates were then incubated at 37 ◦C for 24 h (h) and the zones of inhibition were measured in millimeters. Readings were done in triplicates (Owusu et al., 2021; Balouiri et al., 2016).

Chapter 3: Results

3.1. Quantitative analysis of total phenol content in selected plants.

Gallic acid was used to estimate total phenolic content Figure (3.1), which was expressed as mg/L of Gallic acid equivalent (GAE). Tables (3.1) and (3.2) showed the quantitative estimation of total phenol of the studied wild Palestinian plants: *Chiliadenus iphionoides*, *Hyoscusem aureus* and *Ruta graveolens*, were we observed that these plants do contain a significant amount of the total phenols.

Table (3.1) Absorbance value of Gallic acid standard (mg/L) n=3

GA mg/L	1	2	3	Mean ± SE
50	0.039	0.104	0.0715	0.026±0.0325
100	0.081	0.103	0.092	0.07±0.011
200	0.156	0.152	0.154	0.116±0.002
300	0.182	0.224	0.203	0.1575±0.021
500	0.333	0.256	0.2945	0.2305±0.0385
1000	0.545	0.694	0.6195	0.4823±0.0745

Figure (3.1): Calibration curve of Gallic acid, the point represents the mean of triplicates.

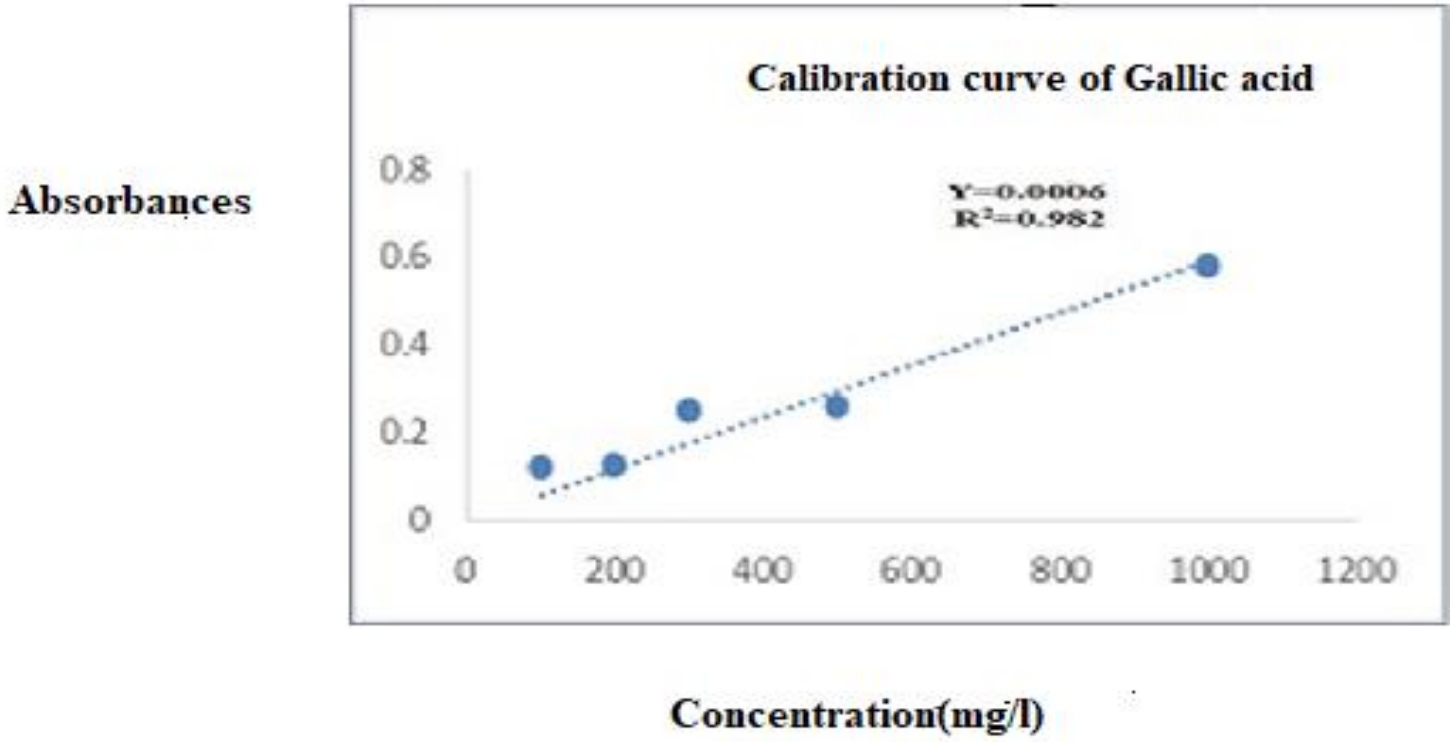


Table (3.2) Total phenol content in *Chiliadenus iphionoides, Hyoscusem aureus,* and *Ruta graveolens*) leaves.

plant	**1**	**2**	**3**	**4**	**Mean ±SD (mg GAE**
Chiliadenus iphionoides	1.722	1.799	1.518	1.680	1.67975± 0.102675
Ruta graveolens	0.742	0.832	0.690	0.774	0.7595± 0.051485
Hyoscumues aurues	0.641	0.931	1.601	0.821	0.9985±0.362931

Methanolic crude extract (n = 4) was estimated quantitatively based on the equation established from Gallic acid standard curve (Y=0.0006X, R^2 = 0.982).

3.1. Antioxidant activity of the selected WPP

3.1.1. DPPH$^{\bullet}$ scavenging capacity

Figure (3.2) showed the diluted methanolic extract (1:10) of the selected wild Palestinian plant leaves, as mentioned in the figure our results showed a pronounced antioxidant activity as assessed by DPPH$^{\bullet}$ free radical scavenging assay. The percentage of scavenging of diluted methanolic extracts of plant leaves were 33.7%, 44.9%, 59.2% for *Chiliadenus iphionoides, Hyoscusem auerues and Ruta graveolens*, respectively. The highest percentage of antioxidant activity was found in *R. graveolens* leaves according to DPPH$^{\bullet}$ scavenging assay.

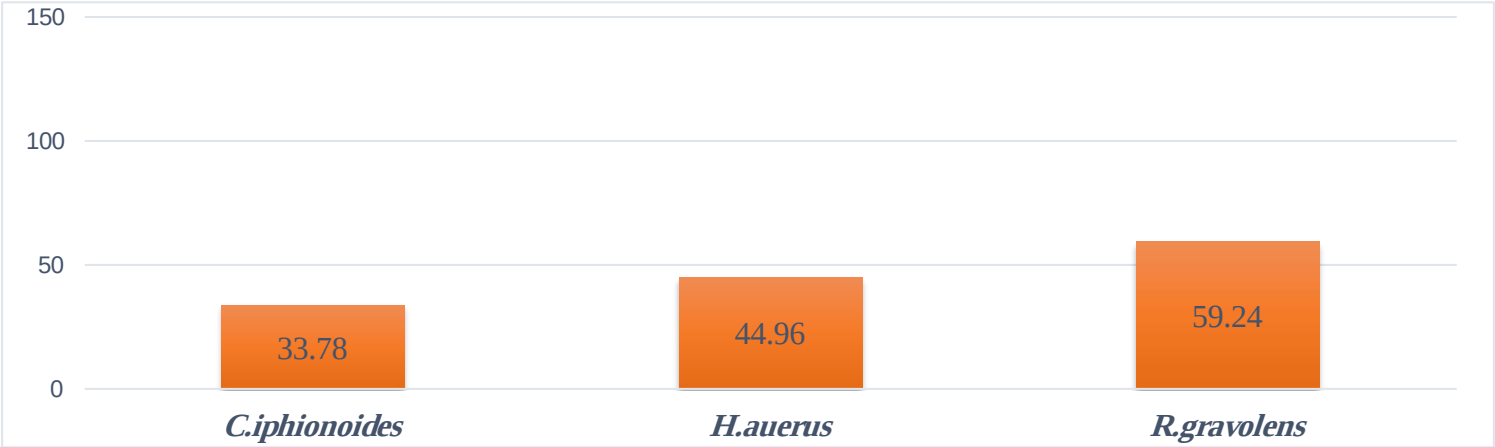


Figure (3.2): Antioxidant activity % of methanolic extracts of the selected WPP leaves of (*Chiliadenus iphionoides*, *Ruta gravenoles* and *Hyoscusem aureus*) using DPPH$^{\bullet}$ free radical scavenging assay.

3.1.2. $ABTS^{\bullet+}$ scavenging capacity

Figure (3.3) Showed the diluted methanolic extracts (1:5) of the selected wild Palestinian plant leaves, our results showed a pronounced antioxidant activity of these plants as assessed by $ABTS^{\bullet+}$ free radical scavenging assay**.** The percentage of $ABTS^{\bullet+}$ scavenging assay of the diluted methanolic extracts of the plant leaves were 43%, 55.57%, 61% for *Chiliadenus iphionoides, Hyoscusem aureus and Ruta graveolens*, respectively. Our results showed high percentage of antioxidant activity found in *Ruta graveolens* according to $ABTS^{\bullet+}$ scavenging assay.

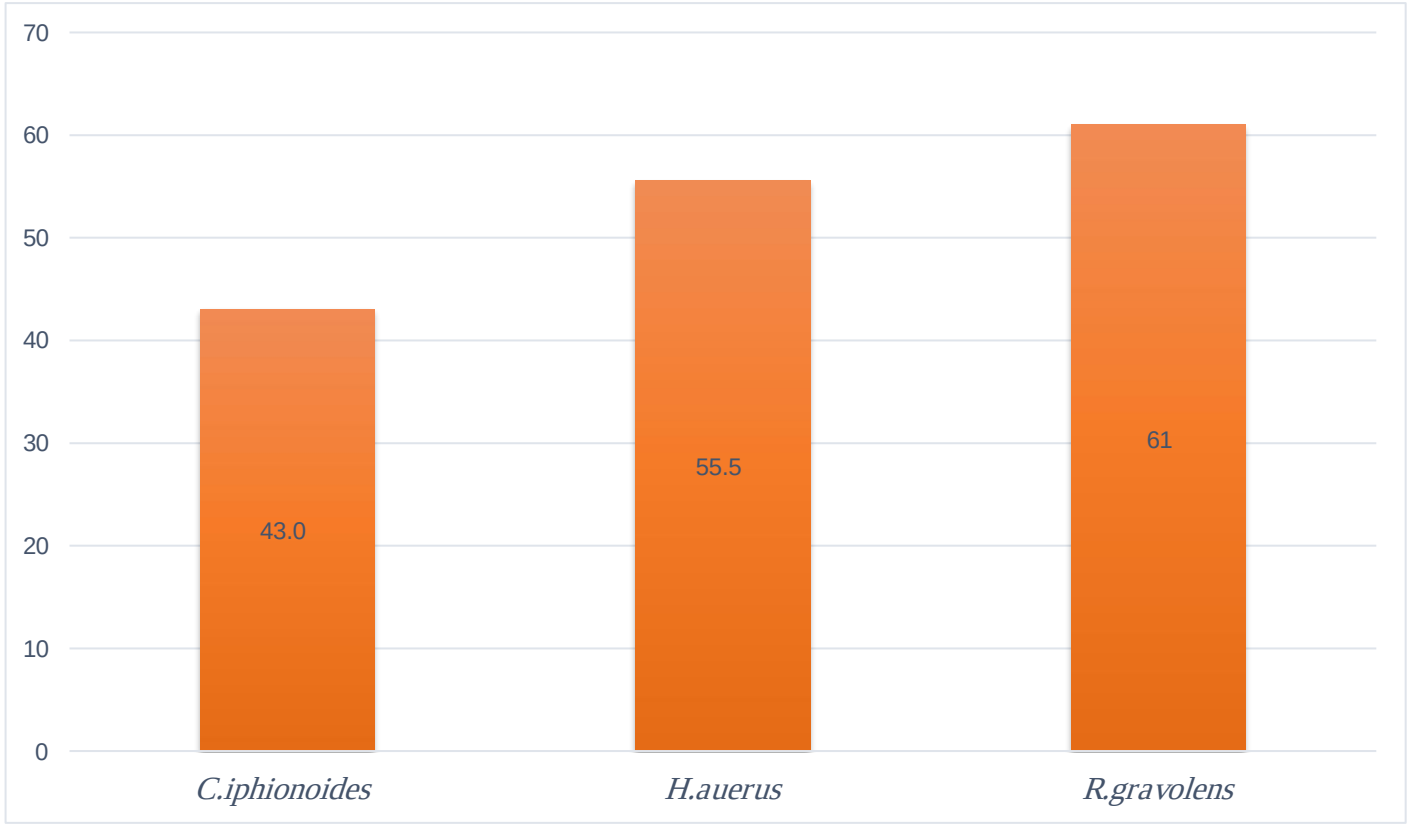


Figure (3.3) Antioxidant activity % of the methanolic extract of the selected WPP (*Chiliadenus iphionoides, Ruta gravenoles* and *Hyoscusem aureus*) plant leaves using $ABTS^{\bullet+}$ free radical scavenging assay.

3.2. Qualitative screening of phytochemicals of the selected WPP

As shown in table (3.3) the phytochemical screening of the selected plants of *Chiliadenus iphionoides, Hyoscusem aureus,* and *Ruta graveolens* leaves revealed the presence of a wide range of phytochemical groups such as alkaloids, flavonoids, phenols, tannins, Quinone's, saponins, steroids, and terpenoids present in the methanolic extracts.

It is well documented that phytochemicals are known to show many important medicinal as well as physiological actions in human body. For example, cardiac glycosides like digoxin and digitalis are phytoestrogens that are used as cardiac tonics and treat heart failure, and supraventricular arrhythmias (atrial fibrillation/flutter) (Karasneh et al., 2017). They were also recommended for the prophylaxis and treatment of some arrhythmias, such as paroxysmal atrial tachycardia and cardiogenic shock (Schneider et al.,2017).

Whereas for Coumarin phytochemicals they have shown pronounced evidence of biological activity and in medicine they are used in pharmaceutical industry, for example some Coumarins are used in the treatment of lymphedema and their ability to increase plasma ant thrombin levels (Jain & Joshi, 2012). Flavonoids such as the catechins are "the most common group of polyphenolic compounds in the human's diet and are found ubiquitously in plants. Flavonoids and their metabolites exert countless health-promoting effects both in humans and animals. They possess multiple biological effects such as antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, and antioxidant activities (Jain & Joshi, 2012), Moreover, they can cross the blood-brain barrier (BBB) and may exhibit neuro pharmacological activities at the molecular level, influencing protein function and gene expression. Importantly, dietary intake of flavonoids up-regulates the brain-derived neurotrophic factor (BDNF) and thus improves the performance of spatial memory.

Phenolic compounds are recognized antioxidants and anti-inflammatory agents which can protect intestinal cells from pro-oxidant and inflammatory injuries (Qawasmeh et al., 2012; Sharma et al., 2019).Quinone imines and the Quinone methods in quinones of the quinoid family, are well mentioned to induce cyto-protection through the induction of detoxification enzymes, anti-inflammatory activities, and modification of redox status (del Río et al.,2013 ; Mujeeb et al., 2014),The mechanisms by which Quinone's induce their effects can be quite complex. The various biological targets of Quinone's depend on their rate and site of formation and reactivity.

For Saponins and their biosynthetic intermediates which are widely distributed in plant natural products have a huge range of structural and functional biodiversity and display a variety of biological activities of interest to the pharmaceutical, cosmetic, and food sectors (Biswas & Dwivedi, 2019; Maulucci et al., 2016) . Unsaturation fatty acid as important components of cell membranes that alter membrane fluidity; and secondly they are considered as signaling molecules (Maulucci et al., 2016). it should be mentioned that the absence or presence of certain phytochemicals in some plants is attributed to various physiological reactions that take place inside the plant, as well as environmental effects on the plants (Mujeeb et al., 2014).

Table (3.3): phytochemical screening of the methanolic extracts of the selected wild plant leaves (*Chiliadenus iphionoides, Hyoscusem aureus, and Ruta graveolens*):

Type	*Chiliadenus iphionoides*	*Ruta graveolens*	*Hyoscyamus aureus*
Phenolic group	**+ve**	**+ve**	**+ve**
Phlobatninas	**+ve**	**+ve**	**+ve**
Alkaloid	**+ve**	**+ve**	**+ve**
Anthraquinon	**-ve**	**-ve**	**+ve**
Anthcyanin	**-ve**	**-ve**	**+ve**
Cardic glycosides	**+ve**	**+ve**	**+ve**
Coumarin	**+ve**	**+ve**	**+ve**
Flavonoids	**-ve**	**+ve**	**+ve**
Glycosides	**+ve**	**+ve**	**+ve**
Quinones	**-ve**	**-ve**	**+ve**
Saponins	**-ve**	**-ve**	**+ve**
Steroids	**+ve**	**+ve**	**+ve**
Tannins	**-ve**	**+ve**	**+ve**
Terpenoids	**+ve**	**+ve**	**-ve**

3.3. GC-MC analysis

Figures (3.5), (3.6), and (3.7) showed the results of the GC-MS analysis of the methanolic extract of *Hyoscusem aureus, Chiliadenus iphionoides, and Ruta graveolens* plant leaves. According to the mentioned GC-MS spectral analysis, numerous bioactive chemicals were found. Tables (3.4), (3.5), and (3.6), showed the presence of bioactive compounds present in the studied WPPs together with their retention times and molecular weights, accordingly.

The WPP leaves of *Chiliadenus iphionoides, Hyoscusem aureus, and Ruta graveolens* used in this GC-MS analysis revealed the presence of several bio-active and predominant compounds. This indicates the traditional significance of these ethnobotanical wild Palestinian plants used by traditional Palestinian practitioners. From a pharmacological perspective, isolating the various phytochemical components of these medicinal plants and subjecting them to biological activity will be yielding fruitful results and will open a new area of investigation of the individual components and their pharmacological potency locally in the Palestinian territories, where these herbs have long been used for many medicinal uses. It is advised that these plants' value for pharmacological purposes be extensively assessed. We found that several phytochemicals appear repeatedly in our investigation. For instance, phytol has a number of significant properties, including antibacterial, anti- Nociceptive (lowering sensitivity to painful stimuli), anti-inflammatory, antidiuretic, immunological stimulatory, anti-diabetic, and antioxidant activities. Contrarily, natural substances with varying degrees of potency, like thymol, carvacrol, eugenol, and menthol, have harmful effects on the growth of all fungi in vitro. Future research into the prevention of the growth of foodborne fungal diseases in vivo may use the in vitro findings collected in this work as a reference (Kulkarni et al., 2015; Tedoneet al., 2014).

Whereas, natural compounds such as thymol, carvacrol, eugenol, and menthol have shown toxic effects on fungal growth in vitro for all fungal species but with different levels of potency. The in vitro data obtained in this study may serve as a guide for future studies in vivo into the growth inhibition of foodborne fungal pathogens (Panahi et al., 2014; Qawasmeh et al., 2012).

Table (3.4): List of major compounds detected in the methanolic extracts from *Hyoscusem auerues* leaves with their retention times (Rt), molecular masses (M/Z), molecular weight (MW), and molecular formula (MF).

#	Rt	M/Z	Compound identification	MW	MF
1.	5.71	55/84/105	Ethinylestradiol	296	C20H24O2
2.	5.87	55/70/82	Phyrrolidinomophinan	296	C20H28N2
3.	6.22	55/73/105	Vadimezan	296	C17H14O4
4.	6.48	55/91/149	2-acetyltritycene	281	C22H16O
5.	6.56	57/77/95	Ethenyl estraiol	296	C20H24O2
6.	6.63	55/83/91	Cryptotanshinone	296	C19H20O3
7.	7.27	57/71/83	Cyclotriol	281	C20H26O3
8.	7.34	55/95/123	Cannabidiol	314	C21H30O2
9.	7.37	55/69/81	Alnusediol	314	C19H22O4
10.	7.45	55/70/91	Guaiacol	124	C7H8O2
11.	7.64	55/81/95	Methyl salicylate	1281	C8H8O3

Table (3.5): List of major compounds detected in the methanolic extracts from *Chiliadenus iphionoides* leaves with their retention times (Rt), molecular masses (M/Z), molecular weight (MW), and molecular formula (MF)

#	Rt	M/Z	Compound identification	MW	MF
1.	4.74	56/73/85	O-Nitrosobenzaldehyde	254	C16H14O3
2.	5.00	57/73/85	Naphthalene	254	C15H14O2N2
3.	5.11	56/73/85	Disperse violet 57	254	C18H22O
4.	5.47	66/73/85	Tetrahydron	272	C18H24O2
5.	5.58	55/85/91	Santolinea triene	136	C10H16
6.	5.69	85/89/178	A-pinene	136	C10H16
7.	7.79	55/68/82	Sabinene	136	C10H16
8.	8.11	55/74/87	Camphor	152	C10H16O
9.	8.74	55/71/81	borneol	154	C10H18O
10.	8.79	55/67/73	Γ-Eudesmol acetate	264	C17H28O2
11.	8.84	57/73/91	Neryl acetate	196	C12H20O2
12.	7.79	55/68/82	α- terpineol	154	C10H18O
13.	8.11	55/74/87	Humulene	204	C15H24

Table (3.6): List of major compounds detected in the methanolic extracts from *Ruta graveolens* leaves with their retention times (Rt), molecular masses (M/Z), molecular weight (MW), and molecular formula (MF)

#	Rt	M/Z	Compound identification	MW	MF
1	5.87	71,59, 58, 57	Nonanone	142	$C_9H_{18}O$
2	9.42	71,59,58,57	Tridecanone	198	$C_{13}H_{26}O$
3	12.64	71,59,58,57	Pentadecanone	226	$C_{15}H_{30}$
4	17.28	232,136,135,131	3 Methyl,4 piperonyl-5 Isoxazalone	233	$C_{12}H_{11}O_4N$
5	20.09	248,148,136,135	Ethyl piperonyl cyanoacetate	247	$C_{13}H_{13}O_4N$
6	20.53	216,201,188,173,145	1,4 naphtholinol dione, 2 acetyl, 3 hydroxy	216	$C_{12}H_8O_4$
7	21.86	254,239,211,199,135	Isopravifuran	254	$C_{16}H_{14}O_3$
8	24.75	260,259, 244, 201	1-Carbethoxy,3-Acetyl-4(H)-Quinolizine 4-One	259	$C_{14}H_{13}O_4N$
9	26.68	314,299,281,255	Hexahydrophenanthren derivative	314	$C_{19}H_{22}O_4$
10	27.70	296,281,253,241	Phenantofuran dione derivative	296	$C_{19}H_{20}O_3$

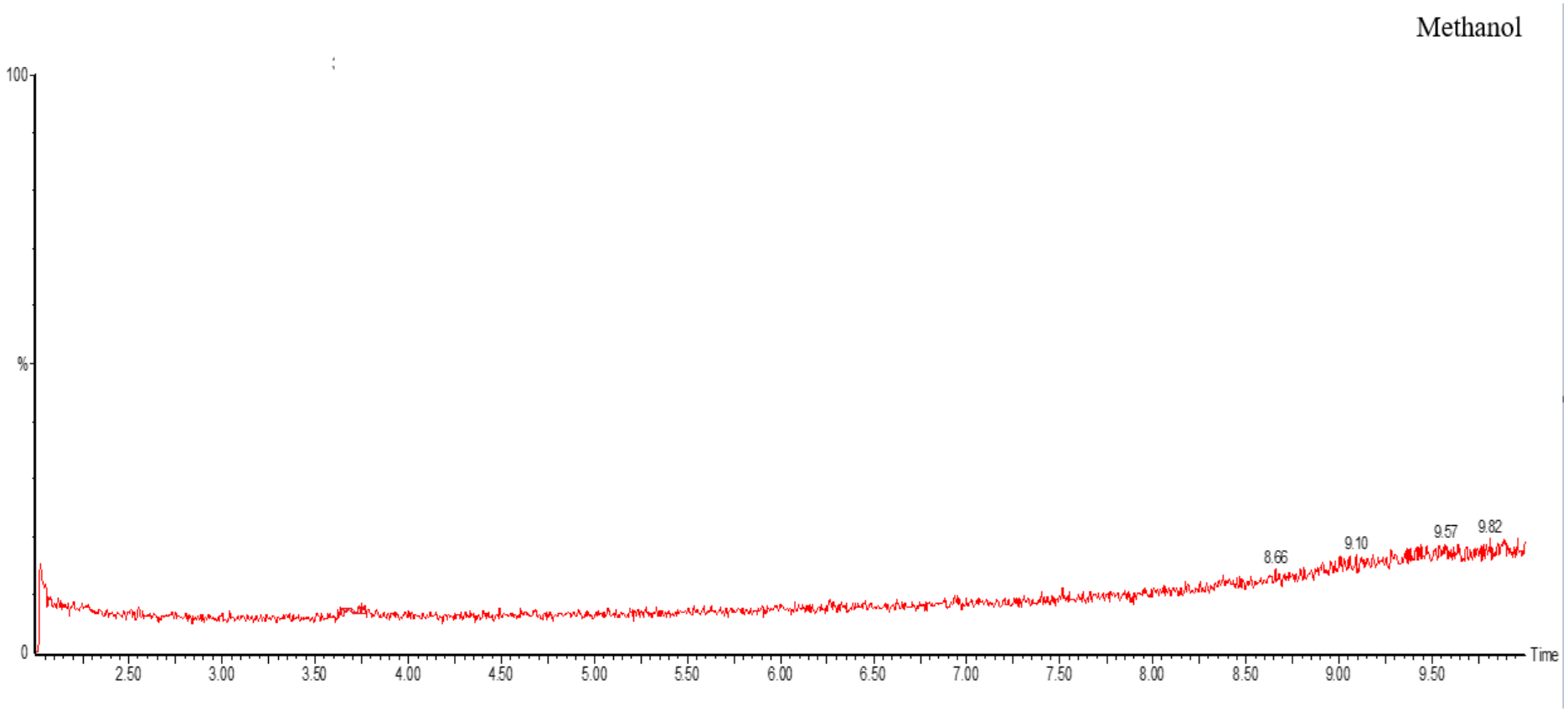


Figure (3.4) Representative total ion chromatogram methanol (80%)

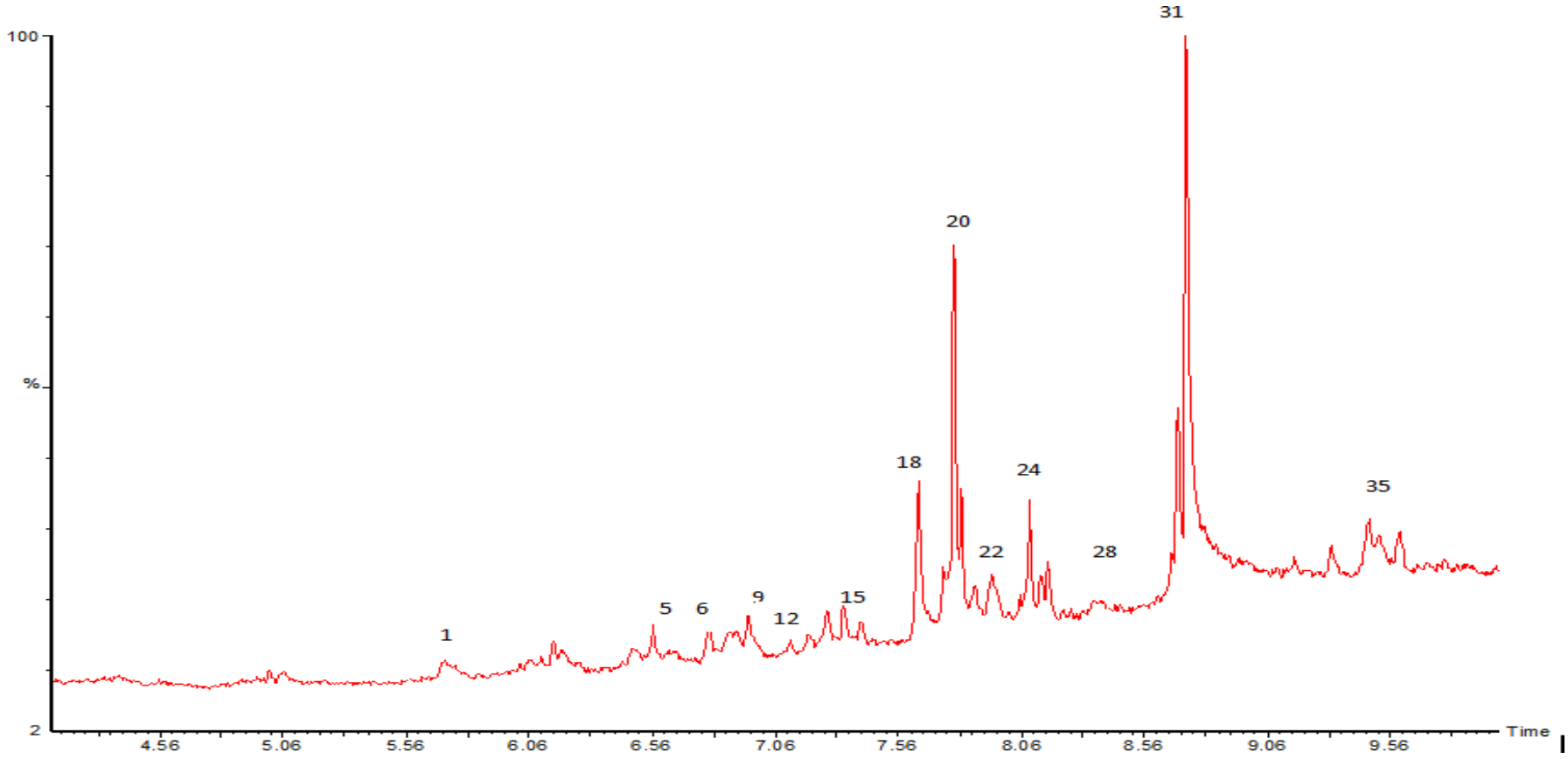


Figure:(3.5) Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of *Hyoscumues aurues* leaves. X axis represent time in min, Y axis represent peak intensity (arbitrary unit). Number above each peak represent their respective retention time (Rt) in min.

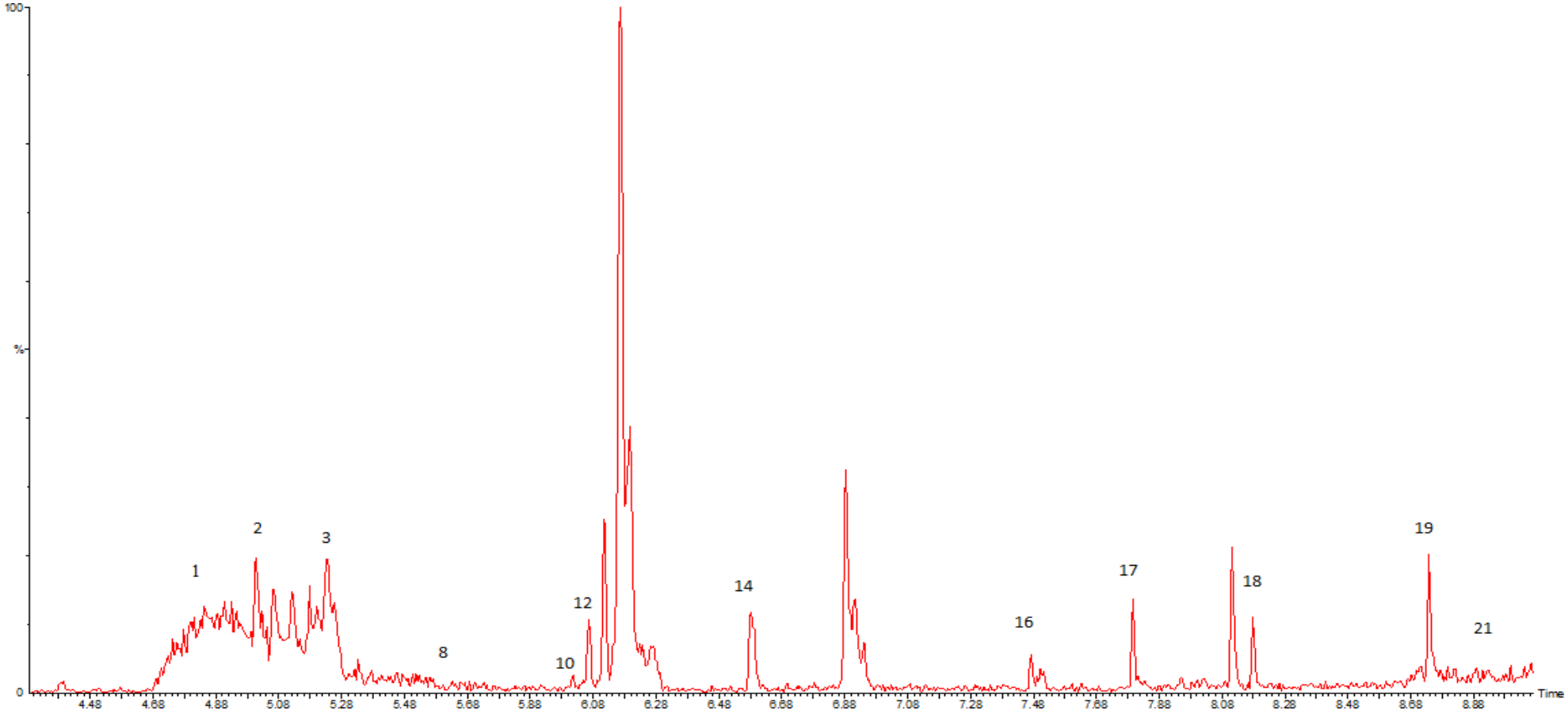


Figure: (3.6) Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of *Chiliadenus iphionoides* leaves. X axis represent time in min, Y axis represent peak intensity (arbitrary unit). Number above each peak represent their respective retention time (Rt) in min.

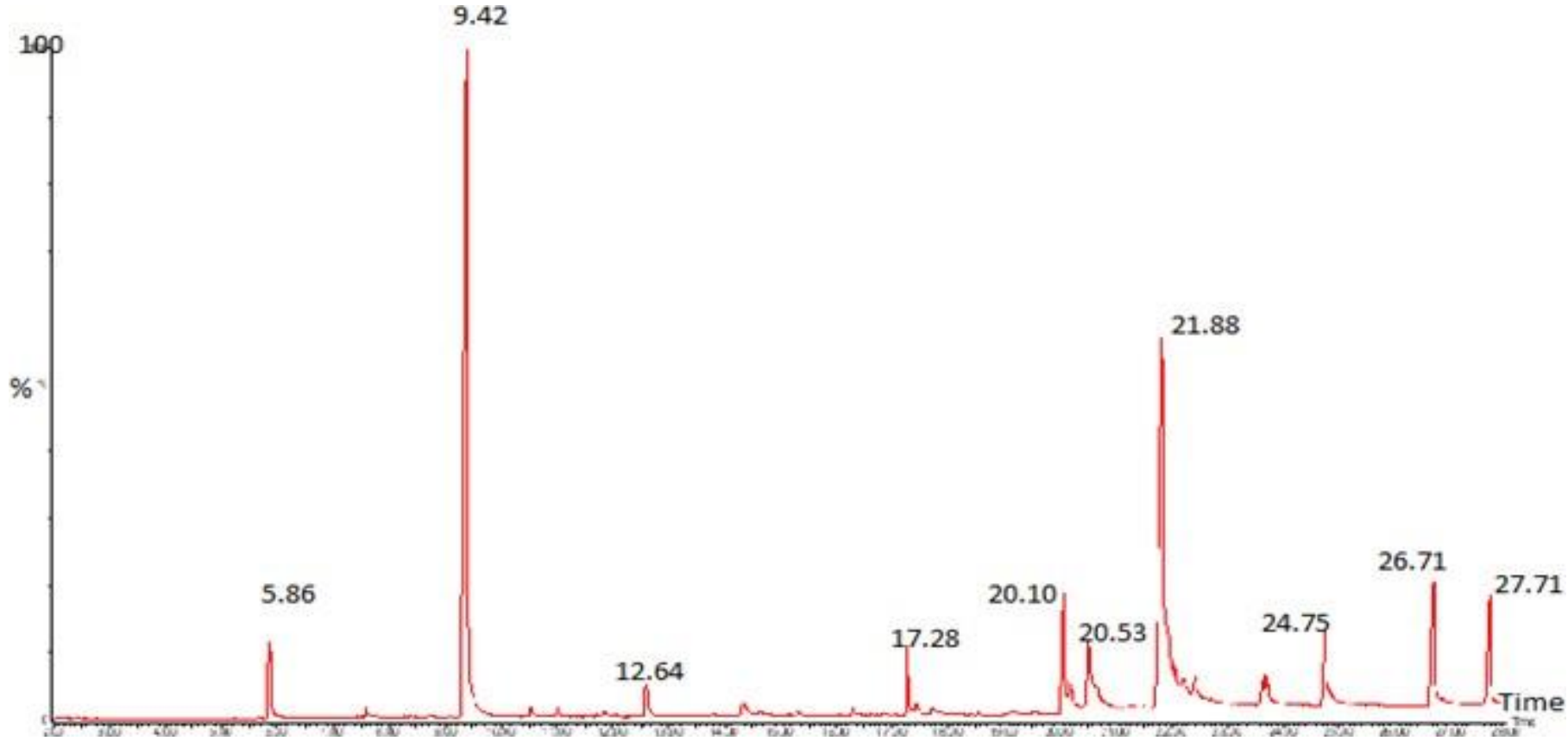


Figure: (3.7) Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of *R. graveolens* leaves. X axis represent time in min, Y axis represent peak intensity (arbitrary unit). Number above each peak represent their respective retention time (Rt) in min.

3.4. Antibacterial Activity

The agar well diffusion method is considered to be the most efficient and sensitive method used in our laboratories to test antibacterial activity. As indicated in Tables **(3.7), (3.8), and (3.9).** the disc diffusion method was used to determine the antibiotic sensitivity pattern by applying standard antibiotic discs to a variety of gram-positive and gram-negative bacterial strains (Valgas et al., 2007).

The gram-positive *Staphylococcus aureus* bacteria as well as Gram - negative *Pseudomonas aeruginosa* and *Proteues mirabilis* bacteria were all showed significant antibacterial effect when treated with the methanolic extract of the examined wild Palestinian plants of (*Ruta graveolens, Hyoscusem aureus,* and *Chiliadenus iphionoides*). The methanolic extract (80%) for the wild plant leaves of *Ruta graveolens* showed strong antibacterial activity against of Gram negative of *Proteues mirabilis* and *Pseudomonas aeruginosa* reaching 68.6 and 80.1activity respectively, and the Gram positive of *Staphylococcus aureus* observed to be highest reaching 89.9%activity in disc diffusion method compared with the positive control. (Vancomycin for gram- positive bacteria and Meropenem for gram- negative bacteria). The methanolic extract for the wild plant leaves of *Chiliadenus iphionoides* showed strong and highest antibacterial activity against Gram–negative *Proteues mirabilis* bacteria reaching 96% activity, followed by Gram -negative bacteria *Pseudomonas aeruginosa* then gram-positive bacteria *Staphylococcus aureus* reaching 70.4% and 21% activity respectively in disc diffusion method compared with the positive control. Whereas, the methanolic extract of the wild plant leaves of *Hyoscusem aureus* showed strongest antibacterial activity against Gram-negative bacteria *Proteues mirabilis* reaching 94%, followed by gram-positive bacteria *Staphylococcus aureus* then Gram -negative bacteria *Pseudomonas aeruginosa* reaching 51% and 60% respectively in disc diffusion method compared with the positive control.

Our findings made it obviously clear that the studied wild Palestinian plants have a significant antibacterial effect, reflecting the antibacterial nature of the phytochemicals present in these ethnobotanical plants, which is supported by their use as a traditional Palestinian folk remedy. These findings also suggested that these plants may represent new sources of medicine for treating various microbial infections (Dowek et al.,2020).

Most probably, the phenolic compounds are the main antimicrobial agents present in these wild studied plants, since their components in the essential oils (EOS) of spices, such as thymol,

carvacrol, and eugenol, have been identified, phenolic compounds are most likely the primary antibacterial agents present in these wild investigated plants (López et al.,2002).

Table (3.7): Antibacterial activity of *Chiliadenus iphionoides* methanolic extract against some bacterial strains. Data were presented as percentage of the positive control (vancomycin).

Bacterial species	Stain	Positive control (mm)	Antibacterial activity(%)*
P. mirabilis	G-	25	96±3.7
P. aeruginosa	G-	25	70.4±2.8
S. aureus	G+	30	50±1.5

*values are mean of replicate determination (n=3) ± standard. G- gram-negative: G+, gram-positive.

Table (3.8): Antibacterial activity of *Ruta graveolens* methanolic extract against some bacterial strains. Data were presented as percentage of the positive control (vancomycin).

Bacterial species	Stain	Positive control (mm)	Antibacterial activity(%)*
P. mirabilis	G-	25	68.6±3.5
P. aeruginosa	G-	25	80.1±4
S. aureus	G+	30	89.9±2

*values are mean of replicate determination (n=3) ± standard deviation. G- gram-negative: G+, gram-positive.

Table (3.9): Antibacterial activity of *Hyoscyamus aureus* methanolic extract against some bacterial strains. Data were presented as percentage of the positive control (vancomycin).

Bacterial species	Stain	Positive control (mm)	Antibacterial activity(%)*
P. mirabilis	G-	25	94.6±3
P. aeruginosa	G-	25	60±3
S. aureus	G+	30	51±2.5

*values are mean of replicate determination (n=3) ± standard deviation. G- gram-negative: G+, gram-positive.

Chapter 4: Discussion

Chapter 4: Discussion

This main goal of this study was to demonstrate the methanolic extract of some WPP and to identify the bioactive compounds present in these plants. The study focused on the following plants: *Ruta graveolens*, *Hyoscusem aureus*, and *Chiliadenus iphionoides*, all of which are widely found in Palestinian territorial habitat. These plants were used frequently by local healers to treat a wide range of illnesses. Numerous investigations, however, revealed that these plants exhibited a range of pharmacological characteristics, such as antioxidant, antibacterial, and antimicrobial activity. (Ali-Shtayeh et al., 2002)

Our research demonstrated the advantages of human prototypes and improved new drugs made from the bioactive substances discovered during this study. *Hyoscusem aureus* and *Ruta graveolens*, for instance, both exhibited beneficial antibacterial activity against a variety of bacterial strains like: *Pseudomonas aeruginosa*, *Proteues mirabilis*, and *Staphylococcus aureus* using the methanolic extract of these plant leaves. This was achieved by using the techniques of agar well diffusion and disc diffusion strategies to examine the antimicrobial effect of *Chiliadenus iphionoides* on both Gram-positive and Gram-negative bacteria.

These wild Palestinian plants used in this study clearly exhibited and harvested from the Tulkarm district in Palestine a pronounce amount of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, free radical scavenging value.

4.1 Qualitative evaluation of the total phenols found in the selected WPP

In the current study, total Phenols of certain WPP were measured and assessed as milligram of Gallic acid per gram of dried plant material (mg GA/g). A high level of the phenols part (mg GA/g) was detected in every extract taken from carefully chosen WPP. The highest amount of powerful reactive secondary metabolites in food are polyphenols. Humans can consume as much as 1g of natural foods each day, which is noticeably higher than the total amount of phytonutrients classified as antioxidants from food. As standpoint, this is 100 times greater than the recommended daily allowance of the antioxidants vitamin E and carotenoids and 10 times more than the consumption of vitamin C,(Manach et al ., 2004; Scalbert & Williamson, 2000).

The negative health impacts of many illnesses, including cardiovascular, pulmonary, and neurological diseases, as well as foraging, are directly linked to oxidative stress. Such adverse impacts were caused by of damaging free radicals destruction of proteins, lipids, and DNA. The combined actions of numerous antioxidants result in protection from these damages (Ranjbar et al., 2006). Total phenols have been shown in Table (3.1), significant quantities in the identifiable estimates of *Hyoscusem auras* leaves, *Ruta graveolens*, and *Chiliadenus iphionoides*. Phenols work physiologically in general as antioxidants. The abundance of significant quantities of phenols in plants showed the resistance of these crops to illnesses (Akintola et al., 2020).

The total phenol content of the identifiable content estimates (*Ruta graveolens, Hyoscusem aurues*, and *Chiliadenus iphionoides*). is shown in Table (3.2), Equation derived from the Gallic acid standard curve was used to quantitatively estimate the methanolic crude extract (n = 4). (R2 = 0.982, Y = 0.0006X). The methanolic extract values of these plants *Ruta graveolens, Hyoscusem aurues*, and *Chiliadenus iphionoides* were (0.74579,0.9803,1.6494), respectively.

4.2 Selected WPPs showed antioxidant activity

Free radical scavenging activity can be measured using a variety of techniques. Because they offer quick and reliable outcomes, $ABTS^{\bullet+}$ and DPPH• free radical scavenging assay have been widely used to assess the antioxidant properties of plant extracts (Parray et al., 2012). Our laboratory results of the selected WPPs methanolic extract (diluted 1:10) showed a pronounced antioxidant capacity using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. Using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, the leaves of three wild Palestinian plants—*Ruta graveolens, Hyoscusem aurues*, and *Chiliadenus iphionoides* showed a marked antioxidant capacity. The mean proportion of scavenging values for the mentioned plants were: 59.49±35%, 44.9 ±23%, and 33.7 ± 26% respectively for $DPPH^{\bullet}$ free radical scavenging assay, whereas, for $ABTS^{\bullet+}$ free radical scavenging assay the antioxidant activity values of these plants were: 61.90±24%, 55.57±41%, and 43.1± 37% respectively. The pronounced antioxidant activity of the selected plants mainly attributed to the presence of phenolic compounds like alkaloids, tannins, flavonoids, and phenols, those containing hydroxyl group capable of scavenging the free radical (Dowek et al., 2020; Stanković et al., 2016). Our findings are in consistent with other study on *Pelargonium*

graveolens essential oil, according to this study the essential oil from *Pelargonium graveolens* displayed strong antioxidant activity (Jaradat et al., 2022).

According to (Hemmami et al., 2023), the amount of total phenols in plants affects the antioxidant capacity to some extent. In contrast to DPPH• assay, the current study discovered a greater relationship among total phenolic content and $ABTS^{\bullet+}$. assay. Based on the decrease of $ABTS^{\bullet+}$. radicals by the examined plant extracts' antioxidants, the capacity of $ABTS^{\bullet+}$ to scavenge free radicals is determined. In addition, the wavelength of absorption at 734 nm in the $ABTS^{\bullet+}$. assay, removes color interference. In accordance with, figure (3.3) while tested by the $ABTS^{\bullet+}$. assay, the antioxidant properties of methanolic extract of leaves from plants showed an extremely potent ability to neutralize free radicals.

The DPPH• regardless of the plant special test, the antioxidant capacity of the plant's methanolic extract using the DPPH• assay showed to be strong but less than $ABTS^{\bullet+}$. These results strongly indicate that the phytochemical composition of these plants has a high capacity for eliminating free radicals as shown in figure (3.2). This could assist to explain why these plants have such respect in Palestinian folklore as both food and medical remedies, and it might additionally render them of great assistance when utilized by the medicine industry (Abdelhalim & Al-Munawarah, 2020).

Furthermore, because there are numerous phenolic compounds present, including flavonoids, tannins, alkaloids, and phenolic compounds with hydroxyl groups that can scavenge free radicals (Dowek et al., 2020; Stanković et al., 2016; Khater & Elashtokhy, 2015).

4.2 Phytochemical screening of the selected WPP

The most valuable present from nature to people is plants. They generate an extensive variety of phytochemicals and are able to generate a wide range of secondary metabolites. The idea that each combination of secondary products in a particular plant is legally distinct is consistent with the medicinal properties of plants that are specific to particular plant species or groups. According to reports, 85 to 90 percent of people worldwide utilize traditional herbal medicines (Diab et al., 2021; Paul et al., 2021; Alkowni et al., 2018).

Complexes that could be used as pharmacologically effective yields come from plants (Barros et al, 2011). The antioxidant activity of flavonols, flavones, and anthocyanin’s spread on the

shifting of oxygen radicals was consistently demonstrated in vitro. The findings of this thesis have advanced our knowledge of the antioxidant compounds found in WPP, such as flavonols, flavones, phenols, and anthocyanin's, our study is well supported by other findings that demonstrated a significant antioxidant activity present in WPPs (Jaenicke & Böhm, 1998). Other research showed an association between the overall phenolic content of the methanolic and water extracts of *C. iphionoides* and its antioxidant activity. Since certain salts present in the plant extract might have an impact regarding the DPPH free radical scavenging activity (Abdelhalim & Al-Munawarah, 2020)

In both humans and animals, alkaloids have a wide range of metabolic and pharmacological effects, including antibacterial, antimalarial, anticancer, and anti-hyperglycemic effects. These traditional Palestinian plants are a valuable source for the pharmaceutical industry due to the presence of alkaloids in them. (Ezeonu & Ejikeme, 2016; Kilari et al., 2015). The high content of flavonoids was additionally discovered in this research; flavonoids show antioxidant activity, prevent the beginning, progress, and development of cancers, and are associated with prevent coronary artery disease, aggregation of platelets, inflammatory processes, and asthma (Paul et al., 2021).

Several chemical compounds have been isolated from Ruta species as a result of phytochemical screening, such as alkaloids, flavonoids, Coumarins, such as bergapten, tannins, volatile oils, glycosides, sterols, and triterpenes, glycosides, flavonoids, and tannins are thought to be effective inhibitors of inflammatory mediator's molecule signaling, which may partially account for the biological properties noticed According to (Coimbra, Ferreira, & Duarte, 2020).

Fortunately, individuals have distinct requirements for antioxidant intake, especially high-risk ones. Evidence suggests that taking supplements of minerals, vitamins, and phenolic compounds is helpful, especially for older people, obese, and individuals with depression (Trujillo et al.,2020), Antioxidants (like vitamins C and E), which may avoid oxidative stress, have had an extended record of use as a treatment for sepsis, acute lung injury, and adverse drug reactions, Consequently, it is thought that antioxidants can also benefit COVID-19 patients According to Soto, (Soto et al.,2020).

4.3 Volatile compounds Identification determined by GC-MS for selected WPP

For accurate determination of phytol-compounds, gas chromatography-mass spectroscopy (GC-MS) is a useful tool. This approach has been used to determine several of for medical purposes essential active components in the extracts of the investigated plant species (Diab et al., 2021).

Each of the examined WPPs contains volatile substances, which are usually unique to their species. Compounds that we found via GC-MS analysis of plants in nature corresponded to the GC-MS analysis and have been identified in multiple research articles for the same kind of plant.

Also, this study indicated the presence of alkaloids, flavonoids, coumarins, cardiac glycosides and many other valuable phytochemicals present in three wild Palestinian plants :(*Chiliadenus iphionoides, Hyoscusem aurues,* and *Ruta graveolens*) was which also considered as main constituents of essential oil component of the wild plant and could be used in multiple pharmacological uses such as: in treating certain neurological disorders like: neurodegeneration and glioblastomas (Colucci et al ., 2020), antibacterial, antitumor, antiulcer, anti-inflammatory, antidiarrheal, anti-mutagenic, preventing cancer and cardiovascular disease (Sovova et al., 2017).

The GC-MS profile of the methanolic extract of this study found to contain multiple volatile compounds. Table 3.6 provides a tentative identification of these compounds. Researchers Nahar and others from the Czech Republic as well as Asgarpanah from Iran have examined volatile compounds found in the leaves of *R. graveolens*. We found some parallels between the volatile compound identities in this investigation and earlier research (Benali et al., 2020; Parray et al., 2012). The majority of these investigations reported nonanone, parvifuran, ethyl piperonyl cyanoacetate, and numerous other volatile compounds, which contradicts our findings. Also, *C. iphionoides* according to table (3.5) plants were utilized to extract the essential oil, which was subsequently evaluated using GC and GC-MS analysis (Abdelhalim & Al-Munawarah, 2020). The main constituents are borneol (49.3%), in addition to its acetyl and formyl esters. In addition, Table 3.4 showed the presence of scopolamine a compound present in *H. aureus* which is used during surgery and or in medication (Sharma et al., 2019). These results clearly indicated that the tested WPPs are rich in various volatile compounds which could be used in pharmaceutical use. However, further research should be conducted to

uncover the exact pharmacological importance, their use in pharmaceutical industry, and their benefits to cure various ailments.

4.4 Antibacterial activity in selected wild Palestinian plants

A significant public health concern is the increase in bacterial infections around the world (Khan et al., 2013). However, different methanolic extract concentrations have been evaluated for their antibacterial activities toward some gram-positive (Staphylococcus a*ureus*) and gram-negative (*Proteues mirabilis and Pseudomonas aeruginosa*) bacterial strains. Hospital-acquired infections attributed to microbes that are resistant to drugs have increased over the past few decades, which has increased use of disinfectants. Due to their easy access to medications, antibacterial therapeutic plants, especially those against Gram-positive strains, are more effective against these bacteria as shown in Tables (3.7), (3.8), and (3.9).

The studied wild Palestinian plants, showed significant antibacterial activity against the tested bacterial strains (*Pseudomonas aeruginosa* and *Proteues mirabilis, and Staphylococcus aureus*). It is believed that there are secondary substances have effective antimicrobial activity against both Gram-positive and Gram-negative bacteria may be present in these plants. More investigation is needed to determine the compounds at fault. Studies around the world have demonstrated that these plants have potency against bacteria and do not harm cells, and that the water that comes from them is a safe source that is resistant to anti-oxidants and preservatives This is the potency of these plants as antibacterial, and it serves as an alternative to preservatives made of chemicals in medicinal products and food chemical preparations (Sbieh et al., 2022). Also, a recent study showed that the most effective antibacterial pathogen is the solvent methanol (Azalework et al., 2017). And in *H. aurues* is very strong against of pesticide and organism (Atalla & Dardona, 2023). Essential oils as a strong antioxidant may eliminate bacteria that cause plant diseases or repel insects in particular, because of their unpleasant odor. Additionally, some researchers believed that essential oils play a key role in biological communication, or the transfer of messages within plants. Some volatile oils have uses for biological disinfection. These plants are derived from an organic source, so it is essential to know how much of an effect they have on bacteria. The kind of disinfectant used depends on the degree of its saturation with air molecules, and its boiling points.

Overall, using both Gram positive and Gram negative bacterial strains, the antibacterial activity of methanolic extract (80%) of the wild Palestinian plants (*Chiliadenus iphionoides,*

Hyoscusem aurues, and *Ruta graveolens*) was displayed in tables (3.7), (3.8), and (3.9) respectively. (*Ruta graveolens, Hyoscusem aurues,* and *Chiliadenus iphionoides) methanolic* extracts demonstrated significant antibacterial activity against Gram-negative *P. mirabilis and P. aeruginosa*, reaching (96%±3.7,68.6%±3.5,94.6%±3) and (70.4%±2.8, 60%±3, 80.1%±4) activity, respectively, in the disc diffusion method compared to the positive control. Using a well and disc, the zone inhibition was measured and expressed as a percentage of the positive control, which was vancomycin for gram positive bacteria and Our results showed that the investigated wild plant *Ruta graveolens* leaves showed potential antimicrobial activities against Gram Negative *P. mirabilis*, and Gram Positive *S. aureus*, and the zone of inhibition recorded was more than half of that recorded for positive control antibiotics (Meropenem and vancomycin respectively).

Overall, the antibacterial properties of the 80% methanolic extract of the studied plants results are in accordance with earlier research conducted by our pharmacy laboratory, which demonstrated the strong antibacterial activity of wild Palestinian plants like Malva sylvestris (Dowek et al., 2020), as well as additional research conducted by (Salman et al., 2018) on *R. graveolens*. This study also in accordance with another study carried by Salman et al on *R. graveolens* against strains of the bacteria *Streptococcus mutans* and *Streptococcus sobrinus* (Salman et al., 2018). Additionally, studies show that *C. iphionoides* works against a variety of bacteria, including *MRSA, E. Coli,* and *Enterococci (Faecium*). (Sbieh et al., 2022). Also, many studies showed *Hyoscyamus auerues* exhibited antimicrobial activity against *Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas stutzeri.* (Atalla & Dardona, 2023)

Proteues mirabilis has a strong connection to rheumatoid arthritis and is able to analyze peptides that are resistant to bacteria. Showed significance of these plants as antibiotics. *Pseudomonas aeruginosa* is attack on the immune system is an additional feature that makes modern medicine incapable of curing it. It is thought to be extremely dangerous and can even cause death two days after taking antibiotics, although (*R. graveolens and C. iphionoides*) may avoid infection in 80.1% and 70.4% of cases, respectively.

These findings are in consistent with previous work in our pharmacy laboratory on wild Palestinian plants (Dowek et al., 2020).

Conclusion:

Based on the results achieved in this study, it was revealed that laboratory investigation of the methanolic extract of the selected wild Palestinian plants: *Ruta gravenoles, Hyoscusem aurues and Chiliadenus iphionoides* harvested from territorial mountains of Palestine exhibit the following valuable effects:

1. The three studied wild plants have high antioxidant activities, this is probably due to high content of phenolic compounds flavonoids, glycosides, phenolic group, alkaloid, saponins, steroids, tannins and terpenoids, in addition these compounds exhibit anti-aging activity, anticancer, antioxidant, antibacterial, and anti-inflammatory activity.

2. Phytochemical screening of the studied plants clearly indicated the presence of various secondary metabolites present in the methanolic extract of plant leaves like; cardiac glycosides, glycosides, phenolic group, saponins, steroids, tannins and terpenoids detected by various phytochemical screening tests and GC-MS analysis.

3. The antimicrobial studies of the plant leaves showed remarkable antimicrobial activity against some gram-negative and gram-positive bacterial strains, suggesting that these Palestinian folkloric medicinal plants possess broad-spectrum antibacterial activity.

4. This study provided enough background information for additional research and the identification of WPP. It has also aided in the exploration of the medicinal values and creation of a database of the medicinal plants that are available in Palestinian pharmacopeia.

5. Our wild Palestinian plants have an interesting zone inhibition against Gram +ve bacteria. These results need further t investigation to enhance pharmacological industry.

Recommendation:

The followings are some suggestions that may be taken into consideration for future investigation:

- The current research used the methanolic solvent in the plant. It is recommended to examine the components using different solvents with the plant to note the differences with the application of the same protocols and compare the results.

- Also the current research used plant leaves. It is recommended to use another study parts to known more details and information.

- Work on more tests on these plants, for example, testing the anti-lipase, anti-amylase anti-cancer and antifungal activities.

- As for the antioxidant test, it is recommended to do several tests, such as IC50, Trolox equivalent antioxidant capacity (TEAC) assay, the ferric reducing ability of plasma (FRAP) assay, and the copper reduction (CUPRAC) assay. Moreover, compare them with the results we obtained with ABTS● & DPPH• antioxidant activity assays, and measure the total oxidant scavenging capacity (TOSC).

- It is recommended to raise the temperature, increase the separation period of vehicles inside the GC-MS, and monitor the exit of new compounds if they appear.

- It is recommended to use the headspace to separate the volatile compounds and compare them with the results we got in this study.

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Appendix 1: Published Article

Biochemical, GC-MS Analysis, antioxidant, and Phytochemical Screening of the Wild Palestinian Plant Ruta graveolens

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Biochemical, GC-MS Analysis, antioxidant, and Phytochemical Screening of the Wild Palestinian Plant *Ruta graveolens*

Mohannad Jazzar, Inas Abu Eid, Abdel Qader Qawasmeh, Seema Fallah
Hebron University
mohannadj@hebron.edu, abedq@hebron.edu, seemaf@hebron.edu,

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Abstract:

Ruta graveolens is a wild plant commonly grown in the mountains of Palestine that belongs to *Rutaceae* family and widely used in the traditional Palestinian

Introduction:

Palestine is located in the middle of Mediterranean basin and its moderate climate all over the year enhances the growth of diverse flora. (Ali-Shtayeh et al., 2015; Jaradat et al., 2017; Saad et al., 2005). Wide range of edible and medicinal plants are often used in Palestinian folkloric culture to treat human ailments and used in daily food consumption. The wild plants in Palestinian territories are widely used by Palestinian local healers since the past, especially in rural areas to treat various ailments like: headache, diarrhea, constipation, diabetes mellitus, cough, cold, and many others ailments (Ali-Shtayeh et al., 2016; Saad et al., 2005).The focus on the wild plants use in medicine and food consumption is due to the presence of various pharmacological properties of bioactive compounds produced by these plants, for example wild plants act as: antioxidant (Salama et al., 2020), antibacterial (Stanković et al., 2016), anticancer (Jaradat et al., 2017), and antispasmodic (Qabaja et al., 2013).

Phytochemicals are identified as active compounds produced from plants and exhibit important medicinal activities. Cardiac glycosides (digoxin and digitalis) are phytoestrogens used to treat heart failure, and supraventricular arrhythmias (atrial fibrillation/flutter) (Karasneh et al., 2017). They were also recommended for the prophylaxis and treatment of some arrhythmias, such as paroxysmal atrial tachycardia and cardiogenic shock (Schneider et al., 2017).

Ruta graveolens is a small shrub plant belongs to the *Rutaceae* family, the plant in common is called as rue or herb of grace, in English, and called as sudab, sadab, or (فيجن Faijan) in Arabic (Jaradat, 2016; Reddy & Al-Rajab, 2016). Concerning the plant habitat, this plant is growing in the hills and mountains of Palestine, and in many parts of the world like: Egypt, Jordan, Iraq, and southern Europe. This plant is growing on the waste stony ground places. The plant is rich in essential oils and many important bioactive compounds with important medicinal activities like: abortifacient, diuretic, emmenagogue stimulant, antiulcer, epilepsy, skin inflammation, headache, anti-diarrhetic, anti-rheumatism, vermicide, and anti-diabetic. Moreover, the plant is used in pharmacological trials like: anthelminthic, abortive, anti- parasitic, anti-inflammatory, anti-diarrhetic, anti-rheumatic, antifebrile, antiulcer, vermicide repellent, anti-diabetic, anti-rheumatism, and antimicrobial activities.(Di Saverio et al., 2020; Noori et al., 2019).

Other studies showed that the plant is used in vitiligo to enhance melanin production and other active compounds such as: coumarins, alkaloids, volatile oils, flavonoids and phenolic compound mainly for vitiligo treatment (Al Qaisi et al., 2022). In traditional medicine the plant is used in different forms for example: nasal drop, suppository, powder seed, orally and as a decoction (Parray et al., 2012). In a recent study (Colucci-D'Amato & Cimaglia, 2020) carried out on zebrafish, a genetic model animal, it has been shown that *R. graveolens* administration results in a decline in eggs exhibition and fertilization due to a disorder of gonadal or thyroid hormones.

Biochemical, GC-MS Analysis, antioxidant, and Phytochemical Screening of the Wild Palestinian Plant *Ruta graveolens*

Mohannad Jazzar[1], Inas Abu Eid[2], Abdel Qader Qawasmeh[3], Seema Fallah[3],
[1]College of Science and Technology, [2]College of Graduate Studies, [3]College of Pharmacy and medical Sciences
Corresponding Author: Mohannad Jazzar. mohannadj@hebron.edu

Abstract:

Ruta graveolens is a wild plant commonly grown in the mountains of Palestine belongs to *Rutaceae* family and widely used in the traditional Palestinian medicine. The aim of this investigation is to study the biochemical, antioxidant, antibacterial, and GC-MS analysis for *Ruta graveolens* plant. The antibacterial activity of the methanolic extract (80%) of *R. graveolens* leaves was conducted by well diffusion method. The antioxidant activity was performed using both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging assays. Phytochemical screening and gas chromatography-mass spectroscopy (GC-MS) analysis were done following standard protocols. Methanolic extract (80%) of *R. graveolens* leaves showed pronounced antibacterial activity against Gram negative *Proteus mirabilis* and *Pseudomonas aeruginosa* bacterial strains reaching 68.6% ± 3.5 and 80.1% ± 4 activity respectively, and against Gram positive *Staphylococcus aureus* reaching 89.9% ± 2 activity in well diffusion method compared with the positive control. Methanolic extract of *R. graveolens* also demonstrated a pronounced scavenging capacities of 60.90% ± 2.4 and 59.49% ± 3.5 activity using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging assays respectively. Phytochemical screening and GC-MS assays of *R. graveolens* leaves revealed the presence of a variety of wide range of phytochemical compounds such as cardiac glycosides, coumarins, phenolic groups, flavonoids, alkaloids, steroids, and Phlobatannins. The present study of the methanolic extract of *R. graveolens* leaves evidently confirmed the presence of a variety of phytochemical compounds such as cardiac glycosides, coumarins, alkaloids, and other phytochemicals in this wild plant that could open further valuable research to investigate its pharmacological benefits.

Keywords: *Ruta graveolnes*, $ABTS^{\bullet+}$, Antibacterial bioactive compounds, GC-MS analysis.

Materials and methods

Plant material of *Ruta graveolens*

Ruta graveolens leaves were collected in the month of July 2021 from Tulkarm district (Latitude: 32°18'37" N. Longitude: 35°01'43" E. Elevation above sea level: 117 m = 383 ft).

Botanically, the plant identification was carried out by referring to prof. Nidal Jaradat from Al Najah university as *R. graveolens* with a voucher specimen number Pharm-PCT 2084, then was preserved for phytochemical analysis at the pharmacological laboratories at Hebron university, and it was confirmed that it conforms to the specifications of scientific research. Then the plant was cleaned, shade-dried at room temperature, grounded to powder and preserved in container at appropriate room/lab conditions for further biochemical investigation.

Ruta graveolens Extract Preparation

The wild plant leaves of *R. graveolens* were prepared as described by Qawasmeh et al., protocol, in which 200 mg of grounded leaves of *R. graveolens* were extracted using 4 ml methanol 80% using a shaker (Labtech, Korea) for 24h at 80 rpm, at 25 °C (Qawasmeh et al 2012). A 1.5 ml plant extract transferred to Eppendorf tubes, then centrifuged down for 5 minutes at 4000 rpm using Micro Cl 17, Thermoscientific Germany. The resulting supernatants were transferred into clean Eppendorf tubes and used for antibacterial, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, phytochemical screening and GC-MS assays.

Antibacterial Activity

Bacterial samples

The bacterial samples were obtained from the Microbiology laboratory, Hebron university. Three pathogenic bacterial strains were used in this study, two strains are Gram negative *Proteus mirabilis* and *Pseudomonas aeruginosa,* and one is Gram positive *Staphylococcus aureus* bacteria, all strains were cultured on nutrient agar and incubated at 37 °C for 24 hours (Heratherm incubator, thermos Scientific, Germany) authenticated by secondary culture of the archived reference samples of isolates. Following incubation, all the cultured plates of all bacterial strains were preserved in a refrigerator at 4 °C to be used for investigation.

Media preparation

The media used in this study were as the following: Eosin methylene blue (EMB), Mannitol salt agar (MSA), (all from HiMedia Laboratories, India), nutrient agar, (NA, BioMaxima, Poland). The media were prepared according to the manufacturer instruction. The prepared media were autoclaved at 121 °C for 15 minutes (Labtech, Korea). All sterile differential media were poured into sterile Petri dishes (90 x 16 mm) and kept in a refrigerator at 4 °C until needed.

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Bacterial cultures and subcultures

The bacterial samples used in this study were sub cultured on nutrient agar plates, incubated at 37 °C for 24 hours. Further subculturing for the grown bacteria in differential media as the following: *P. aeruginosa* on nutrient agar, *S. aureus* on MSA, and *P. mirabilis* on EMB. Then all the media plates were incubated at 37 °C for 24hours.

Antimicrobial activity evaluation of plant extracts using agar well diffusion method

For bacterial samples used, the sensitivity testing was performed using well diffusion method on Muller Hinton Agar plates as described by Dowek et al. The bacterial suspensions were prepared to a density of 0.5 McFarland units (equivalent to $15*10^8$ CFUs/ml) from 18-24 hr old colonies of bacteria in saline solution. Then the bacterial suspensions were spread on Mueller Hinton agar plates by a sterile cotton swab. Following this, four holes in each plate were made in which 10 µl extract was added on to each of the first three holes, the fourth hole was used as a negative control (methanol). In this study, the positive control disks used were Meropenem (10 µg BioMaxima, Poland) for *P. mirabilis* and *P. aeruginosa,* while Vancomycin (30 µg BioMaxima, Poland) used for *S. aureus*, methanol was the negative control. The inhibition zone of the positive controls of the studied wild plant *R. graveolens*

extracts were measured (mm) after 24 hours incubation at 37 °C, results were expressed as a percentage (%) of the positive control, (Dowek et al., 2020).

Antioxidant Activity

Diphenyl-1-picrylhydrazyl (DPPH•) assay

Ruta graveolens methanolic leaves extract was tested for its ability to scavenge free radicals using the free radical 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH•), a molecule contains a stable free radical (Sharma & Bhat, 2009), DPPH• scavenging capacity as described by (Barros et al., 2011; Sharma & Bhat, 2009) with minor modification.

A stock solution was prepared by dissolving 2.3 mg of DPPH• (Sigma Aldrich-STBD4146V) with 5.57 mL of methanol 80%. A 200 µl of DPPH• stock solution was mixed with 2 ml 80% methanol and 20 µl of diluted plant extract (1:5, Sample) or 20 µl of methanol (80%, control) in plastic cuvettes. All the cuvettes were mixed by vortex and incubated in a dark at room temperature for 1h. The absorbance's of plant extracts (A sample) and the methanol (A control) were measured at 734 nm using a Genway UV/Visible spectrophotometer (Cole-Parmer Ltd UK) at 517 nm (Dowek et al., 2020). The radical scavenging activity was calculated as a percentage of DPPH• discoloration using the following equation:

$$\text{DPPH• Scavenging (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100\%$$

ABTS•+ Assay

ABTS•+ stock solution was prepared following the protocol described by (Qawasmeh *et al.*, 2012) with minor modification, in which 18 mg of ABTS•+ (Sigma Aldrich, Palestine) dissolved in 5 mL distilled water to get a final concentration of 7 mmol. An aliquot (88 µl) of potassium persulfate solution (2.45 mmol) was added to ABTS•+ solution. The mixture was incubated in the dark overnight before use. The working solution of ABTS•+ was prepared by diluting a stock solution of ABTS•+ with methanol (80%) to final absorbance of 0.7000 ± 0.02 at 734 nm. A 30 µl of diluted plant extracts (1:5) solutions were mixed with 3 ml ABTS•+ working solution in micro cuvettes. For control, 30 µl methanol's' (80%) were mixed. All cuvettes were mixed by a vortex (Lab net international Inc. U.S.A) and incubated in a dark for 30 min at room temperature. The absorbance's of *R. graveolnes* extracts (A sample) and the methanol (A control) were measured at 734 nm using the Genway UV/Visible spectrophotometer (Cole-Parmer Ltd UK) (Joseph et al 2020., Cao et al 2019). The percentage scavenging of ABTS•+ was calculated according to the equation:

$$\text{ABTS•+ Scavenging (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100\%$$

Results and Discussion

Palestine geographically is considered as one of the global biodiversity hot spots as it lies at the crossroads between three continents of Asia, Africa and Europe that makes it rich in various ethnobotanical plant species of biodiversity (Al-Salem & Lettieri, 2010). Many studies carried out in Palestine on various medicinal plants that clearly showed that these plants have valuable antioxidant and antibacterial activity beside their nutritive and pharmaceutical value (Eid et al., 2022; Qabaja et al., 2013).

Antibacterial activity

Methanolic extract of *R. graveolens* showed pronounced antibacterial activity against Gram negative *P. mirabilis* and *P. aeruginosa* reaching 68.6% ± 3.5 and 80.1% ± 4 activity respectively (Table 1), and the Gram positive of *S. aureus* reaching 89.9% activity using well diffusion method compared with the positive control (Table 1). Our results on crude methanolic extracts strongly indicated that *R. graveolens* leaves showed pronounced antibacterial activities against both Gram negative and Gram positive bacterial strains tested in this study, and the zone of inhibition recorded was more than half of that recorded for positive control antibiotics (meropenem and vancomycin respectively). These findings are in consistent with previous work of our pharmacy laboratory showing that the Wild Palestinian plants like *Malva sylvestris* possess a pronounced antibacterial activity (Dowek et al., 2020), and other work carried by Salman et al on *R. graveolens*, this study evidently proved that the methanolic extract of *R. graveolens* contains significant antibacterial activity against *Streptococcus mutans* and *Streptococcus sobrinus* bacterial strains (Salman et al., 2018).

Table 1: Antibacterial activity of *Ruta graveolens* methanolic extract against some bacterial strains. Data were presented as percentage of the positive control (vancomycin).

Bacterial species	stain	Positive control (mm)	Antibacterial activity (%)*
P. mirabilis	G-	25	68.6±3.5
P. aeruginosa	G-	25	80.1±4
S. aureus	G+	30	89.9±2

*Values are mean of replicate determination (n=3) ± standard deviation. G-: Gram negative, G+: Gram positive.

Antioxidant activity

Our laboratory results of *R. graveolens* methanolic extract (diluted 1:5) showed a pronounced antioxidant capacity using DPPH$^{\bullet}$ and ABTS$^{\bullet+}$. The average percentage of scavenging was 61.90 ± 24% and 59.49 ± 35% for ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ respectively. The pronounced antioxidant activity of *R. graveolens* mainly attributed to the presence of phenolic compounds like alkaloids, tannins, flavonoids, and phenols, those containing hydroxyl group capable of scavenging the free radical (Dowek et al., 2020; Stanković et al., 2016). Our findings are in consistent with other study on *Pelargonium graveolens* essential oil, according to this study the essential oil from *Pelargonium graveolens* displayed strong antioxidant activity (Jaradat et al 2022).

Quantitative estimation of total phenols in *Ruta graveolens*

Total phenol content in *R. graveolens* leaves methanolic crude extract (n = 4) was estimated quantitatively based on the equation established from gallic acid standard curve (Y=0.0006X, R^2 = 0.982). The average A values for the methanolic extract was 0.7595 which corresponds to 1.265 ± 0.043mg GAE/g.

Phytochemical and GC–MS Screening

The phytochemical screening for the methanolic extract of *R. graveolens* leaves are shown in Table 2. The methanolic extract of *R. graveolens* revealed the presence of a wide range of phytochemical compounds such as alkaloids, phenols, coumarins, flavonoids, cardiac glycosides and many others as indicated in Table 2. These findings are in consistent with other studies performed on different Ruta species (Szewczyk et al., 2022). According to these results the phytochemical analysis of *R. graveolens* leaves revealed presence of phenolic compounds and alkaloid compounds, rutin, quercetin, furocoumarin lemonins and flavonoids. These compounds proved pharmaceutically to be effective in treating arthritis, inhibiting oxidative stress, and fighting worse diseases like cancer (Jaradat et al., 2022; Szewczyk et al., 2022).

The GC–MS analysis was performed for the methanolic extract of *R. graveolens* and the analysis revealed the presence of many compounds as shown in Table 3. Whereas, Figure 1 represents the GC–MS total ion mass chromatograms for the volatile compounds detected in the methanolic extract of *R. graveolens* leaves.

The GC-MS profile of the methanolic extract of *R. graveolens* of this study obviously confirmed the presence of several volatile compounds. These compounds were tentatively identified as described in Table 3. Volatile compounds in *R. graveolens* leaves have been studied by Nahar and others from Czech Republic and by Asgarpanah and Khoshkam from Iran. We observed some similarities in the identity of the volatile compounds of this study and previous studies (Nahar et al 2021, Asgarpanah and Khoshkam 2012). 2- Nonanone,

scavenging) activity to flavonoids. Quercetin is one of the greatest common native flavonoids occurring mostly in glycosidic forms for example rutin (Sovova et al., 2017). The number of phytochemical Compounds is very important for promoting health such as: furacridone, coumarins, isorutarin, rutacultin, suberenone, Saponins, tannins, glycosides, chalppensins, furanocoumarins, 5-methoxypsoralen (bergapten), acidone, alkaloids, coumarins, essential oils and furoquinolines and 8-methoxypsoralen (Szewczyk et al 2022., Shahrajabian 2022).

Table 2: phytochemical screening for the methanolic extract of *R. graveolens* Leaves

Type	*R. graveolens*
Phenolic group	+ve
Phlobatannins	+ve
Alkaloid	+ve
Anthraquinones	-ve
Anthocyanin	-ve
Cardiac glycosides	+ve
Coumarins	+ve
Flavonoids	+ve
Glycosides	+ve
Quinones	-ve
Saponins	-ve
Steroids	+ve

Table 3: List of major compounds detected in the methanolic extracts from *R. graveolens* leaves with their retention times (Rt), molecular masses (M/Z), molecular weight (MW), and molecular formula (MF).

Rt	M/Z	Compound identification	MW	MF
5.87	71,59, 58, 57	Nonanone	142	$C_9H_{18}O$
9.42	71,59,58,57	Tridecanone	198	$C_{13}H_{26}O$
12.64	71,59,58,57	Pentadecanone	226	$C_{15}H_{30}$
17.28	232,136,135,131	3 Methyl,4 piperonyl-5 Isoxazalone	233	$C_{12}H_{11}O_4N$
20.09	248,148,136,135	Ethyl piperonyl cyanoacetate	247	$C_{13}H_{13}O_4N$
20.53	216,201,188,173,145	1,4 naphtholinol dione, 2 acetyl, 3 hydroxy	216	$C_{12}H_8O_4$
21.86	254,239,211,199,135	Isopravifuran	254	$C_{16}H_{14}O_3$
24.75	260,259, 244, 201	1-Carbethoxy,3-Acetyl-4(H)-Quinolizine 4-One	259	$C_{14}H_{13}O_4N$
26.68	314,299,281,255	Hexahydrophenanthren derivative	314	$C_{19}H_{22}O_4$
27.70	296,281,253,241	Phenantofuran dione derivative	296	$C_{19}H_{20}O_3$

scavenging) activity to flavonoids. Quercetin is one of the greatest common native flavonoids occurring mostly in glycosidic forms for example rutin (Sovova et al., 2017). The number of phytochemical Compounds is very important for promoting health such as: furacridone, coumarins, isorutarin, rutacultin, suberenone, Saponins, tannins, glycosides, chalppensins, furanocoumarins, 5-methoxypsoralen (bergapten), acidone, alkaloids, coumarins, essential oils and furoquinolines and 8-methoxypsoralen (Szewczyk et al 2022., Shahrajabian 2022).

Table 2: phytochemical screening for the methanolic extract of *R. graveolens* Leaves

Type	*R. graveolens*
Phenolic group	+ve
Phlobatannins	+ve
Alkaloid	+ve
Anthraquinones	-ve
Anthocyanin	-ve
Cardiac glycosides	+ve
Coumarins	+ve
Flavonoids	+ve
Glycosides	+ve
Quinones	-ve
Saponins	-ve
Steroids	+ve

Table 3: List of major compounds detected in the methanolic extracts from *R. graveolens* leaves with their retention times (Rt), molecular masses (M/Z), molecular weight (MW), and molecular formula (MF).

Rt	M/Z	Compound identification	MW	MF
5.87	71,59, 58, 57	Nonanone	142	$C_9H_{18}O$
9.42	71,59,58,57	Tridecanone	198	$C_{13}H_{26}O$
12.64	71,59,58,57	Pentadecanone	226	$C_{15}H_{30}$
17.28	232,136,135,131	3 Methyl,4 piperonyl-5 Isoxazalone	233	$C_{12}H_{11}O_4N$
20.09	248,148,136,135	Ethyl piperonyl cyanoacetate	247	$C_{13}H_{13}O_4N$
20.53	216,201,188,173,145	1,4 naphtholinol dione, 2 acetyl, 3 hydroxy	216	$C_{12}H_8O_4$
21.86	254,239,211,199,135	Isopravifuran	254	$C_{16}H_{14}O_3$
24.75	260,259, 244, 201	1-Carbethoxy,3-Acetyl-4(H)-Quinolizine 4-One	259	$C_{14}H_{13}O_4N$
26.68	314,299,281,255	Hexahydrophenanthren derivative	314	$C_{19}H_{22}O_4$
27.70	296,281,253,241	Phenantofuran dione derivative	296	$C_{19}H_{20}O_3$

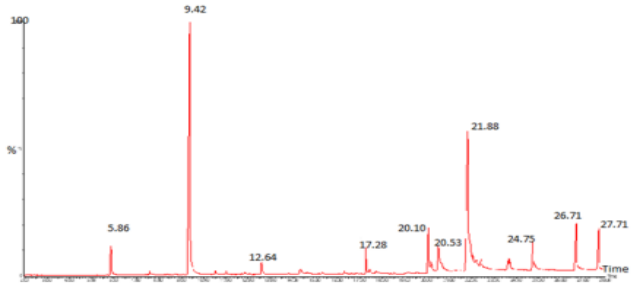

Figure 1: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of *R. graveolens* leaves. X axis represent time in min, Y axis represent peak intensity (arbitrary unit). Number above each peak represent their respective retention time (Rt) in min

Conclusion:

The present study on the wild Palestinian plant *R. graveolens* clearly showed that this plant has a potential antibacterial activity against all studied bacterial strains, in addition this plant showed an effective antioxidant activity. The phytochemical screening and GC-MS analysis revealed the presence of many phytochemical compounds such as coumarins, flavonoids, cardiac glycosides and alkaloids in *R. graveolens* leaves extract. which could be valuable for pharmacological testing. The presence of a pronounced amount of variable phytochemical groups in *R. graveolens* could open the doors widely in the field of pharmacological industry and ethnomedicinal treatments, since such phytochemicals are considered as a main source of antimicrobial, antioxidant, and anti-inflammatory agents. Further studies should be conducted on Ruta species to explore various pharmacological activities hidden in this precious wild Palestinian plant.

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AUTHORS CONTRIBUTION

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CONFLICT OF INTEREST

This statement is to declare that all authors involved in manuscript have no conflict of interest.