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Biochemical Studies on Some Palestinian Medical Plants

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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…….....….

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30/ 5/ 2022

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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List of abbreviations

WPP	Wild Palestinian plant
WHO	World Health Organization
T.B	Tuberculosis
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-performance liquid 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
M. sylvestris	*Malva sylvestris*
M. fruticosa	*Micromeria fruticosa*
A. palaestinum	*Arum palaestinum*
E. alata	*Ephedra alata*
A. officinalis	*Asparagus officinalis*
U. urens	*Urtica urens*
S. pneumoniae	*Streptococcus pneumoniae*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
P. aeruginosa	*Pseudomonas aeruginosa*
K. pneumoniae	*Klebsiella pneumoniae*
S. cearevisie	*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
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
TIC	total-ion mass chromatograms
COVID-19	Coronavirus disease
MD	Mediterranean diet
TEAC	Trolox equivalent antioxidant capacity
FRAP	Ferric reducing ability of plasma
CUPRAC	copper reduction
EOs	essential oils
TOSC	total oxidant scavenging capacity

Abstract

Malva sylvistris, *Urtica urens*, *Micromeria fruticosa*, *Asparagus officinalis*, *Ephedra alata*, and *Arum palaestinum* plants are widely used in Palestinian folkloric culture as food and as a remedy for curing different ailments. Methanolic extracts of these plants' leaves were evaluated for their antibacterial, and antioxidant activities, and were screened for the presence of major secondary metabolites, and volatile compounds using GC-MS analysis. The antibacterial analysis using the agar well diffusion method showed that the methanolic extract of *U. urens*, *E. alata*, and *M sylvistris* leaves produced 47.8%, 47.44%, and 47.2% mm inhibition zone against *K. pneumonia, and S. aureus* respectively. Methanolic extracts of *E. Alata*, *A. palaestinum*, and *A. officinalis* leaves showed a 96.19%, 95.94%, and 93.31% scavenging capacity using $ABTS^{\bullet+}$ assay. Using $DPPH^{\bullet}$ assay, the plant of *E. Alata, A. Palestinian*, and *M. fruticosa* showed 89.26%, 62.94%, and 53.04% scavenging capacity respectively. Phytochemical analysis of the plants revealed the presence of major phytochemical compounds such as saponins, coumarins, cardiac glycosides, steroids, tannins, quinones, and terpenoids. GC-MS analyses of *M. sylvistris*, *U. urens*, and *M. fruticosa* leaves showed the presence of 47, 35, and 30 peaks respectively. Major volatile compounds detected in *M. sylvistris* include vitamin A aldehyde, transbenzyl cyclohexanol, oxirane, tridecandial, and phytol, in *U. urens* include menthol, and phytol, in *M. fruticosa* include menthol, pulegone, and thymol. The findings of this study indicate the presence of promising antioxidant activity of the plants and potential antibacterial activity. This study was the first to screen and evaluate phytochemical compounds in some wild Palestinian plants for their antibacterial and antioxidant activity.

الملخص

إن استخدام النباتات البرية في فلسطين شائع بشكل كبير ومتداول بين الناس، ومن بين هذه النباتات البرية الخبيزة، والقريص، والهليون، واللّوف، والزعتر بلاط، والعلندة. إن استخدام النباتات البرية في فلسطين مرتبط بتقاليد متوارثة عن الأجداد كغذاء وكعلاج الأمراض المختلفة أكثر من الاعتماد على الأبحاث والأسس العلمية. تم قياس مضادات البكتيريا ومضادات الاكسدة والبحث عن وجود الوسائط الأيضية الرئيسية وأيضا المركبات المتطايرة بوساطة جهاز كروماتوغرافيا الغاز للمستخلص الميثانولي لتلك النباتات. تم باختبار المضاد للبكتيريا بطريقة الانتشار على أقراص الآجار (Agar Disc Diffusion Method) وأظهرت النتائج للمستخلص الميثانولي. إن نبات القريص والعلندة والخبيزة كانت لهم أعلى القيم في تثبيط النشاط البكتيري (47.80% و 47.44% و 47.2%) ملم في حالة كليبسيلا رئوية , ومكورات عنقودية ذهبية على التوالي, وعند دراسة التأثير المضاد للأكسدة من المستخلص الميثانولي لأوراق تلك النباتات بوساطة اختبار $ABTS^{+\bullet}$ تم الحصول على أعلى قيم لدى العلندة و اللّوف والهليون بقيم 96.19% و95.94% و 93.31% على التوالي , وبوساطة اختبار DPPH• تم الحصول على أعلى قيم لنبات العلندة واللّوف والزعتر بلاط بقيم 89.26% و 62.94% و 53.04% على التوالي , وكشف أيضا التحليل الكيميائي للنباتات عن وجود مركبات كيميائية نباتية رئيسية مثل الصابونين والكومارين وجليكوسيدات القلب والستيرويدات والعفص والكينون والتربينويدات, و بجهاز كروماتوغرافيا الغاز تم تحديد هوية أربعة وأربعين مركباً أساسياً متطايراً من نبات الخبيزة و ثلاثة وثلاثين مركباً في نبات القريص و تسعة وعشرين مركباً في نبات زعتر بلاط و أربعة وعشرين مركباً في نبات الهليون و ثمانية عشر مركباً في نبات العلندة وخمسة عشر مركباً في نبات اللّوف وتم رصد المركبات المتطايرة الأساسية في الخبيزة والتي تتضمن: vitamin A aldehyde و transbenzyl cyclohexanol و oxiraneو tridecandial و mannitol و phytol وبنبات القريص تم تحديد بعض المركبات المتطايرة ومنها: menthol وphytol وبنبات الزعتر بلاط menthol وpulegone وthymol . تشير نتائج هذه الدراسة إلى وجود نشاط واعد لمضادات الأكسدة والجراثيم للنباتات المذكورة. هذه الدراسة هي الأولى من نوعها لفحص وتقييم المركبات الكيميائية النباتية في بعض النباتات البرية الفلسطينية من حيث نشاطها المضاد للبكتيريا ومضادات الأكسدة.

Chapter 1: Introduction

1.1 Introduction

The use of plants by humans as a source of remedies to treat and prevent diseases has increased over the recent years. It has been estimated by the WHO that approximately 80% of the world's population from developing countries rely mainly on traditional medicines for their primary health care (Alves & Rosa, 2005). Herbs have been considered a significant part of traditional medicine representing an integral part of the health care systems in many Middle Eastern countries such as Palestine (Jaradat, et al., 2016), Jordan (Alzweiri, et al., 2011), Iraq (Ahmed, 2016), and Yemen (Al-Fatimi, 2019). People living in these countries mainly in rural areas rely heavily on the use of 'selected' herbs to treat illnesses (Jaradat, et al., 2016; Licata et al., 2016).

Nowadays, traditional medicine is considered a vital branch in pharmacy and medicine since several plants are considered vital sources of pharmacologically active chemicals that can be developed into a pharmaceutical dosage form (Sen & Chakraborty, 2017). For example, plants offer a diverse range of active pharmacological drugs that may play vital roles in the treatment/management of viral infections such as Coronavirus (Zhang, et al., 2020), cancer (Lee & Houghton, 2005), malaria (Stangeland, et al., 2011), cardiovascular (Rouhi-Boroujeni, et al., 2017), neurological disorders (Saki et al., 2014) and as a source of a 'novel' antimicrobial agents (Patwardhan, 2004).

The medicinal properties of plants are due to the presence of chemically-diverse secondary compounds. These compounds based on their structure are classified into different groups such as alkaloids, saponins, volatile oils, and phenolic compounds including tannins, flavonoids, coumarins, and anthraquinones. These compounds are created in plants by two principal pathways including the (I) shikimic acid/aromatic amino acid, and (II) mevalonic acid pathways (Doughari, 2012; Devitt, 2006).

Many studies have highlighted several pharmacological properties in medicinal plants such as *Euphorbia tirucalli, Mandevilla velutina, Phyllanthus spp., Euterpe oleracea, Vitis labrusca, Hypericum caprifoliatum and Hypericum polyanthemum, Maytenus ilicifolia, Protium kleinii and Protium heptaphylium and Trichilia catigua* (Dutra, 2016) or their isolated constituents including antioxidant such as DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP, SOD, and ORAC assays (Dudonne, et al., 2009), anti-diabetes such as glucose tolerance test

(Widharna, et al., 2010), antibacterial against bacteria's: *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus faecalis* and *Escherichia coli* (Prusti et al., 2008) , antiviral against viruses: herpes simplex-1 virus (HSV), poliomyelitis-1 virus (POLIO) and vesicular stomatitis virus (VSV) (Soltan, & Zaki, 2009), and anti-ulcer activities in many parts of the world (Meléndez & Capriles, 2006). Given the fact the richness of the Palestinian lands with herbs that are commonly used as part of their folkloric medicine (Jaradat & Zaid, 2019) this study was designed to assess the medicinal values of some 'selected' herbs wildly grown in Palestinian regions and to identify the major secondary compounds present in these herbs.

1.2 Palestine as a source of unique medicinal plants

Palestine (including the occupied Palestinian territory) has a unique and rich natural flora and biodiversity despite its limited area due to its geographical location at the crossroads of Africa, Asia, and Europe (Isaac & Hilal, 2011). Palestine is also one of the 25 countries in the world designated as "global biodiversity hotspots (Salem, 2008) 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, 2006).

In the hills and mountains of Palestine (West Bank and Gaza), around 2780 plant taxa were covered and recorded as local or naturalized (Awaja, 2015). From the native medicinal benefits (Shtayeh & Jamous, 2002), The diverse topography of the country has allowed the survival of traditional knowledge regarding the plant resources that the local people use as food. However, only a few ethnobotanical studies have been conducted on medicinal plants in some parts of the country (Shtayeh, et al., 2000; Azaizeh, et al., 2003; Said, et al., 2002), with little or no emphasis on edible wild plants (Abu-Rabia, 2005).

1.3 Wild plants in Palestine (WPP)

Edible wild Palestinian plants (WPP) have always been significant in the folklore of the Palestine region (Shtayeh et al., 2008), however, the nutritional and medicinal uses of these plants have been some of the most relevant and consistent reasons for the popular

administration of plants. For this reason, ethnic-oriented research is very useful in discovering and developing new drugs and food resources (Heinrich & Gibbons, 2001; Khafagi & Dewedar, 2000). It is crucial 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; Pieroni, et al., 2005)

Through the last decade, several studies have analysed the consumption and collection of edible plants in specific countries in the Mediterranean region including (Alawamy, et al, 2020) for example, Greece (CChristophoridis, et al., 2019), Spain, and Italy (Paoletti, et al., 1995; Tardío, et al., 2006), Turkey (Ertuğ, 2004), Cyprus (Della, et al., 2006), or in the whole of the Mediterranean region. Many studies dealt with the therapeutic benefit of the main compounds of some wild plants in Palestine and Jordan, these plants therapeutic benefits are summarized in the those studies (Al-Aboudi & Afifi, 2011; Jaradat, 2005; Said et al., 2002).

Some examples of these plants and their biological activity include *Achillea santolina* Leaves, its flowering branches used for reduction of blood glucose level, cold, anti-colic, and kidney stones (Yazdanparast, et al., 2007), *Ajuga iva* herb used to stimulate nervous and cardiovascular systems as well as for the treatment of female sterility (Eissa, et al., 2014), *Ambrosia maritima* has hypoglycemic activity (Helal, et al., 2014), *Artemisia herba* possessed a hypoliposis effect (Slighoua,, et al., 2019). *Achillea millefolium* leaves 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, et al., 2019), *Arctium lappa* leaves used for anti-diarrhea, antiseptic (Saravani, et al., 2020), *Astragalus gummifera* gums for protection of the kidney (Noreen, et al., 2019), *Capsella bursapastori* entire plant used for kidney stone(Jaradat, 2005), *Convolvulus arvensis*, and entire organism used for leg corns, skin inflammation, anticough, sedative (Al-Snafi, 2018) *Cynoglossuf officinalis* root's bark used as antispasmodic, anti-inflammatory, demulcent (Joshi, 2016), *Valeriana officinalis* roots and flower stems used as antispasmodic, antiseptic, relief stomach pains, sedative, and arthritis (Wang, et al., 2010).

1.4 Traditional use of WPP

There are about 4,200,000 flowering plants have been estimated, out of these there are about 50,000 plants species used for therapeutic purposes that have been reported around the world (Govaerts, 2001). There are 396 different species of plants utilized in Traditional Arabic Palestinian Herbal Medicine (TAPHM) to cure various ailments in Palestine. (Shtayeh, et al., 2014). Traditional Palestinian plants are considered as important part of the Palestinian culture and religion, there are about 96 species native to Palestine, out of these, 84 species introduced from Asian nations. Locally obtained medicinal plants include those that grow in Palestine's natural environment and are harvested by individuals or therapists; 43 plant species are utilized in Palestinian traditional food, as mentioned by Jaradat (Jaradat, 2005), Palestinian traditional therapists use 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, 2005; Said et al., 2002).

Among these WPP, *Majorana syriaca, Foeniculum vulgare, Malva sylvestris, Salvia fruticosa, Cyclamen persicum, Micromeria fruticosa, Arum palaestinum, Trigonella foenum-graecum, Gundelia tournefortii,* and *Matricaria aurea* are most reported Palestinian traditional medicinal plants (cultural importance index) (Shtayeh et al., 2008; Tardío & Pardo-de-Santayana, 2008)

The traditional remedies are widely used throughout the West Bank of Palestine, particularly in rural regions. This could be related to the country's political unrest and the high expense of traditional medications (Shtayeh, et al., 2016; Jaradat, et al., 2016; Jaradat, et al., 2016). Antipyretics, analgesics, diuretics, laxatives, antimicrobials, antidiarrheals, antiemetics, and tonics for the heart are all made from a variety of trees, shrubs, and herbaceous species. (Jaradat, 2005). Because they grow wild or are farmed, these plants are readily available and affordable. (Abu-Rabia, 2005; Alkowni & Sawalha, 2012).

1.5 Medicinal value of WPP

Over the past decade, many studies have emerged to provide evidence proving or declining the traditional uses of WPP (Azaizeh, , et al., 2010, Hawash, et al., 2020). Among using their efforts many WPP have been tested for their argued antimicrobial (Abu-Shanab, et al., 2007), antioxidant (AI Husein et al., 2014), and anticancer effects in vitro (Jaradat, et al., 2016).

Because of its biodiversity and moderate climate, Palestine is a wealthy source of medicinal plants that have been used in treating diseases for centuries practiced by many practitioners and clinicians to effectively cure patients. (Jaradat, 2005). For example: *Rosmarinus officinalis* (*R. officinalis*), which is used in curing respiratory disorders, renal colic (as antispasmodic), rheumatoid arthritis (as antirheumatic), as well as general analgesic, diuretic, and expectorant (Al-Sereiti, et al., 1999). As well as antimicrobial and antioxidant activities (Wang, et al., 2012). Another one is *Teucrium polium (T. polium)* that has been used a anti-inflammatory, anti-diabetic, analgesic, antioxidant, antispasmodic properties, and antimicrobial properties against several clinical pathogens (Darabpour, et al., 2010; Qabaha, 2013).

1.5.1 Evidence implicated WPP as an antioxidant

The antioxidant activity of WPP has been got attention in many studies (Jaradat et al., 2017; Jaradat, et al., 2016; Salameh et al., 2020), many studies have confirmed WPP as antioxidants activity (Abu-Qaoud, et al., 2018; Al-Rimawi et al., 2017; Hawash et al., 2020). The antioxidant capacity of plants refers to the ability of the plants to extract mainly methanolic extracts to scavenge free radicals. In vitro testing on plants, the antioxidant capacity involved the use of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals (Jamuna, 2012).

Al-Rimawi and his team (Al-Rimawi et al., 2017) were analyzed the phenolic content, flavonoid content, antioxidant activity by using $DPPH^{\bullet}$, $ABTS^{\bullet+}$, Ferric reducing antioxidant power, Cupric reducing antioxidant power assays, and phenolic and flavonoid content of *Ephedra alata*, in this study three different solvents were used to extract the *Ephedra alata*؛ 100% water, 80% ethanol, and 100% ethanol, by HPLC/PDA/MS, extract

results indicated that antioxidants, phenolics, and flavonoids are abundant in the *Ephedra alata* grown in Palestine. The antioxidant of ethanol (80 %) extracts 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 66.0a±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 significantly correlated with each other.

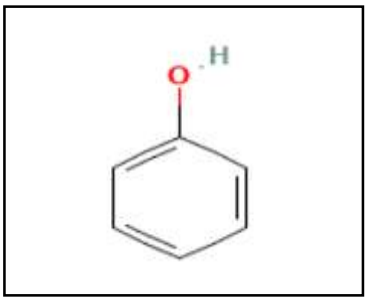


Fig 1.1: Phenol structure.

In a study by Jaradat and colleagues. (Jaradat, Damiri, et al., 2016) on Urtica urens, Rumex cyprius, and Borago officinalis, the results of free radical scavenging activity evaluated by DPPH•, results 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. However, Jahajha performed a study on the *Calamintha genus* and has shown the presence of flavonoids, terpenoids, tannins, and phenolic compounds, these compounds clearly showed important pharmacological and antioxidant activities (Jahajha., 2017).

Another study by (Hassan, et al., 2018), on *Borage. officinalis* 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*, results of the study in wild leaves extract, 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.

Salameh and his colleagues aimed to examine the antioxidant properties of *Micromeria fruticosa serpyllifolia* volatile oils from different areas in Palestine (North, Central, and South). The volatile oils were extracted from three samples of *M. fruticosa serpyllifolia* using a microwave ultrasonic device. The antioxidant activity of volatile oils was evaluated by inhibiting DPPH$^{\bullet}$ free radicals examination of the biological activity of plant extracts, revealed that the sample from Ramallah (central region) possessed the strongest antioxidant activity with an IC_{50} value of 0.45 μg/ml (Salameh et al., 2020). Another study of Husein and colleagues evaluated the antioxidant activity for six wild Palestinian plants namely: *Arum palaestinum Boiss (Araceae), Urtica pilulifera L. (Urticaceae), Coridothymus capitatus (L.) Reichb (Lamiaceae), Majorana syriaca (L.) Teucrium creticum L. (Lamiaceae), Teucrium creticum L. (Lamiaceae),* The results of their study showed that the antioxidant capabilities of the six plants studied were closely linked to their phenolic and flavonoid levels. Total phenols are the most abundant in *U. Pilulifera*, and total flavonoids are the second most abundant. (AI Husein, et al., 2014)

1.5.2 Evidence implicating WPP as antibacterial

The microbial activity of Palestinian wild plants has been an interest for many studies (Jaradat, et al., 2017) (Hamarsheh , et al., 2021; Salameh et al., 2020; Saleh , et al., 2013). Several 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). In their study, 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.

The study of Saleh and colleagues (Saleh, et al., 2013) on five Palestinian wild medicinal plants namely *Echinops adenocaulos, Parietaria judaica, Urtica urens, Verbascum fruticulosum,* and *Vitex agnus-castus* extract from holly Mecca Zamzam water, all plants extract exhibit antibacterial activity against a multidrug-resistant strain of *Streptococcus*

pneumoniae, the findings 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*.

Abu-Shanab and his team have studied the ethanol extracts and water extract of *Rosa damascene, Althaea officinalis, Mentha longifolia, Melissa officinalis*, 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 mm 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). By using well diffusion method a study by (Qabaha, 2013) found that plant extracts from *Rosmarinus Officinalis* and *Teucrium polium* exhibit 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 colleagues (Naseef, et al., 2017) 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). Naseef and his team 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*.

1.6 WPP as a source of new pharmacological active drugs

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

Table 1:1 summarizes some secondary compounds and biological activities of selected WPP used in wild Palestinian Folkloric Medicine in Table (1.1).

Table 1.1: Secondary compounds and biological activities of some wild Palestinian plants used in Palestinian Folkloric Medicine.

Latin name	family	Part used	Secondary compound	Biological activity	Study country	REF
Malva sylvestris	*Malvaceae*	Area part Seed	Malvone Malvidin malvin	Antiseptic Antimicrobial antifungal	Iran	(Mousavi et al., 2021; Pirbalouti & Koohpyeh, 2011; Razavi, et al., 2011)
Ephedra alata	*Ephedraceae*	aerial parts,	Polyphenolic alkaloids derived from phenyl-alanine	Against human breast cancer antioxidant activity antibacterial cocci and Candida	Tunisia	(Corina Danciu et al., 2019)
Arum Palestinum	*Araceae*	aerial parts	Piperazirum Isoorientin	Anticancer, urinary disorders	Egypt Palestine	(Farid, et al., 2016; Zaid, et al., 2010; Zaid, et al., 2012)
Asparagus officinalis	*Liliaceae*	aboveground part	Poly saccharides, polyphenols, anthocyanins, and saponins	anti-cancer, anti-tumor, antioxidant, immunomodulatory, hypoglycemic, antihypertensive, anti-epileptic	China	(Guo et al., 2020)

Latin name	family	Part used	Secondary compound	Biological activity	Study country	REF
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, 2011; Paul & Pillai, 2021)
Micromeria fruticosa	*Lamiaceae*	aerial part	phenolic acids, flavonoids, coumarin, and tannins	Stomach, intestine pain and inflammation, fever, asthma, and respiratory system	Turkey Palestine	(Said , et al., 2002; Taskin, et al., 2021)
Majorana syriaca	*Labiates or Lamiaceae*	Leaves	α-phellandrene, α-pinene, β-myrcene, m-cymene, p-cymene, γ-terpinene, thymol, and carvacrol	Intestinal pain, inflammation, high blood pressure	Palestine	(Abu-Lafi et al., 2007; Said et al., 2002)
Foeniculum vulgare	*Umbelliferae*	The whole plant, roots, seeds, and oil of the seed	saponins, flavonoids, cardiac glycosides, sterols, triterpenes, coumarins, proteins	antidiabetic, antioxidant, anticancer, antimicrobial, cardiovascular, immunological,	Iraq	(Al-Snafi, 2018)

Latin name	family	Part used	Secondary compound	Biological activity	Study country	REF
Salvia fruticosa	*Lamiaceae*	Leaves	1,8-cineole Thujone Carnosic acid	Stomach ache, intestinal gas and inflammation, diabetes and sexual weakness, antiseptic,	Tunisia Palestine	(Ben Farhat et al., 2009; Said, et al., 2002)
Cyclamen persicum	*Primulaceae*	Leaf and bulb	An alkaloid, Phenolic Compounds, Saponin Glycosides, Tannins, Starch, Flavonoids	antibacterial effects	Palestine	(Alkowni, et al, 2018)
Trigonella foenum-graecum	*Fabaceae*	Seeds	phenol, flavonoid	antioxidant activity against α-amylase	Palestine	(Jaradat, et al., 2021)
Gundelia tournefortii	*Asteraceae*	Roots, the stems, and head sections	sitosterol,stigmasterol, lupeol, gitoxigenin, α-amyrin,and artemisinin	Anticancer Laxative, emollient	Palestine Jordan	(Abu-Lafi, et al., 2019; Al-Khalil, 1995)
Matricaria aurea	*Asteraceae*	Leaf flower	coumarins, phenols, esters, and ketones	antimicrobial activity Fever, coughing, and heart diseases	Saudi Arabia Palestine	(Rizwana, et al, 2016; Said, et al., 2002)
Artemisia judaica	*Asteraceae*	Leaf, flower, and root	Total phenolic and flavonoid content	Fever, menstruation regulator, and nerve system (calming)	Saudi Arabia Palestine	(Nasr, et al., 2020; Said, et al., 2002)

Latin name	family	Part used	Secondary compound	Biological activity	Study country	REF
Varthemia iphionoides	*Asteraceae*	Leaf and stem	1,8-Cineole, borneol, and α-cadinol	Nerve system (tremors), heart diseases, stomach and intestine pain and inflammation, Abdominal pain	Palestine Jordan	(Abbas, et al, 2019; Issa, et al., 2019; Said, et al., 2002)

1.7 Literature review of the studied traditional WPP

Studies on total phenolic content of *Malva sylvestris* extracts of leaves and flowers showed effectiveness against pathogenic microorganisms like : (*E. coli* ATCC 25922, E. coli ATCC 8739, *Salmonella sp*. (clinical isolate), and *St. aureus* ATCC 6538-P), and showed no antifungal activity against *S. cearevisie, A. niger,* and *P. vulgaris* (Mihaylova, et al., 2015). Another study performed in Algeria showed that *M. sylvestris* showed significant scavenging activity of some radicals such as DPPH (Tizi-Ouzou, 2020).

A recent study from Iran showed that the main components of essential oil of *Urtica urens* were phytol (27.34%), α-limonene (19.73%), γ-terpinene (17.21%), and p-cymene (16.41%).(Bagheri, et al., 2021), Another study showed that the secondary metabolites pertinence to the classes of terpenoids, phenolics, flavonoids, and choline, In addition, *U. urens* leaves extract was tested for several pharmacological activities and found to have positive effects as antibacterial, antioxidant, and immunomodulatory activities due to the presence of components of phenolics and flavonoids (Grauso, et al., 2020).

A study carried out in Turkey on the methanolic extract of *Micromeria fruticosa* and analyzed by GC-MS showed that it has 29 compounds, the most important compounds being piperitenone (50.61%) and pulegone (29.19%), in addition, the essential oil exhibited antibacterial activity against 14 different bacterial strains, three fungi and yeast, and antioxidant activity. (Güllüce, et al., 2004) (Dudai, et al., 2001).

Other studies performed on *Ephedra alata* leaves methanolic extract showed high antioxidant and antibacterial activity (Jerbi, et al., 2016; Danciu et al., 2019).

A study on the crude aqueous methanolic extracts of *Arum palaestinum* species revealed the presence of several biologically active phytochemicals with the highest quantity of Alkaloids, Phenols, Saponin, Carbohydrates, Phenols, Tannin, and Flavonoid. *A. palaestinum* was also evaluated for free radical scavenging activities, and was found to be the best source for free radical scavenging. (Jaradat & Abualhasan, 2016). Another study on *Arum palaestinum* carried to evaluate the exhaustive extract yields percentages for Solomon's Lily (Arum palaestinum Boiss.) leaves from different regions in the West

Bank/Palestine including (Nablus, Jenin, Tubas, Tulkarm, Salfeet, Qalqilya, Ramallah, Jericho, Jerusalem, Bethlehem, Hebron) to consider the best area in Palestine that have powerful anticancer activity. It has been found that the best aqueous and organic yields were in Salfeet district (4.8%, 3.24 %, respectively), while the lowest aqueous and organic yield was in Jericho (2.64%, 0.76%, respectively), Nidal, et al., 2015. A study established in Iraq showed that the main compounds in both Asparagus officinalis and Asparagus sprengeri, were myrtanol, pinocarveol, 2-ethylhexanol, perillaldehyde, Octanol, Eugenol, Pregnane-11,20-dione,3,17-dihydroxy, αmyrcene, sabinene, carvone, 4-[1-hydroxyethyl] benzaldehyde, hexanal, camphor, acetic acid, octyl ester. By using gas chromatography equipped with flame ionization detector (GC-FID) and gas chromatography joined to mass spectrometry (GC–MS), the antibacterial activity of two types of *Asparagus* against six bacterial species was tested by using microdilution (MIC) determination and agar well diffusion assay. The results showed the essential oil in dilution 80% has more inhibitor activity, and most activities were shown that the bacterial species tested against the essential oils of *Asparagus officinalis*, *Staphylococcus aureus* was found to be highly sensitive to it is action followed by *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus sp.*, *Klebsiella pneumoniae*, and *Brucella* species, while *Asparagus sprengeri* essential oil showed the highest antibacterial activity against *Escherichia coli* and lowest activity against *Brucella* species. In both Gram-positive and Gram-negative bacterial strains were shown to be sensitive to the strength of two species of *Asparagus* essential oils upon extensive recent literature search, it turned out that there are very limited numbers of investigations that have been carried out (Kadhim & Salah, 2014). It has been shown that *Asparagus* roots have many bioactive compounds including polyphenols, saponins, and polysaccharides that is recommended in study of H. Zhang and his team (Zhang, et al., 2019).

All required information were obtained by searching keywords such as antioxidant, antibacterial, medicinal wild plant extracts, or volatile compounds in published articles in authentic scientific databases such as Science Direct, Wiley-Blackwell, Springer, Google Scholar, Scientific Information Database, and PubMed. We obtained 59 research articles for *Malva sylvestris*, 12 for *Ephedra alata*, 20 for *Urtica urens*, 7 for *Micromeria fruticosa*, 6 for *Arum Palaestinum*, and 730 for *Asparagus officinalis*

1.8 The WPP used in this study

The traditional folkloric Palestinian plants used in this study include: *Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria fruticosea,* and *Arum Palaestinum.* 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.

Based on recommended traditional local reports, plant parts were precisely selected (Shtayeh et al., 2008; Jaradat, 2005; Said et al., 2002), history of toxicity or poisoning was not documented for the selected plants, as they are used by local people for medical purposes, or as food. Furthermore, the plants with the desired activities were deemed safe since they are used by local people for medical purposes or as food. (Hamarsheh et al., 2021). This study included the following traditional Palestinian ethnomedicinal plants:

1. *Malva sylvestris* (L) Pharm PCT- 2743, commonly called as Mallow, grows wildly in Palestine and in many other countries, and used in the Mediterranean and European folkloric drugs and ethnoveterinary for the cure of internal and external inflammation-related diseases such as rheumatism, and tissue damage. Moreover, its use is not only restricted to medicinal purposes; but also the species is locally considered as a wild food plant in different folkloric cultures (Barros, et al., 2010)

2. *Urtica urens* (L). Pharm PCT- 2562, (stinging nettle), an immortal herb of the family *Urticaceae* is a local flowering wild plant that grows broadly in Palestine, North Africa, Europe, Asia, and North America. It is known by the stinging hairs that line its stem, leaves, and flowers, and provoke irritation to the skin (Fu, et al., 2006), The common olden healing usage of *Urtica urens* L mainly in ancient Egyptians is the treatment of rheumatism, lumbago, muscular paralysis, and arthritis (Upton, 2013)

3. *Micromeria fruticosa* (L) Pharm PCT- 1575, (White Micromeria) (*Lamiaceae*]: is a dwarf shrub set up in northern and central Palestine, Lebanon, Syria, Jordan, and Turkey. The aeriform parts of the plant are broadly used in the eastern

Mediterranean region as therapeutic teas, and infusions, and to cure many diseases such as abdominal pains, diarrhea, eye infections, heart disorders, high blood pressure, weariness, exhaustion, colds, and open wounds (Sharma, et al., 2021; Dudai, & Yaniv, 2014)

4. *Ephedra alata* Decne Pharm PCT- 904, (alenda in Arabic) (*Ephedraceae*): 40 species of the family Ephedraceae grow in Palestine, China, India, Egypt, the Middle East, Europe, and the Americas. They are an evolutional original group of shrubs that grow on arid, stony, or sandy terrain in desert or dry areas, *E. alata* flowers during late winter and spring, and seeds start to ripe from mid-May to early August. The plant contains powerfully aromatic, with a bitterish taste. The part that can be used is dried stem, it is used in weight loss and energy formulas. It is available in the bulk herb, capsules, hydroalcoholic extract and is often found in weight loss and energy formulas. It is accepted for illnesses respiratory tract with mild bronchospasms, as a bronchodilator, anti-asthmatic, allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, and nasal congestion, the product is the source of numerous components contain, alkaloids, tannins, saponins, proanthocyanidins, phenolic acids, flavonoids, and essential oils. (Blumenthal, 1999)

5. *Arum Palaestinum*, Pharm PCT- 246, also called as Lufe in Arabic, other name include: Solomon's Lily, Black Calla, and Priest's Hood of the plant family *Araceae*), is a perennial herbal plant, this plant is eatable after steeping, about 31 species of plants that belong to genus Arum were identified in nature. One of the most common is Arum palaestinum Boiss. This family has about 1000 members spread mostly in Mediterranean regions, this plant grows wildly in Palestine, and in other countries like: Jordan, Lebanon, and Syria. It is used by herbal practitioners and local provincial therapists in treating many diseases like: cough, constipation, heart burn, urinary tract infections, cancer, diabetes, hemorrhoids, atherosclerosis, and worms in the GIT and skin diseases. This plant is well documented in Palestinian traditional medicine. (Shtayeh & Jamous, 2011; Farid, et al., 2014; Oran & Al-Eisawi, 2014; Sakthivel & Guruvayoorappan, 2012)

6. *Asparagus officinalis* L (*Asparagus*.), Pharm PCT- 4686, also called as Halayoun in Arabic, is a long-term perennial plant with a low-input, and high-market value, the vegetable harvest with a production cycle of up to 15 years or more (Yergeau, et al., 2006), It has a rich source of phytochemical compounds such as flavonoids (e.g., rutin and anthocyanins), other phenolic and polyphenolic complexes, saponins, etc., which have a biological and therapeutic effect on human health (Chitrakar, et al., 2019).

This study is important because it has given the massive loss of traditional knowledge regarding wild edible plants, we aimed to document and assess the indigenous knowledge, diversity, and cultural indication of these plant species in two areas in the northern and southern West Bank, and to compare the cultural indication of edible wild plants historically collected as food and the socio-economic and anthropological context in which they were collected and studied**.**

1.9 Antioxidant activity of the selected WPP

Plants with high antioxidant activity are likely to possess beneficial effects on humans and treat/protect humans from diseases. (Tripoli, et al., 2005).

Rosmarinus officinalis is used in traditional medicine in the treatment of respiratory disorders, antispasmodic, rheumatoid arthritis as well as general analgesics, diuretic, expectorant. *(*Al-Sereiti, et al., 1999), Additionally, *Pisidium guajava* has been used in the treatment of ulcers, cholera, diarrhea, and bowels. Guava leaves are used in folk medicine as an antipyretic and antispasmodic (Sanda, et al, 2011), Moreover *Punica granatum* has been used in folk medicine to treat diarrhea, dysentery, and respiratory and bleeding diseases. It also has antioxidant activity (Choi, et al., 2011), Additionally *Teucrium polium* was used as an anti-inflammatory, antidiabetic, antispasmodic, analgesic, and antioxidant properties (Darabpour, et al., 2010), Finally, *Salvia officinalis* (sage) has been used to treat several cardiovascular disorders (Esmaeilizadeh & Šener, 2017).

Antioxidants are stable natural chemical compounds capable of neutralizing reactive oxygen species (ROS) and subsequently reducing their harmful effects in humans. In

general, the antioxidant efficiency of herbal extracts depends largely on the content of phenolic compounds and the reaction activity of the phenol because of the chain-carrying peroxyl radicals and on the stability of the phenoxyl radicals formed in the reaction. it is identified that typical phenolics that possess antioxidant activity (Ćavar, et al., 2013).

The pharmacological value of plants lies in the presence of some organic compounds such as alkaloids, saponins, flavonoids, tannins, anthraquinones, steroids, and terpenoids (Dhandapani & Sabna, 2008). These compounds are synthesized by the secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure functions (Asgarpanah & Mohajerani, 2012). Amongst the most important functions of some of the secondary compounds is their ability to scavenge ROS due to the presence of structurally diverse phenolic compounds.

In Palestine, several edible/cultivated plants having healthy effects on humans have potent antioxidant activities. The antioxidant activity of the plant is commonly assessed in vitro by its ability to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals. These plants are widely used commercially in foodstuffs for their antioxidant properties (Al-Rimawi, et al., 2017). Although a variety of herbs are known to be sources of phenolic compounds. Polyphenols have recently received a lot of attention due to their diverse biological activities, from pharmacological and therapeutic perspectives, and the antioxidant properties of polyphenols, such as free radicals scavenging and inhibiting lipid peroxidation, are the most important (Anderson, et al., 2001).

However, to the best of our knowledge, few studies have focused on plants that grow in the wild. Accord, to this study, we aimed to evaluate antioxidant activities in methanolic extracts and to determine the phenolic contents of some Palestinian medicinal wild plants that are commonly used in Palestinian folkloric traditional medicine by using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ tests, and Folin–Ciocalteu for determining total phenols.

1.10 Antibacterial Activity of selected WPP

Therapeutic plants long before have been used as a source of treatment for different ailments in almost all nations (Petrovska, 2012). During the latter decennium, the usage of traditional medicine (TM) has extended globally and is earning popularity. Persons use herbal medicines due to their effectiveness, folklore, and low price (Alonso-Castro, et al., 2012). Medicinal plants are significant components of indigenous medical systems in Palestine as well as in other developing countries. Complementary and alternate medicine (CAM) use in Palestine are common, specially the use of herbal medicine (Abou-Elkhair, et al., 2010).

Increased numbers of bacterial strains showing resistance to antibiotics have received much interest in recent years. According to the World Health Organization (WHO), antibiotic resistance is a serious threat affecting every country with the potential to affect anyone, at any age, in the world, and Palestine (Adwan, et al., 2005; Maillard, et al., 2020; Organization, 2014). Antimicrobial Drug Resistance (ADR) limits access to infectious diseases and poses a threat in cases of infectious disease cases and during routine surgery, cancer treatments, and organ transplants (Fleming, et al., 1995), therefore a large number of sicknesses and deaths that are caused by fighting bacteria worldwide and this needs global commanding actions to prevent ADR (Aslam, et al., 2015), At present, there is an insistent need to explore new antimicrobial medications with various mechanisms of action. That is at most endorses to the upgrowth of infections affected by bacterial strains that are greatly strong to antibiotics and the presence of chronic diseases that could not be cured effectively in all cases with the existing modern medications (Guven, et al., 2009; Okoli, et al., 2009).

Even though the healing of patients cured with therapeutic plants appears to be a slow progression, they are becoming more common due to their specific side effects associated with current medications (Pimpliskar, et al., 2012).It is difficult to restrict the influence of antibiotic resistance in terms of death rate and budget. The US Center for Disease Control and Prevention estimated that more than two million people every year are affected with antibiotic-resistant infections, with at least 23 000 dying as a result of the infection" (Health & Services, 2013), In Europe every year, the number of infections and deaths due to the most frequent multidrug-resistant bacteria (*S. aureus, Escherichia coli,*

Enterococcus faecium, Streptococcus pneumoniae, Klebsiella pneumoniae, and *Pseudomonas aeruginosa*) was estimated to be ~400 000 and 25 000, respectively (Garau, 2010). Infections caused by multidrug-resistant bacterial strains are the core causes of output morbidity and death rate in patients enduring these actions. A report from the University of Texas, published in 2014, displayed high antibiotic resistance rates in infections in cancer patients with chemotherapy-related neutropenia (Nesher & Rolston, 2014). The development of medicine resistance and the side effects of antibiotics have study to the examination of other new antimicrobial agents mostly among plant extracts to find out new chemical structures which overcome the above difficulties (El-Bashiti, et al., 2016).

Ali-Shtayeh and his team (Shtayeh, et al., 2014), analyzed the *Rosmarinus officinalis*, the results have shown that the plant has wide antimicrobial and antioxidant activities. Essawi & Srour study on *Teucrium polium* has been found to possess antimicrobial properties against various clinical pathogens (Essawi & Srour, 2000).

The present study is designed to evaluate the antibacterial activities (inhibition zone) using the agar well diffusion method for the methanolic extract of selected wild Palestinian plants against gram-positive bacteria *Staphylococcus aureus*, and four gram-negative bacteria *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*). Palestinian herbal medicine is used to treat diseases like gastrointestinal problems, urinary tract infections, infertility, and cutaneous abscesses that occur in the West Bank and Gaza Strip (Palestine) (Essawi & Srour, 2000), Antimicrobial resistance has increased in Palestine and become a major problem due to methicillin-resistant *Staphylococcus aureus* (MRSA) (Abu-Shanab, et al., 2007)

1.11 Phytochemical Screening and Volatile Compounds in selected WPP

There are around 300,000 plant kinds whose phytochemicals with various structures and properties are explained (Lattanzio, 2013). Information of the chemical components of plants is necessary, not only for the finding of healing agents but also because such information may be of value in disclosing new sources of such commercial materials as oils, tannins, alkaloids, flavonoids, phenolic, and ancestors for the synthesis of complex chemical substances, etc. There is consistent evidence that the knowledge of the chemical

constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mustafa, et al., 2017).

These phytochemicals are distributed in two main classifications, firstly primary metabolism like lipid, proteins, carbohydrates; which is liable for the growth and improvement of plants; and secondly metabolisms such as terpenoids, alkaloids, and phenolic compounds, that are responsible for resistance mechanism against insects, ecological pollutants, and other danger to the plant (Bernards, 2010). The wild eatable plants always have a vital role in the folkloric traditions of the Mediterranean region. On the other hand, food has complementary alternative and pharmaceutical uses (Hadjichambis, et al., 2008).

Phytochemicals and Volatile oils abbreviated as VO_S, are well known to have antioxidant, anti-inflammatory, anticancer, anthelmintic, antimalarial, antiviral, antibacterial, cholesterol suppression, and pesticide activities (Salameh, et al., 2020; Shakya, 2016), The chemical constructions of VO_S locate their medicinal activities (Djilani & Dicko, 2012). Recently, there has been great attention in VOs extracts of therapeutical wild plants to avoid or slow the growth of a bacteria, virus, or other microorganisms have potential in causing disease (Çetin, et al., 2011). Many scientific studies provided information about the chemical configuration, overall product. It is well documented that the smell of VOs, differences are due to growing settings like: weather, kind of soil and structure, height, plant age, geoclimatic position, and environmental circumstances of collection time and place (Andrade, et al., 2011; Brockett, et al., 2012).

Palestine is one of the countries with the highest biodiversity in the world due to its diverse geographical situation, several climatic, phytogeographic, and zoogeographic regions covered Palestine, which has 2780 plant taxa that were noted as native or naturalized (Shtayeh & Jamous, 2002; Djamali, et al., 2012).

Up to date, there were no previous studies on Leaves *of Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria fruticosea*, and *Arum palaestinum*, on their antioxidant, antibacterial activities, phytochemical screening, their total phenol, and Volatile compounds contents. Therefore, the biochemical and biological investigations of these plants could be of great scientific value. The main objectives of this study were to

screen the presence of major phytochemical compounds and analyze the phytochemicals as plant secondary metabolites of some WPP, Phytochemical analysis was performed to investigate the plant's secondary metabolites such as saponins, coumarins, cardiac glycosides, steroids, tannins, quinones, and terpenoids by using Phytochemical analysis. And screening the volatile compounds by using GC-MS analyses.

1.12 Aim of the study

This study aimed to investigate and document certain biochemical, phytochemical, and pharmacological data on selected WPP usually and traditionally used by Palestinian healers in the rural areas of the West Bank for treating of many diseases, using antioxidant, antibacterial, phytochemical screening, and GC-MS methods, and to the best of our knowledge, there are no studies in the literature reporting about these wild plants used this analysis**.**

1.13 Objectives of the study

1. To evaluate the antioxidant activity of the volatile compounds of the above selected WPP leaves using $ABTS^{\bullet+}$ & $DPPH^{\bullet}$ assays.
2. Determine total phenols content using Folin Ciocalteu's
3. To determine the inhibitory effects of the volatile compounds of the above selected WPP leaves on the growth of selected four gram-negative bacterial strains namely (*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 analyze these phytochemicals as plant secondary metabolites present in plant leaves extract.
5. To identify the chemical components of the phytochemical and volatile compounds from selected wild Palestinian plant leaves using GC-MS.

Chapter 2: Materials and Methods

2.1 Study area

West Bank placed between the 31°21` and 32°33` latitude and between 34°52` and 35°32` longitude, this location made the zone highly affected by the Mediterranean climate. The Mediterranean climate 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 high ecosystem variety due to its geographical location between three continents: Africa, Asia, and Europe and due to different climatic, zoogeographic, and phytogeographic zones, this generates large biological diversity (Shtayeh, et al., 2008; Saad, 2015; Dowek, et al., 2020),

2.2 Plant samples

The aerial parts of the selected WPP (*Malva sylvistris, Urtica urens, Arum palaestinum, Asparagus officinalis, Ephedra Alata, and Micromeria fruticosea*) cultivated from Palestine and harvested during January 2019, from Hebron district of Palestine (31°31'45.66"N, 35°5'37.68"E.), plants (*Malva sylvistris, Urtica urens,* and *Arum palaestinum*) were cultivated. from Tulkarem district of Palestine (32°18′37″ N, 35°01′43″ E) plants (*Asparagus officinalis, Ephedra Alata,* and *Micromeria fruticosea*) were cultivated. Leaves were separated, washed, and dried at room temperature until no change in weight was observed. Dried 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

Extracts from WPP were prepared following the protocol described by (Qawasmeh, et al., 2012). Briefly, 200 mg of grounded leaves from each selected WPP were extracted with 4 mL methanol (80% v/v) on a shaker (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.

2.3.1.1 $ABTS^{\bullet+}$ Assay

$ABTS^{\bullet+}$ stock solution was prepared following 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 (1:4) 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 (Cole-Parmer Ltd, UK). The percentage scavenging of $ABTS^{\bullet+}$ was calculated according to the equation:

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

Data analysis: Data were expressed as means ± standard error (n=4).

2.3.1.2 $DPPH^{\bullet}$ assay

The extracts were tested for their ability to scavenge free radicals using the free radical 2,2-diphenyl-1-picrylhydrazyl hydrate ($DPPH^{\bullet}$), which is a molecule containing a stable free radical (Sharma & Bhat, 2009), $DPPH^{\bullet}$ scavenging capacity of extracted solutions was assayed based on the methods described in (Barros, et al., 2007) with minor modification, $DPPH^{\bullet}$ 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 (1:4, 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 absorbances of plant extracts (A_{sample}) and the methanol ($A_{control}$) were measured at 734 nm using a

Genway UV/Visible spectrophotometer at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH• discoloration using the following equation:

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

Data analysis: Data were expressed as means ± standard error (n=4).

2.4 Quantitative Phytochemical Screening

2.4.1 Preparation of crude phenolic extracts

The samples were extracted following the protocol by (Qawasmeh, et al., 2012). A 2g of dried plant leaves were extracted with 20 mL methanol (70%, v/v) at room temperature for 30 min with continuous stirring. After filtration, the resulting raffinate was re-extracted with 10 mL methanol (70%) for 15 min with continuous stirring and filtrated. The combined filtrate was defatted twice with 20 mL of n-hexane. The defatted extract was filtered twice by GF/F filter and 0.45 μm nylon syringe filter. All resulting crude was stored at −20°C in the refrigerator until analysis.

2.4.2 Determination of total phenols using Folin–Ciocalteu

Folin-Ciocalteu reagent was used to measure the total soluble phenols in ethanol extracts of plants (Katsube, et al., 2004)**,** Total phenols were measured using the method (Qawasmeh, et al., 2012). A 20 μL of plant extract was mixed with 1.58 mL distilled water and 100 μL Folin–Ciocalteu reagent, all tubes were mixed with a vortex of 30 μL of aqueous sodium carbonate (20 %, w/v). The flask was shaken, and the volume was made up to 10 mL with distilled water and left the sample 1h. The absorbance of the resulting solution was measured at 760 nm results were expressed as milligrams of Gallic acid per gram of dried plant (mg GAE/g dry). Each crude extract from each plant sample was analyzed in triplicate. (as the protocol of preparation of Gallic acid).

2.5 Antibacterial activity

2.5.1 Plants extraction

The methodology of the leaf extracts was prepared following the protocol of (Qawasmeh, et al., 2012) with minor modifications. Briefly, 10 g of *Malva. Sylvistris, Urtica urens, Asparagus officinalis, Ephedra alata, Micromeria fruticosa,* and *Arum palaestinum* were extracted in 100 mL methanol (100%) individually at 30°C for 24 h at room temperature by using a shaking incubator. The extracts were then filtered through vacuum technique and evaporated at a temperature below the solvent's boiling point, finally concentrated to a final volume of 20 mL and subjected to antibacterial analysis.

2.5.2 Microbial sample

Five pathogenic bacterial strains were obtained from the microbiology department of Hebron Governmental Hospital. A gram-positive bacterium (*Staphylococcus aureus*), and four gram-negative bacterial strains namely (*Escherichia coli, Proteus Vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*). All the strains were cultured on nutrient agar and incubated at 37 °C for 24 h in Thermo Fisher Scientific, Heratherm incubator (OGS60, Germany). The cultured bacterial strains were preserved in the refrigerator at 2–8 °C until use for testing**.**

2.5.3 Media preparation

Muller Hinton agar (HIMEDIA, 000381656) (19g M.H were dissolved in 500ml H_2O), EMB (HIMEDIA, 0000289212) (8.99g EMB were dissolved in 250ml H_2O), Mannitol Salt agar (HIMEDIA, 0000335310) (27.755g M.S were dissolved in 250ml H_2O) and nutrient agar (Bio Maxima S.A, FM4704) (7g N.A were dissolved in 28ml H_2O) until boiling. The media were autoclaved (LAC-5065SP, LABTECH) under these conditions (121 °C for 15 minutes). The sterile media were poured into sterile Petri dishes (90 X 16 mm). A 25ml Muller Hinton agar medium was poured in sterile Petri dishes with a height of 4 mm. All the Petri dishes were stored in the refrigerator at 2–8°C for later use.

2.5.4 Bacterial culture and subculture

Bacterial isolates were sub-cultured on nutrient agar plates and incubated at 37 °C for 24 h. The grown bacteria were sub-cultured into different differential media as the following:

*Staphylococcus aureu*s was cultured on Mannitol Salt Agar, *Escherichia coli*, and *Klebsiella pneumonia* was cultured on EMB, *Proteus Vulgaris*, and *Pseudomonas aeruginosa* on nutrient agar. All the plates were incubated at 37 °C for 24 h.

2.5.5 Sensitivity testing

Sensitivity of bacterial species to selected WPP extracts was performed using the well diffusion method on Muller Hinton agar plates. Bacterial suspensions were prepared to a density of 0.5 McFarland units (0000304103) which is equivalent to 15*108 CFUs/mL from 18-24 hours old bacteria colonies in saline solution and spread on Muller Hinton agar plates by a sterile cotton swab. Four holes in each plate were made, three holes for extract (10 µL), one hole for negative control (methanol), and in middle for the positive controls Vancomycin was the positive control for *Staphylococcus aureus* bacteria and Meropenem was the positive control for other bacterial strains. All sensitivity testing plates were incubated at 37 °C for 24 h.

2.6 Qualitative phytochemical screening

2.6.1 Extract preparation

Contents were measured by following the protocol described by (Mujeeb, et al., 2014). A 3g of powdered dried Leaves of *Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria fruticosea*, and *Arum palaestinum* were extracted in 60ml methanol (80%) for 24 h at room temperature with continuous stirring in a shaking incubator (Lab Tech Dihan labtech co ltd). The extracts were then filtered through the vacuum.

2.6.2 Tests methods

2.6.2.1 Alkaloids

1ml of HCl (1%) was added to 2ml of extract, followed by a few drops of Mayer's reagent, a white precipitate was taken as evidence of the presence of alkaloids.

2.6.2.2 Anthraquinones.

1ml of NH_3(10%) solution was added to the 2ml of extract Then mixed with benzene. A red, pink, or violet color indicates the presence of anthraquinones.

2.6.2.3 Anthocyanin.

1ml of NaOH (2N) was added to the 2ml extract and heated for 5min. A bluish-green color was taken for the presence of anthocyanin.

2.6.2.4 Cardiac Glycosides.

2ml of glacial acetic acid was added to 1ml of concentration H_2SO_4. Followed by few drops of $FeCl_3$ were added to the 2ml extract. a formation of a brown ring indicates the presence of cardiac glycosides.

2.6.2.5 Coumarins.

A 1ml of NaOH (1N) was added to 1ml of extract and kept in a boiling water bath for a few minutes. The formation of yellow color indicates the presence of coumarins.

2.6.2.6 Flavonoids.

A few drops of NH_3(1%) solution was added to 2ml of extract. a yellow color indicates the presence of flavonoids.

2.6.2.7 Glycosides.

2ml of H_2SO_4 (50%) was added to 2ml of extract. after 5minutes of heating in water, a bath was added 10ml Fehling's solution and boiled. The appearance of a brick precipitate indicates the presence of glycosides.

2.6.2.8 Phenolic groups.

2ml of distilled water was added to a few drops of $FeCl_3$ (10%). A 1ml of the extract was followed. blue or black color indicates the presence of the phenolic group.

2.6.2.9 Phlobatannins.

A 1ml of NaOH (10%) solution was added to the 2ml extract. A yellow color indicates the presence of phlobatannins.

2.6.2.10 Quinones.

A 1ml of concentrated H_2SO_4 was added to 1ml of extract. A red color indicates the presence of quinones.

2.6.2.11 Saponins.

5ml of distilled water was shaken with 2ml extract. The formation of foam indicates the presence of saponins.

2.6.2. 12 Steroids.

2ml of $CHCl_3$ and H_2SO_4(50%) were added to the 1ml extract. A reddish-brown ring indicates the presence of steroids.

2.6.2.13 Tannins.

A 1ml of distilled water and 1-2 drops of $FeCl_3$ were added to 2ml extract. A green or blue-black color indicates the presence of tannins.

2.6.2.14 Terpenoids.

2ml of $CHCl_3$ and 3ml of concentrated H_2SO_4 were mixed with 2ml extract. A reddish-brown layer indicates the presence of terpenoids.

2.7 GC–MS analysis

Plants extracts from WPP of *Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria fruticosa,* and *Arum palaestinum* were analyzed using GC–MS analysis(Clarus SQ 8S, Perkin Elmer, USA) fitted with a BD-5ms capillary column (30 m, 0.25 µm film thickness, 0.25 µm bore diameter) based on the method described by (Qawasmeh, et al., 2011) with minor modifications as described below.

The injection volume was 1 µl. The Oven temperature was maintained at 80°C for 2min and was programmed to rise to 280°C at the rate of 30°C min^{-1}. 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^{-1} and 43.1 cm s^{-1}, respectively. Mass spectrometry (MS) scan speed was 1000 $amus^{-1}$ and the molecular masses (m/z) of the compounds between 50 and 500 m/z were acquired. The analysis for each sample was repeated 5 times. Compounds were tentatively identified using the NIST05 mass spectral library, and when applicable, their mass spectra were compared with those published in the literature.

Chapter 3: Results

3.1. Antioxidant activity

3.1.1 $ABTS^{\bullet+}$ scavenging capacity

Diluted methanol extracts 1:4 for selected WPP showed pronounced antioxidant activity as assessed by $ABTS^{\bullet+}$ free radical scavenging assay, the average percentage $ABTS^{\bullet+}$ of methanolic extracts of *Ephedra Alata, Arum palaestinum, Asparagus officinalis, Micromeria fruticose, Urtica urens, and Malva Sylvistris* scavenging capacities were: 96.19 ± 0.08, 95.94 ± 0.12, 93.31 ± 0.98, 80.41 ± 1.31, 78.81 ± 1.15 and 72.78±0.59; respectively as shown in Table 3.1.

3.1.2 $DPPH^{\bullet}$ scavenging capacity

Diluted methanol extracts (1:4) for selected WPP showed pronounced antioxidant activity as assessed by $DPPH^{\bullet}$ free radical scavenging assay**,** the average percentage $DPPH^{\bullet}$ of methanolic extracts of *Ephedra Alata, Arum palaestinum, Micromeria fruticosa, Asparagus officinalis, Urtica urens,* and *Malva Sylvistris* scavenging capacities were: 89.26±0.49, 79.49±0.4, 62.94±0.69, 53.04±1.47, 51.09±1.38, 27.52±0.88, and 20.86±0.85; respectively as shown in Table 3.2.

Table 3.1: Antioxidant capacity (%) of the methanolic extracts *of Malva. sylvistris, Urtica. urens, Asparagus officinalis, Ephedra. Alata, Micromeria. fruticosa,* and *Arum palaestinum* leaves (n=4) using $ABTS^{\bullet+}$ free radical scavenging assay.

	1	**2**	**3**	**4**	**Mean ± SE**
M.sylvistris	69.76	75.31	73.79	72.26	72.78±0.59
U. urens	71.98	81.69	80.03	81.55	78.81±1.15
A.officinalis	87.52	94.59	95.98	95.15	93.31±0.98
E. Alata	95.98	96.12	95.98	96.67	96.19±0.08
M.fruticosa	72.54	83.08	83.08	82.94	80.41±1.31
A palaestinum	96.53	95.42	95.70	96.12	95.94±0.12

Table 3.2: Antioxidant capacity (%) of the methanolic extracts of *Malva. sylvistris, Urtica. urens, Asparagus officinalis, Ephedra. Alata, Micromeria. fruticosa,* and *Arum palaestinum* leaves (n=4) using DPPH$^{\bullet}$ free radical scavenging assay.

	1	**2**	**3**	**4**	**Mean ± SE**
M.sylvistris	15.78	22.12	23.29	22.25	20.86±0.85
U. urens	22.38	30.27	28.85	28.59	27.52± 0.88
A.officinalis	50.84	54.08	43.47	56.02	51.09± 1.38
E. Alata	87.84	91.46	90.43	87.32	89.26± 0.49
M.fruticosa	53.69	57.96	55.89	44.63	53.04± 1.47
A .palestinian	60.93	60.93	63.13	66.75	62.94± 0.69

3.2 Quantitative estimation of Total Phenols in selected WPP.

Total phenolic content was estimated by Gallic acid (Fig.3.1) and expressed as mg_s of Gallic acid equivalent (GAE). The quantitative estimation of Total Phenols of plant leaves of *M. sylvistris*, *U. urens*, *A. officinalis*, *E. Alata*, *M. fruticosea*, and *A. palaestinum* (Tables 3.3 and 3.4) were observed to contain a significant amount of total phenols.

Table 3.3: Absorbance values of Gallic acid standard (mg/L) n=3

GAE (mg/L)	1	2	3	Mean ±SE (mg/GAE)
100	0.033	0.050	0.048	0.04 ±0.00
200	0.249	0.635	0.292	0.39±0.12
300	0.689	0.800	0.587	0.69±0.06
500	1.328	1.013	1.132	1.16±0.09
1000	2.208	1.883	2.481	2.19±0.17

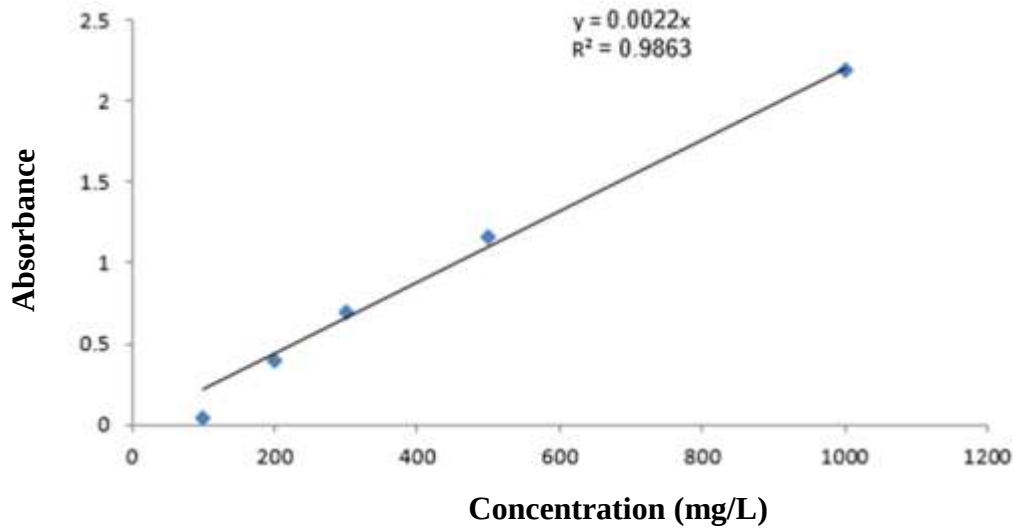


Fig 3.1: Calibration curve of gallic acid. Each point represent the mean of triplicates.

Table 3.4: Determination of total phenols from *Micromeria fruticosa (M. fruticosea), Urtica urens (U. urens), Malva sylvistris (M. sylvistris), Ephedra alata (E. Alata), Arum palaestinum (A. palaestinum*), and *Asparagus officinalis (A. officinalis)* leaves

	Absorbance			**Polyphenol mg GAE/1g dry matter**			
	1	**2**	**3**	**C1**	**C2**	**C3**	**Mean ± SE**
M. fruticosea	0.65	0.84	1.13	158.11	199.85	262.02	206.66±30.19
U. urens	0.12	0.18	0.47	43.76	57.02	119.41	73.39±23.32
M. sylvistris	0.27	0.22	0.37	75.72	64.19	96.80	78.90±9.55
E. Alata	0.32	0.19	0.19	86.80	58.54	57.89	67.74±9.53
A. palaestinum	0.31	0.48	0.71	85.06	122.02	170.93	126.00±24.87
A. officinalis	0.48	0.39	0.50	122.67	102.45	127.02	117.38±7.57

* values expressed are mean ± standard deviation (n=3)

3.3 Antibacterial activity

Various methods of testing antibacterial activity were performed in our laboratory, the most effective and sensitive method was the agar well diffusion method (Valgas, et al., 2007). The antibiotic sensitivity pattern was performed using the disc diffusion method by applying standard antibiotic discs against some Gram-negative bacterial strains like *E. coli, K. pneumonia,* and *P. aeruginosa,* and a gram-positive bacteria *S. aureus*. As shown in Tables: 3.5, 3.6, 3.7, 3.8, 3.9, 3.10

It was clearly observed that methanol extract of antibacterial activity of *Malva sylvistris, Urtica urens, asparagus officinalis, Ephedra Alata, Micromeria fruticosa*, and *Arum palaestinum* to be high in *S. aureus* strain, *for Malva. Sylvistris, Asparagus officinalis, Ephedra Alata, and Arum palaestinum.* and high for *Escherichia Coli* in *Urtica urens, Ephedra alata,* and *Micromeria fruticosa* and high for *Klebsiella pneumonia* in *Malva Sylvistris, Urtica urens, Ephedra Alata,* and *Pseudomonas aeruginosa* is high for *Ephedra Alata*. Other strains showed lesser activity, results shown in mm zone inhibition.

These results clearly showed that these plants have a significant antibacterial effect which reflects the antibacterial nature of phytochemicals present in these ethnobotanical plants supported by their use as a traditional remedy in Palestinian folkloric medicine, and suggested to be new medicinal resources for various microbial infections.
We have used *Proteus Vulgaris* Gram-negative bacteria, but it did not show any result and the most likely that it was damaged.

Phenolic compounds are probably the main antimicrobials, since their components in the essential oils (EO_S) of the spices have been recognized. The active components of EOs are such as thymol, carvacrol, and eugenol (López-Malo, et al., 2002).

Table 3.5: Antibacterial activity of methanolic (Me OH) of *Malva sylvistris* leaves against some gram-negative bacteria: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa),* and gram-positive bacteria *Staphylococcus aureus (S. aureus).*

M. sylvistris		**Percentage%**			
Bacterial Species	**Positive control (mm)**	**1**	**2**	**3**	**Antibacterial activity (%)* ± SD**
E. Coli	G^- 25	33.33	18.18	21.21	24.2 ± 4.6
K. pneumonia	G^- 10	58.82	41.17	41.17	47.1 ± 5.9
P. aeruginosa	G^-25	20.68	31.03	37.93	29.9 ± 5.0
S. aureus	G^+30	57.89	52.63	31.57	47.2 ± 4.6

*****Values are mean of replicates determination (n=3) ± standard deviation. G–, gram-negative; G+, gram-positive.

Table 3.6: Antibacterial activity of methanolic (Me OH) of *Urtica urens* leaves against some gram-negative bacteria: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa),* and gram-positive bacteria *Staphylococcus aureus (S. aureus).*

U. urens		**Percentage%**			
Bacterial Species	***Positive control (mm)***	**1**	**2**	**3**	**Antibacterial activity (%)* ± SD**
E. Coli	G– 25	40	46.66	36.66	41.1 ± 2.9
K. pneumonia	G– 10	65.21	34.78	43.47	47.8 ± 9.1
P. aeruginosa	G–25	22.22	22.22	22.22	22.2 ± 2.5
S. aureus	G+30	57.89	26.31	36.84	40.4 ± 9.1

*****Values are mean of replicates determination (n=3) ± standard deviation G–, gram-negative; G+, gram-positive.

Table3.7: Antibacterial activity of methanolic (Me OH) *Asparagus officinalis* leaves against some gram-negative bacteria*: Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa*), and gram-positive bacteria *Staphylococcus aureus (S. aureus).*

A. officinalis		**Percentage%**			
Bacterial Species	***Positive control (mm)***	**1**	**2**	**3**	**Antibacterial activity (%)* ±SD**
E. Coli	G– 25	43.33	26.66	26.66	32.22 ± 5.55
K. pneumonia	G– 10	20.68	20.68	24.13	21.84 ± 1.15
P. aeruginosa	G–25	7.142	21.42	50	26.19 ± 12.59
S. aureus	G+30	58.82	58.82	52.94	56.86 ± 1.96

*Values are mean of replicates determination (n=3) ± standard deviation. G–, gram-negative; G+, gram-positive.

Table 3.8: Antibacterial activity of methanolic (Me OH) of *Ephedra Alata* leaves against some gram-negative bacteria*: Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa*), and gram-positive bacteria *Staphylococcus aureus (S. aureus*).

E. Alata		**Percentage%**			
Bacterial Species	***Positive control (mm)***	**1**	**2**	**3**	**Antibacterial activity (%)* ±SD**
E. Coli	G– 25	51.72	31.03	34.48	39.08 ± 6.39
K. pneumonia	G– 10	50	42.30	50	47.44 ± 2.56
P. aeruginosa	G–25	27.58	34.48	34.48	32.18 ± 2.29
S. aureus	G+30	65	50	40	51.66 ± 7.26

*Values are mean of replicates determination (n=3) ± standard deviation. G–, gram-negative; G+, gram-positive.

Table 3.9: Antibacterial activity of methanolic (Me OH) of *Micromeria fruticosa* leaves against some gram-negative bacteria: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa*), and gram-positive bacteria *Staphylococcus aureus (S. aureus).*

M. fruticosa		**Percentage%**			
Bacterial Species	***Positive control (mm)***	**1**	**2**	**3**	**Antibacterial activity (%)* ±SD**
E. Coli	G– 25	43.75	34.37	28.12	35.42 ± 4.54
K. pneumonia	G– 10	28	40	20	29.33 ± 5.81
P. aeruginosa	G–25	20	44	20	28 ± 8.00
S. aureus	G+30	5.55	27.78	27.77	20.37 ± 7.41

*Values are mean of replicates determination (n=3) ± standard deviation. G–, gram-negative; G+, gram-positive.

Table 3.10: Antibacterial activity of methanolic (Me OH) of *Arum palaestinum* leaves against some gram-negative bacteria: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa*), and gram-positive bacteria *Staphylococcus aureus (S. aureus).*

A palaestinum		**Percentage%**			
Bacterial Species	***Positive. Control (mm)***	**1**	**2**	**3**	**Antibacterial activity (%)* ±SD**
E. Coli	G– 25	22.58	25.80	48.38	32.26 ± 8.11
K. pneumonia	G– 10	28	24	28	26.66 ± 1.33
P. aeruginosa	G–25	20.68	31.03	37.93	29.88 ± 5.01
S. aureus	G+30	33.33	55.55	77.77	55.55 ± 12.83

*****Values are mean of replicates determination (n=3) ± standard deviation. G–, gram-negative; G+, gram-positive.

3.4 Qualitative phytochemical screening

The phytochemical screening assays *of M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea,* and *A. palaestinum* leaves revealed the presence of a wide range of phytochemical groups such as alkaloids, flavonoids, phenols, tannins, quinones, saponins, steroids, and terpenoids present in the methanolic extract. as shown in Table 3.11 methanolic extract.

Phytochemicals are known to exhibit important medicinal as well as many physiological activities. Cardiac glycosides (digoxin and digitalis) are phytoestrogens, and are 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).

Coumarins have shown some evidence of biological activity and have limited approval for few medical uses as pharmaceuticals, such as in the treatment of lymphedema and their ability to increase plasma antithrombin levels. Flavonoids (specifical 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 (Gurung, et al., 2013). Moreover, they can cross the blood-brain barrier (BBB) and may exhibit neuropharmacological 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 (Rendeiro, et al., 2013).

Phenolic compounds are recognized antioxidants and anti-inflammatory agents which can protect intestinal cells from pro-oxidant and inflammatory injuries (Rodríguez-Ramiro, et al., 2013). Quinones is a subset of the quinoid family which also contains the quinone imines and the quinone methides, quinones can induce cytoprotection through the

induction of detoxification enzymes, anti-inflammatory activities, and modification of redox status.

The mechanisms by which quinones cause these effects can be quite complex. The various biological targets of quinones depend on their rate and site of formation and reactivity (Bolton & Dunlap, 2017). Saponins are widely distributed plant natural products with vast structural and functional diversity. The saponins and their biosynthetic intermediates display a variety of biological activities of interest to the pharmaceutical, cosmetic, and food sectors (Moses, et al., 2014).

Steroids have two principal biological functions: as important components of cell membranes that alter membrane fluidity; and as signaling molecules. It should be mentioned that the absence or presence of certain phytochemicals in some plant is attributed to various physiological reactions that take place inside the plant, as well as environmental effects on the plant (Ezeonu & Ejikeme, 2016; Re, et al., 1999; Shrestha, et al., 2015)

Table 3.11: Phytochemical screening for the methanolic extracts from *Malva sylvistris*, *Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria fruticosa,* and *Arum palaestinum* leaves**.**

test	***M. sylvistris***	***U. urens***	***A. officinalis***	***E. Alata***	***M. fruticosa***	***A. palaestinum***
alkaloids	+	+	-	-	+	+
anthraquinones	-	+	-	-	-	-
anthocyanin	-	-	-	-	-	-
cardiac glycosides	-	-	-	-	-	-
coumarins	-	-	-	-	+	+
flavonoids	+	++	+	-	+	-
glycosides	-	-	-	+	-	+
phenolic groups	+	++	+	-	+	+
phlobatannins	-	-	-	-	+	-
quinones	+	-	+	-	-	-
saponins	+	+	+	-	+	-
steroids	+	-	-	-	-	-
tannins	++	++	+	+	+	+
terpenoids	+	+	-	-	-	-

3.5 GC-MS analysis

The GC-MS analysis of a methanolic extract of *Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra Alata, Micromeria. fruticosa* and *Arum palaestinum* leaves are illustrated in Figures (3.3, 3.4, 3.5, 3.6, 3.7, 3.8). The GC-MS analysis revealed the presence of many bioactive compounds. The bioactive compounds identified with their retention time and molecular weight are shown in tables (3.12, 3.13, 3.14, 3.15, 3.16, 3.17).

The most predominant bioactive compounds of these plants are the presence of various bio-active compounds detected by GC-MS analysis using the methanolic extract of the plant's leaves of *M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea*, and *A. palaestinum* justifies the traditional importance of these ethnobotanical plants used by traditional practitioners. Locally in Palestinian territories, these herbs were used long before for many medicinal uses such as *Malva sylvistris* used to increase milk production in lactating mothers, indigestion problems, skin disorders, and as food (Zakhireh, et al., 2013). *Arum palaestinum* is the stewed leaves used for cancer, kill intestinal worms of animals, boost bones of the body, remedy for open injuries, and kidney stones (Mayer-Chissick & Lev, 2014)

In Iraq *Arum palaestinum* was used medically to treat skin sores, syphilis, rheumatism, tuberculosis, diarrhea, and stomach worms (Ben-Ya'akov, 1992). *Urtica urens* is used as a laxative diuretic, dog bites, gangrenous wounds, swellings, nose bleeding, relieving menstruation, spleen-related disease, pleurisy, pneumonia, and mouth sores (Randall, 2001). Ephedra alata is used in healing kidneys, cancer, bronchi, circular system, and to relieve an asthma attack (Jaradat, et al., 2015). *Pricky asparagus is* especially used as a diuretic drug, and relieves pain, enhances fertility by increasing sperm number (Lev, 2002). *Micromeria fruticosa (L.)* Druce was utilized to relieve stomachache and abdominal colic, intestinal ache and inflammation, fever, asthma, and respiratory system; whereas *M. myrtifolia* L. was used to cure skin and heart diseases, digestive systems, and asthma (Kilari, et al., 2015)

From a pharmacological point of view, isolation of individual phytochemical constituents of these medicinal plants and subjecting them to biological activity will be giving fruitful

results and will open a new area of investigation of individual components and their pharmacological potency. Therefore, it is recommended that these plants should be fully evaluated for their pharmaceutical importance.

Some compounds were repeated in more than one wild plant of the selected plants we studied, which called us to search for their biological activities.

1. Phytol activity is Antimicrobial, anticancer, anti-inflammatory, antidiuretic, immunostimulatory, and anti-diabetic Antinociceptive (reducing sensitivity to painful stimuli) and antioxidant properties, antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immune-stimulatory, and anti-diabetic (Kulkarni, et al., 2015).
2. Natural compounds like: thymol, carvacrol, eugenol, and menthol have shown to be effective against various fungal growth in vitro for all fungal species, with different levels of potency. According to this study, the *in vitro* data obtained may serve as a guide for future studies *in vivo* in growth inhibition of foodborne fungal pathogens (Abbaszadeh, et al., 2014)

Table 3.12: Major compounds detected in *Malva sylvistris* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF).

#	**Rt**	**M/Z**	**Compound ID**	**MW**	**MF**
1.	4.98	61/70/144	Cyclohexanecarboxylic acid,2-hydroxy,ethyl ester	172	$C_9H_{16}O_3$
2.	5.75	55/84/105	1-Pentene,2,3,3-trimethyl-5-phenyl	188	$C_{14}H_{20}$
3.	6.04	58/84/105	1-Oxaspiro(2.2)pentane,5-isopropylidene-tetramethyl	166	$C_{11}H_{18}O$
4.	6.07	93/105/132	5,9-Tetradecadiyne	190	$C_{14}H_{22}$
5.	6.08	55/73/84	cyclopentane,1-hexyl-3-methyl	168	$C_{12}H_{24}$
6.	6.13	55/73/127	Caprylic anhydride	270	$C_{16}H_{30}O_3$
7.	6.42	55/91/107	Vitamine A aldehyde	204	$C_{15}H_{24}$
8.	6.47	55/91/147	Hexanoic acid	280	$C_{16}H_{24}O_4$
9.	6.57	57/191/206	Phenol,3,5-bis(1,1-dimethylethyl	206	$C_4H_{22}O$
10.	6.78	55/70/111	5,5,8A-trimethyldecalin-1-one	194	$C_{13}H_{22}O$
11.	6.79	55/111/137	2(4h)-benzofuranone,5,6,7,7a-tetrahydre-4,4,7a-trimethyln(R)	180	$C_{11}H_{16}O_2$
12.	6.83	137/156/180	3-Ehyl-4-hydroxybicyclo(3.3.1)non-3-one	180	$C_{11}H_{16}O_2$
13.	6.95	73/117/156	Pentanedioic acid 3,3-dimethyl-dimethyl ester	188	$C_9H_{16}O_4$
14.	7.11	55/91/133	Megastigmatrienone	190	$C_{13}H_{18}O$
15.	7.25	55/70/105	6-methyl-3,5,8,8a-tetrahydro-1h-2-benzopyran. 1h-2-benzopyran,3,5,8,8a-tetrahydro-6-methyl	150	$C_{10}H_{14}O$

#	Rt	M/Z	Compound ID	MW	MF
16.	7.28	55/71/82	3-methyl-2-(3-methylpentyl)-3-buten-1-ol	170	$C_{11}H_{22}O$
17.	7.34	55/105/123	benzoic acid,pentyl ester	192	$C_{12}H_{16}O_2$
18.	7.55	55/70/105	2,5-octadecadiynoic acid,methyl ester	290	$C_{19}H_{30}O_2$
19.	7.65	55/71/110	1,1-bicyclooctyl	222	$C_{16}H_{30}$
20.	7.76	57/68/95	Hexadecen-1-ol, tetramethyl	296	$C_{20}H_{40}O$
21.	7.78	55/68/95	Cyclohexane,1,1(oxydi-2,1-ethanediyl)bis(4-methyl)	266	$C_{18}H_{34}O$
22.	7.79	57/68/95	cyclododecanol	184	$C_{12}H_{24}O$
23.	7.80	57/68/95	Tetradecen-1-ol, methylpropionate	282	$C_{18}H_{34}O_2$
24.	7.82	57/71/95	3-t-butyl-oct-6-en-1-ol	184	$C_{12}H_{24}O$
25.	7.86	55/69/91	3-eicosyne	278	$C_{20}H_{38}$
26.	7.94	69/81/95	Cyclopropaneoctanal,2-octyl	280	$C_{19}H_{36}O$
27.	7.95	57/81/95	Oxirane, heptadecyl	282	$C_{19}H_{38}O$
28.	8.03	57/73/147	1,2-15,16-Diepoxyhexadecane	254	$C_{16}H_{30}O_2$
29.	8.1	55/74/87	benzene(1-cyclohexylethyl)	188	$C_{14}H_{20}$
30.	8.11	74/87/143	Nonadecanoic acid, methyl ester	312	$C_{20}H_{40}O_2$
31.	8.13	55/91/105	tricyclo(4.3.0(7,9))non-3-ene,2,2,5,5,8,8-hexamethyl,(1.alpha,63be	204	$C_{15}H_{24}$
32.	8.17	57/147/227	benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)4-hydroxy,met	292	$C_{18}H_{28}O_3$

#	Rt	M/Z	Compound ID	MW	MF
33.	8.27	55/70/82	cycloteradecene,1,2-dimethyl	222	$C_{16}H_{30}$
34.	8.32	55/70/91	17-octadecene-9,11-diynoic acid,8-hydroxy,methyl ester	304	$C_{19}H_{28}O_3$
35.	8.45	55/91/105	isoledene	204	$C_{15}H_{24}$
36.	8.54	55/105/133	5-isopropylidene-6-methyldeca-3,6,9-trien-2-one	204	$C_{14}H_{20}O$
37.	8.70	55/67/79	cis,cis,cis-7,10,13-hexadecatrienal	234	$C_{16}H_{26}O$
38.	8.71	55/67/79	11,14,17-eicosatrienoic acid,methyl ester	320	$C_{21}H_{36}O_2$
39.	8.74	57/71/95	1,2-15,16-diepoxyhexadecane	254	$C_{16}H_{30}O_2$
40.	8.75	55/71/123	Phytol	296	$C_{20}H_{40}O$
41.	8.84	55/119/157	Benzene,1,2,4,5-tetraethyl	190	$C_{14}H_{22}$
42.	8.91	55/161/175	1R,4R,7R,11R-1,3,4,7 tetramethyltricyclo (5.3.1.0(4,11) undec-2-ene	204	$C_{15}H_{27}$
43.	9.68	55/105/189	cycloprop(e)indene-1a,2(1h)-dicarboxaldehde,3a,4,5,6,6a,6b-hexa	232	$C_{15}H_{20}O_2$
44.	9.86	55/91/189	Tetracyclo(6.1.0.0)(2,4).0(5,7)nonane,3,6,9-trimethyl	246	$C_{18}H_30$

Table 3.13: Major compounds detected in *Urtica urens* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF).

#	Rt	M/Z	Compound ID	MW	MF
1.	5.71	55/84/105	1-pentene,2,3,3-trimethyl-5-phenyl	188	$C_{14}H_{20}$
2.	5.87	55/70/82	3-methyl-2-(3-methylpentyl)-3-buten-1-ol	170	$C_{11}H_{22}O$
3.	6.22	55/73/105	11,11-dimethyl-spiro(2,9)dodeca-3,7-dien	190	$C_{14}H_{22}$
4.	6.48	55/91/149	Bicyclo(3.3.1)non-2-one,1-methyl-9-(1-methylethylidene)	192	$C_{13}H_{20}O$
5.	6.56	57/77/95	Oxirane,(4-(1,1-dimethylethyl)phenoxy)methyl)	206	$C_{13}H_{18}O_2$
6.	6.63	55/83/91	(3,7,7-trimethyl-bicyclo(2.2.1)hept-2-yl)-methanol	168	$C_{11}H_{20}O$
7.	6.79	55/67/91	1H-naphthalen-2-one,3,4,5,6,7,8-hexahdro-4A,8A-dimethyl	180	$C_{12}H_{20}O$
8.	6.95	55/77/129	Cyclopentanecarboxylic acid,4-methylene-2-phenyl-methyl est	216	$C_{14}H_{16}O_2$
9.	7.05	55/69/91	Tricyclo(4.3.0.0(7,9))non-3-ene,2,2,5,5,8,8-hexamethyl-(1,alpha,6.be	204	$C_{15}H_{24}$
10.	7.12	55/71/91	spiro(2.7)dec-4-ene,1,1,5,6,6,9,9-heptamethyl-10-methylene	246	$C_{18}H_{30}$
11.	7.19	55/77/105	Vitamin A aldehyde	284	$C_{20}H_{28}O$

#	Rt	M/Z	Compound ID	MW	MF
12.	7.27	57/71/83	3-cyclopentylpropionic acid,2,2-dimethylpropyl ester	212	$C_{13}H_{24}O_2$
13.	7.34	55/95/123	Decahydro-4,4,8,9,10-pentamethylnaphthalene	208	$C_{15}H_{28}$
14.	7.37	55/69/81	menthol	156	$C_{10}H_{20}O$
15.	7.45	55/70/91	Octahydro-2,2-dimethyl-5,8-epoxy-4,10-methano-1,3-dioxolo(4,5-H)(3)	266	$C_{14}H_{18}O_5$
16.	7.64	55/81/95	Menthyl isovalerate	240	$C_{15}H_{28}O_2$
17.	7.68	55/91/110	3,7,7-Trimethyl-8-(2-methyl-propenyl)-bicyclo(4.2.0)oct-2-ene	204	$C_{15}H_{24}$
18.	7.79	57/68/95	Butanoic acid,3-methyl-6-octenyl ester	240	$C_{15}H_{28}O_2$
19.	7.82	58/71/95	2-Pentadecanone,6,10,14-trimethyl	268	$C_{18}H_{36}O$
20.	7.94	55/81/95	Z-8-methyl-9-tetradecen-1-ol formate	254	$C_{16}H_{30}O_2$
21.	8.09	55/74/87	Pentadecanoic acid,methyl ester	256	$C_{16}H_{32}O_2$
22.	8.14	55/95/138	p-menthane,3-allylperoxy	212	$C_{13}H_{24}O_2$
23.	8.17	57/91/147	Benzenepropanoic acid,3,5-bis(1,1-dimethyl)-4-hydroxy,met	292	$C_{18}H_{28}O_3$
24.	8.26	55/91/149	E,Z-2,15-octadecadien-1-ol acetate	308	$C_{20}H_{36}O_2$
25.	8.33	55/69/91	10,12-docasadyndioic acid	362	$C_{22}H_{34}O_4$
26.	8.41	55/70/91	isopinocarveol	152	$C_{10}H_{16}O$

#	Rt	M/Z	Compound ID	MW	MF
27.	8.67	55/67/81	2H-pyran,2-(7-heptadecynyloxy)tetrahydro	336	$C_{22}H_{40}O_2$
28.	8.69	67/79/95	Nonanoic acid,9-(hexenylidenecyclopropylidene),2-hydroxy-1	352	$C_{21}H_{36}O_4$
29.	8.73	57/71/81	phytol	296	$C_{20}H_{40}O$
30.	8.80	55/71/95	Oxirane,dodecyl	212	$C_{14}H_{28}O$
31.	9.12	55/69/73	1,10-hexadecanediol	258	$C_{16}H_{34}O_2$
32.	9.19	55/73/79	Nonanoic acid,9-(3-hexenylidenecyclopropylidene),2-hydroxy-1	352	$C_{21}H_{36}O_4$
33.	9.47	55/83/95	Succinic acid,di-(0)-menthyl ester	394	$C_{24}H_{42}O_4$

Table 3.14: Major compounds detected in *Asparagus officinalis* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF).

#	Rt	M/Z	Compound ID	MW	MF
1.	4.25	55/61/70	1,5-cyclooctanediol	144	$C_8H_{16}O_2$
2.	4.97	57/73/101	1,2-cyclobutanedicarboxylic acid,cis	144	$C_6H_8O_4$
3.	5.01	57/71/101	D-glucuronic acid	194	$C_6H_{10}O_7$
4.	5.28	55/73/91	Neo-inositol	180	$C_6H_{12}O_6$
5.	5.54	55/73/84	2-propenoic acid, octyl ester	184	$C_{11}H_{20}O_2$
6.	5.60	55/67/83	octanoic acid,2-hexenylvester, (E)	226	$C_{14}H_{26}O_2$
7.	5.62	55/82/91	8-octadecenal	266	$C_{18}H_{34}O$
8.	5.64	55/73/82	cycloheptanone,2-methyl	126	$C_8H_{14}O$
9.	5.72	77/135/150	2-methoxy-4-vinylphenol	150	$C_9H_{10}O_2$
10.	5.90	55/84/91	12-methyl-oxa-cyclododecan-2-one	198	$C_{12}H_{22}O_2$
11.	5.94	55/77/96	P-menthane-3,8-diol,cis-1,3,trans-1,4-	172	$C_{10}H_{20}O_2$
12.	6.29	55/69/82	Z-9-pentadecenol	226	$C_{15}H_{30}O$
13.	6.49	55/73/91	2-propenoic acid,tridecyl ester	254	$C_{16}H_{30}O_2$
14.	6.56	57/91/191	7,9-di-tertbutyl-1-oxaspiro(4,5)deca-6,9-dien-8-one	262	$C_{17}H_{26}O_2$
15.	6.73	57/73/95	1,10-hexadecanediol	258	$C_{16}H_{34}O_2$
16.	6.84	77/137/180	4-methyl-2,5-dimethoxybenzaldehyde	180	$C_{10}H_{12}O_3$

#	Rt	M/Z	Compound ID	MW	MF
17.	7.79	55/68/95	3-eicosyne	278	$C_{20}H_{38}$
18.	7.82	55/71/95	E-8-methyl-9-tetradecen-1-ol acetate	268	$C_{17}H_{32}O_2$
19.	7.95	55/68/82	2H-benzocyclohepten-2-one,decahydro-9A-methyl-trans	180	$C_{12}H_{20}O$
20.	8.10	55/74/87	Heptadecanoic acid,methyl ester	284	$C_{18}H_{36}O_2$
21.	8.17	57/147/277	Benzenepropanoic acid,3,5-bis(dimethyl)-4-hydroxy,met	292	$C_{18}H_{28}O_3$
22.	8.71	55/67/79	11,14,17-eicosatrienoic acid,methyl ester	320	$C_{21}H_{36}O_2$
23.	8.74	57/71/81	phytol	296	$C_{20}H_{40}O$
24.	9.33	57/71/83	1-dodecanol,2-methyl-(S)	200	$C_{13}H_{28}O$

Table 3.15: Major compounds detected in *Ephedra Alata* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF).

#	Rt	M/Z	Compound ID	MW	MF
1.	4.74	56/73/85	Ethanol,2-(2-(tetrahydro-2H-pyran-2-YL)oxy)ethoxy)	190	$C_9H_{18}O_4$
2.	5.00	57/73/85	4-methylpentyl pentanoate	186	$C_{11}H_{22}O_2$
3.	5.11	56/73/85	1-butanol,4-((tetrahydro-2-yl)oxy)	174	$C_9H_{18}O_3$
4.	5.47	66/73/85	2-(2-(5-norbornenyl)oxy)-tetrahydropyran	194	$C_{12}H_{18}O_2$
5.	5.58	55/85/91	2-octyn-1-ol,7-(tetrahydro-2H-pyran-2-yl)oxy	226	$C_{13}H_{22}O_3$
6.	5.69	85/89/178	Pentanoic acid,4,4-dimethoxy, ethyl ester	190	$C_9H_{18}O_4$
7.	5.73	55/57/85	Valeric anhydride	186	$C_{10}H_{18}O_3$
8.	5.86	55/85/107	Cyclohexene,1-methyl-4-(1-tetrahydropyran-2-yloxy)ethyl)	224	$C_{14}H_{24}O_2$
9.	5.91	55/65/73	1,2-pentanediol	104	$C_5H_{12}O_2$
10.	6.07	56/73/91	1,3-dioxolane,2-(2-phenyl-2-propyl)	192	$C_{12}H_{16}O_2$
11.	6.58	57/77/191	Pentanedioic acid,(2,4-di-T-butylphenyl)mono-ester	320	$C_{19}H_{28}O_4$
12.	6.94	55/73/147	Methyl 14-methoxyhexadecanoate	300	$C_{18}H_{36}O_3$
13.	7.47	55/73/173	1,3,5,trioxane,2,4,6-tripropyl	216	$C_{12}H_{24}O_3$
14.	7.79	55/68/82	Nonamethylene glycol	204	$C_{11}H_{24}O_3$
15.	8.11	55/74/87	Octadecanoic acid,methyl ester	298	$C_{19}H_{38}O_2$
16.	8.74	55/71/81	Phytol	296	$C_{20}H_{40}O$
17.	8.79	55/67/73	2-propenoic acid 1,6-hexanediyl ester	226	$C_{12}H_{18}O_4$
18.	8.84	57/73/91	Benzene 1-(1,1-dimethylethyl)4-(2-ethoxyethoxy)	222	$C_{14}H_{22}O_2$

Table 3.16: Major compounds detected in *Micromeria fruticosa* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF)

#	Rt	M/Z	Compound ID	MW	MF
1.	4.79	59/79/121	2-naphthalenol,decahydro	154	$C_{10}H_{18}O$
2.	4.89	55/69/97	Cyclohexanone,5-methyl-2-(1-methylethyl)	154	$C_{10}H_{18}O$
3.	5.05	55/71/81	menthol	156	$C_{10}H_{20}O$
4.	5.32	67/81/109	pulegone	152	$C_{10}H_{16}O$
5.	5.58	55/91/135	thymol	150	$C_{10}H_{14}O$
6.	5.71	55/71/87	Trans-sabinenehydrate	154	$C_{10}H_{18}O$
7.	6.02	55/67/81	(1S,15S)-bicyclo(13.1.0)hexadecan-2-one	236	$C_{16}H_{28}O$
8.	6.26	69/79/93	caryophylene	204	$C_{15}H_{24}$
9.	6.48	55/69/81	1-oxaspiro(2.5)octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)	206	$C_{14}H_{22}O$
10.	6.53	55/81/91	isoledene	204	$C_{15}H_{24}$
11.	6.57	57/91/191	2H-indeno(1,2-B)furan-2-one,3,3A,4,5,6,7,8,8B-octahydro-8,8-dimethy	206	$C_{13}H_{18}O_2$
12.	6.63	67/81/137	Mint furanone	166	$C_{10}H_{14}O_2$
13.	6.77	55/67/81	Trans,cis-2-ethylbicyclo(4.4.0)decane	166	$C_{12}H_{22}$
14.	6.88	73/91/149	2-ethyl-5-N-propylphenol	164	$C_{11}H_{16}O$
15.	6.96	55/69/79	(-)-spathulenol	220	$C_{15}H_{24}O$

#	Rt	M/Z	Compound ID	MW	MF
16.	6.99	69/79/93	Caryophyllene oxide	220	$C_{15}H_{24}O$
17.	7.09	55/91/105	Cyclodecacyclotetradecene,14,15-didehydro-1,4,5,8,9,10,11,12,13	296	$C_{22}H_{32}$
18.	7.20	55/69/91	Tricyclo(4.4.0.0(2,7))dec-3-methyl,1-methyl-8-(1-methyeth	220	$C_{15}H_{24}O$
19.	7.32	55/79/91	Androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha)	318	$C_{21}H_{34}O_2$
20.	7.41	55/77/91	6,9-octadecadiynoic,methyl ester	290	$C_{19}H_{30}O_2$
21.	7.79	55/68/95	3-eicosyne	278	$C_{20}H_{38}$
22.	7.84	55/95/137	Naphthalene,1,1-methylenebis(decahydro)	288	$C_{21}H_{36}$
23.	7.94	55/81/95	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
24.	8.09	55/74/87	Octadecanoic acid,11-methyl,methyl ester	312	$C_{20}H_{40}O_2$
25.	8.17	57/71/91	Tricyclo(6.1.0.0(2,4)0(5,7))nonane,3,6,9-triethyl-3,6,9-trimethyl	246	$C_{18}H_{30}$
26.	8.74	55/71/81	1,2-15,16-diepoxyhexadecane	254	$C_{16}H_{30}O_2$
27.	8.85	55/67/119	Ursodeoxycholic acid	392	$C_{24}H_{40}O_4$
28.	9.18	55/69/81	5.alpha-pregnan-20-one,3.beta,11,alpha,12beta-trihydroxy	350	$C_{21}H_{34}O_4$
29.	9.32	55/69/73	10,12-decasadiyndioic acid	362	$C_{22}H_{34}O_4$

Table 3.17: Major compounds detected in *Arum palaestinum* extracts with their retention time (Rt) and molecular weight (MW), Molecular masses (M/Z), and the molecular formula (MF).

#	Rt	M/Z	Compound ID	MW	MF
1.	4.09	56/70/84	2-ethyl-5-propylcyclopentanone	154	$C_{10}H_{18}O$
2.	5.01	57/71/83	Cyclopropane,1-(2-methylbutyl)-1-(methylpropyl	168	$C_{12}H_{24}$
3.	6.58	57/73/98	Oxirane,((4-(1,1-dimethylethyl)phenoxy)methyl)	206	$C_{13}H_{18}O_2$
4.	7.13	55/91/133	9-heptadecene-4,6-diyn-8-ol,(Z)	246	$C_{17}H_{26}O$
5.	7.79	57/68/82	E-10-methyl-11-tetradecen-1-ol propionate	282	$C_{18}H_{34}O_2$
6.	7.83	58/71/85	2-pentadecanone,6,10,14-trimethyl	268	$C_{18}H_{36}O$
7.	7.96	55/69/82	Oleyl alcohol	268	$C_{18}H_{36}O$
8.	8.10	74/87/95	Hexadecanoic acid, methyl ester	270	$C_{17}H_{24}O_2$
9.	8.17	57/147/277	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)4-hydroxy,met	292	$C_{18}H_{28}O_3$
10.	8.68	55/67/81	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$
11.	8.71	55/57/79	11,14,17-eicosatrienoic acid,methyl ester	320	$C_{21}H_{36}O_2$
12.	8.74	57/71/81	phytol	296	$C_{20}H_{40}O$
13.	9.04	55/69/83	9-octadecenal,(Z)	266	$C_{18}H_{34}O$
14.	9.33	57/71/85	Octadecane,1-(ethenyloxy)	296	$C_{20}H_{40}O$
15.	9.59	55/69/111	Z-9-pentadecenol	226	$C_{15}H_{30}O$

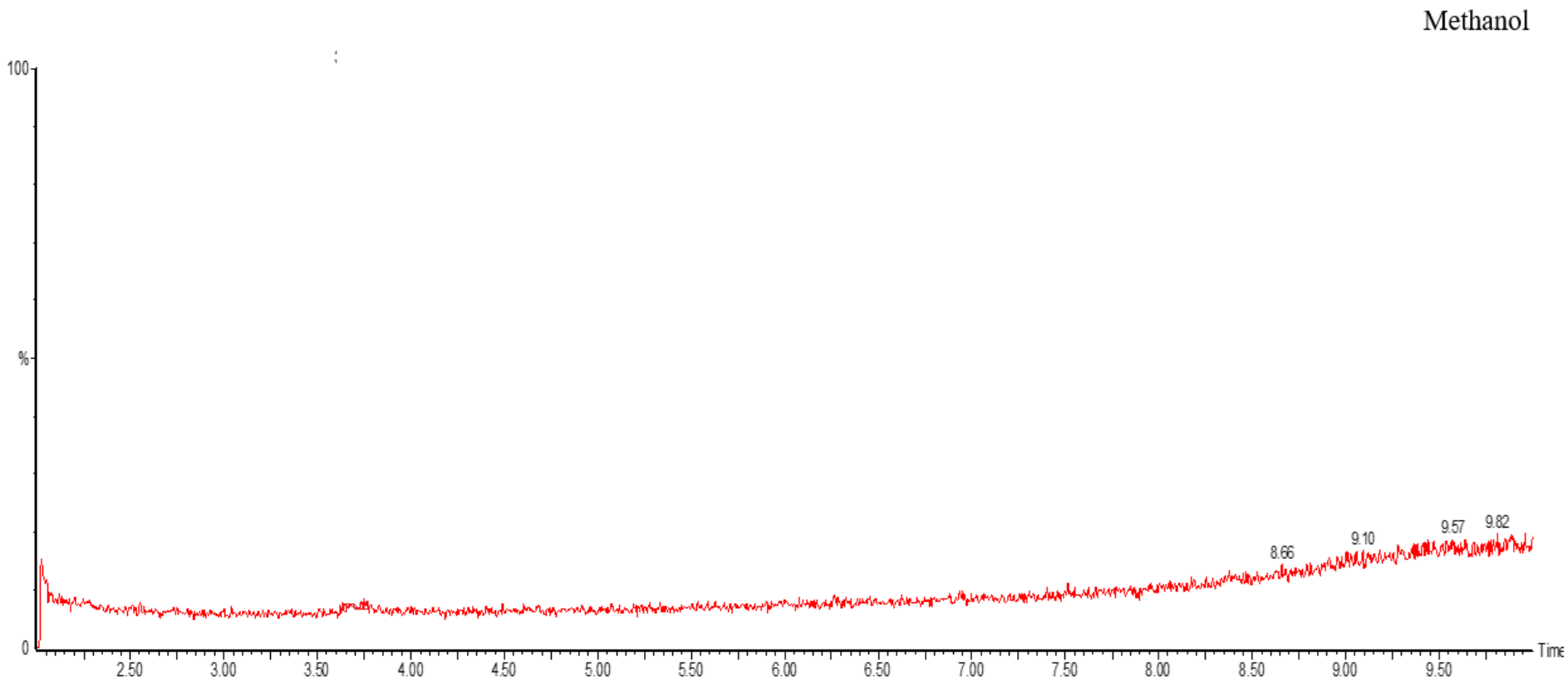


Fig 3.2: Representative total-ion mass chromatograms (TIC) of the methanol (100%)

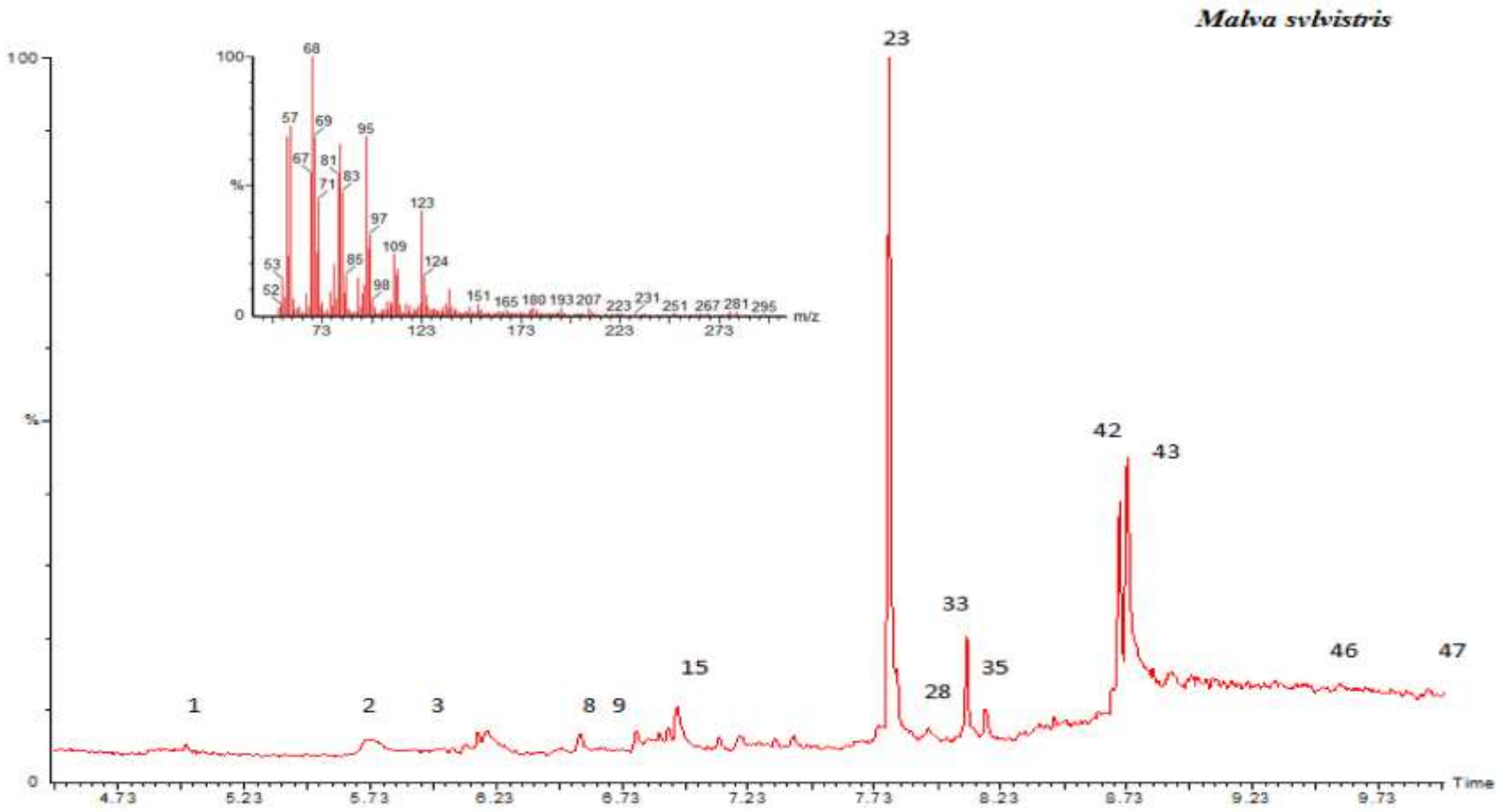


Fig 3.3: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Malva sylvistris*) leaves (A) and control (B). numbers under peaks represent the retention time (Rt) in minutes for each peak.

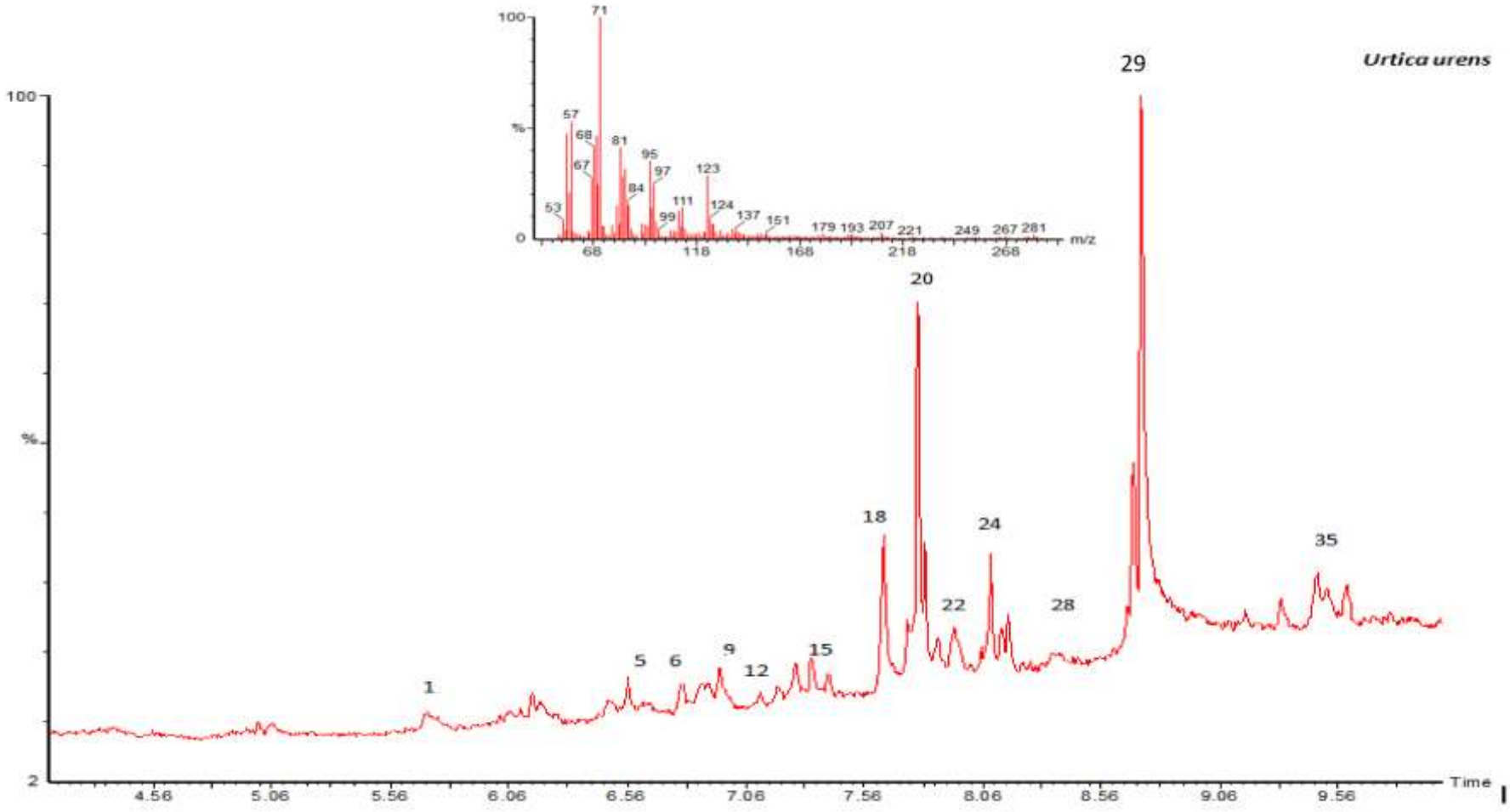


Fig 3.4: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Urtica urens*) leaves (A) and control (B). numbers under peaks represent the retention time (Rt) in minutes for each peak.

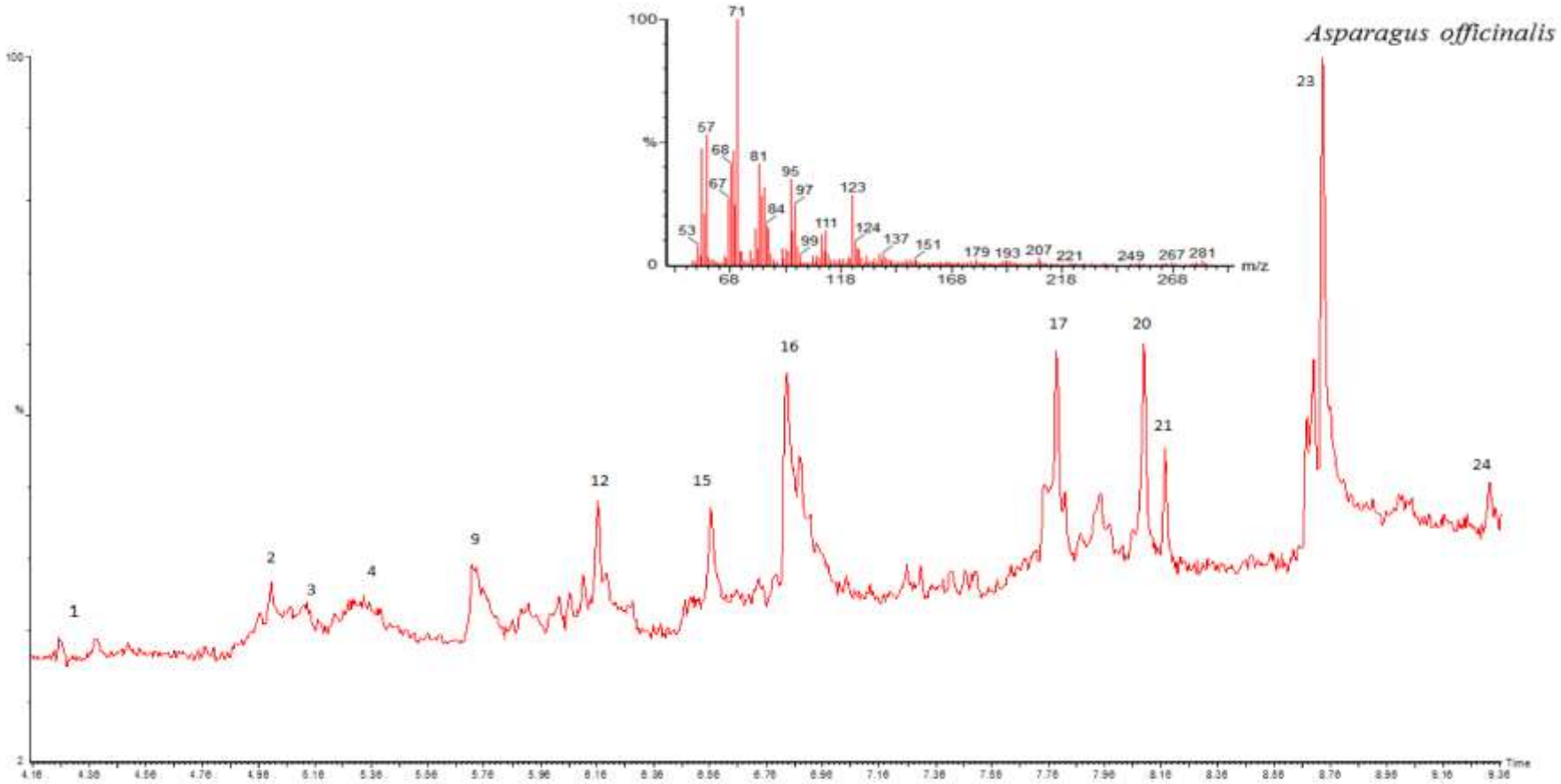


Fig 3.5: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Asparagus officinalis*) leaves (A) and control (B)**.** numbers under peaks represent the retention time (Rt) in minutes for each peak.

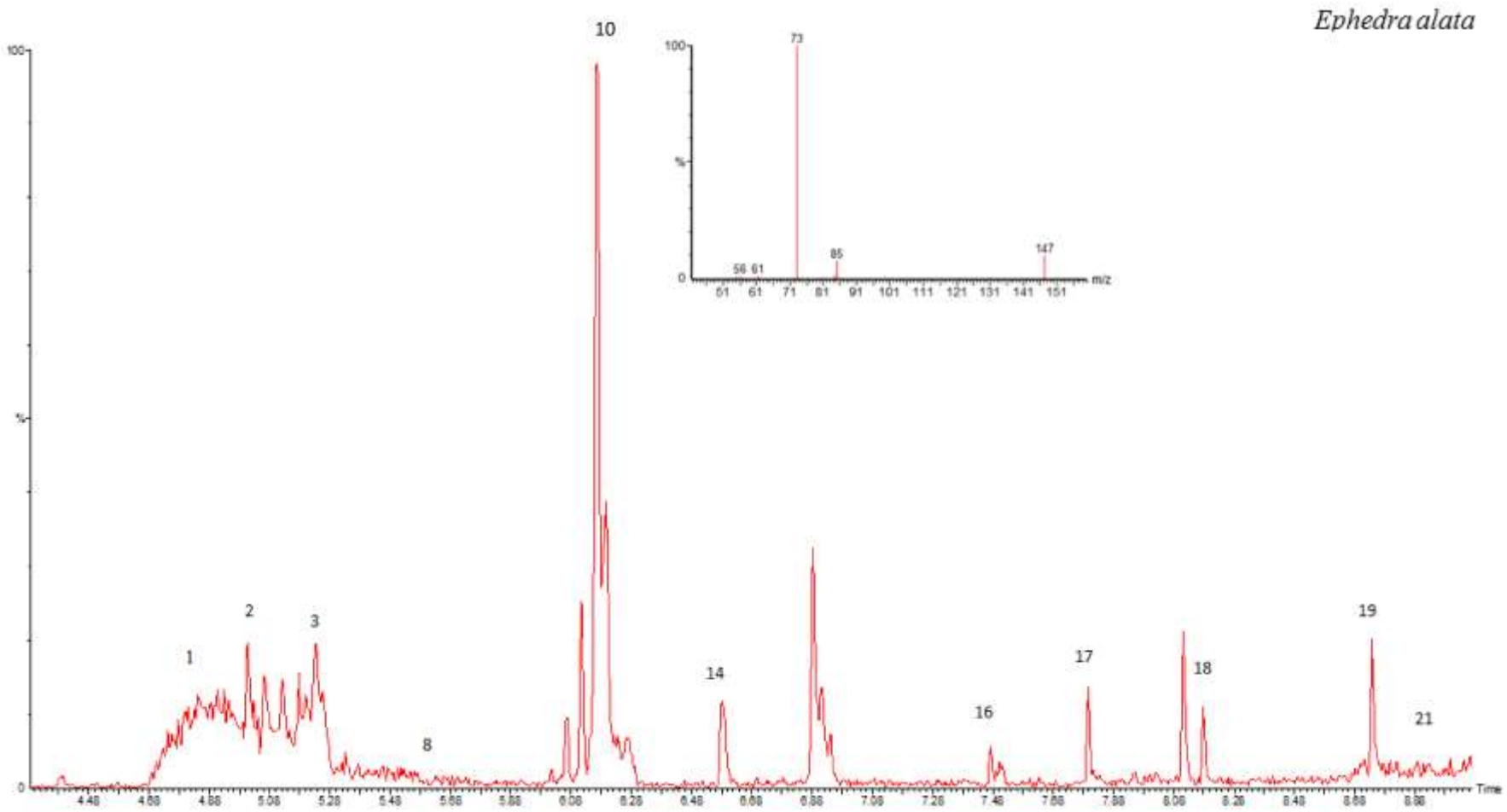


Fig 3.6: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Ephedra alata*) leaves (A) and control (B). numbers under peaks represent the retention time (Rt) in minutes for each peak.

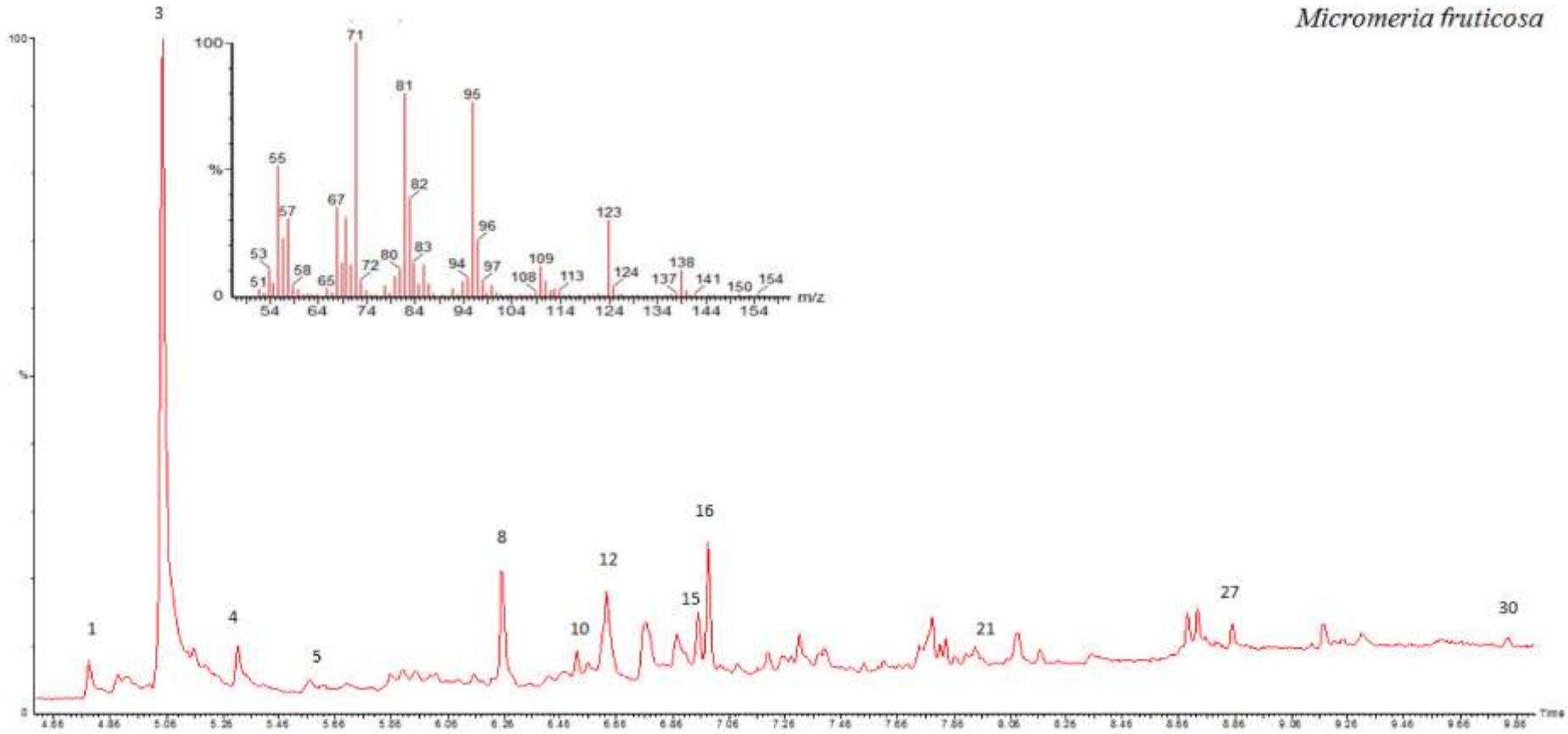


Fig 3.7: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Micromeria fruticosa*) leaves (A) and control (B). numbers under peaks represent the retention time (Rt) in minutes for each peak.

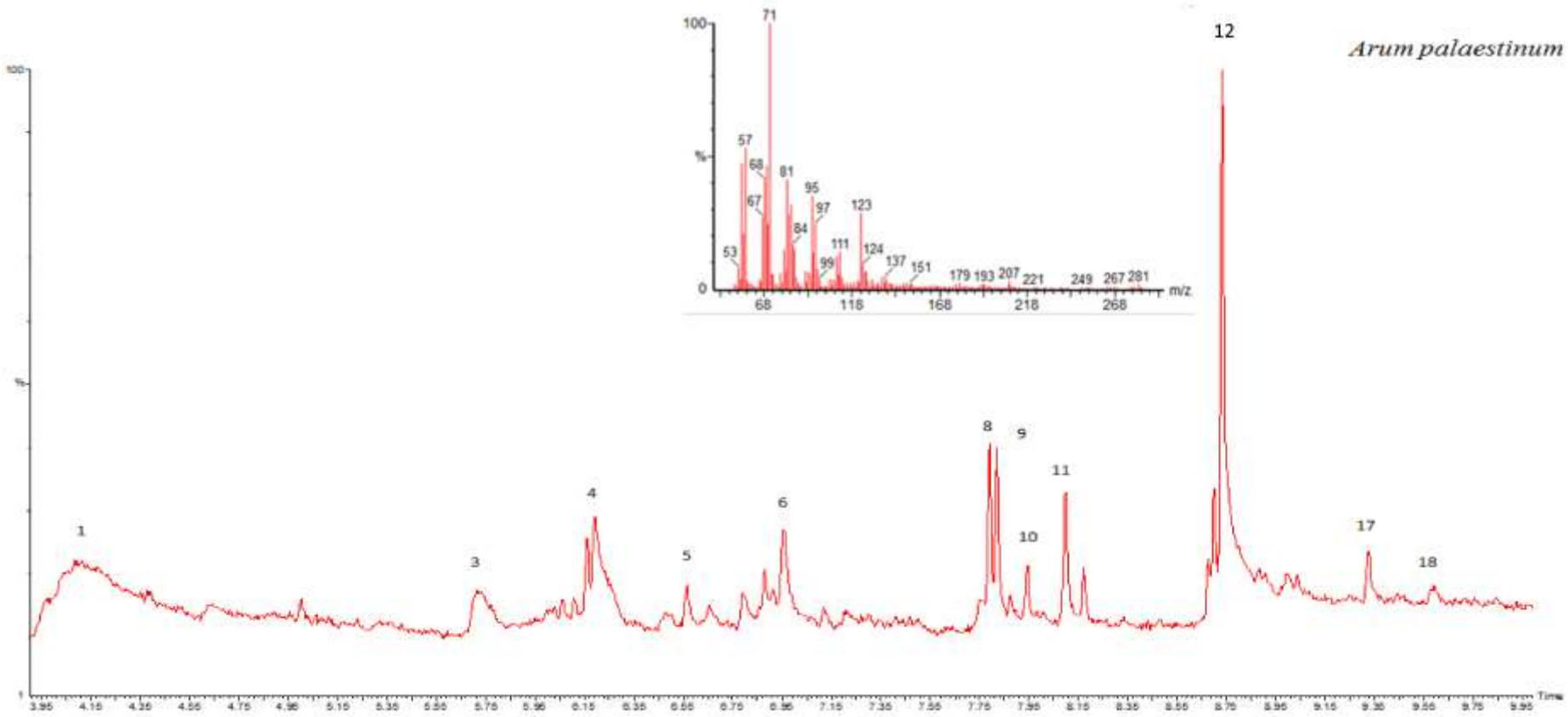


Fig 3.8: Representative total-ion mass chromatograms (TIC) of the volatile compounds detected in the methanolic extracts of (*Arum palaestinum)* leaves (A) and control (B). numbers under peaks represent the retention time (Rt) in minutes for each peak.

Chapter4: Discussion

The overall aim of this thesis was to identify the main volatile compounds and phenolic compound present in six Wild Palestinian Plants (WPP), the effect of these plants on some pathogenic bacterial strains, estimate the antioxidant effect using $ABTS^{\bullet+}$ $DPPH^{\bullet}$ assays, evaluate the phenolic content, and show some phytochemical screening characters. Our investigation showed that *Ephedra. Alata, Arum palaestinum,* and *Asparagus officinalis* Wild Palestinian medicinal plants have a strong antioxidant activity compared to other plants tested. *Micromeria fruticosa, Arum palaestinum,* and *Urtica urens* shown to have a great polyphenol content.

Malva sylvistris showed high antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus, Urtica urens* showed a high antimicrobial against *Klebsiella pneumoniae, Asparagus officinalis* showed great antimicrobial activity against *Staphylococcus aureus, Ephedra Alata* showed a high value for antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, and *Arum palaestinum* showed a great antimicrobial value against *Staphylococcus aureus*. All six plants have phytochemicals, where, *M. sylvistris, U. urens, M. fruticosa,* and *A. palaestinum* have alkaloids and phenolic groups, *M. sylvistris, U. urens, A. officinalis,* and *M. fruticosa* have flavonoids, and only *U. urens* has anthraquinones.

Due to its unique biodiversity, Palestine has been classified as one of the most global biodiversity hot spots, this is due to its unique presence at a crossroads between three continents of Asia, Africa, and Europe, which provide a wide variety of plant species biodiversity (Salem, et al., 2010). It has been estimated that Palestine has around 2780 plant taxa covered in the hills and mountains of Gaza and the west bank, out of these, there are more than 700 recorded to have medicinal value (Shtayeh, et al., 2002). In Palestinian folkloric medicine, there are more than 182 medicinal plants are used in treating range of disorders related to skin, kidney, diabetes, hypertension, digestive problems, and many other diseases (Jaradat, 2005; Said, et al., 2002). An investigation carried on some of these Palestinian medicinal plants clearly showed that these plants have valuable antioxidant, antibacterial activities, besides their high nutritive and pharmaceutical value (Hamarsheh, et al., 2017; Qabaha, 2013; Saleh, et al., 2013). WPP showed rich source of biochemicals whose biological activities are not known yet, this research is the first to scan the volatile chemical constituents, estimate the antioxidant activity, assess antimicrobial activity, and screen the presence of major phytochemical

compounds in some wild Palestinian plants namely; *Malva sylvistris, Urtica urens, Asparagus officinalis, Ephedra alata, Micromeria fruticosea,* and *Arum palaestinum*.

4.1 Antioxidant activity in selected WPP

There are several methods by which free radical scavenging activity can be measured. $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays have been widely used to determine the antioxidant capacities of the plant extracts because they give fast and reproducible results (Bhandari, 2012).

Investigation the diluted 1:4 methanolic extracts of the selected WPP showed a remarking but variable scavenging capacity for both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging assays. All selected WPP also showed a high level of phenolic compound (mg/GAE).

The high antioxidant capacity of the selected WPP are consistent with the literature, (Floegel, et al., 2011; Mihaylova, et al., 2015; Mzid, et al., 2017) the antioxidant capacity is partly dependent on total phenols content in plants. The present study found a stronger correlation between total phenolics content and $ABTS^{\bullet+}$ assay than $DPPH^{\bullet}$ assay.

Ephedra alata has been reported to contain a large amount of phenolic compounds that compared with the study that has phenolic compound with high levels (Hegazi & El-Lamey, 2011), the phenolic compound showed a high antioxidant capacity using $ABTS^{\bullet+}$ assay. Although we are not sure of the identity of the phenolic compound in our *E. alata* sample, it is likely that the highest antioxidant capacity.

The antioxidant capacity of selected plants have been consistently associated with the therapeutic potential of the plant, for example, *Ephedra aphylla* is reported in vitro to have strong antiproliferative acting as a model in the treatment of breast cancer (Al-Awaida, et al., 2018). In this study, *Ephedra aphylla* is also showed high antioxidant capacity assessed by H_2O_2 and NO scavenging activity assay. In another study by (Khan, et al., 2017) *Ephedra gerardiana* (root and stem) is showed high antioxidant capacity assessed by $DPPH^{\bullet}$ assay. According to WPP may potentially treat some diseases that are unfamiliar imposing.

The free radical scavenging capacity by $ABTS^{\bullet+}$ is based on the reduction of $ABTS^{\bullet+}$ radicals by antioxidants of the plant extracts tested. Also, in $ABTS^{\bullet+}$ assay the wavelength absorption at 734 nm eliminates the color interference. As shown in (Table 3.1), the antioxidant activity of methanolic extract of plant leaves exhibited a very strong free radical scavenging capacity tested by $ABTS^{\bullet+}$ assay**,**

The $DPPH^{\bullet}$ method is a UV method in which free radicals have a purple color and are strongly absorbed at 517 nm. When $DPPH^{\bullet}$ is radicalized with hydrogen to remove free radicals, the color of the antioxidant is reduced to $DPPH^{\bullet}$ and turns yellow and $DPPH^{\bullet}$ is absorbed at 517 nm reduction (Prakash, et al., 2001).

The antioxidant capacity of methanolic extract of the plant using $DPPH^{\bullet}$ assay showed also strong but less than $ABTS^{\bullet+}$ irrespective of plant special test, these results strongly indicate that the phytochemical composition of these plants has a high free radical scavenging capacity as shown in (Table 3.2). This could explain why these plants are strongly recommended in Palestinian tradition as food and drug remedies, and this also may make them highly beneficial in the pharmaceutical industry.

4.2 Quantitative estimation of Total Phenols of the selected WPP

This investigation aimed to assess and evaluate Total Phenols of selected WPP, as milligrams of Gallic acid per gram of dried plant (mg GAE⁄g). All Extracts of the selected WPP showed a high level of phenols compound (mg/GAE).

The quantitative assessments of Total Phenols of plant leaves of *M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea,* and *A. palaestinum* table 3.4 were observed to contain a significant amount of total phenols. Total Phenols physiologically act as antioxidants. The presence of a valuable amount of phenols in this study revealed the potency of these plants in defeating diseases (Akintola, et al., 2020). Alkaloids carry out many metabolic and pharmacological activities in humans and animals like: antibacterial, antimalarial, anticancer, and antihyperglycemic activities. The Presence of alkaloids in these traditional Palestinian plants exploits them to be a very good source in the pharmaceutical industry (Ezeonu & Ejikeme, 2016; Kilari, et al., 2015). This work also pointed out the presence of flavonoids, flavonoids also exhibit antioxidant activity, they inhibit initiation, promotion, and progression of the tumor, they also inhibit coronary heart diseases, platelet aggregation, inflammation, and allergies (Rahman, 2007)

Polyphenols are the most abundant antioxidant secondary metabolites in the diet. Their whole food intake can be up to 1g/day, which is significantly higher than all other classes of phytochemicals identified as dietary antioxidants. From a perspective point, this is 10 times higher than the intake of vitamin C and 100 times higher than the intake of vitamin E and carotenoids. (Manach, et al., 2004; Scalbert & Williamson, 2000).

Oxidative stress is closely related to the adverse health effects of many diseases, including cardiovascular, respiratory, and nervous diseases, as well as foraging. Free radical damage to lipids, proteins, and DNA has led to such harmful effects. Protection from damage occurs through the action of multiple antioxidants led to protection from these damages (Ranjbar, et al., 2006).

Plants are the origin of complexes that may be utilized as pharmacologically active products.(Barros, et al., 2011), There is consistent evidence in vitro that flavonols, flavones, and anthocyanins have antioxidant activity relayed on the scavenging of oxygen radicals. (Seeram & Nair, 2002), The findings that emerged from this thesis have enhanced our understanding about the WPP consist of these antioxidant compounds like flavonols, flavones, phenols, and anthocyanins that support these WPP contains a huge antioxidant activity.

Phenolic compounds are generally set up in both edible and nonedible plants, and they have been described to have multiple biological effects, including antioxidant activity. Phenolic compounds have increased interest in the food industry as they delay oxidative degradation of lipids, thereby getting better quality and nutritional value of food. (Loliger, 1991), After observing the results, we found that the wild plants harvested from Tulkarm district, Palestine (*Asparagus officinalis, Micromeria fruticosa,* and *Ephedra alata*) were higher in the $ABTS^{\bullet+}$ $DPPH^{\bullet}$ free radical assay, the reason is that the Tulkarm district located in the north of Palestine and is characterized by more rainfall in winter than Hebron District, this what explained the increase in the amount of phenol compounds.

4.3 Antibacterial activity in selected wild Palestinian plants

During the past decades, there has been a significant increase in hospital-acquired infections caused by multiple drugs resistant microbes have occurred. As a result, the risk of bacterial contamination and infection led to an increase in the use of disinfectants (Anderson, et al., 2018), Most antibacterial therapeutic wild plants offensive Gram-

positive strains while rare are active against Gram-negative bacteria (Chopra, et al., 2008; Saravolatz & Eliopoulos, 2003) Gram-positive bacteria More effective goals for antibiotics than Gram-negative bacilli due to the lack of an outer membrane and therefore there is easy access to the drug. However, Gram-positive organisms often have naturally high antimicrobial resistance. In addition, these bacteria can acquire resistance to many drugs use quickly (Epand, et al., 2016), Interestingly, our current results showed a remarkable antimicrobial activity on a higher range of Gram-negative antibiotic-resistant isolates in six wild Palestinian plants that used, *E. Coli* (*U. urens*=41.1%, *E. alata*= 39.08%), *K. pneumonia* (*U. urens*= 47.80%, *E. alata*=47.44%, *M. sylvistris*= 47.10%), *P. aeruginosa* (*E. alata*= 32.18%, *M. sylvestris= 29.9%, A. palaestinum*= 29.88%)

our results on WPP clearly showed that these plants have the potential to be a source of a secondary compound having antimicrobial activity against Gram-positive and Gram-negative bacteria, Indeed, more studies are required to identify which compound is responsible for the Gram-negative action in *U. urens, E. alata, M. sylvistris,* and *A. palaestinum*

4.4 Phytochemical screening of the selected WPP

Medicinal plants are of magnificent value to the health of individuals and communities. The medicinal value of these plants is placed in some chemical compounds that make a certain physiological action on the human body. The greatest importance of these bioactive components of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Edeoga, et al., 2005), additionally, plants are rich in free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Cai , et al., 2003; Wang & Zheng, 2001), Many research studies has revealed that several of these antioxidant complexes possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities(Nile & Park, 2014; Rice, 1995; Sala, et al., 2002)

There is consistent evidence that steroidal compounds are of significance and attention in pharmacy due to their association with such compounds as sex hormones (Okwu, 2001), Tannins have rigorous properties, speed up the curing of wounds, and inflamed mucous

membranes. Apart from tannin and phenolic compounds, additionally the alkaloids, saponin, and flavonoids. Flavonoids, on the other hand, are potent water-soluble antioxidants and free radical scavengers, which avoid oxidative cell damage, have powerful anti-cancer activity (Okwu, 2004)

Many nutrients have a proven ability to enhance immunity, antiviral, antioxidant, and anti-inflammatory effects (Mrityunjaya, et al., 2020). They include zinc, vitamin D, vitamin C, curcumin, cinnamaldehyde, probiotics, selenium, lactoferrin, quercetin, etc. It spreads, preventing disease progression to the severe stage, and further suppressing excessive inflammation providing preventive and curative support against COVID-19 (Mrityunjaya, et al., 2020). There may be scope for adjuvant therapies including nutritional interventions (Lammi & Arnoldi, 2021)

High production of reactive oxygen species can lead to decreased immunity in the living organisms, by increasing susceptibility to viral infections, and infection. To combat this imbalance, getting natural antioxidants from food becomes important.

Typically, a traditional balanced diet aims to provide all the nutritional and antioxidant requirements needed to maintain well-functioning enzymatic and non-enzymatic antioxidant responses that support the immune system. In particular, MD has been shown to contain most of the essential micronutrients and phenolic compounds required for normal functioning.

However, requirements for antioxidant intake vary, especially among high-risk populations. Evidence suggests that supplementation with minerals, vitamins, and phenolic compounds has positive results, especially in frail, obese adults and depressed individuals. (Trujillo-Mayol, et al., 2021), The use of antioxidants (e.g., vitamins C and E), which can combat oxidative stress, has been well proven as a treatment for Adverse Drug Reactions, acute lung injury, and sepsis.(Soto, et al., 2020), Consequently, it is believed that antioxidants can also help patients with COVID-19 (Grant et al., 2020; Soto et al., 2020)

4.5 Phytochemical and Volatile Compounds Identification determined by GC-MS for selected WPP

This study is considered to be the first study to provide evidence for methanolic extracts of *M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea,* and *A. palaestinum* leaves, and were the models used to determine the profile of alkaloids, phenolic, and volatile compounds, which suggested that these plants possess broad-spectrum phenolic and volatile compounds. Therefore, these results could be of relevance to the seed industry, and many other medicinal and pharmaceutical use, which makes them highly recommended in the pharmaceutical industry. The GC-MS technique was utilized and found to be precise, accurate, and reliable in the separation and identification of the components of *M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea,* and *A. palaestinum l*eaves complex volatile mixtures. Phytochemical and Volatile compounds in WPP *M. sylvistris, U. urens, A. officinalis, E. Alata, M. fruticosea,* and *A. palaestinum* methanolic extracts were examined by GC–MS and identified by comparing their mass spectrum with NIST05 mass spectral library. A 44, 33, 24, 18, 29, and 29 volatile compounds and phenolic compounds were detected *in M. sylvistris, U. urens, A. officinalis, E. alata, M. fruticosea,* and *A. palaestinum* respectively. The compound's identity, retention time, molecular formula, and molecular masses values are summarized in Tables (3.12, 3.13, 3.14, 3.15, 3.16, 3.17). The identified peaks of all tested WPP were depicted in the GC–MS TIC chromatogram figures (3.3, 3.4, 3.5, 3.6, 3.7, 3.8). All tested WPPs contain volatile compounds that are in most cases unique to their species. Compounds that were found when we analyzed wild plants in GC-MS, which matched the GC-MS analysis and were published in various scientific papers for the same type of plant. were: Phytol present in *Malva sylvistris, Urtica urens, Arum palaestinum* showed, anxiolytic, metabolic rate, cytotoxic, antioxidant, autophagy, apoptosis-stimulating, anti-nociceptive, anti-inflammatory, immunomodulatory, and anti-microbial activities (Al-Rubaye, et al., 2017; Farid, et al., 2015; Islam, et al., 2018).

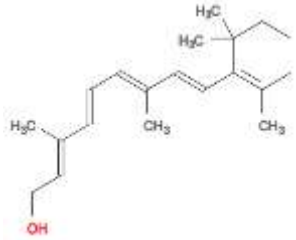
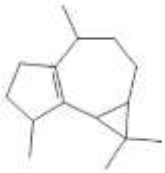
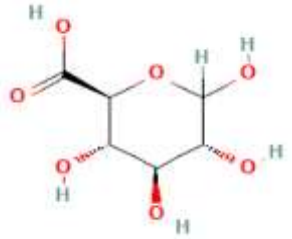
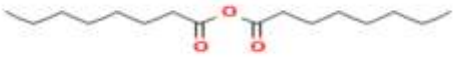
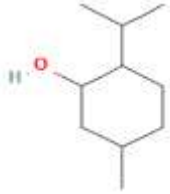
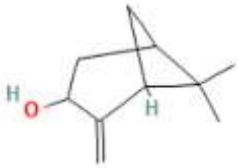
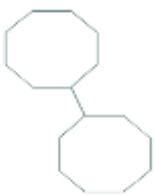
Megastigmatrienone present in *Malva sylvistris* is used as an aroma compound (Delfine, et al., 2017; Slaghenaufi, et al , 2014). Menthol present in *Urtica urens* has biological activity as antibacterial, antifungal, antipruritic, anticancer, and analgesic effects, it is also an efficient vaporizer. In addition, menthol is one of the most effective terpenes medicine used to boost the permeation of pharmaceutical preparations through the skin, (Kamatou,

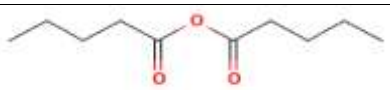
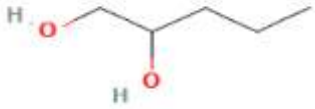
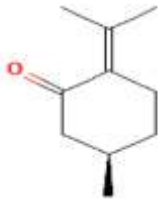
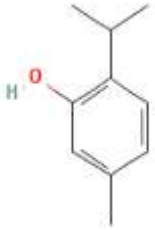
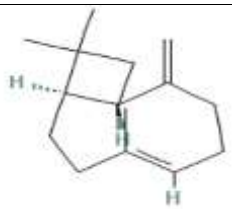
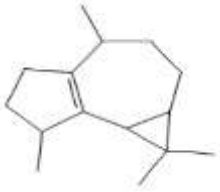
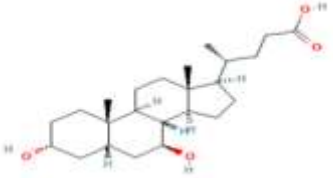
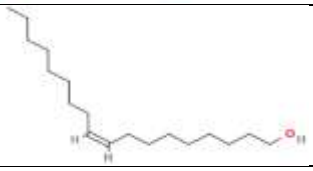
et al., 2013; Gül, et al., 2012) There are many important compounds present in *Micromeria fruticosa* as Pulegone has antimicrobial, antifungal activities, insecticides, antifeedants, repellents or fumigants, and oil used as novel bioherbicides for weed management (Al-Hamwi, et al., 2011; Božović & Ragno, 2017; Duru, et al, 2004; Güllüce, et al., 2004; Telci & Ceylan, 2007), The bioactive thymol has antibacterial, antioxidant, antifungal, and antiparasitic agents. (Piombino, et al., 2020) , Mint furanone is present in *Micromeria fruticosa* like research (Duru, et al., 2004), and the biological activity is affecting the antimicrobial activity (Benahmed, et al., 2019), Spathulenol has antioxidant, anti-inflammatory activities (Al-Hamwi et al., 2011; do Nascimento et al., 2018; Güllüce, et al., 2004; Telci & Ceylan, 2007)

Hexadecanoic acid, methyl ester present in *Arum palaestinum* is in consistent with the study of (Farid, et al., 2015), that has saturated fatty which acid are being marketed as a new health supplement, and their methyl ester derivatives are of interest, and have high biological significance, as they have antioxidant, anticancer properties (Melariri, et al , 2012).

The following tables (4.1, 4.2, 4.3) summarized the results we obtained and compared with other studies carried out in this field.

Table 4.1: Representative the main compounds detected by GC-MS with chemical structures and class

Compound	Chemical structure	Class
Vitamine A aldehyde		Vitamine
3-Eicosyne		Terminal alkynes
5,9-Tetradecadiyne		Alkynes
Isoledene		Cyclic alkene
Phytol		Isoprenoid alcohol
D-Glucuronic acid		Fatty acid
Caprylic Anhydride		Fatty acid
Menthol		Monoterpenoid
Isopinocarveol		Bicyclic monoterpenoid
1,1-Bicyclooctyl		Bicyclo alkane

Compound	Chemical structure	Class
Valeric anhydride		Ester
1,2-Pentanediol		Aliphatic diol
Pulegone		Monoterpene
8-Octadecenal		Fatty aldehydes.
Thymol		Monoterpenoid phenol
Caryophylene		Natural bicyclic
Isoledene		Biogenic aliphatic hydrocarbon
(-)-Spathulenol		Alcohol
Ursodeoxycholic		Epimer of chenodeoxycholic acid
Oleyl alcohol		Fatty alcohol

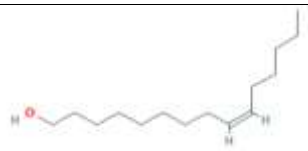
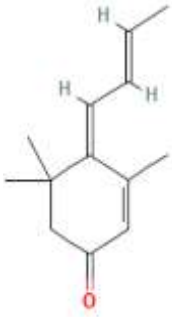
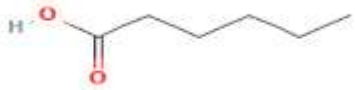
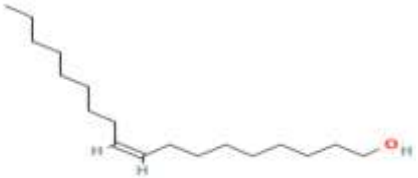
Compound	Chemical structure	Class
Z-9-Pentadecenol		Fatty Alcohols
Megastigmatrienone		Carbonyl compounds
Hexanoic acid		Fatty acid
Oleyl alcohol		Fatty alcohol

Table 4.2: Comparison between the main compounds detected by GC-MS

Plant	Compounds	Ref.
Malva sylvistris	Phytol, Megastigmatrienone	(Al-Rubaye, et al., 2017; Slaghenaufi, et al. , 2014; Delfine et al., 2017)
Urtica urens	Phytol	(Lapinskaya, & Kopyt'ko, 2009)
Asparagus officinalis	_____	
Ephedra Alata	_____	
Micromeria fruticosa	Pulegone, Spathulenol, Menthol, Thymol, Mint furanone.	(Güllüce, et al., 2004; Telci, & Ceylan, 2007; Duru, et al., 2004; Baser, et al., 1996; Duru, et al., 2004)
Arum palaestinum	Hexadecanoic acid methyl ester	(M. Farid, et al.,2015)

Table 4.3: Comparison between selected tests (Antioxidant, Antibacterial, and Total Phenol)

Plant	Antioxidant mg/ml	Antibacterial mg/ml (methanolic extract)	Total Phenol	Ref.
Malva sylvistris	$DPPH^{\bullet}$: 1,87±0,01 $ABTS^{\bullet+}$: 25,87±0,21	*S. aureus*: 12.5 *E. coli*: 6.25 *P. aeruginosa*: 6.25	20.47 ± 1.48 mg EGA/g DM	(Mihaylova, et al., 2015; Shadid, et al., 2021)
Urtica urens	$DPPH^{\bullet}$: 91.2 $ABTS^{\bullet+}$: 95.2	*S. aureus*: 5 *E. coli*: 5 *P. aeruginosa*: NA *K. pneumonia*: NA	14.42±0.51 mg tannic acid/g dry plant	(Jimoh, et al., 2010)
Asparagus officinalis	$DPPH^{\bullet}$: 55.8-69.9% $ABTS^{\bullet+}$: 43.0- 52.0%	————————	25.1- 26.2 mg GAE/g dry	(Zhanga, et al., 2018)
Ephedra Alata	$DPPH^{\bullet}$: 0.557± 0.028 $ABTS^{\bullet+}$:0.143± 0.014	*S. aureus*: no inhibition *E. coli* : no inhibition	125.73 ± 1.68 GAE/g dry	(Dbeibia, et al., 2022; Ghanem, S., & El-Magly, 2008)
Micromeria fruticosa	$DPPH^{\bullet}$: 0.047 ± 0.003	*S. aureus*: 14.33 ± 0.6 *E. coli*: 10 *P. aeruginosa* :15.66 ± 1.5	56.78±0.49 mg GAE/g	(Sadeq, et al., 2021)
Arum palaestinum	$DPPH^{\bullet}$: 24.3± 1.00 $ABTS^{\bullet+}$: 116.0± 1.00	*S. aureus*: no inhibition *E. coli:* no inhibition *P. aeruginosa* : no inhibition	27.6±2.4 mg GAE /g dry	(Naseef, et al., 2017; Al-Mustafa, , & Al-Thunibat, 2008)

4.6 Conclusions

Based on the results obtained in this study, it was revealed that the methanolic extract of the selected WPP: *Malva sylvistris, Urtica urens, Micromeria fruticosa, Asparagus officinalis, Ephedra alata,* and *Arum palaestinum* harvested from different location in Palestine have the following valuable effects:

1. The methanolic extract of the selected WPP revealed high antioxidant activity due to high content of phenolic compounds like alkaloids and flavonoids present in these selected plants, these compounds exhibit antimalarial, anticancer, antioxidant, antibacterial, and anti-inflammatory activity.
2. The antimicrobial studies of the selected WPP 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.
3. Phytochemical screening of the selected WPP revealed the presence and detection of various plant secondary metabolites present in the methanolic extract of leaves of these selected plants like coumarins, phenolic compounds, flavonoids, saponins, steroids, and quinones detected by different phytochemical screening tests and GC-MS analysis like phytol, menthol, thymol, pulegone, mint furanone and other chemicals.
4. The phytochemical screening and phytochemical composition of these traditional WPP showed strong free radical scavenging capacity, which makes them highly recommended in the pharmaceutical and manufacturing industry.
5. This research has laid sufficient background for further study and identification of WPP, and assisted in exploring the medicinal values and creating data base of medicinal plants available in Palestine.
6. *Ephedra alata* has an interesting value in the field of research needs further investigation.
7. Our plants have an interesting zone inhibition against G-ve bacteria. These results to be investigated to enhance pharmacological industry.

4.7 Recommendation

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

1. The current research used the compounds found in the plant while it is dried. It is recommended to examine the components in the different stages of the plant to note the seasonal differences with the application of the same protocols and compare the results.
2. Work on more tests on these plants, for example, testing the anti-cancer, and antifungal activities.
3. As for the antioxidant test, it is recommended to do several tests, such as IC_{50} , Trolox equivalent antioxidant capacity (TEAC) assay, the ferric reducing ability of plasma (FRAP) assay, and the copper reduction (CUPRAC) assay. and compare them with the results we obtained with $ABTS^{\bullet+}$ & $DPPH^{\bullet}$ antioxidant activity assays. additionally, measure the total oxidant scavenging capacity (TOSC).
4. In the antimicrobial test, it is recommended to use filter paper disc method, and compare with results of Well Diffusion method.
5. 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.
6. Use of hexane leaf extract in antibacterial test recommended and comparing the results with what we got in this investigation study.
7. It is recommended to measure the minerals contents of dried leaves.
8. It is recommended to Use the headspace to separate the volatile compounds and compare them with the results we got in this study.
9. As WPP proved to have a valuable antioxidant activity, it is recommended that these plants could be used as a therapeutic agent to treat various infectious diseases including COVID-19 infection, the effect of WPP on the COVID-19 virus, and its signs and symptoms are yet to be understood.

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Appendix

Appendix 1: Published Article

Antibacterial, Antioxidant and Phytochemical Screening of Palestinian Mallow, Malva Sylvestris L

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Original Article

ANTIBACTERIAL, ANTIOXIDANT AND PHYTOCHEMICAL SCREENING OF PALESTINIAN MALLOW, *MALVA SYLVESTRIS* L.

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ABSTRACT

Objective: To evaluate antibacterial, antioxidant activities, the existence of the major secondary metabolites, and volatile compounds in methanolic extracts from *M. sylvestris* leaves.

Methods: Antibacterial activity was assessed using a well diffusion method. Antioxidant activity was assessed using $ABTS^{+\cdot}$and DPPH• free radical scavenging assays. Phytochemical screening for secondary metabolites and volatile compounds were done following standard techniques and gas chromatography-mass spectrometry (GC-MS).

Results: Methanolic extracts exhibited moderate antibacterial activity compared with the positive control against the gram-negative *Klebsiella pneumoniae* and the gram-positive bacteria's *Staphylococcus aureus* by 47.2 and with 47.1% respectively. The average percentage of scavenging was 97.82±0.05 and 79.49±0.4 for $ABTS^{+\cdot}$and DPPH•, correspondingly. Total phenols were quantitatively estimated and found to be 78.9±9.55 mg GAE/g. Phytochemical screening assays revealed the presence of a wide range of phytochemical groups such as alkaloids flavonoids, phenols, tannins, quinones, saponins, steroids, tannins, terpenoides with at least sixteen volatile compounds detected in the plant.

Conclusion: The present study confirmed the antioxidant activity of the methanolic extract of *M. sylvestris* and the existence of is the volatile compounds (phytol), which mediate, even partially, the antioxidant and the claimed analgesic activity of the plant.

Keywords: Mallow Mallow, Antioxidant, DPPH, Phytol

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INTRODUCTION

Medicinal plants also called medicinal herbs have been frequently used over the years for anticipation and treatment of many diseases as well as for healthiness. Statistically, it is estimated that there are 750,000 plant species on Earth, of which 1-10% are used as food and medicine by both humans and animals [1, 2]. Medicinal plants display several pharmacological properties such as antioxidants [3], anti-diabetes [4], antibacterial [5], antiviral [6], anticancer [7], and anti-ulcer activities [8]. The medicinal value of these plants lies in a group of bioactive organic compounds (metabolites, secondary compounds) generally classified into alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids, and terpenoids and present at different quantities and qualities within the plants [9].

Mallow (*Malva sylvestris* L., Malvaceae) is a biennial-perennial ethnobotanical herb native to Northern Africa, Europe, and Asia. The plant generally grows naturally in moist areas such as near marshes, ditches, riverbanks, and meadows [10]. For many decades, this plant is widely used in the traditional Palestinian culture as food and in curing a large number of diseases [11]. Indeed, leaf extracts have been used traditionally as medicine for their anti-inflammatory, analgesic, antioxidant, neurogrotective, antibacterial, and antifungal activities [12]. For example, decoction from aerial parts of *M. sylvestris* has demonstrated remarkable anti-ulcerogenic activity against an ethanol-induced gastric ulcer in rat models [13]. However, hexane extract of *M.* leaves is reported to play a critical role in diabetes management by inhibiting insulin resistance, lipid abnormalities, and oxidative stress [14]. Successive petroleum ether extraction of *Malva* leaves has been reported to exhibit a counter-irritant effect on the rabbit's ear [15]. Due to these pharmacological activities of *Malva* species and others reported by Gasparetto and colleagues (2012), the interest in finding other pharmacological activities or confirming the traditional use of *Malva* species continues to grow given the large scale at which *Malva* species grow and the recent existing literature highlighting its effectiveness in treating diseases in Palestine and other parts of the world [5, 8, 11]. The present study was designed to assess the antimicrobial and antioxidant activities of the methanolic extracts from *M. sylvestris* leaves, and to determine the profile of the secondary and volatile compounds.

MATERIALS AND METHODS

Collection of plant materials

Leaves of mature local mallow plants were collected in April/2018 from Hebron city (Lat: 31.538629, Lon: 35.085769). Botanically, the plant was identified as *M. sylvestris*. Morphological identification of the plant was carried out by referring to the Traditional Arabic Palestinian Herbal Medicine, TAPHM by Ali-Shtayeh, and Flora of Israel Online by Avinoam Danin (http://flora.org.il/en/plants/ephfoe/). A voucher specimen (Pharm-PCT-2743) was preserved for identification in the phytochemical analysis laboratories at Hebron University. Leaves were isolated from plant stems, then cleaned, shade-dried at room temperature, grounded to a coarse powder, and stored in airtight containers.

Antibacterial activity

Extract preparation

The methanolic extract of *M sylvestris* leaves was performed based on the method described by [16] with minor modifications as described below. A 10 g of *M. sylvestris* grounded leaves were extracted in absolute methanol (100 ml) for 24 h at room temperature. The extracts were filtered and concentrated to a final volume of 20 ml and subjected to antibacterial analysis.

Bacterial samples

Four pathogenic bacterial strains were obtained from the microbiology department of Hebron Governmental Hospital. A gram-positive bacteria (*Staphylococcus aureus*), and three strains of

gram-negative bacteria including *Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*, were cultured on nutrient agar and incubated at 37 °C for 24 h (Heratherm incubator, Thermo Scientific, Germany). Cultured plates from all bacterial strains were preserved in a refrigerator at 2–4 °C until further use.

Media preparation

Differential media Muller Hinton agar (MHA), Eosin methylene blue (EMB), Mannitol Salt agar (MSA) (all from HiMedia Laboratories, India), and nutrient agar (NA, BioMaxima, Poland) were prepared based on manufacturers recommendations. All prepared media samples were autoclaved at 121 °C for 15 min (Labtech, Korea). The sterile media were poured in sterile Petri dishes (90 × 16 mm) and stored in the refrigerator at 2–4 °C for later use.

Bacterial culture and subculture

All bacterial strains were subcultured on nutrient agar plates and incubated at 37 °C for 24 h. The grown bacteria were further subcultured in differential media as the following: *S. aureus* on MSA, *E. coli,* and *K. pneumoniae* on EMB and *P. aeruginosa* on nutrient agar. Accordingly, all plates were incubated at 37 °C for 24 h.

Sensitivity testing

Sensitivity testing was performed for all bacterial strains using well diffusion method on Muller Hinton agar plates as described in [17]. Bacterial suspensions were prepared to a density of 0.5 McFarland units which is equivalent to $15*10^8$ CFUs/ml from 18-24 h old bacteria colonies in saline solution and spread on Muller Hinton agar plates by a sterile cotton swab. Then, four holes in each plate were made, in which 10 µl extract were added onto each of the first three holes, whereas the fourth hole was used for negative control (methanol). Positive control disks used in this study include vancomycin (30 µg, Biomaxima, Poland) for *S. aureus* bacteria and meropenem (10 µg, Biomaxima, Poland) for all other bacterial strains. The zone of inhibition of the positive controls and *M. sylvestris* extracts were measured (mm) after 24 h incubation at 37 °C, and expressed as a percentage (%) of positive control.

Antioxidant activity

Extract preparation

The leaf extract was prepared following the protocol by [16]. A 200 mg of grounded leaves of *M. sylvestris* were extracted using 4 ml methanol 80% on a shaker (Labtech, Korea) for 24 h at 80 rpm, at 25 °C. The extract (1.5 ml) was transferred into Eppendorf tubes and spun down for 5 min at 4000 rpm) using (MicroCl 17, Thermo Scientific, Germany). The supernatants were transferred to another clean Eppendorf tube for ABTS•⁺and DPPH• assays.

ABTS•⁺assay

The ABTS•⁺solution was prepared by mixing a stock solution of ABTS•⁺(7 mmol; prepared by dissolving 18 mg of ABTS•⁺reagent in 5 ml distilled water) with 88 µl potassium persulfate solution (2.45 mmol, prepared by dissolving 75 mg in 2 ml distilled water). The ABTS•⁺solution was incubated overnight in a dark place. The working solution of ABTS•⁺was prepared by diluting a stock solution of ABTS•⁺with 80% methanol to final absorbance 0.7000±0.02 at 734 nm. A 30 µl of diluted plant extracts (1:4) solutions were mixed with 3 ml ABTS•⁺working solution in micro cuvettes. For control, 30 µl methanol (80%) was mixed. All cuvettes were mixed and incubated in a dark place 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 (Cole-Parmer Ltd, UK). The percentage scavenging of ABTS•⁺was calculated according to the equation:

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

Data Analysis: Data were expressed as means of triplicates±standard deviation.

DPPH• assay

A stock solution of DPPH• was prepared by dissolving 2.3 mg of DPPH• with 5.57 ml of 80% methanol. A 200 µl of DPPH• stock solution was mixed with 2 ml 80% methanol and 20 µl of diluted plant extract (1:4, Sample) or 20 µl of methanol (80%, control) in plastic cuvettes. All cuvettes were mixed and incubated in a dark place at room temperature for 1 h. The absorbances of plant extracts (A_{sample}) and the methanol ($A_{control}$) were measured at 734 nm using Genway UV/Visible spectrophotometer (Cole-Parmer Ltd, UK) at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH• discoloration using the following equation:

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

Phytochemical screening

Malva sylvestris powder (3 g) was extracted in 60 ml methanol 80% for 24 h at room temperature with continuous stirring in a shaking incubator. The extracts then filtered through the vacuum and used to screen for the presence of alkaloids, anthraquinones, anthocyanins, cardiac glycosides, coumarins, flavonoids, glycosides, phenolics, phlobatannins, quinones, saponins, steroids, tannins and terpenoids based on the methods described in [18].

Determination of total phenols using folin–ciocalteu

Leaves samples were extracted following the protocol described in [16]. Dried leaves (2 g) were extracted with 20 ml methanol (70%) at room temperature for 30 min with continuous stirring. After filtration, the resulting raffinate was re-extracted with 10 ml methanol (70%) for 15 min with continuous stirring and filtrated. The combined filtrate was defatted twice with 20 ml of *n*-hexane. Consequently, the defatted extract was filtered twice by GF/F filter paper and 0.45 µm nylon syringe filter. Obtained extracts were stored at-20 °C until analysis.

Total phenols were assessed based on the method described in [16]. Plant extracts (20 µl) were mixed with 1.58 ml distilled water, 100 µl Folin–Ciocalteu reagent (Sigma, Israel), and 30 µl of aqueous Na_2CO_3 (20 %, w/v) in plastic macro-cuvettes. All cuvettes were mixed and incubated in the dark for 1 h. The absorbance of resulting solutions was measured at 760 nm, data were expressed as milligrams of gallic acid per gram of dried plant leaves (mg GAE/g). The assay was done in triplicate.

GC–MS analysis

Volatile compounds in *M. sylvestris* leaves (3 g) were extracted in absolute methanol (10 ml) overnight and analyzed using a GC–MS (Clarus SQ 8S, Perkin Elmer, USA) fitted with a BD-5 ms capillary column (30 m, 0.25 µm film thickness, 0.25 µm bore diameter) based on the method described by Qawasmeh and others [19] with minor modifications as described below. 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 3 times. Compounds were tentatively identified using the NIST05 mass spectral library, and when applicable, their mass spectra were compared with those published in the literature.

Table 1: Mean antibacterial activity (% of positive control) of *Malva sylvestris* leaves methanolic extracts against several bacterial species

Bacterial species		Positive control (mm)	Antibacterial activity (%)*
Escherichia Coli	G⁻	25	24.2±4.6
Klebsiella pneumonia	G⁻	10	47.1±5.9
Pseudomonas aeruginosa	G⁻	25	29.9±5.0
Staphylococcus aureus	G⁺	30	47.2±4.6

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

RESULTS

Antibacterial activity

Methanolic extract of *M. sylvestris* leaves displayed antibacterial activity against the gram-negative *K. pneumoniae* (47.1%) and the gram-positive *S. aureus* (47.2%) bacteria compared with the positive control (table 1). Other bacterial species were little affected by the methanolic extract table 1.

Antioxidant activity

The diluted methanolic extract of *M. sylvestris* leaves displayed antioxidant capacity using the two stable free radical scavenging assays, namely, ABTS•-and DPPH•. The average percentage of scavenging was 97.82±0.05 and 79.49±0.4 for ABTS•-and DPPH•, respectively.

Phytochemical screening

The Phytochemical screening assays for the methanolic extracts of *M.* sylvestris leaves revealed the presence of a wide range of phytochemical groups such as alkaloids flavonoids, phenols, tannins, quinones, saponins, steroids, tannins, terpenoides. Other groups were not detected as summarized in table 2.

Determination of total phenols using folin–ciocalteu

The total phenols in the methanolic extracts of *M. sylvestris* leaves were quantitatively estimated and found to be 78.9±9.55 mg GAE/g (n=3).

GC–MS analysis

The GC–MS analysis revealed the presence of at least 16 volatile compounds fig. 1. Major volatile compounds detected in the methanolic extract of *M. sylvestris* leaves were tetradecenol (*Rt* = 7.8), oxirane (*Rt* = 7.958.2), Octadecatrienoic acid (*Rt* = 8.71) and Phytol (*Rt* = 8.75). Other volatile compounds identified include but not limited to vitamin A aldehyde (retinal), hexadecenol, nonadecanoic acid, and trans benzyl cyclohexanol table 3.

Table 2: Phytochemical screening for the methanolic extracts from *M. sylvestris* leaves

Phytochemical groups	Extract
Alkaloids	+
Anthraquinone	-
Anthocyanin	-
Cardiac Glycoside	-
Coumarins	-
Flavonoid	+
Glycosides	-
Phenols	+
Phlobatannins	-
Quinones	+
Saponins	+
Steroids	+
Tannins	++
Terpenoids	+

+, present,-, absent

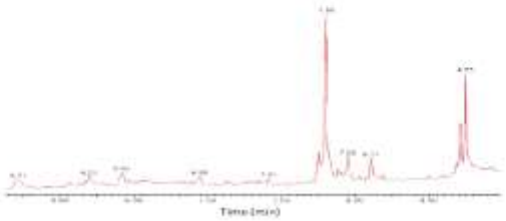

Fig. 1: Representative GC–MS total-ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Malva sylvestris leaves. Numbers on peaks represent the retention time (*Rt*) in minutes for each peak

Table 3: Major compounds detected in *M. sylvestris* extracts with their retention time (*Rt*) and molecular weight (MW), Molecular masses (M/Z) and the molecular formula (MF)

t	M/Z	Compound identification	MW	MF
5.71	55,84,91	1-Pentene,2,3,3-trimethyl-5-phenyl	188	$C_{14}H_{20}$
6.04	58,84,105	1-Oxaspiro(2.2)pentane,5-isopropylidene-tetramethyl	166	$C_{11}H_{18}O$
6.07	93,105,132	5,9-Tetradecadiyne	190	$C_{14}H_{22}$
6.13	55,73,127	Caprylic anhydride	270	$C_{16}H_{30}O_3$
6.20	73/98/147	N-phenethyl-2-methylbutylidenimine	189	$C_{13}H_{19}N$
6.42	55,91,107	Vitamine A aldehyde	204	$C_{15}H_{24}$
6.47	55,91,147	Hexanoic acid	280	$C_{16}H_{24}O_4$
6.95	73,117,156	Pentanedioic acid 3,3-dimethyl-dimethyl ester	188	C9H1604
7.41	55,117,173	Transbenzylcyclohexanol	242	$C_{16}H_{27}N_2$
7.76	57,68,95	Hexadecen-1-ol, tetramethyl	296	$C_{20}H_{40}O$
7.80	57,68,95	Tetradecen-1-ol, methylpropionate	282	$C_{18}H_{34}O_2$
7.95	57,81,95	Oxirane, heptadecyl	282	$C_{19}H_{38}O$
8.03	57,73,147	1,2-15,16-Diepoxyhexadecane	254	$C_{16}H_{30}O_2$
8.11	74,87,143	Nonadecanoic acid, methyl ester	312	$C_{20}H_{40}O_2$
8.71	57,67,79	Octadecatrienoic acide	320	$C_{18}H_{32}O$
8.75	55,71,123	Phytol	296	$C_{20}H_{40}O$

DISCUSSION

Although Palestine is a small country, it has a wide range of agro-ecological concerns and hosts a large variety of plants [20-22]. Such plant richness might relate to its unique geographical and ecological environment preciously to its location between the continents Asia, Europe, and Africa, and between the eastern Mediterranean Sea, Red Sea, Dead Sea, and Jordan River; in addition to its elevation span ranges from-430 meters (Dead Sea) to+1100 meter above the sea level). Such a situation allowed the survival of a wide range of plants, some of which are edible as a food and/or as a medicinal plant. *Malva sylvestris* is among the traditional edible and medicinally important plants in Palestine. However, only a few ethnobotanical studies on *M. sylvestris* plant have been accomplished [23, 24] with a slight emphasis on its biological activities and phytochemical constituents [12]. Given the large scale at which the plant is wildly grown, from sea level up to the high mountains and its multiple usages among Palestinians, it is crucial to evaluate its antimicrobial and antioxidant activities, profile the major phytochemical groups and appraise the existence of the volatile compounds.

Our results showed that *M. sylvestris* leaves showed potential antimicrobial activities against *K. pneumoniae* and *S aureus*. The zone of inhibition recorded for both strains was almost half of that recorded for the positive control antibiotics (meropenem and vancomycin, respectively). These findings are consistent with the study of Dulger and Gonus (2004) reporting moderate activity of *M. sylvestris* aerial parts (methanolic extract) against *K. pneumoniae* and *S aureus* using disc diffusion methods [25]. In this study, *E. coli* and *P. aeruginosa* were little affected by the methanolic extracts of *M. sylvestris* leaves (zone of inhibition<30% of the positive control). These findings are in accordance with other studies reporting *E. coli* particularly is either resistant [25, 26] or moderately sensitive [10] to the methanolic extract of *M. sylvestris* aerial parts. Although some studies have reported antimicrobial activities against some bacterial strains such as *Streptococcus mutans* [27], based on our research findings, the antibacterial activities of *M. sylvestris* leaves *in-vitro* remain inconclusive and warrant further investigations.

The antioxidant activity of *M. sylvestris* was evident using $ABTS^{\bullet+}$and $DPPH^{\bullet}$ assays. The percentage scavenging activity was 97.82±0.05 and 79.49±0.4 for $ABTS^{\bullet+}$and $DPPH^{\bullet}$, respectively. This antioxidant activity has been consistently attributed to the presence of phenolic compounds (flavonoids, phenols, and tannins) containing a hydroxyl group capable of scavenging the free radical [16, 28, 29]. Phytochemical screening revealed the presence of phenols, flavonoids, and tannins in the methanolic extract of *M. sylvestris*, confirming the existence of the antioxidant activity. Detection of alkaloids in *M. sylvestris* is of particular interest due to the potential pharmacological activities of these compounds. In this study and others [30, 31] alkaloids have been detected qualitatively in *M. sylvestris* and the related species such as *M. parviflora* [32], whereas only two alkaloids, sanguinarine, and berberine, were reported to occur in the flowers of *M. sylvestris* plant at concentrations (w/w %) 0.10126% and 0.00059%, respectively [31]. Whether these alkaloids or others exist in the leaves of Palestinian *M. sylvestris* has not been established and further studies are needed.

Here, we confirmed for the first time the presence of 16 volatile compounds in the methanolic extract of Palestinian *M. sylvestris* leaves using GC-MS. These compounds were tentatively identified as described in table 3. Volatile compounds in *M. sylvestris* have been studied by Tabaraki and others from Iran involving plants' flowers [33] and by Al-Rubaye and others from Iraq involving plants' leaves [34]. Notably, some similarities in the identity of the volatile compounds have been observed between this study and previous studies [33, 34]. Phytol, hexadecenol (ipalmitic acid analog), and octadecatrienoic acid (linolenic acid analog) derivatives were all reported in the leaves of *M. sylvestris* plants [34]. Although it is difficult to associate the pharmacological activities of *M. sylvestris* with a single volatile compound, phytol has been reported to exhibit antioxidant, anticancer, diuretic, and antinociceptive (analgesic) activities.[34, 35]

CONCLUSION

Malva sylvestris leaves are a source of chemically diverse compounds, some of which may represent a starting compound in drug development. Identifying phytochemical compounds in *M. sylvestris* leaves-plant commonly used in Palestinian herbal medicine-remains in its early stages. Several experimental models are required to approve its ethnopharmacological uses and identifying the underlying compounds responsible for the plants' activity. To the best of our knowledge and despite the plants' antioxidant activity, there are no pharmaceutical dosage forms that have been designed where *M. sylvestris* is part of their active constituents.

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AUTHORS CONTRIBUTIONS

All authors contributed equally in writing the manuscript.

CONFLICT OF INTERESTS

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

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