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# Biochemical Studies on Some Wild Palestinian Plants Roots

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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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**19/ 1/ 2024**

## Dedication

This thesis is dedicated:

To my father and mother, a special feeling of gratitude to my loving parents, whose words of encouragement and push for the tenacity to finish this thesis. To my dearest husband the first supporter and for endless encouragement. To my brother and sisters who have never left my side and are very special.

**Noor Muhyee Mohammad Batat**

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**List of Abbreviations**

| | |
|---|---|
| $ABTS^{\bullet+}$ | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid |
| ADR | Antimicrobial Drug Resistance |
| $C^{\circ}$ | Celsius |
| CVD | Cardiovascular disease |
| DNA | Deoxyribonucleic Acid |
| $DPPH^{\bullet}$ | 2,2-diphenyl-1-picrylhydrazyl hydrate |
| *E. coli* | *Escherichia coli* |
| G | Gram |
| GC-MS | Gas chromatography-mass spectrometry |
| HAV | Hepatitis A virus |
| HMG-CoA | 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase |
| HIV | Human immunodeficiency virus |
| WPPR | Wild Palestinian Plant Roots |
| AD | Alzheimer diseases |
| *K. pneumoniae* | *Klebsiella pneumonia* |
| LDL | Low-density lipoprotein |
| MIC | Minimal inhibitory concentration |
| Mm | Millimeter |
| *P. aeruginosa* | *Pseudomonas aeruginosa* |
| *P. mirabilis* | *Proteus mirabilis* |
| ROS | Reduce Oxidative Stress |
| Rt | Retention time |
| *S. aureus* | *Staphylococcus aureus* |
| $SO_2$ | Sulfur dioxide |
| TPC | Total phenolic content |
| WHO | World Health Organization |
| *A. officinalis* | *Asparagus officinalis* |
| *M. sylvistris* | *Malva sylvistris* |

| | |
|---|---|
| *S. arvensis* | *Sinapis arvensis*, |
| *C. spinosa* | *Capparis spinosa* |
| *U. diocia* | *Urtica diocia* |
| *E. alata* | *Ephedra alata* |
| TNF-α | Tumor Necrosis Factor alpha |
| IL-6 | Interleukin-6 |
| BHA | Butylated hydroxyanisole |
| MDA | Molandialdehyde |
| TEAC | Trolox equivalent antioxidant capacity |
| GRE | Ginger root extract |
| $NF_{\kappa}B$ | Nuclear factor kappa B |
| IL-1β | Interleukin-1β |
| MRHF | Human fibroblast cells |
| TB | Tuberculosis |
| LDH | Lactate dehydrogenase |
| ALT | Alanine amino transferase |
| AST | Aspartate amino transferase |
| ALP | Alkaline phosphatase |
| *V. officinalis* | *Valeriana officinalis* |
| IC | Inhibitory concentration |
| GA | Gallic acid |
| KG | Killo gram |
| DW | Distilled water |
| MW | Molecular weight |
| M/Z | Molecular masses |
| MF | Molecular formula |
| µl | Micro liter |
| GAE | Gallic acid equivalent |
| CC | Column chromatogram |

| | |
|---|---|
| TLC | Thin layer chromatogram |
| NMR | Nuclear magnetic resonance spectroscopy |
| IR | Infrared |
| PTP1B | protein-tyrosine phosphatase 1B |
| FRAP | Ferric reducing ability of plasma assay |
| CUPRAC | Cupric Reducing Antioxidant Capacity |
| TOSC | Total oxidant scavenging capacity |

**Abstract**

In most cases, people rely on herbs as an alternative or combination with essential drugs. The use of plant roots as treatment for many diseases was increasing gradually until today and that confirms the efficacy of the root in disease curing. The aerial parts of *Asparagus officinalis, Malva Sylvestris, Sinapis arvensis*, *Capparis spinosa*, *Urtica dioica*, and *Ephedra alata* have been used widely in Palestinian folkloric culture as remedy for wide range of diseases. In this study, the roots of these wild Palestinian plants were evaluated for their antioxidant, and antibacterial activities. Furthermore, the roots were screened for the presence of bioactive secondary constituents, and volatile compounds using GC-MS analysis. The methanolic root extracts of *A. officinalis,* and *E. alata* showed a 57.7% and 69.5% scavenging capacity using $DPPH^{\bullet}$ assay, respectively. While using $ABTS^{\bullet+}$ assay showed 69.6% and 66.1% scavenging capacity respectively. The antibacterial activity of the methanolic root extracts of 'selected' WPP using well and disk diffusion method showed there is no zone of inhibition against gram-negative and gram-positive bacteria. The phytochemical screening of these plant showed the presence of the major phytochemical like saponins, cardiac glycosides, quinones, steroids, terpenoids, and flavonoids. The GC-MS analysis showed the presence of at least seven different volatile compounds in each root sample, The main volatile compounds detected in the methanolic root extracts of 'selected' WPP were *D*-glucose, 4-O-alpha-d-glucopyranosyle, naphthalene-decahydro, vitamin A aldehyde, 11-hexadecynal, 1-heptatriacotanol, Rebitol, Aromadendrene oxide-2, and benzoic acid. This study is the first to investigate the roots of these plants and the findings indicate the presence of promising bioactive compounds and antioxidant activity.

**الملخص**

يعتمد الناس في معظم الحالات المرضية على الاعشاب الطبية كبديل أو مع الأدوية الأساسية، وكان استخدام جذور النباتات كعلاج للعديد من الأمراض يتزايد تدريجياً حتى يومنا هذا، مما يؤكد فعالية الجذر في علاج الأمراض. الأجزاء العلوية من نبات الهليون المخزني، الخبيزة، الخردل، القبار، القريص، والعلندة قد تم استخدامها على نطاق واسع في الثقافة الشعبية الفلسطينية كعلاج لمجموعة واسعة من الأمراض. في هذه الدراسة، تم تقييم جذور هذه النباتات البرية الفلسطينية لنشاطها المضاد للأكسدة والمضاد للبكتيريا، وتم فحصها للتأكد من وجود المكونات الثانوية النشطة بيولوجيا والمركبات المتطايرة باستخدام تحليل GC-MS. أظهرت مستخلصات الجذور الميثانولي لـنبات الهليون والعلندة نسبة عالية من مضادات الاكسدة بنسبة 57.7% و69.5% باستخدام مقايس $DPPH^{\bullet}$، على التوالي. بينما أظهر استخدام مقايس $ABTS^{\bullet+}$ النسب التالية 69.6% و66.1% على التوالي. أظهر النشاط المضاد للبكتيريا لمستخلصات الجذر الميثانولي للنباتات البرية الفلسطينية المستخدمة باستخدام طريقة الانتشار والقرص عدم وجود منطقة تثبيط ضد البكتيريا سالبة الجرام وإيجابية الجرام. أظهر الفحص الكيميائي النباتي لهذه النباتات وجود المواد الكيميائية النباتية الرئيسية مثل الصابونين، جليكوسيدات القلب، الكينونات، الستيرويدات، التربينويدات، والفلافونويدات. أظهر تحليل GC-MS وجود ما لا يقل عن سبع مركبات متطايرة مختلفة في كل عينة جذر، وكانت المركبات المتطايرة الرئيسية المكتشفة في مستخلصات الجذر الميثانولي للنباتات البرية الفلسطينية المستخدمة هي D-glucose، O-alpha-d-glucopyranosyle4-، النفثالين-ديكاهيدرو، فيتامين أ ألديهيد، 11-هيكساديسينال، 1-هيبتاترياكوتانول، ريبيتول، أكسيد أروماديندرين-2، وحمض البنزويك. هذه الدراسة هي الأولى التي تبحث في جذور هذه النباتات وتشير النتائج إلى وجود مركبات نشطة بيولوجيا واعدة ونشاط مضاد للأكسدة .

# Chapter 1: Literature Review

## 1.1 Introduction

The intensive efforts of scientific research about medicinal plants are increasing with time. In the last decades, more than 110,000 studies regarding herbal medicine have been published from 1960 to 2019 (Salmerón-Manzano et al., 2020). Studies and research on roots are not recent, they started in the 19$^{th}$ century. However, most studies are not deep and superficial. The roots of the plants have been largely neglected compared with other plant parts, despite their crucial importance (Novoplansky, 2019). Recently, plant roots have started to receive much attention in research. This interest includes the understanding of the clear and vital roles in plant metabolism and function (Ephrath et al, 2020).

In most cases, people rely on herbs as an alternative or in combination with other primary drugs (Petrovska, 2012), whatever the parts of the plant they use, leaves, stem, fruit, root, or even the bark (Shrestha & Dhillion, 2003). The use of plant roots as treatment for many diseases is increasing gradually due to the efficacy in curing of diseases, little or no side effects, obtainability, and an inexpensive (Tapsell et al., 2006). Rather than, that most drugs used against pathogenic bacteria and fungi (antibiotics) become less active after frequent use due to resistance to the drugs from microorganism (Obeidat, 2011). Accordingly, roots may provide a source of novel drugs against bacteria and fungi.

The people in the Middle East, in particular, have a wide knowledge on the uses of medicinal plants (Abu-Odeh & Talib, 2021). In Palestine, wild plants are numerous and vary in all regions, more than 2700 species are growing in Palestine. Some plants are edible, others are used as ornamental plants, and other are used as medicine. In Palestinian folk medicine, *Thymus vulgaris* (Lamiaceae) is a wild plant used for cough, *Malva sylvestris* (Malvaceae)

used to reduce constipation and treat irritable bowel syndrome, and *Arum palaestinum* (Loof, Araceae) is used in abundance in Mediterranean diet which contain phytochemicals like: (flavonoids, phenolic compound) and used as ant-diabetic agent (Mohammed S. Ali-Shtayeh et al., 2008)

Most of the current research on wild plants has involved the aerial parts to confirm the medicinal uses of such plant (Dowek et al., 2020). However, the underground parts -roots- have less attention in biochemical studies (Silberbush, 2013). The main functions of the roots are to anchorage the plant in the soil, transport the water and nutrients to the all-plant parts, and as food source. Due to its function, roots may represent a rich source of secondary metabolites that may have the potential to treat many diseases some of which remains intractable. Studies on biological activities of root extracts are still few and limited, and mostly focused on the function, structure, morphology of the roots, and how interact with the microorganisms exist in the soil (Lux & Rost, 2012).

The interest in biological activities of roots extracts and their secondary compounds came in 2016 by Ododo and colleagues. In their study, β-sitosterol- isolated from the roots of *Malva parviflora (Malvaceae*)- has showed some antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (M. Ododo et al., 2016). More recently, root extract from *Miscanthus capensis*, (Poaceae) -an evergreen, flowering grass plant- have been reported to inhibit protein glycation and to increase collagen production in human dermal fibroblast (MRHF) cells explaining their traditional uses in wound and pimple healing (Sagbo IJ et al., 2020).

*Zingiber officinale* (ginger, Zingiberaceae) is used traditionally to treat many diseases, such as, neurosis, nervous diseases, Alzheimer disease (AD). *In vivo* study, Zeng, G. F. et al, (2013) have confirmed the efficacy of ginger root extract (GRE) on behavioral dysfunctions (memory defect) that relevant with AD; the study had been conducted on operated rat model of AD, which administered different doses of ginger root extract (5% gingerol). The AD rat model that receives high dose (4 g/kg) of (GRE) appear less level in inflammatory cytokines nuclear factor kappa B ($NF_{\kappa}B$), interleukin-1β (IL-1β), and molandialdehyde (MDA). Moreover, the latency in memory defect takes short time to show response (Zeng et al., 2013).

The biochemical activity of Palestinian wild plants' roots hadn't been thoroughly investigated. In this project, we aimed to evaluate the extract of some Palestinian wild plant roots in many aspects, particularly, the antioxidant, antibacterial, total phenol, phytochemical screening, in addition to GC-MS.

### 1.2 Pharmacological activities reported about roots

The root of many plants holds a group of secondary compounds that are associated with pharmacological activities involved in the treatment of many diseases. This secondary compound can act as antimicrobial, antioxidant, anti-inflammatory, and antiulcer. Table 1 summarizes the

major medicinal plants collectively with their root-related pharmacological activity.

### 1.2.1 *In vitro* and *In vivo* studies implicating roots as a source of biological active compounds

The decoction and maceration of the roots of the *Combretum hartmannianum* plant (bush willows, Combretaceae), have been used traditionally for cough and tuberculosis (TB) (Eldeen & Van Staden, 2007). In a recent study involving *C. hartmannianum* roots, both methanolic and ethyl acetate root extracts have exhibited inhibitory effects on *Mycobacterium smegmatis* with minimum inhibitory concentration (MIC) 312.5 and 625 µg/ml, respectively and that effects were relates to the presence of novel potent compounds such as luteolin, corilagin, gallic acid, and castalagin (Salih et al., 2021).

The hydroalcoholic extract of the root bark of *Premna. integgrifolia* (Agnimanth, Verbenaceae) has been reported to be anti-atherosclerosis agent by decreasing the biochemical parameters – hepatic enzymes- that indicate to atherosclerosis and cardiovascular diseases (Chitra et al., 2017). In vivo study carried on 60 Wister rats (6-8 weeks) involve the impact of different doses of the hydroalcoholic extract of the root bark of *P. integgrifolia* on the diagnostic cardiac biomarkers such as lactate dehydrogenase (LDH), alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP). The 60 rats were fed with high fat diet and divided into 6 group each group include 10 rats: group 1standerd pallet diet, group 2 high fat diet, group 3 fed with 100 mg/kg root extract, group 4 fed with 200 mg/kg root extract, group 5 fed with 500 mg/kg root extract, and the final one medicated with 10 mg/kg Atorvastatin. After 30 days, the results of the study showed a significant alteration in the cardiac parameters in the groups treated with the hydroalcoholic extract of the root bark of *P. integgrifolia* after 4 ml of the blood serum was analyzed. In general, the effect of the root extract of *P. integgrifolia* on the liver enzymes

was significantly observed, there are a notable reduction in the level of cardiac biomarkers in the groups 3,4,5 that treated with 100, 200, and 500mg/kg respectively (Chitra et al., 2017).

### 1.2.2 Clinical studies implicating roots as a source of biological active compounds

The traditional extract of *Valeriana officinalis* (Caprifoliaceae) roots was used to activate and treat the nervous problems. The root of *V. officinalis* is reported to contain many pharmacological active compounds, more than 200 active compounds were discovered in the whole plant including roots (Jugran et al., 2019). Valerenic acid (Figure 1) – sesquiterpene derivative – is a major secondary compound detected in *V. officinalis* roots and implicated to have CNS sedative effects attributed to the plant's sedative/hypnotic therapeutic properties in addition to other phytochemicals belongs to flavonoids, alkaloids, and lignans secondary compounds (Nandhini et al., 2018; Sermukhamedova et al., 2017)

The clinical studies also have been performed on *V. officinalis* root extract, Azizi H, and et al (2020) conducted a clinical trial on 88 patients (34.9 ± 8.7 years old) suffering from daily headache, the participants classified into two groups, placebo group which receive 500 mg of breadcrumbs twice daily, and intervention group that take capsules contain powder of *V. officinalis* root extract with concentration of 530 mg twice daily for 1 month. The impact of valerenic acid on the daily headache patients were gave positive effects, which significantly reduces the stress, weakness, inability, and tension-type headache (Azizi et al., 2020).

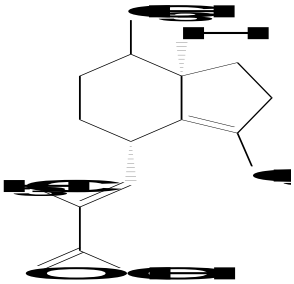

**Figure 1: Structure of valerenic acid**

A clinical study included 64 participants with history of stress and anxiety was performed to assess the efficacy and safety of *Withamia somnefira L* (ashwagandha, Solanaceae) root extract. The study carried was as follows: two groups of 64 participants have been classified; placebo control group and treatment group that received capsule with high dose (300 mg twice a day) of ashwagandha root extract for 60 days. The results of the study were taken by evaluating the serum cortisol levels, the score stress-assessment scale and any adverse effects observed after 60 days. The results of this study showed that there was a reduction in the levels of serum cortisol ($P = 0.0006$), and the scores of stress-assessments was ($p < 0.0001$) in the ashwagandha group with no adverse effects taken in consideration compared with control group (Kartik Chandrasekhar et al., 2012).

*Rauwolifia. serpentana* (Apocynaceae) *is* a flowering plant that traditionally used to treat many diseases include insomnia, epilepsy, and hypertension (HP). The pharmacological activities of *R. serpentana* are attributed to the alkaloids that exist in the root of the *R. serpentan*. More than 80 alkaloids were isolated from the root of *R. serpentan*, the most bioactive and prominent one was is reserpine – an indole alkaloid (Figure 2) –, this bioactive compound was approved in 1955 as a drug that used in the management of cardiovascular diseases (CVD) and hypertension (Soni et al., 2016).

Many successful clinical trials have investigated and documented the pharmacological effects of reserpine as a compound can be used as antihypertensive and sedative agent (Rustom, 1949; Smith et al., 1964; Vakil, 1955).

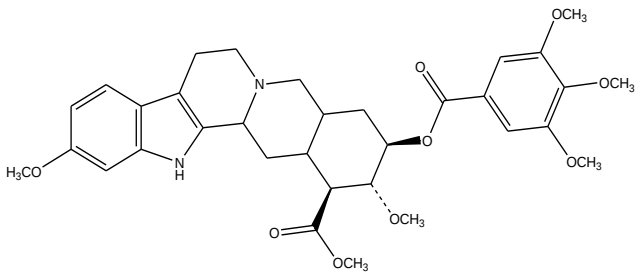


**Figure 2: Structure of reserpine**

**Table 1: The major medicinal plants with their pharmacological activities related to the roots**

| # | Common name | Arabic name | Scientific name | Family | Uses | Ref |
|---|---|---|---|---|---|---|
| **1** | Ashwagandha | العبعب المنوم | *Withamia somnefira L* | Solanaceae | Reducing stress and anxiety in adults | (Kartik Chandrasekhar, Jyoti Kapoor, & Sridhar Anishetty, 2012) |
| **2** | Rosy milkweed | | *Oxystelma. esculentum* | Apocynaceae | Laxative<br>Anti-ulcer<br>Diuretic<br>Used in treatment of hepatitis | (Pandya & Anand, 2011) |
| **3** | Valeriana | الناردين المخزني | *Valeriana officinalis* | Caprifoliaceae | Commonly used as sedative and treatment for nervous disorders and tension-type headache. | (Azizi et al., 2020)<br>(Jugran et al., 2019) |
| **4** | Ginger | الزنجبيل | *Zingiber officinale* | Zingiberaceae | Control the pressure.<br>Enhance gastrointestinal disorders.<br>Anti-inflammatory.<br>Weight loss. | (Nikkhah Bodagh, Maleki, Hekmatdoost, & nutrition, 2019) |
| **5** | Ginseng | الجنسة | *Panax ginseng* | Araliaceae | Improve blood glucose.<br>Reduce blood pressure. | (Vuksan et al., 2019) |
| **6** | Turmeric | الكركم | *Curcuma longa* | Zingiberaceae | Strong antioxidant<br>Anti-inflammatory<br>Anti-cancer | (Chanda & Ramachandra, 2019) |
| **7** | Beetroot | الشمندر الأحمر | *Beta vulgaris* | Brassicaceae | Anti-tumor effects<br>Anti-cancer | (Kapadia, Tokuda, Konoshima, & Nishino, 1996) |
| **8** | White mulberry | التوت الأبيض | *Morus alba* | Moraceae | Antibacterial activities against oral bacteria *(Streptococcus mutans*) | (Palombo & Medicine, 2011) |
| **9** | Indian Kudzu | | *Pueraria tuberosa* | Fabaceae | Immunomodulatory<br>Anti-Ulcerogenic<br>Anti-Diabetic,<br>Anticancer,<br>Antioxidant,<br>Anti-Stress. | (Bharti, Chopra, Raut, & Khatri, 2021) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **10** | Thump or Madanaphala | | *Catunaregam spinosa* | Rubiaceae | Reduce rheumatic fever and joint pain | (Rout, Panda, & Mishra, 2009) |
| **11** | Golden berry | الحرنكش | *Physalis peruviana* | Solanaceae | Hepato- renal protective agent | (El-Gengaihi, Hassan, Hamed, Zahran, & Mohammed, 2013) |
| **12** | Green chiretta | | *Andrographis paniculate* | Acanthaceae | Antidiabetic activity.<br>Reduce nephropathy complications. | (Rao, 2006) |
| **13** | Spike thorn | | *Maytenus senegalensis* | Clastraceae | Anti-plasmodial,<br>Anti-toxicological<br>Anti-inflammatory<br>Anti-nociceptive effects | (Umar et al., 2019) |
| **14** | Indian sarsaparilla | عشبة النار الهندية | *Hemidesmus. indicus* | Apocynaceae | Protective agent against gentamicin-induced renal toxicity | (Kotnis, Patel, Menon, & Sane, 2004) |
| **15** | Agnimanth | شوجب منشاري الأوراق | *Premna. Integgrifolia* | Verbenaceae | Anti-atherosclerosis activity | (Chitra et al., 2017) |
| **16** | Syrian mesquite | ينبوت | *Prosopis. farcta* | Fabaceae | Anti-hyperlipidemic effect | Saidi, M. R., et al. (2016) |
| **17** | Snake root Sarpagandha | جذر الثعبان | *Rauvolfia. serpentina* | Apocynaceae | Anti-hypertensive activity | (Soni et al., 2016) |
| **18** | Moghat | مغاث عجمي | *Glossostemon. Bruguieri* | Malvaceae | Aid in relief gout pain<br>Increase the bone calcium and vitamin D mass | (Ghareeb, El-Rashidy, & El-Mallawany, 2014) |
| **19** | Shatavari | الهليون العرق | *Asparagus. racemosus* | Asparagaceae | Anti-dyspepaia<br>Regulate sexual behavioral<br>Rich in anioxidants | (Mishra & Verma, 2017) |
| **20** | Peyr (Balle) | | *Phragmanthera. Glaucocarpa* | Loranthaceae | Anti-oxidant activities | (Fernandes, Canelo, dos Santos Mata, de Mendonça, & de Mendonça, 2018) |
| **21** | Dooki Kantakara | | *Combretum glutinosum* | Combretaceae | Anti-oxidant,<br>Anti-inflammatory,<br>Antiproliferative, and<br>Anti-bacterial activities | (Muhammad, Shaban, Elrashidy, Ghareeb, & Antioxidants, 2019) |

### 1.3 Root structure

Roots in plant physiology play the most vital function for optimum metabolic reaction in all plant parts, that because of their important role in the transport of water and micronutrient, in addition plant roots function in supporting and anchorage rule in the soil**,** communication by signals and chemicals; regulate plant growth, and defense line to fight enemies in the ground (Shabala et al., 2015)

Root exudates are natural chemicals secreted by the root to fight pathogenic microorganisms in the ground and so keeping the plant in a healthy state. Concurrently, these exudates stimulate in building soil environment in a form allows the plant to grow naturally without trouble (Aiken & Smucker, 1996; Baetz & Martinoia, 2014)

The root (net of hairy branches) system in general, represents the underground part of most plants providing their support, water, and nutrients. The hidden part root system started when the first fraction initiated and grow under the ground after seeds germination this tiny part (radical) develop and go down to the soil, then the root elongated deeply and branched horizontally through the soil to become a complex network of non-axillary buds' branches, its main jobs are water and nutrients absorption, anchoring the plant, and other functions (Lopez & Barclay, 2017).

Most of botanists and researchers agree that the root system is very complicated to study, but according to the *Arabidopsis thaliana* (mouse-ear cress, Brassicaceae) plant which has the simplest genetic map, the external morphology of the root is composed of three main parts: primary root (tap root) which grows deeply in the soil and make up the main body of the root.

The main functions of the tap root are anchoring the plant, storing food, and conducting water and minerals to the other parts of the plant. The secondary root (lateral root) system which initiates from the parent tap root then branches and expands crosswise to achieve maximum water and minerals absorption (Eshel & Beeckman, 2013).

The final type is the adventitious root, which usually forms from other plant organs such as stem, leaf, and root: it give the plant another function in addition to known roles of absorption and anchoring, like reproduction vegetatively to produce new plant without seeds, this process is called as propagation vegetatively, and developed from certain place in stem-like rhizomes such as ginger (*Zingiber officinale*), corms (fleshy stem) that seen in gladiolus (*Gladiolus italicus,* iridaceae) species plant, and tubers that usually grow underground in starchy plant like potato (*Solanum tuberosum*) (Geiss et al., 2009).

Root hairs are not from the main root types, but they are very important parts that takes a tubular shape extended from the surface of the root (epidermal cells) and has the huge surface area to absorb nutrients and water by osmosis. The interaction with the microorganisms in the soil is attributed to the root hairs (Grierson et al., 2014). The contribution of root hairs in nutrient uptake may reach 80% in some species of plant; that depends on multiple factors including the genetics of the plant species and the availability of the nutrients (Jungk, 2001).

The inner longitudinally composition of any root consists of a root cap which considers the director and falls at the bottom of the root and it reform continuously due to the penetration process inside the soil. The root cap function represents the protection of the active dividing

region meristematic cells which are placed directly behind the root cap. There are three different zones in roots: the meristematic tissue where the new cells were divided, the zone of elongation where the root becomes longer due to the new cells formed and increasing in length, and finally, the zone of differentiation where the cells become mature and functionally clear, such as xylem, phloem, and root hairs.

The composition of the crosswise section from the mature root appears more complex and has many parts and details. Simply, it composes of a cortex and pith, the pith is in the middle of the root where the vascular system, xylem and phloem surround it, and the cortex forms a big ring around the vascular system. From outside to the inside, the cross-section of the root consists of multiple layers; the cuticle is the first outer waxy thin layer, which forms a protective wrap to the plant from microorganisms' infections and drought; another protective layer is epidermal cells or the skin, that composed of the layer from one thick cell, its function is preservation the inner vascular tissues. The cortex is the next part (food and water storage) then followed by endodermis; a thick single-cell layer consisting of structural compounds lignans and suberin. Among the endodermal cells, there are very important tissues that induce the production of the lateral root called pericycle. The overall functions of the root are attributed to the vascular system xylem and phloem; which placed in the center of the root. The conduction of carbohydrates through plant parts is done by the phloem, while the transport of water and nutrients is achieved by xylem tissues (https://bio.libretexts.org/@go/page/59220).

### 1.4 Differences between roots and rhizomes

Rhizomes (rootstalk) are underground parts of some plants bearing both root and stem characteristics. Anatomically, most of the rhizomes have the same structure and carry nodes and internodes capable of producing new stems or new roots (Sl & Sajo, 2008). In some plants, rhizomes store foods as starch and proteins like *Bambusoideae* (bamboos, poaceae), another plant, that could propagate vegetatively by using rhizomes such as *Populus tremula* (salicaceae). So, rhizomes are distinguished from roots by their function as food storage and for propagation, not the absorption of water and nutrients. *Asparagus officinalis* (asparagaceae), *Zingiber officinala* (zingiberaceae, ginger), and *Curcuma longa* (zingiberaceae, turmeric), all are other valuable examples of rhizomes (Al-Snafi, 2015; Krishi et al., 2018; Kumariet al., 2020).

### 1.5 Palestine as a source of medicinal plants

Around the world, herbal folk medicine is very common. There are a large number of confirmed scientific studies that have results backing the traditional use of herbal drugs (Orhan et al., 2012). In Palestine, and until today, traditional folk medicine has been used for the treatment of numerous diseases including chronic diseases, injuries, and burns. The parts of plants that have active compounds may ease, prevent, or cure several human diseases (Mohammed S Ali-Shtayeh, & Jamous, 2011).

Essawi, and Srour (2000) previously reported that 15 Palestinian medicinal plants have antibacterial agents and showed that these plants are widely used in curing diseases among

Palestinians in West Bank and Gaza Strip, the work has been done by extracting 15 Palestinian plants (from Ramallah and Jerusalem), using two different solvents; mixture of methylene chloride and methanol (1:1) as organic extract and aqueous extract. The antibacterial test was carried by disc diffusion and holes-plate methods. The bacterial strains used in this study are very pathogenic and relevant to common diseases, these strains are *Bacillus subtilis*, three species of *S. aureus*, two species of *E. coli*, *Enterococcus fecalis*, and *Pseudomonas aeruginosa*. The results of the study showed eight plant species among 15 have antibacterial activities against some bacterial strains, these plants are *Thymus vulgaris* (Lamiaceae), *salvia officinalis* (Lamiaceae), *Teucrium polium* (Lamiaceae), *Majorana syriaca* (Lamiaceae), *Thymus origanum* (Lamiaceae), *Rosmarinus officinalis* (Lamiaceae), *Foeniculum vulgare* (Apiaceae), and *Commiphora opobalsamum* (Burseraceae). The diameter of the zone of inhibition varies according to the extraction way and the method used for antibacterial activity (Essawi & Srour, 2000).

Another biochemical study was conducted on eleven Palestinian plants that were used traditionally as remedies for skin infections in the past, and reach to strong antidermatophytic, antibacterial, and antioxidant Palestinian plants. The 11 Palestinian plants from different regions have been extracted with methanol (80%) and then screened for antibacterial activities (6 bacterial strains), antioxidant ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) assays, antidermatophyte (*Microsporum canis* and *Trichophyton rubrum*), and anticandidal activities (5 candida albicans strains). Two plant species *Rhus coriara* (sumac, Anacardiaceae) and *Epilobium hirsutum* (hairy willowherb, Onagraceae) of 11 reported to have a potent antibacterial, antideramtophytic, antioxidant, and anticandidal phytochemicals from the leave extract. According to the antioxidant activities, the

free-radical scavenging appeared clearly in *E. hirsutum* ($IC_{50}$: 33µg/ml) compared with butylated hydroxyanisole (BHA) control (9 µg/ml), also it has a higher content of flavonoids than *R. coriara* which exhibits a high concentration of phenolic compounds (14.7 mg/g dried plant material). *Rhus coriara* showed the best antimicrobial activities versus all types of microorganisms (Jamous et al., 2015).

### 1.6 Selected Wild Palestinian Plants (WPP) involved in this study

The traditional folkloric WPP used in this study include: *Asparagus officinalis*, *Malva Sylvestris*, *Sinapis arvensis*, *Capparis spinosa*, *Urtica dioica*, and *Ephedra alata*, the roots of these Palestinian plants are not widely explored in the literature, as compared to the arial parts.

### The studied WPP plant roots

1. *Asparagus officinalis*: (*A. officinalis*) is a perennial plant, in Arabic this plant is called as Halayoun هليون, the plant belongs to Asparagaceae family, grows widely on western coasts and in Mediterranean region, this plant includes three types according to the color differences; green asparagus, purple asparagus, and white asparagus. *In vivo* and *in vitro* study, the ethanolic extract of *A. officinalis* exhibit a strong phytochemical compound (polyphenol) that confirmed the activity of anti-cancer, antioxidant, hypoglycemic, and anti-hypertensive (Guo et al., 2020)

*2. Malva sylvestris*: (*M. sylvestris*) also known as Mallow (Khubbaizeh in Arabic خبيزة), is an annual, short plant with leaves that grows close to the ground which belongs to Malvaceae

family, it native to the south-west Asia countries; Mallow is used as a famous edible herbal food in the Palestinian cuisine. The leaves are considered as a type of vegetable that enters many food recipes. The leaves and flowers of the plant have been reported to have antibacterial activities against a wide range of pathogenic bacteria such as *S. aureus* in addition to other medical uses like remedies for wound healing (Razavi et al., 2011; Dwek et al., 2020).

*3. Sinapis arvensis*: (*S. arvensis*) also known as Wild Mustard or charlock (Khardal in Arabic خردل), is an annual plant that grows broadly in Eurasia and belongs to the Brassicaceae family. In literature the whole plant parts of *S. arvensis* are considered as a good source of secondary compounds, and antioxidants like: kaempferol, catechin, and hydroxycinnamic acid found in leaves, seeds, and flowers; whereas the stems and fruits contain vanillic acid, thus, it acts as anti-inflammatory, and antitoxic agent (Ashwini et al., 2022; Başyiğit et al., 2020)

*4. Capparis spinosa L.*: (*C. spinosa* L.) in Arabic known as kobbar (كبار) belongs to capparaceae family, the plant's native back to the Mediterranean regions, it is a perennial plant with thick leaves (store water) and has white flower, the plant with all parts has been used in folk medicine as anti-inflammatory for rheumatism, this plant has a wide range of bioactive compounds that identified especially in the root; for example rutin (quercetin) a flavonoid compound that has no toxicity and very potent antioxidant used as anti-carcinogenic (Rahnavard & Razavi, 2017).

*5. Urtica dioica L.*: (*U. dioica*) is a perennial plant with sharp nettle around the leaves (Kurrais in Arabic قريص), belongs to the Urticaceae family with the common name stinging nettle, and is widely distributed in South Asian countries. Since the past, the stinging nettle known as a

medicinal herb and was used in treating many diseases such as rheumatism, anti-inflammatory, analgesic, and others (Joshi et al., 2014).

*6. Ephedra alata*: (*E. alata*) is a small, shrub (Alanda in Arabic علندة), perennial plant belongs to the Ephedraceae family, the plant is native to Middle East regions (Palestine, Lebanon, Egypt, Jordan, Saudi Arabia, Tunisia, and Iraq). Ephedra species include more than 40 different species, the stems are used in folk medicine in many countries such as China, India, Palestine, and others for respiratory problems, kidney diseases, and stimulants. *E. alata* is rich in secondary compounds compared with other species, amongst alkaloids, flavonoids, phenolic acids, and essential oils (Chebouat et al., 2016; Jaradat et al., 2021).

### 1.7 Antibacterial activity of roots

Plants usually synthesize antimicrobial secondary compounds naturally, that occur as a result of the metabolic reactions to continue growth or in response to other external forces like infection, the antimicrobials are classified as antibacterial and antifungal, the healthy plants produce various secondary compounds for defense (Morrissey & Osbourn, 1999). *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* consider the most common bacterial strains that cause skin inflammation if injury found (Luseba et al., 2007). The diversity of the active compounds in the plant parts confirms the strong ability to inhibit microbial infections; particularly the opposite of these strains. For instance, the concentrated phytochemical catechin in the seeds of *Nymphaea nouchli* (Nymphaeaceae) has been reported as a natural antibacterial agent (Parimala & Shoba, 2014).

In addition, the root's extract *of Hydnora africana* (Aristolochiaceae) have shown zone of inhibition with mean diameter from 0 to 25 mm against wide arrange strains of bacteria like: *S. aureus* and *E. coli* (Wintola & Afolayan, 2015*).* Moreover; the active compound β-sitosterol in the root bark of *Malva parviflora* (Malvaceae) after extracted with chloroform, acts as an antibacterial versus *S. aureus* and *E. coli* (M. Ododo et al., 2016*).* Different methods of root extract of *Landilphia owerrience* (Apocynaceae) exhibit some action against common bacterial strains with different proportions; whereas the ethanolic extract exhibits about 66.7% percent antibacterial activity against *S. aureus* (Okeke et al., 2001).

### 1.8 Antifungal activity of roots

Fungal species that infect the human body especially the skin is multiple and vary, more than 100 fungal species are involved in severe skin infections such as invasive mycosis diseases of hair or nails, at some time the fungal infections reach the subcutaneous layers of the skin and affect deep tissues like muscles. *Trichoderma sp, Aspergillus fumigatus, Candida albicans,* and other fungal species consider the main causes and are capable of superficial infections (Chetri & Gupta, 2019). Fungal infections affecting the body may lead to immunodeficiency, an example of fungal skin infection is oral thrush caused by Candida species (A. Singh et al., 2014)

A lot of strong secondary compounds secreted by plant roots have antifungal activity; in a study conducted on many plant species belonging to the two families mycotrophic and non-mycotrophic present in the root extract of some species have chemicals act as antifungal

agents, these chemicals are capable to prevent spores germination, for example *Brassica kaber* of (Brassicaceae) family (Paul Schreiner & Koide, 1993)

The root bark extract of *Periploca sepium* (Asclepiadiadaceae) has been investigated to isolate three of five polyphenolic antifungal compounds (sepiumol A-E), these compounds show significant antifungal activity against *Alternaria longipes* and *Gibberella saubinetii* fungal species ( MIC: 3.13µg/ml and 1.56µg/ml, respectively) (W. Zhao et al., 2019).

Flavonoids are a group of active compounds, amongst are ferrerol, hildegardiol, and 2-hydroxymaackiain; that have been isolated from the root of *Hildegardia barteri* (Malvaceae) by using NMR analysis to exhibit a strong antifungal activity (77% of inhibition at 250µg/ml) against some strains of *Candida* (Meragelman, Tucker et al., 2005)

### 1.9 Antiviral activity of roots

Using of the medicinal plants as antiviral is common and relevant in ancient folk medicine for thousands of years. The initial exploit was back to the Boost drug company (Nottingham, England), which screened more than 280 plants and classified them as anti-influenza agents (Chantrill et al., 1952). Following studies focused on how these plants work as antiviral and which parts (leaves, stem, root, bark, and seeds) are the most active. The phytochemicals in the plants act as inhibitory effects on the replication of viruses such as HIV (Asres & Bucar, 2005), hepatitis B virus (HBV) (K.-L. Huang et al., 2006).

In many plants, the root is the only active part used in the treatment of infections. The hidden part roots are a rich source of phytochemicals that have antiviral activity, some of these plants enhance the health status and improve the immune system during HIV infection, for example: *Panax ginseng* (ginseng, Araliaceae) the root extract of ginseng recover the destroyed cells usually ravaged among infection, *Zingiber officinale* (ginger, Zingiberaceae) aids in relief nausea causes by antiviral drugs, goldenseal (*Hydrastis canadensis*) root extract contain alkaloid and berberine which alleviate from diarrhea and weight loss complications associated with HIV infection, and licorice (*Glycyrrhiza glabra*) prevents viral production due to the active compound glycyrrhizin (Mukhtar et al., 2008). People who are infected with hepatitis B virus suffer from severe complications causes by the virus itself or treating drugs. The ethanolic extract of *Boehmeria nivea* (Urticaceae) root has been reported significantly to inhibit HBV DNA secretion production (K.-L. Huang et al., 2006)

### 1.10 Antioxidant activity of roots

Researchers more interested in investigating the effects of antioxidant activity on chronic diseases, diabetes, atherosclerosis, cancer, and other diseases (Ajitha, 2001). The naturally occurring health substances nutraceuticals or antioxidants are a large term that include different potent compounds which act against free radicals (Sharma & Bhat, 2009). The free radicals consider the most dangerous byproducts come from many sources in the body such as during metabolism, infection, inflammation, smoking, and illnesses. So, the main action of antioxidants is probably to delay or prevent illness and improve health. Anti-oxidants can inhibit or reduce the oxidative stress inside the cell. Plants include the biggest part of active compounds that have antioxidant properties and can scavenge free radicals, amongst phenolic compounds, carotenoids,

flavonoids, and vitamins. This variety of compounds is invited to using them in prophylactic and curative phytotherapy (Munteanu & Apetrei, 2021).

The importance in measuring the antioxidant activity has been increased until today, and in continuous development to invent a new protocol. A lot of methods of antioxidant assays were applied, the most common stable free radical scavenging assays include: (2,2-diphenyl-1-picryl-hydrazy) or $DPPH^{\bullet}$ and 2,2'-zaino-bis (3-ethylbenzothiozolin-6-sulfonic acid ($ABTS^{\bullet+)}$ free radical scavenging assay (Munteanu & Apetrei, 2021).

Many studies have been succeeded in confirming the antioxidant activity of the root. For example, the root extract of *Arctium lappa* (burduck) have been reported to have tannins and a phenolic active compound which detoxify the blood in *vivo* study and enhance the blood circulation (Chan et al., 2011). *Geranium sanguineum* (Geraniaceae) is a known medicinal plant native to Bulgaria, which treats many diseases such as infections and gastrointestinal disorders by using the roots of this plant in the past. *G. sanguineum* root extract has been reported to contain potent antioxidant capacity using $DPPH^{\bullet}$ free radical scavenging assay (IC50 =13.86 ± 0.84 g/ml) (Krishnaiah et al., 2011).

In another study, Badami et al. tested the methanolic and ethyl acetate root extract of *Aporosa lindleyana Baill* (Phyllanthaceae) for antioxidant activity, the test performed *in vivo* and *in vitro* models. By using the $DPPH^{\bullet}$ free radical scavenging assay it is found that *A. lindleyana* root extracts have a pronounced antioxidant capacity with $IC_{50}$: 3.51 ± 0.27 and 6.09 ± 1.00 µg/ml (Badami et al., 2005)

### 1.11 Bioactive secondary compounds present in roots

Different parts of the plant could be a rich source of bioactive compounds for the purpose of investigating of secondary metabolites, including pharmaceuticals, agrichemicals, flavors, and fragrances as well as for commercial drug production. Amongst plant parts that have been under-utilized and under-explored as a source of active secondary compounds are the roots. Several studies have implicated roots to be a source of secondary metabolites potentially active as antioxidant, antibacterial, anticancer antifungal and antiviral (Asnaashari et al., 2018). The bioactive compounds present in the root may exist naturally with high concentration in the tap root, adventitious root, or in the hairy root. The chemicals that are produced by the roots and secreted in the rhizosphere play key roles like attractants or repellants (Walker et al., 2003). The adventitious root of many certain plants has been effectively cultured and stimulated in the lap to get a maximum valuable secondary compound. This new technology also was applied to the hairy roots to produce more bioactive compounds, the hairy roots have been induced in suitable circumstances to obtain high doses of phytochemicals (Nadeem & Ahmad, 2019)

Roots have been implicated to provide a range of structurally diverse secondary compounds (Chizzola & Lohwasser, 2020). For example, *Conium maculatum* (Asteraceae) roots have been reported to contain ten different secondary compounds belonging to furocoumarins, prenylated coumarins, aliphatic C17-polyacetylenes, and the phenylpropanoid elemicin, (Table 2).

Another interesting study conducted *in vitro* to isolate the active compounds in the root of *Glycyrrihza glabra* (Fabaceae) and show how these compounds affect the liver cells as a cytotoxic property, the study was concluded with the isolation of nine potent cytotoxic

compounds in the root of *G. glabra*, some of them, glaring, kandosol, tetrahydroxymethoxychalcon, and glabrene (Çevik et al., 2018)

The methanolic root extract of *Gynochthodes ridsdaleis* has been analyzed and reported to have 26 different compounds, which include: hexadecenoic acid that has antioxidant and anti-inflammatory activity, and phytosterol which play a vital role in cholesterol metabolism (Nair & Gangaprasad, 2017).

**Table 2: Some groups of bioactive secondary compounds extracted from the root of *Conium maculatum*.**

| Groups | | | |
|---|---|---|---|
| **Furocoumarins** | **Prenylated coumarins** | **AliphaticC17-polyacetylenes** | **Phenylpropanoid elemicin** |
| Xanthotoxin (8-methoxy-psoralen) | Osthol | Falcarinol | Phenylpropanoid elemicin |
| Isopimpinellin (5,8-dimethoxy-psoralen) | Trans-superenol | Falcarindiol | |
| Bergapten (5-methoxy-psoralen) | | | |
| Psoralen | | | |
| Marmesin | | | |

## 1.12 Aim of the study

This study aimed to identify and evaluate the major phytochemicals and biochemical compounds in some wild Palestinian plant roots (WPPR) that are used traditionally in Palestine regions, using antioxidant, phytochemical screening, antibacterial, and GC-MS methods. We consider this study is a unique as there are no studies conducted about wild plant roots specially in Palestine.

## 1.13 Objectives of the study

1. To evaluate the antioxidant activity of the methanolic extract of six selected WPPR namely: *Asparagus officinalis, Malva sylvestris, Sinapis arvensis, Capparis spinosa, Urtica dioica,* and *Ephedra alata* using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging assay.
2. Determine the total phenol content using Folin Ciocalteu's method.
3. To screen the major phytochemical compounds in the roots of six wild Palestinian plants.
4. To test the antibacterial activity of the 'selected' root extract against 'selected' different strains of gram-negative bacteria which include: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumo*niae and one strain of gram-positive bacteria: *Staphylococcus aureus*
5. Identify the spectrum of the major volatile compounds present in the roots of 'selected' wild Palestinian plants using GC-MS spectral analysis.

# Chapter 2: Materials and Methods

### 2.1 Collection of roots of selected wild Palestinian plants

Roots of wild Palestinian plants namely *Asparagus officinalis, Malva sylvestris, Sinapis arvensis, Capparis spinosa, Urtica dioica,* and *Ephedra alata* were collected from natural habitat in January 2020 from the northern parts of Palestine (Tulkarm city; Latitude: 32.3186, Longitude: 35.0897). All roots were transferred to the laboratories in the Faculty of Pharmacy and Medical Sciences at Hebron University. Roots have been separated from other plant parts, cleaned, dried at room temperature, grounded, and stored in airtight glass container until used.

### 2.2 Roots extraction

Roots of 'selected' wild Palestinian plants were extracted based on a protocol described by Qawasmeh et al., 2012 with minor modification (Abdelqader Qawasmeh et al., 2012). From each plant, a 1g of the grounded root was extracted in 10 mL methanol (80% v/v) overnight on a shaker (Labtech, Model No. LSI-3016R, Daihan Labtech India Pvt. Ltd., Hyderabad, India) at 80 rpm, at 25 ℃. Roots suspensions were filtered and stored in a refrigerator for $DPPH^{\bullet}$, $ABTS^{\bullet+}$, total phenols and GC–MS analysis.

### 2.3 $DPPH^{\bullet}$ free radical scavenging assay

$DPPH^{\bullet}$ free radical scavenging assay was performed based on the method described by Dowaek et al., 2020 with minor modifications (Dowek et al., 2020). A stock solution of $DPPH^{\bullet}$ (1000 mmol) was prepared by dissolving 6.8 mg of $DPPH^{\bullet}$ (Sigma Aldrich, Palestine-STBD4146V) in 17 mL methanol (80%). A working solution of $DPPH^{\bullet}$ was prepared by diluting an aliquot of $DPPH^{\bullet}$ with methanol (80%) until final absorbance reaching 0.7 at $\lambda_{max}$ 517 nm. The 2 mL of working $DPPH^{\bullet}$ solution, 30 µL of undiluted methanolic root extracts from each Palestinian wild

plant was added and mixed well in cuvettes and incubated for 1 h in dark at room temperature. The control sample was prepared by mixing 2 mL $DPPH^{\bullet}$ working solution with 30 µL methanol (80%). The absorbance of the control (A control) and all root extracts (A sample) were measured using Genway UV/visible spectrophotometer (Manufactured in the UK by Cole-Parmer Ltd Stone, Staffs, UK, ST150 SA, Model- 7205) at 517 nm. The percentage of $DPPH^{\bullet}$ scavenging of the roots was calculated based on the equation below:

$$DPPH^{\bullet} \text{ scavenging } (\%) = [(A_{control} - A_{sample})/A_{control}] * 100\%$$

**2.4 $ABTS^{\bullet+}$ scavenging assay**

The solution of $ABTS^{\bullet+}$ (Sigma Aldrich, Palestine) was prepared with a concentration of 7 mmol by adding 6.9 mg to 3 mL DW. A 3 mL of $ABTS^{\bullet+}$ stock solution was mixed with 3 mL potassium persulfate (6.6 mg of $K_2S_2O_3$ dissolved in 15.9 mL DW, 2.45 mmol). The $ABTS^{\bullet+}$ solution kept overnight in the dark. In the next day, $ABTS^{\bullet+}$ stock solution was diluted with methanol 80% until absorbance reached 0.7000±0.02 at 734 nm. In cuvette, a 1 mL of $ABTS^{\bullet+}$ solution and 15µl of extract was added and then mixed by vortex. For control, 15 µL of methanol 80% was mixed. Finally, all the cuvettes left in dark for 1h at room temperature. The absorbance (A) of all roots samples and the control was determined by using Genway UV/visible spectrophotometer, the absorbance of control (methanol) and sample (extract) was read at 734 nm. The percentage of scavenging of $ABTS^{\bullet+}$ was calculated using to the equation:

$$ABTS^{\bullet+} \text{ scavenging } (\%) = [(A_{control} - A_{sample})/A_{control}] * 100\%$$

## 2.5 Determination of total phenols

The total phenols in the 'selected' roots of Palestinian plants were determined based on the techniques described by Qawasmeh et al., 2012 with minor modifications (Abdelqader Qawasmeh et al., 2012). Briefly, A 2 g of root samples were separately mixed with 20 mL methanol 80% with stirring for 30 min at room temperature. The extract was filtered and extracted successively with an extra 10 mL methanol 80% for 15 min. Both filtrates were combined and defatted with 10 mL of *n*-hexane. The defatting process was repeated 3 times. The defatted methanolic extracts used directly for the determination of total phenols without dilution.

Gallic acid solution (standard) was prepared by dissolving 0.25 g of G.A powder with 5 mL ethanol 96% (gallic acid doesn't dissolve in water), then diluted with DW up to 50 mL and stored in a refrigerator until use. Sodium bicarbonate solution ($NaHCO_3$, 25 %, w/v) was prepared by mixing 25 g of NaHCO3 with 100 mL DW with stirring and heating until dissolved. The filtered aqueous extract was stored in refrigerator up to 2 weeks. Total phenols in roots of selected Palestenian plants were determined by mixing a 20 µL of selected roots extract with 1.58 mL DW, 150 µL Folin-Ciocalteu reagent (102180470, sigma, Palestine), and 30 µL of NaHCO3 in cuvettes. The absorbance (A) of all root samples and the standard gallic acid concentrations were determined using Genway UV/visible spectrophotometer at 760 nm, data were expressed as milligram of gallic acid per gram of dried plant roots.

## 2.6 Phytochemical screening

Phytochemical screening for major secondary compounds (alkaloids, anthraquinones, anthocyanins, cardiac glycosides, coumarins, flavonoids, glycosides, phenolics, phlobatannins, quinones, saponins, steroids, tannins, and terpenoids) were performed based on the protocols described by Harborne, 1998 (Harborne, 1998). Briefly, a 3 g of roots powder were extracted in 80% methanol overnight at room temperature. Filtered extracts were used to qualitatively assess the presences of major secondary compounds (n=2).

### Alkaloids test

Alkaloids test was performed by mixing 2 mL of root extract with 1 mL of Hydrochloric acid (HCL, 1%) and few drops of Meyers reagent (1.36 g of mercuric chloride with 5 g of potassium chloride were dissolved in 100 mL D.W) in a test tube. The formation of white precipitate in the bottom of the test tube indicates the presence of alkaloid.

### Anthraquinone test

Anthraquinone test was performed by mixing 2 mL of root extract with benzene. A 1 mL of Ammonia ($NH_3$, 10%) was added. A red, violet, or pink color solution; indicate positive result.

### Cardiac glycosides test

Cardiac glycosides test performed by mixing 2 mL of glacial acetic acid with 2 mL of root extract, then 1 mL of concentrate sulfuric acid ($H_2SO_4$) was added. A few drops of Ferric chloride $Fecl_3$ reagent was added to obtain brown ring as the result.

**Flavonoid test**

Flavonoid test prepared by mixing 2 mL of root extract with few drops of $NH_3$ 1%. The change to yellow color is the positive result.

**Phenolic group test**

Phenolic group test performed by adding a few drops of $Fecl_3$ 10% to the mixture of 1 mL of root extract and 2 mL of D.W to obtain blue or black color.

**Anthocyanin test**

Anthocyanin test prepared by mixing 2 mL of root extract with 1 mL of 2 N sodium hydroxide NaOH then heated for 5 min to convert the mixture to bluish green as positive result.

**Coumarin test**

Coumarin test performed by boiling the Mixture of 1 mL root extract with 1 mL of 1N NaOH in water bath for A few min. the change to yellow color indicates positive result.

**Saponins test**

Saponins test prepared by shaking 2 mL of root extract with 5 mL DW; the formation of foam indicates positive result.

**Quinones test**

Quinones test performed by mixing 1 mL of $H_2SO_4$ 98% with 1 mL of root extract to change solution to red color.

**Steroids test**

Steroids test performed by mixing 1 mL of chloroform $CHCL_3$ with 1 mL of root extract. 1 mL of $H_2SO_4$ 98% then was added. The presence of reddish-brown ring indicates positive results.

**Terpenoid test**

Terpenoid test performed by mixing 2 mL of root extract with 2 mL $CHCL_3$ and 3 mL $H_2SO_4$ 98%. The formation of reddish-brown layer in the top of the test tube indicates positive result.

**Tannins test**

Tannins test prepared by mixing 2 mL of root extract with 1 mL DW, then, 1-2 drops of $FeCl_3$ was added. The presence of green or blue-black color indicates positive results.

**Phlobatannins test**

Phlobatannins test was performed by mixing 1 mL of NaOH 10% with 2 mL of root extract. The presence of yellow color indicted positive results.

**Glycosides test**

Glycosides test prepared by mixing 2 mL of root extract with 2 mL $H_2SO_4$ 50%. The mixture then heated for 5 min, after that, 10 mL of Fehling solution was added and boiled for few min. The formation of red brick precipitate indicated positive result.

## 2.7 Antibacterial activity

### 2.7.1 Extract preparation

Root extract samples were prepared depend on the protocol followed by (Abdelqader Qawasmeh et al., 2012). Each 10 g of selected root samples were extracted with 100 mL methanol 80%, so, 1 g of the sample mixed with 10 ml methanol 80% with continuous shaking for 24 h at room temperature. All samples were filtered and stored at 4 °C until use.

### 2.7.2 Bacterial samples

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

### 2.7.3 Media preparation

All required media were prepared according to the instructions given in the label that recommended by the manufacturer of nutrient agar (NA, Bio Maxima S.A, FM4704). The media was prepared by dissolving 7 g of N.A powder in 250 mL D.W. MacConkey, was prepared by dissolving 12.885 g of powder in 250 mL D.W. Eosin Methylene Blue (EMB, HIMEDIA, 0000289212) was prepared by dissolving 8.9 g of EMB powder in 250 mL D.W. Mannitol Salt Agar (MSA, HIMEDIA, 0000335310) was prepared by dissolving 27.755 g of MSA powder in 250 mL D.W. Differential media Muller Hinton agar (MHA, HIMEDIA, 000381656) was

prepared by dissolving 28.5 g of powder in 750 mL D.W. All prepared media were autoclaved (LAC-5065SP, LABTECH) at 121℃ for 90 min, after cooling and pouring the media in petri dish (90 X 16 mm), all prepared sterile media were refrigerated at 2-4 ℃.

### 2.7.4 Bacterial culture and subculture

All bacterial strains were cultured on suitable media, *P. aeruginosa* and *P. vulgaris* were cultured on nutrient agar media, whereas, *E. coli* and *K. pneumoniae* were cultured on MacConkey and Eosin Methylene Blue (EMB). The rest strain S. aureus was cultured on Mannitol Salt Agar media. All media were incubated at 37 ℃ for 24 h.

### 2.7.5 Sensitivity test

For well diffusion method, the sensitivity test was conducted on all Muller Hinton Media that cultured with all bacterial strains according to (Irulandi et al., 2017). In a test tube, the bacterial suspension was performed by mixing one colony of 12-18 h old bacteria with 1 mL normal saline and stirring gently, the Genway UV spectrophotometer was used to measure the absorbance of bacterial suspension for a proper density of 0.06-0.08. with sterile cotton swap, the Muller Hinton plates were spread with all bacterial strains, 6 samples with 3 replicates, each plate was having 5 holes; 3 for 10 µl extract samples, whereas the other 2 holes for negative control (methanol) and positive control disks which include Vancomycin (30 µg Biomaxima, Poland) for S. aureus bacteria and Meropenem (10 µg Biomaxima, Poland) for the rest bacterial strains.

For disk diffusion method, the sterile filter papers were saturated with the extracts and used instead of the holes, the zone of inhibition of the positive controls and the extract samples were measured (mm) after 24 h incubation at 37 ℃ (Digrak et al., 1999)

## 2.8 GC-MS analysis

The GC-MS analysis of methanolic root extract of *M. sylvestris, C. spinosa, U. dioica, E. alata, A. officinalis, S. arvensis* was performed using GC-MS) Clarus SQ 8S, Perkin Elmer, USA) fitted with a BD-5ms capillary column (30m, 0.25µ film thickness, 0.25 µm bore diameter) based on the method described by (Abdelqader Qawasmeh et al., 2012) with minor modification as described below. A 1µL of the root extracts were injected. The oven temperature was programed to be started at 80℃ for 2 min and then increased to 280 ℃ at a rate of 30 ℃ $min^{-1}$. The temperature of injector and detector were maintained at 260 ℃ and 260 ℃, 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 50 and 500 m/z were acquired. Each sample was analyzed 2 times. Following that, the compounds identified using NIST05 mass spectral library, then they were compared with those published in the literature.

# Chapter 3: Results

### 3.1 DPPH$^{\bullet}$ scavenging capacity

The methanolic extract of root samples exhibited antioxidant capacity using DPPH$^{\bullet}$ free radical scavenging assay, the average percentage of scavenging showed in Figure 3. The highest level of antioxidant capacity was founded in the root extract of *E. alata* and *A. officinalis* with DPPH$^{\bullet}$ scavenging percent 69.5% and 57.7%, respectively. However, the lowest level of antioxidant scavenging was founded in the root extract of *U. dioica* and *M. sylvistris* with DPPH$^{\bullet}$ scavenging percent 18.9% and 22.8%, respectively as showed in Table 3.

**Table 3: Absorbance values for DPPH● scavenging assay (raw data) of some wild Palestinian plant roots. SE: Standard error**

| Replicates number/plant name | 1 | 2 | 3 | 4 | Mean ± SE |
|---|---|---|---|---|---|
| ***A. officinalis*** | 0.247 | 0.289 | 0.264 | 0.293 | 57.70 ± 1.68 |
| ***M. sylvistris*** | 0.498 | 0.499 | 0.490 | 0.509 | 22.76 ± 0.60 |
| ***S. arvensis*** | 0.454 | 0.502 | 0.387 | 0.490 | 29.06 ± 4.00 |
| ***C. spinosa*** | 0.427 | 0.392 | 0.344 | 0.355 | 21.25 ± 2.92 |
| ***U. diocia*** | 0.504 | 0.568 | 0.551 | 0.472 | 18.92 ± 3.39 |
| ***E. alata*** | 0.177 | 0.256 | 0.181 | 0.174 | 69.50 ± 3.05 |

### 3.2 ABTS$^{\bullet+}$ scavenging capacity

The methanolic (80%) extract of six root samples exhibited antioxidant capacity using the ABTS$^{\bullet+}$ free radical scavenging assay; the average percentage of scavenging showed in Figure 4. The highest level of antioxidant capacity was founded in the root extract of *A. officinalis* and *E. alata* with ABTS$^{\bullet+}$ scavenging percent 69.6% and 66.1%, respectively. However, the root extract of *S. arvensis* exhibited the lowest level of antioxidant capacity with ABTS$^{\bullet+}$ scavenging percent 12.5%.

**Table 4: Absorbance values for ABTS●+ scavenging assay (raw data) of some wild Palestinian plant roots. SE: Standard error**

| Replicates number/plant name | 1 | 2 | 3 | 4 | Mean ± SE |
|---|---|---|---|---|---|
| ***A. officinalis*** | 0.196 | 0.191 | 0.147 | 0.201 | 69.58 ± 4.11 |
| ***M. sylvistris*** | 0.226 | 0.275 | 0.301 | 0.321 | 53.52 ± 6.80 |
| ***S. arvensis*** | 0.591 | 0.555 | 0.502 | 0.466 | 12.5 ± 9.18 |
| ***C. spinosa*** | 0.311 | 0.382 | 0.442 | 0.233 | 43.29 ± 14.9 |
| ***U. diocia*** | 0.363 | 0.373 | 0.307 | 0.495 | 36.34 ± 13.1 |
| ***E. alata*** | 0.276 | 0.328 | 0.111 | 0.103 | 66.14 ± 18.9 |

### 3.3 Total phenol

The total phenol in the methanolic extract of root samples were quantitatively estimated as mgs of Gallic acid equivalent mg GAE /1 g dry weight (n=3) as shown in Figure 5. The highest total phenol was founded in *A. officinalis* with 362.5 mg GAE / g dry weight compared with other samples, the other root samples were showed results closed together ranged from 167. mg GAE / g dry weight to 245.83 mg GAE / g dry weight as showed in Figure 6.

**Table 5: Absorbance values for total phenol (raw data) of some wild Palestinian plant roots. SE: Standard error**

| | Polyphenol mg GAE/ g dry matter | | | | | | |
|---|---|---|---|---|---|---|---|
| **Replicates number/ plant name** | **1** | **2** | **3** | **C1** | **C2** | **C3** | **Mean ± SE** |
| ***A. officinalis*** | 0.168 | 0.226 | 0.308 | 280 | 376.67 | 513.33 | 362.5 ± 63.74 |
| ***M. sylvistris*** | 0.136 | 0.138 | 0.163 | 226.67 | 230 | 271.67 | 245.83 ± 12.33 |
| ***S. arvensis*** | 0.186 | 0.114 | 0.111 | 310 | 190 | 185 | 218.75 ± 35.15 |
| ***C. spinosa*** | 0.082 | 0.066 | 0.193 | 136.67 | 110 | 321.67 | 188.33 ± 54.36 |
| ***U. diocia*** | 0.025 | 0.134 | 0.100 | 41.67 | 223.33 | 166.67 | 169.17 ± 52.65 |
| ***E. alata*** | 0.043 | 0.111 | 0.134 | 71.67 | 185 | 223.33 | 167.5 ± 38.17 |

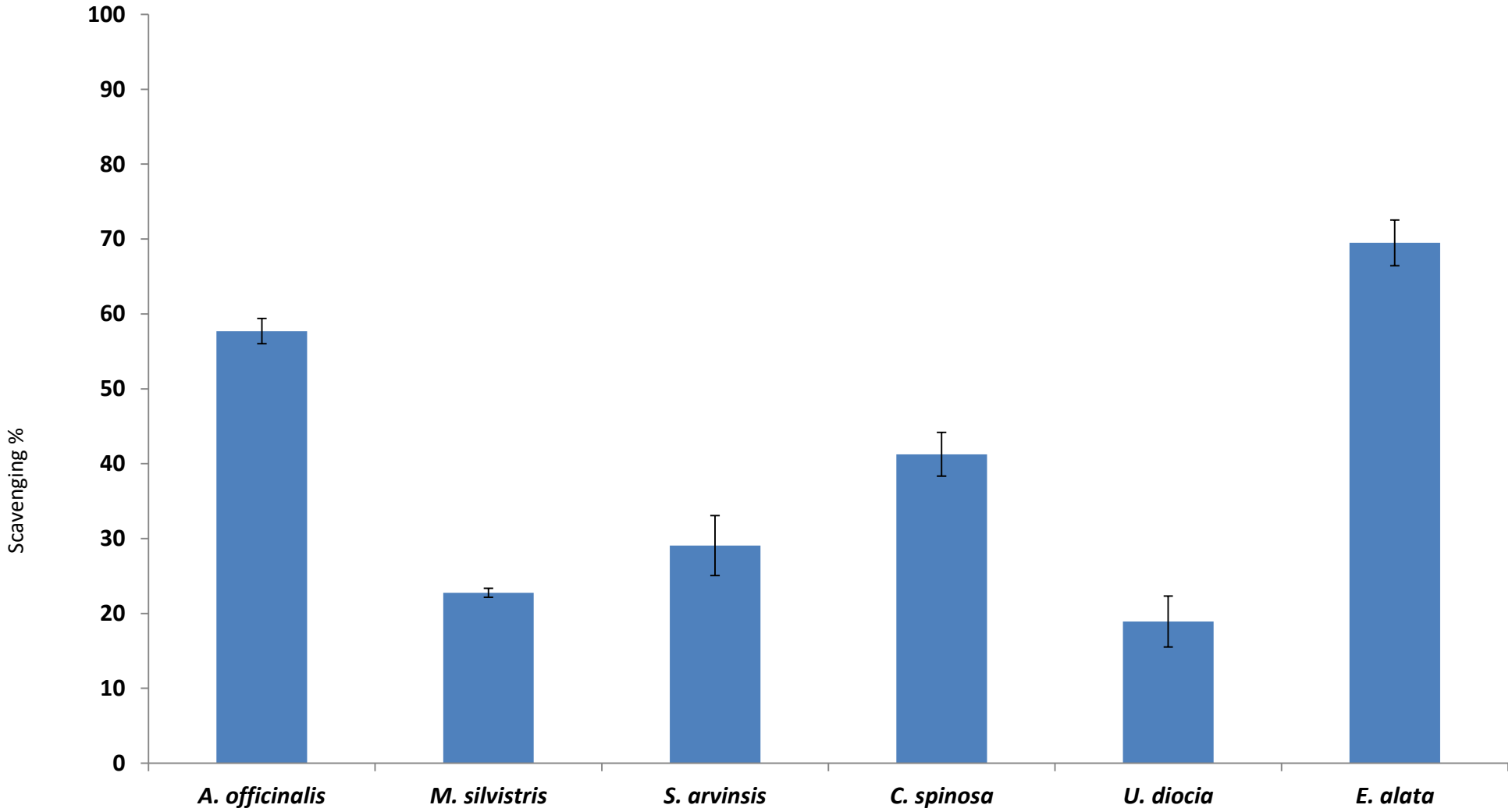


**Figure 3: Mean radical scavenging of six wild Palestinian plant root extracts assayed by DPPH• radical scavenging method using methanolic extract**

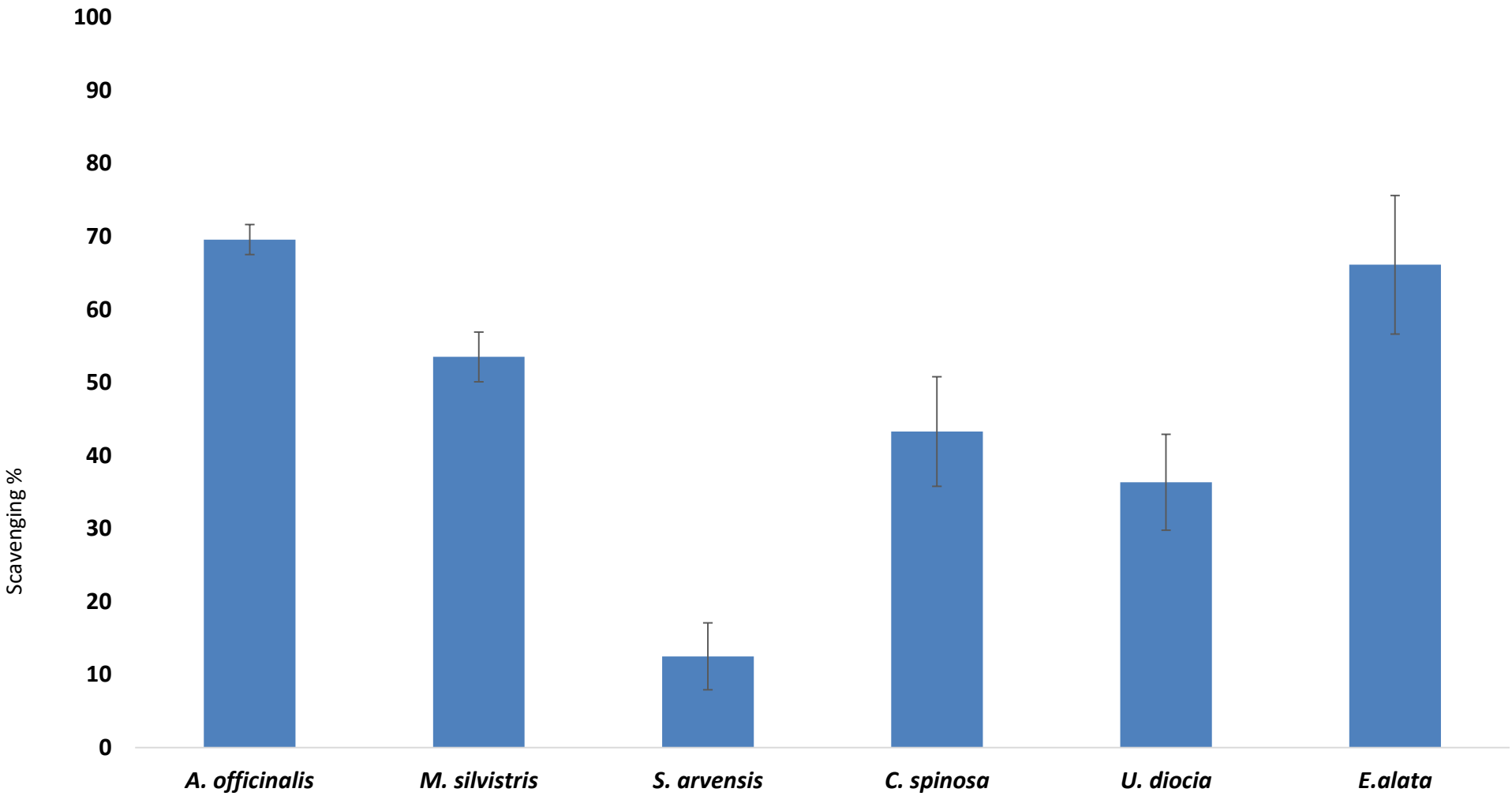


**Figure 4: Mean radical scavenging of six wild Palestinian plant root extract assayed by ABTS$^{\bullet+}$ radical scavenging method using methanolic extract.**

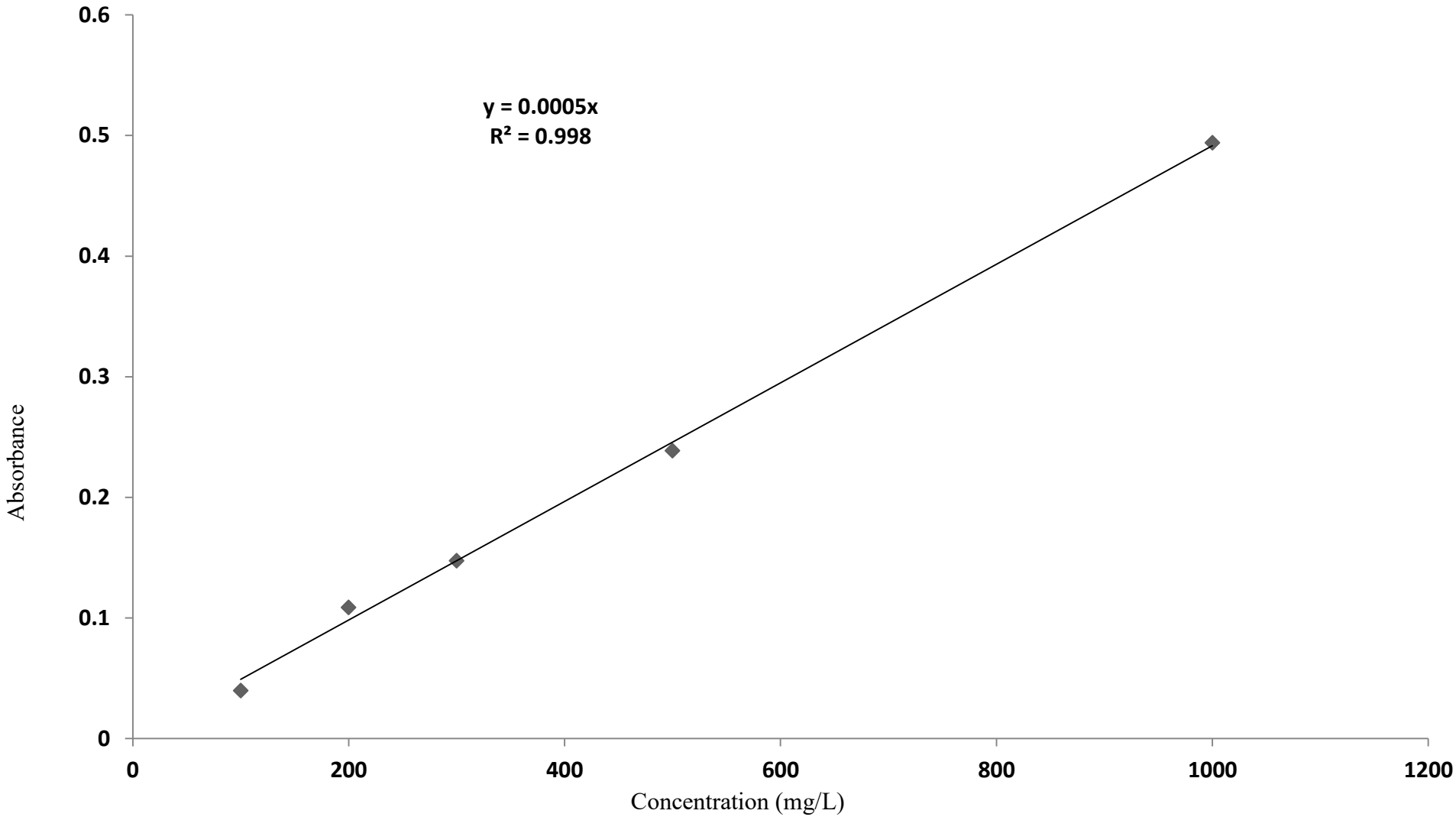


**Figure 5: Calibration curve of gallic acid. Each point represents the mean of triplicates**

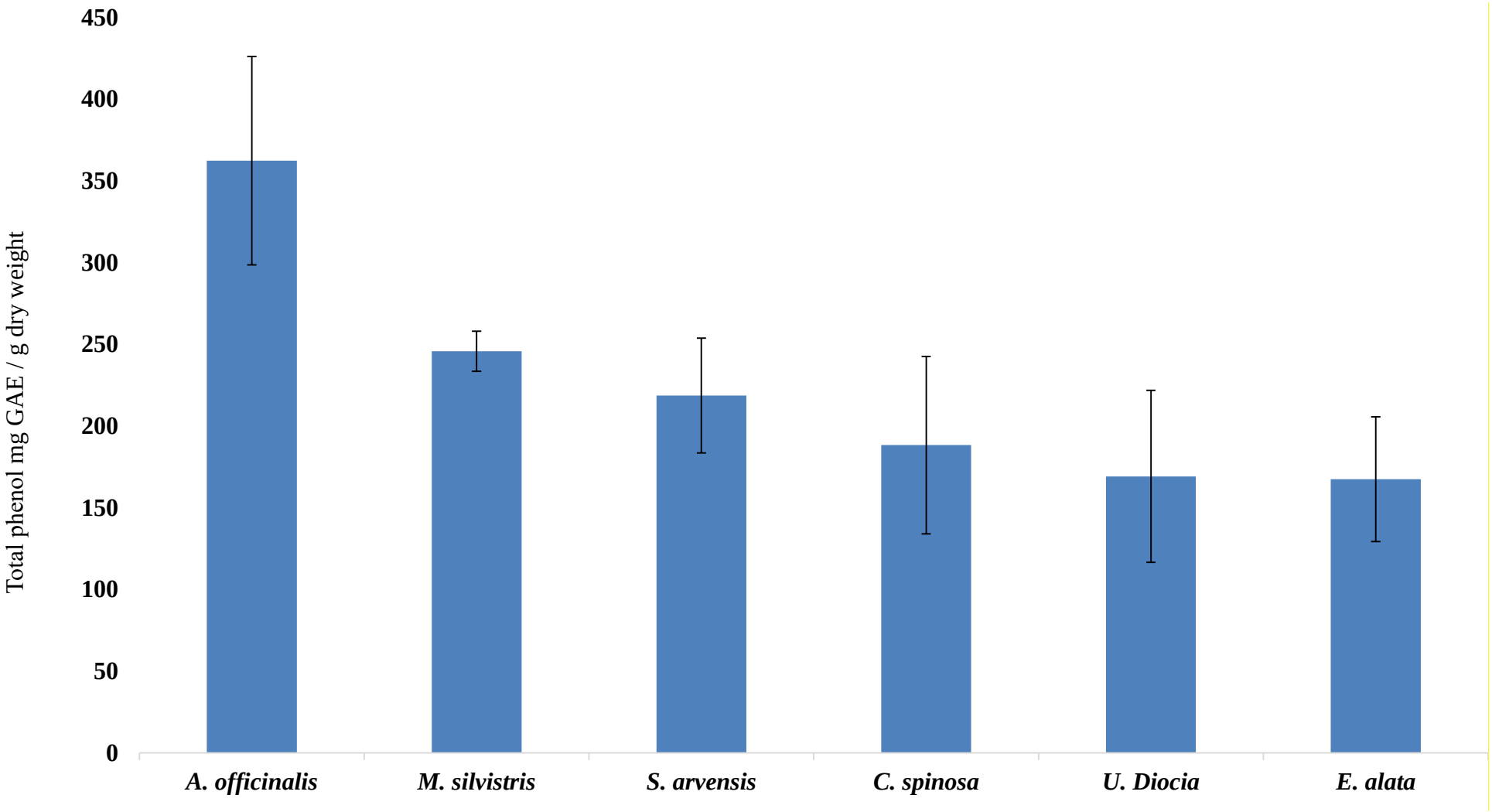


**Figure 6: Total phenol of six wild Palestinian plant root methanolic extracts produced by Folin-Ciocalteu method, n=3**

### 3.4 Phytochemical screening

The methanolic extract of the root of six WPPR evidently showed the presence of a wide range of phytochemical groups such as cardiac glycosides, flavonoids, saponins, terpenoids, quinones, and steroids as shown in Table 6. However, some of phytochemical groups are not detected in any samples like alkaloids, anthraquinones, coumarins, tannins, and Phlobatannins.

**Table 6: Phytochemical screening for the methanolic extracts of root samples**

| Test /Extract | *A. afficinalis* | *M. sylvestris* | *S. arvensis* | *C. spinosa* | *U. diocia* | *E. alata* |
|---|---|---|---|---|---|---|
| **Alkaloids** | - | - | - | - | - | - |
| **Anthraquinone** | - | - | - | - | - | - |
| **Cardiac glycosides** | + | - | - | - | - | + |
| **Flavonoids** | + | - | - | + | - | + |
| **Phenolic groups** | - | - | - | - | - | - |
| **Anthocyanins** | - | - | - | - | - | - |
| **Coumarins** | - | - | - | - | - | - |
| **Glycosides** | + | - | - | + | - | + |
| **Saponins** | + | + | ++ | + | ++ | + |
| **Quinones** | - | - | - | - | - | + |
| **Steroids** | + | - | - | - | - | + |
| **Terpenoids** | + | - | - | + | - | + |
| **Tannins** | - | - | - | - | - | - |
| **Phlobatannins** | - | - | - | - | - | - |

### 3.5 Antibacterial activity

The methanolic extract of the root samples showed negative results against gram-negative and positive bacteria and there is no notable zone of inhibition by using two methods: disk as well as well diffusion Figure 7, 8.

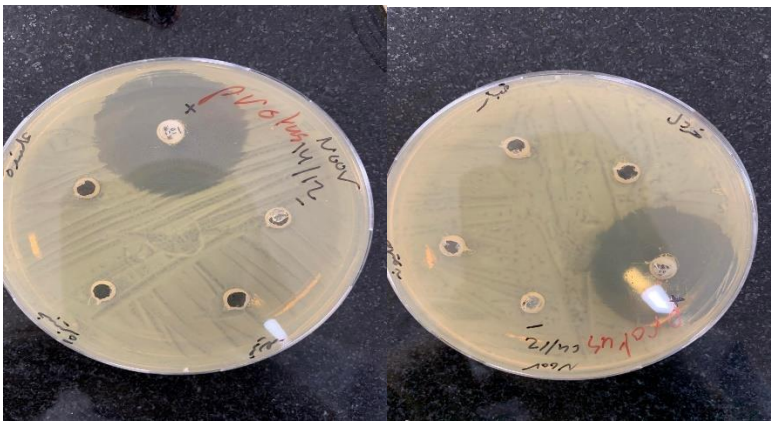

**Figure 7: Antibacterial activity evaluation of root extract by well diffusion method against *Pseudomonas aeruginosa* and *Proteus mirabilis*.**


**Figure 8: Antibacterial activity evaluation of root extract by disk diffusion method against *Proteus mirabilis* and *Klebsiella pneumonia*.**

### 3.6 GC-MS analysis

The GC-MS analysis showed the presence of at least seven different volatile compounds in each root sample, Figures 9,10,11,12,13, 14, and 15. The Major volatile compounds detected in the methanolic root extract of *A. officinalis*, *M. sylvestris, S. arvensis*, *C. spinosa, U. diocia, E. alata* were summarized in Tables 7,8,9,10, 11, and 12.

***Asparagus officinalis***: the methanolic root extract exhibited the presence of eight different volatile compounds that found in the root extract of *A. officinalis* with different RT. The major volatile compounds detected include: Methyl tetradecanoate, Trans, cis-1,8-dimethylspiro [4,5] decane, 1,19-eicosadiene, D-glucose, 4-O-alpha-D-glucopyranosyle, and 1-heptatriacotanol (Table 7)

***Malva sylvestris***: GC-MS method identified several volatile compounds found in the methanolic root extract of M. sylvestris, Nonanoic acid, methyl ester, L-(+)-ascorbic acid 2,6-dihexadeconate, naphthalene, decahydro, and 9,12-octadecadienoic acid methyl ester were the major volatile compounds detected (Table 8)

***Sinapis arvensis***: the major volatile compounds that were identified by GC-MS in the methanolic root extract of *S. arvensis* were d-glucose, 4-O-alpha-d-glucopyranosyle, octanoic acid, 2-methyl, vitamin A aldehyde, 9,12-octadecadienoic acid (z, z)-, methyl ester, and 1-heptatriacotanol (Table9).

***Capparis spinosa***: the methanolic root extract exhibits the presence of different volatile compounds that are found in the root extract of *C. spinosa* with clear RT, which include rebitol, propanoic acid-methyl propyle ester, benzoic acid, myristic acid isobutyl ester, d-glucose, 4-O-alpha-d-glucopyranosyle, heptadecanoic acid (methyl ester), d-mannitol,1-thioheptyle-1-deoxy, 11-hexadecynal, and 6,11-ecosadienoic acid methyl ester (Table 10)

***Urtica diocia***: six of different volatile compounds were identified from eleven that detected by GC-MS method, they include: bicyclo[3.1.1]heptan-2-one, 2,6,6-trimethyl, cyclohexyle isovalerate, homomenthyl salicylate, decanoic acid, 2-methyl, l-ascorbic acid, 6-octadecanoate, and linoleic acid ethyl ester (Table 11).

***Ephedra alata***: the major volatile compounds that were identified by GC-MS in the methanolic root extract of *E. alata* were: furaldehyde, benzoic acid, vitamin A aldehyde, 2,4.4,6-tetramethyle-6-phenyl-2-heptene, aromadendrene oxide-2, d-glucose, 4-o-alpha-d-glucopyranosyle, 5,7-dimethyloctahydrocoumarin, 2-pentanone,1,3-dimethoxy-2-methyl, heptacosanoic acid, 25-methyl-, methyl ester, and cyclo hexen,1-hexyle (Table 12).

**Table 7: Major compounds detected in *A. officinalis* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 18.60 | 55, 74, 87 | Methyl tetradecanoate | 242 | $C_{15}H_{30}O_2$ |
| 2 | 20.61 | 55, 67, 81 | Trans, cis-1,8-dimethylspiro [4,5] decane | 166 | $C_{12}H_{22}$ |
| 3 | 20.68 | 55, 69, 83 | 1,19-eicosadiene | 278 | $C_{20}H_{38}$ |
| 4 | 21.70 | 69, 91, 107 | 1-heptatriacotanol | 536 | $C_{37}H_{76}O$ |
| 5 | 23.11 | 55, 73, 77 | Unknown | | |
| 6 | 24.41 | 55, 69, 93 | D-glucose, 4-O-alpha-D-glucopyranosyle | 342 | $C_{12}H_{22}O_{11}$ |
| 7 | 25.72 | 55, 70, 81 | 11-Hexadecynal | 236 | $C_{16}H_{28}O$ |
| 8 | 27.11 | 60, 73, 93 | Unknown | | |

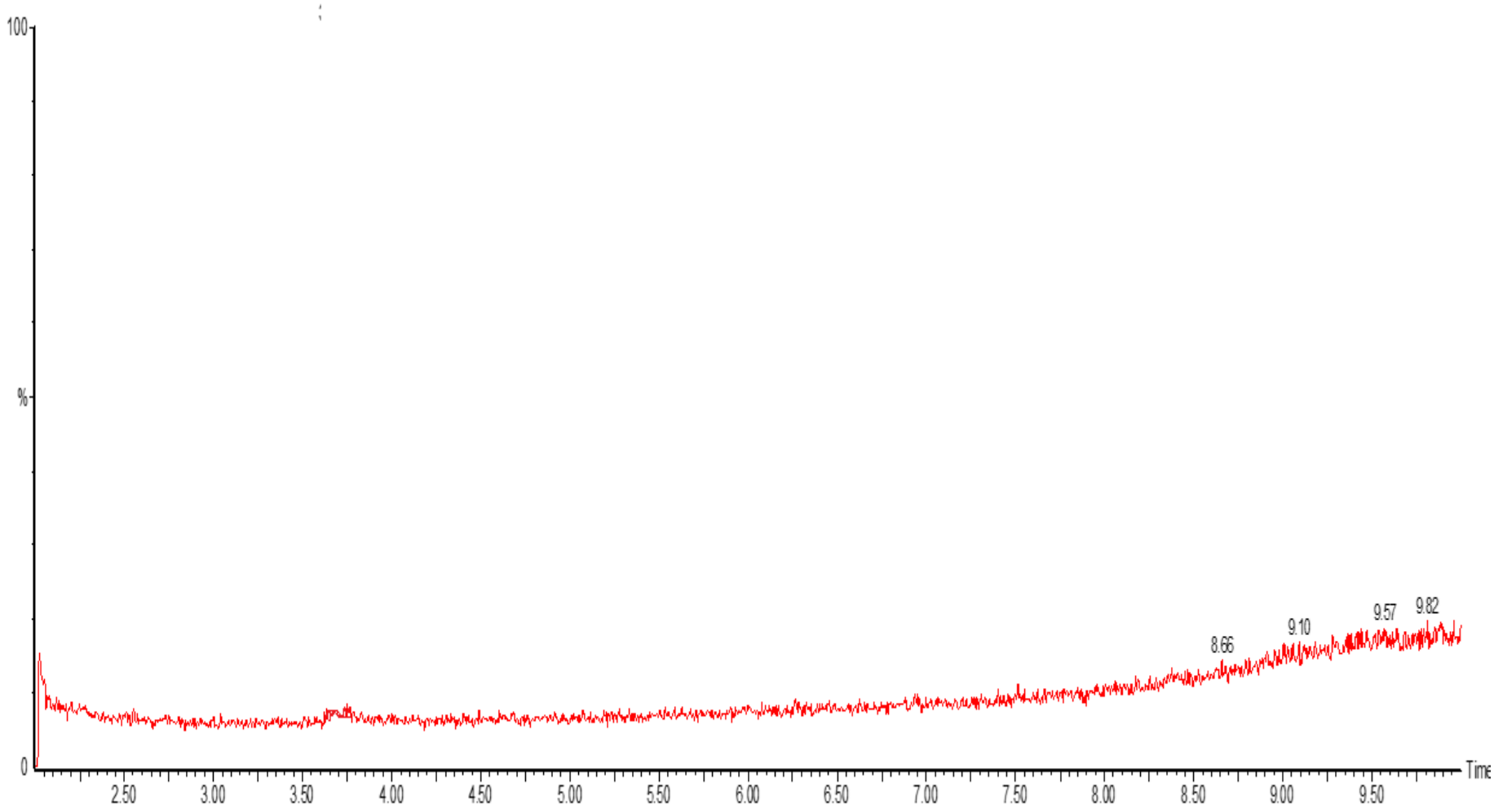


**Figure 9: Representative chromatograms (TIC) of the methanol (80%)**

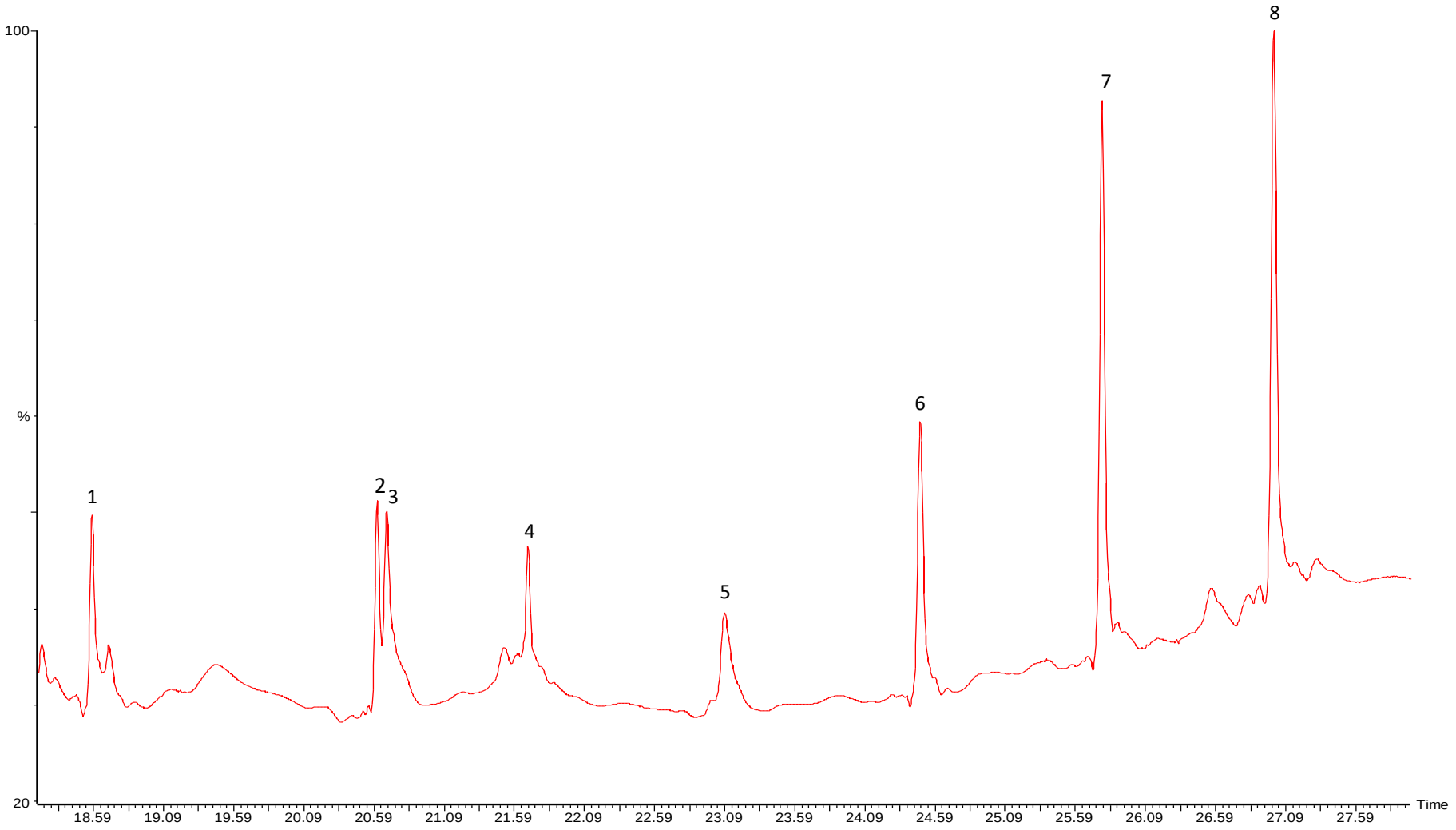


**Figure 10: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *A. officinalis* root.**

**Table 8: Major compounds detected in *M. sylvestris* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 8.83 | 55, 73, 147 | Unknown | | |
| 2 | 11.89 | 73, 147, 281 | Unknown | | |
| 3 | 14.39 | 55, 73, 75 | Unknown | | |
| 4 | 17.29 | 57, 73, 84 | L-(+)-ascorbic acid 2,6-dihexadeconate | 652 | $C_{38}H_{68}O_8$ |
| 5 | 18.23 | 57, 69, 81 | Naphthalene, decahydro | 138 | $C_{10}H_{18}$ |
| 6 | 18.60 | 57, 74, 87 | Nonanoic acid, methyl ester | 172 | $C_{10}H_{20}O_2$ |
| 7 | 20.59 | 54, 67, 81 | 9,12-octadecadienoic acid, methyl ester | 294 | $C_{19}H_{34}O_2$ |

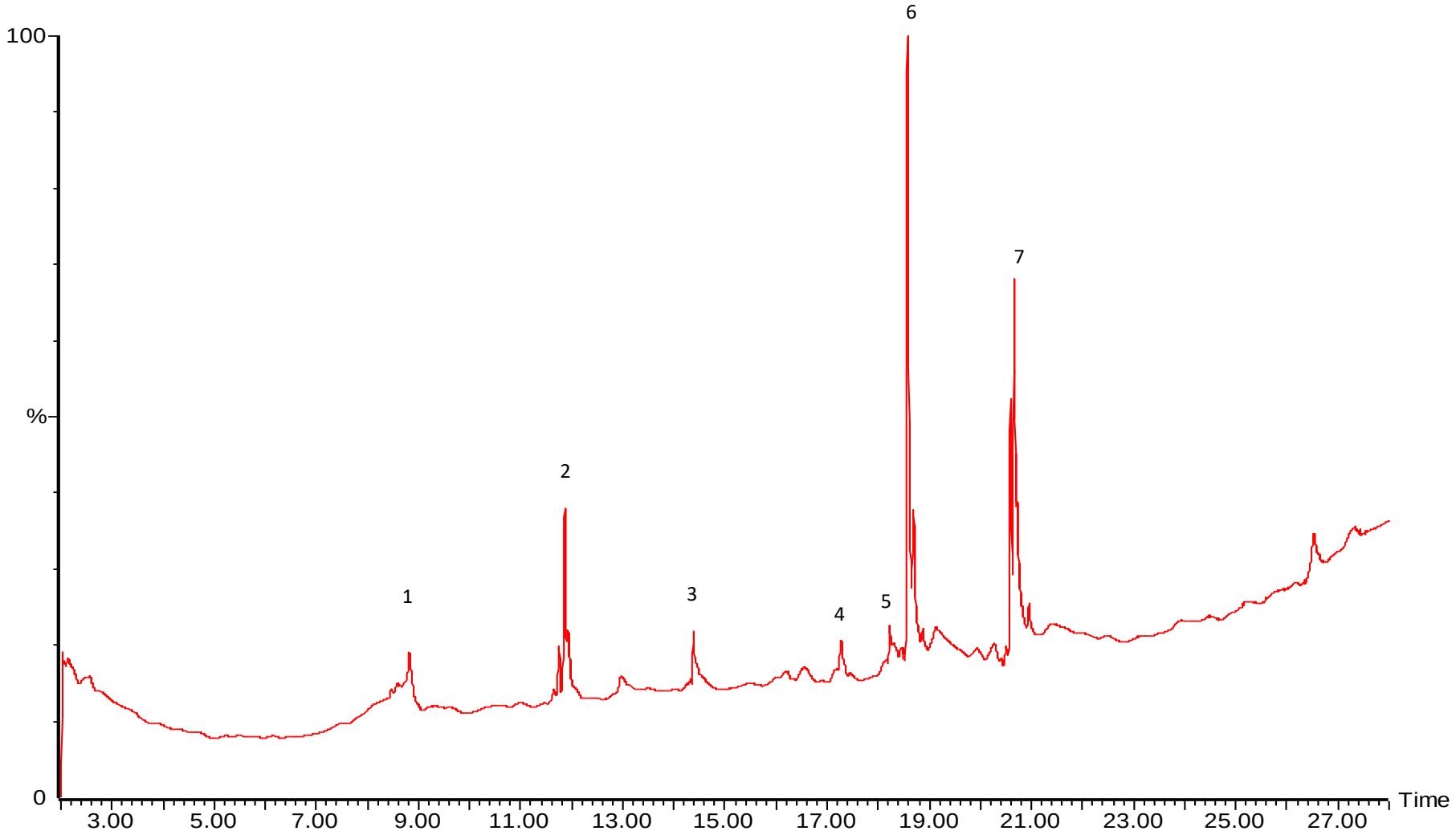


**Figure 11: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *M. sylvestris* root.**

**Table 9: Major compounds detected in *S. arvensis* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 8.66 | 55, 67, 79 | Unknown | | |
| 2 | 11.89 | 55, 73, 147 | Unknown | | |
| 3 | 12.35 | 73, 85, 93 | Unknown | | |
| 4 | 13.42 | 55, 67, 75 | Unknown | | |
| 5 | 14.5 | 54, 67, 81 | Unknown | | |
| 6 | 17.28 | 55, 57, 69 | D-glucose, 4-O-alpha-D-glucopyranosyle | 342 | $C_{12}H_{22}O_{11}$ |
| 7 | 18.21 | 55, 69, 109 | Naphthalene, decahydro | 138 | $C_{10}H_{18}$ |
| 8 | 18.75 | 55, 73, 85 | Octanoic acid, 2-methyl | 158 | $C_9H_{18}O_2$ |
| 9 | 20.31 | 55, 60, 73 | Vitamin A aldehyde | 284 | $C_{20}H_{28}O$ |
| 10 | 20.66 | 55, 67, 79 | 9,12-octadecadienoic acid (z, z)-, methyl ester | 294 | $C_{19}H_{34}O_2$ |
| 11 | 21.70 | 55, 69, 73 | 1-Heptatriacotanol | 536 | $C_{37}H_{76}O$ |

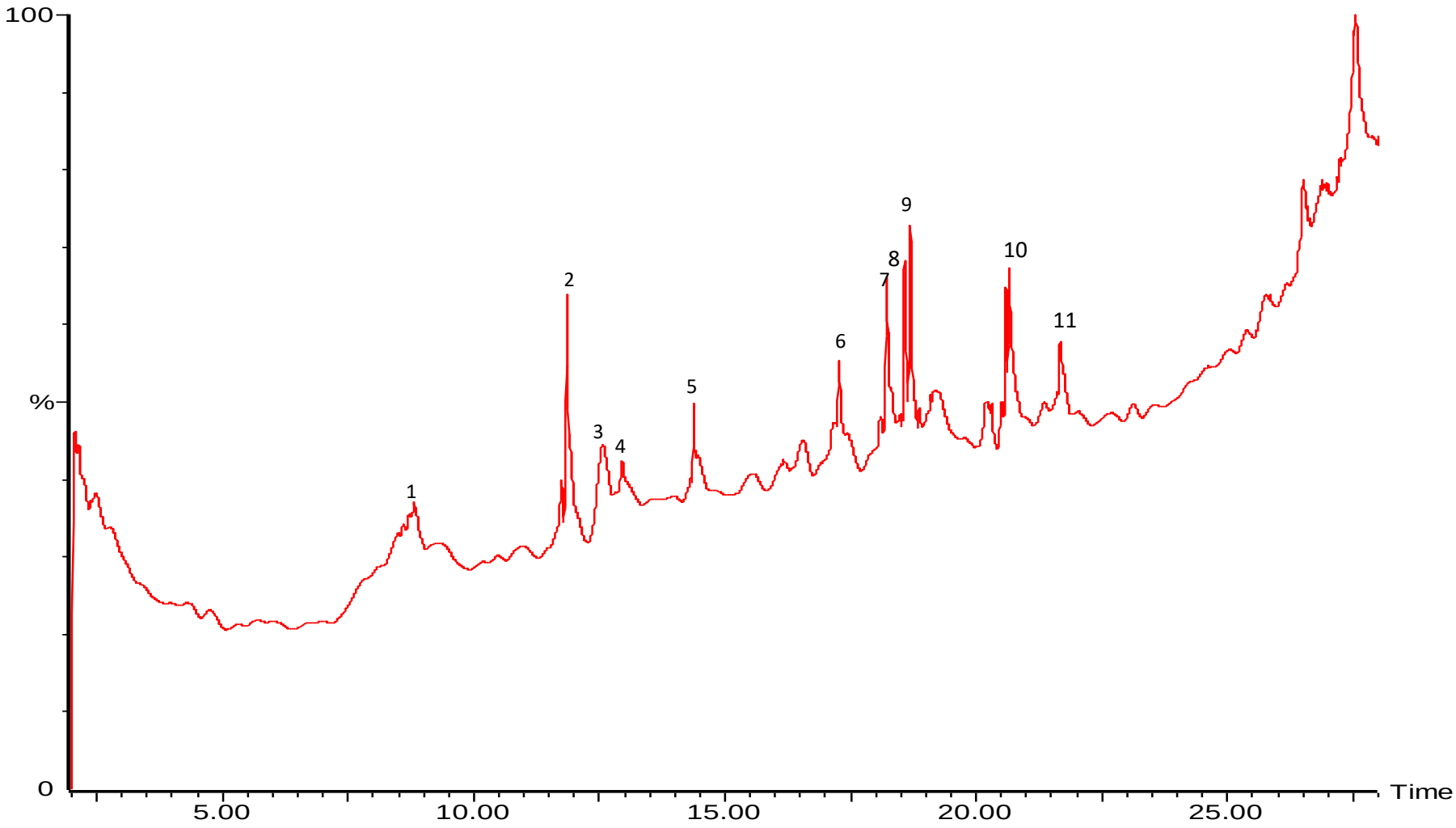


**Figure 12: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *S. arvensis* root**

**Table 10: Major compounds detected in *C. spinosa* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 2.42 | 55, 57, 60 | Rebitol | 152 | $C_5H_{12}O_5$ |
| 2 | 2.38 | 55, 57, 73 | Propanoic acid-methyl propyle ester | 130 | $C_7H_{14}O_2$ |
| 3 | 11.88 | 55, 73, 147 | Benzoic acid | 296 | $C_7H_6O_2$ |
| 4 | 12.95 | 55, 66, 73 | D-glucose, 4-O-alpha-D-glucopyranosyle | 342 | $C_{12}H_{22}O_{11}$ |
| 5 | 14.35 | 55. 75, 93 | Unknown | | |
| 6 | 17.27 | 60, 73, 85 | Myristic acid isobutyl ester | 284 | $C_{18}H_{36}O_2$ |
| 7 | 18.56 | 69, 74, 87 | Heptadecanoic acid (methyl ester) | 284 | $C_{18}H_{36}O_2$ |
| 8 | 18.70 | 57, 74, 147 | D-mannitol,1-thioheptyle-1-deoxy | 296 | $C_{13}H_{28}O3$ |
| 8 | 20.67 | 54, 67, 81 | 11-Hexadecynal | 236 | $C_{16}H_{28}O$ |
| 9 | 20.75 | 55, 67, 95 | 6,11-ecosadienoic acid methyl ester | 322 | $C_{21}H_{38}O_2$ |

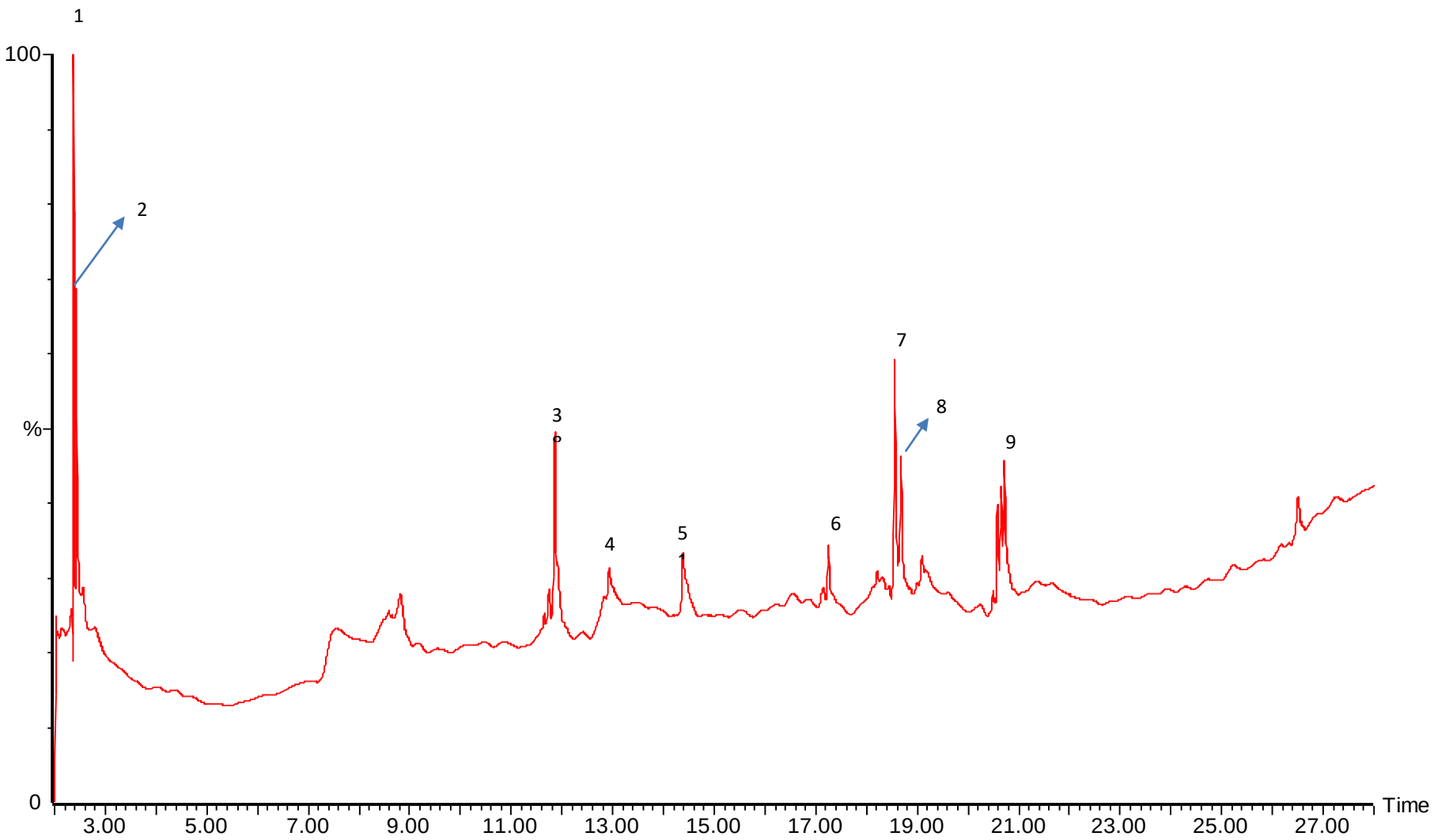


**Figure 13: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *C. spinosa* root.**

**Table 11: Major compounds detected in *U. dioica* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 6.85 | 55, 83, 95 | Bicyclo[3.1.1]heptan-2-one, 2,6,6-trimethyl | 138 | $C_9H_{14}O$ |
| 2 | 16.20 | 73, 84, 102 | Cyclohexyle isovalerate | 184 | $C_{11}H_{20}O_2$ |
| 3 | 18.22 | 56, 69, 81 | Homomenthyl salicylate | 262 | $C_9H_{22}O_3$ |
| 4 | 18.57 | 55, 74, 87 | Decanoic acid, 2-methyl | 186 | $C_{16}H_{22}O_2$ |
| 5 | 19.15 | 55, 69, 73 | Ascorbyl stearate | 442 | $C_{24}H_{42}O_7$ |
| 6 | 20.60 | 54, 67, 81 | Linoleic acid methyl ester | 294 | $C_{19}H_{34}O_2$ |
| 7 | 23.16 | 55, 69, 73 | Unknown | | |
| 8 | 24.52 | 55, 73, 77 | Unknown | | |
| 9 | 25.80 | 55, 69, 73 | Unknown | | |
| 10 | 26.53 | 55, 73, 207 | Unknown | | |
| 11 | 27.20 | 69, 73, 81 | Unknown | | |

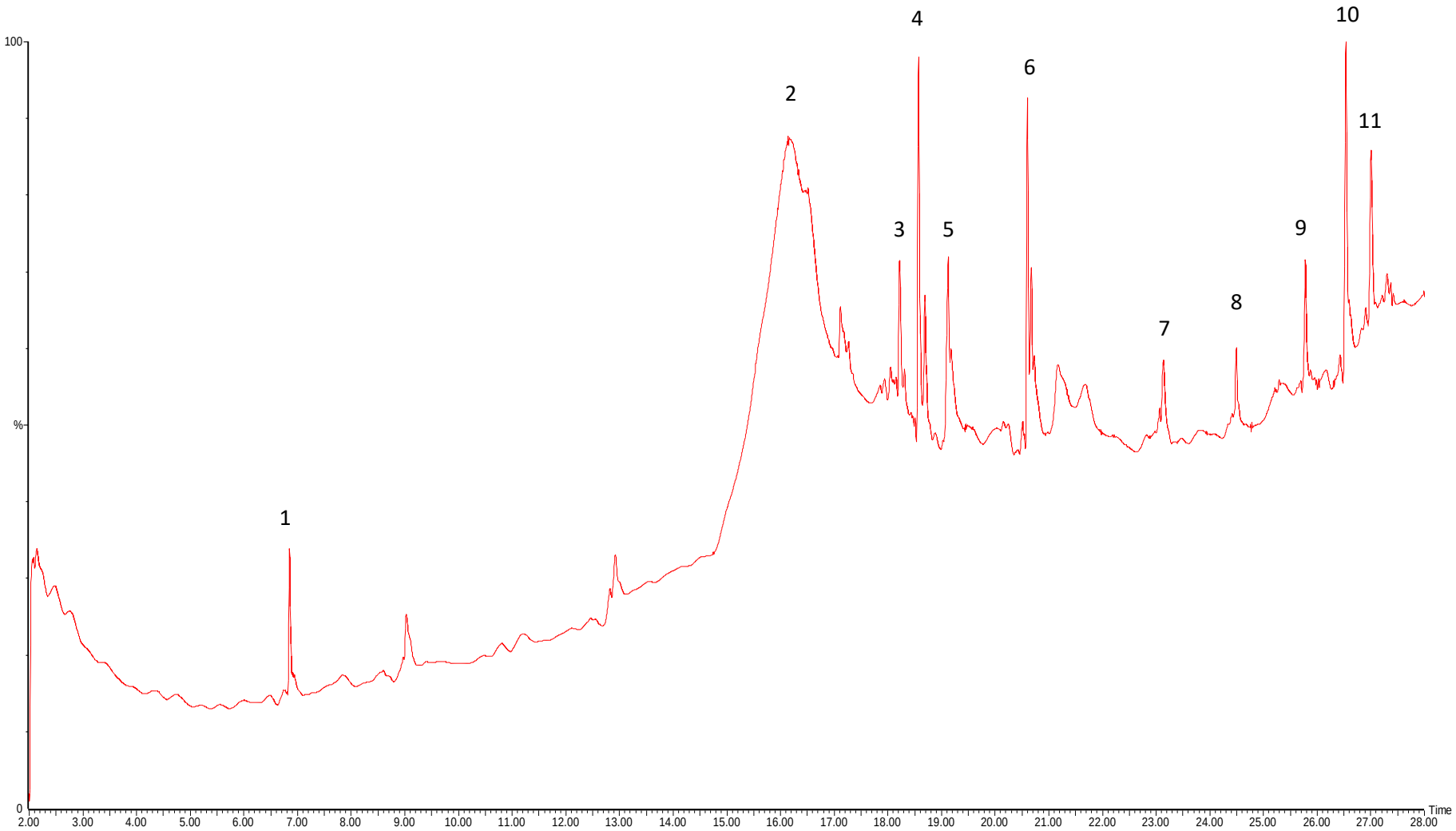


**Figure 14: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *U. dioica* root**

**Table 12: Major compounds detected in *E. alata* with their retention time (RT) and molecular weight (MW), Molecular masses (*M/Z*) and the molecular formula (MF)**

| Peak # | RT | *M/Z* | Compounds identification | MW | MF |
|---|---|---|---|---|---|
| 1 | 2.58 | 55 ,67, 97 | Furaldehyde | 96 | $C_5H4O_2$ |
| 2 | 11.88 | 69, 73, 97 | Benzoic acid | 296 | $C_7H_6O_2$ |
| 3 | 12.55 | 97, 119, 132 | Vitamin A aldehyde | 284 | $C_{20}H_{28}O$ |
| 4 | 12.97 | 97, 105, 126 | 2,4.4,6-Tetramethyle-6-phenyl-2-heptene | 230 | $C_{16}H_{27}$ |
| 5 | 14.88 | 55, 73, 97 | Unknown | | |
| 6 | 16.23 | 55, 71, 91 | Aromadendrene oxide-2 | 220 | $C_{27}H_{44}O3$ |
| 7 | 17.29 | 73, 85, 97 | D-glucose, 4-O-alpha-D-glucopyranosyle | 342 | $C_{12}H_{22}O_{11}$ |
| 8 | 18.26 | 55, 69, 82 | 5,7-Dimethyloctahydrocoumarin | 182 | $C_{11}H_{18}O_2$ |
| 9 | 18.60 | 74, 87, 97 | 2-pentanone,1,3-dimethoxy-2-methyl | 160 | $C_8H_{16}O_3$ |
| 10 | 18.73 | 57, 74, 87 | Heptacosanoic acid, 25-methyl-, methyl ester | 438 | $C_{29}H_{58}O_2$ |
| 11 | 20.69 | 55, 67, 79 | Cyclo hexen,1-hexyle | 160 | $C_{12}H_{22}$ |

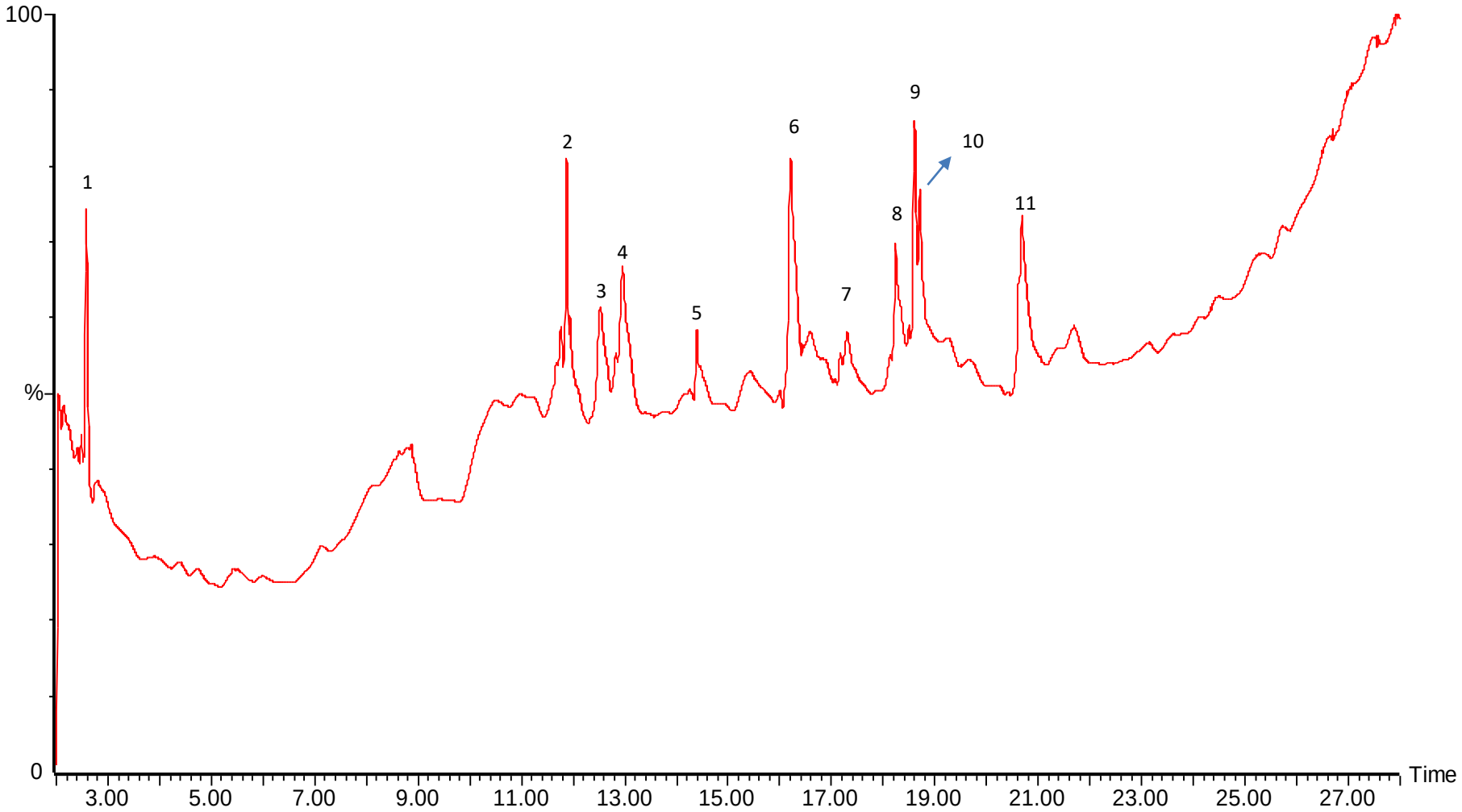


**Figure 15: Representative GC-MS chromatograms of the volatile compounds detected in the methanolic extract of *E. alata* root**

# Chapter 4: Discussion

**4.1 Discussion**

Overall, plants produce and synthesize structurally particular phytoconstituents that have active functional groups like: carboxyl, aldehyde, and hydroxyl, which are active on enzymes, cell receptors, and transporters. These functional groups could intervene in many cellular reactions that involved in the cure of many diseases, such as cancer (Mawalagedera et al., 2019).

The main goal of this thesis was to highlight and to cover the research gap about the plant roots, and to evaluate the antioxidant capacity by using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assay, assess the total phenol content, screen the phytochemical characteristics in the root samples, show the activity of root samples on some pathogenic bacterial strains, and to distinguish the main volatile compounds present in the root of six wild Palestinian plants by using GC-MS analysis.

The results indicated that the methanolic extract of all the root samples of six wild Palestinian plants had no effects on the different pathogenic bacterial strains Figures 7, 8. However, the root extract of *A. officinalis* and *E. alata* showed the highest antioxidant capacity as assessed by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging assays as shown in Figures 3, 4. Whereas, our results showed the presence of a notable phytochemical compounds present in the methanolic extract of the selected plant roots as shown in Table 6. Amongst all tested root extracts, *A. officinalis* showed the highest content of total phenol Figure 6. The methanolic extract of all the roots of six wild Palestinian plants displayed 'novel' chromatograms for volatile compounds Figures 10, 11, 12, 13, 14, and 15

## 4.2 Antioxidant activities in 'selected' root plants

The presence of the secondary compounds like: phenols, flavonoids, alkaloids, glycosides, etc. in the wild Palestinian plant roots are very important for modulating and do a balance in the byproduct chemicals needed for various biochemical processes and metabolism. Free radicals are the main active destructive byproduct which needed to be scavenged by antioxidants. These secondary compounds have vital roles as defense mechanisms against oxidative stress (S. Singh et al., 2016). The most common methods used to measure the antioxidant capacity in research are $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging assays, that is due to their high reproducibility and fast results (Bhandari, 2012). The methanolic extract of some WPPR samples showed a great scavenging capacity for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging assay, and some showed minimal results as represented in Figures 3, 4.

As shown in the literature, there is a strong relationship between antioxidant capacity and phenolic compound content in the plant (Kırca & Arslan, 2008; Mihaylova, et al., 2014). The antioxidant capacity relies on the total phenol content in the plants. The root extract of *A. officinalis* has been reported to contain a large number of phenolic compounds compared with the study that has a phenolic compound with high levels (Symes et al., 2018). Our results showed that the phenolic compounds content in the *A. officinalis* root exhibits a great antioxidant capacity using $DPPH^{\bullet}$ Scavenging assay Figure 3. In this study, the results support the correlation between antioxidant and phenolic compounds, *A. officinalis* show the highest level of total phenol content and antioxidant capacity, 69.6% scavenging capacity by $ABTS^{\bullet+}$ assay and 57.8 % scavenging capacity by $DPPH^{\bullet}$ assay and that matches with the results of the study that

investigate the root of *A. officinalis* using different solvents for extraction, and concluded with great antioxidant activities using 70% ethanol range 38.9-78.1% scavenging capacity by $DPPH^{\bullet}$ assay, and 36.6- 61.2% scavenging capacity by $ABTS^{\bullet+}$) (H. Zhang et al., 2018). (Tables 3, 4)

The genus Ephedra belongs to the plant family Ephedraceae which includes 67 species, has a long medical history back to traditional Chinese medicine which is used to treat many diseases, including allergies, headaches, asthma, and coughs. This is due to the presence of the active compound ephedrine, (Khanal et al., 2022). *E. alata* root extract also showed an interesting antioxidant capacity, 69.5% scavenging capacity using $DPPH^{\bullet}$ free radical scavenging assay, and 66% scavenging capacity by $ABTS^{\bullet+}$ free radical scavenging assay, (Tables 3,4). Previous reports on the root extract of *E. sinica* species (M. Lv et al., 2022; M. Y. Lv et al., 2016) In vivo using ethyl acetate root extract (ERE) on dextran-sulfate-sodium-induced ulcerative colitis in mice. These results concluded that ERE has a strong activity as anti-inflammatory which reducing the oxidative stress induced by TNF-α and IL-6. In vitro the ERE extract of *E. sinica* has been reported to have wonderful antioxidant activities determined using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging assays (M. Lv et al., 2022). Proanthocyanidine is a group of a strongly active compounds natural occurring in the plants called polyphenols which act as a potent antioxidant and anti-inflammatory secondary compounds, like flavonoids and tannins. The ethyl acetate root extract of *E. sinica* exhibited a valuable content of Proanthocyanidine, as has been reported by Lv, Wang et al. (M. Lv et al., 2022). So, these results indicate and confirm the antioxidant activity of the Ephedra species roots.

The total phenol content in plants is very important for antioxidant activities due to redox properties. Phenolic compounds are benzene rings with single or more hydroxyl groups, which include flavonoids (simple or complex), phenolic acids, and anthocyanins, which play many vital roles in neutralizing or inhibiting free radicals (Aryal et al., 2019). The content of phenolic compounds in methanolic root extracts ranged from 362.5 to 167.5 mg of GAE/g Figure 6. *A. officinalis* represent the highest value 362.5 mg of GAE/g, while the smallest value was found in *E. alata* 167.5 mg of GAE/g (Table 5). There is consistent evidence in study showed that flavonoids have antioxidant activity using different solvent in the roots of 7 species of Asparagus (Kapoor et al., 2019). The findings that emerged from this thesis have enhanced our understanding about the WPPR consist of these antioxidant compounds like flavonoids.

### 4.3 Phytochemical screening

Phytochemicals are compounds synthesized naturally in plants that are primarily found in fruits, vegetables, legumes, beans, nuts, plant roots, and whole grains. Phytochemicals include numerous compounds like phytosterols, saponins, flavonoids, terpenes, and others that are responsible for the health benefits of these plant-based foods and beverages (Brindha, 2016). Phytochemicals in plants are known to mediate many pharmacological actions like antioxidant, anti-inflammatory, antibacterial, and antiviral activities. This is the reason why the plants entered many pharmaceutical industries, as they were used as an alternative or complementary medicine to treat many diseases (Kumar et al., 2023)

Some phytochemicals like carotenoids, which are pigments with yellow, red, orange, and purple color, include a large group of very strong antioxidant compounds, more than 50 different types of carotenes exist in nature most of them found in plant parts, amongst α-carotene, β-carotene, lycopene, lutein, peridinin, and others. Thus, phytochemicals are a term that involved a big class and groups of medical bioactive compounds that could reduce or prevent the incidence of chronic diseases (Y.-J. Zhang et al., 2015). For example; Resveratrol is a bioactive phytochemical compound notified as a polyphenolic compound found in grapes, peanuts, and berries, it is approved in many studies that is resveratrol responsible for decreasing platelet aggregation, acts as an anti-inflammatory, and reduces oxidative stress by inhibiting the production of reactive oxygen species (ROS) (Pagliaro, Santolamazza et al., 2015).

In this study, all cardiac glycosides, flavonoids, glycosides, saponins, quinones, steroids, and terpenoids are phytochemicals found in some of 'selected' WPPR (Table 6). The phytochemical profile of the methanolic root extract of *A. officinalis* and *E. alate* was almost the same with expected quinones, which was not the same as in *A. officinalis.* Saponins are observed in all 'selected' root extracts but manifested clearly and uniquely in the methanolic extract of *S. arvensis* and *U. diocia.* The methanolic root extract of *C. spinosa* showed some flavonoids and steroids (Table 6).

Saponins – naturally occurring secondary compounds – either steroidal and/or triterpenoid glycosides are used extensively as surfactants in cosmetics and drugs industry. Saponins have been reported to occur in all plant parts but primary in the plant roots more than the aerial parts, Teng et al., (2009) investigated the variance content of saponins in different parts with different

ages in *Polygala tenuifolia* (Polygalaceae) and found that the roots have the highest content of saponins (Teng et a., 2009). Saponins are known for their antimicrobial activity and Phytoprotectants which mostly secreted in the outer layers of the root tissues especially in the epidermis and phloem to prevent the microbial attack that feeds on the phloem sap such as insects and nematodes. Furthermore, β-amyrin is a triterpene saponin derivative regarding plant development and root nodulation (Ahmad & Geelen, 2013). These results are in consistent with our finding in this thesis. As all roots examined showed the presence of saponins in the respective methanolic extract (Table 6).

The methanolic root extract of *U. diocia L*. has been investigated using column chromatogram (CC), thin layer chromatogram (TLC), and NMR data to isolate two types of triterpenes namely ursolic acid and 7, 24 dihydroxy ursolic acid (Tole, 2023). These compounds have been reported to have biological activities as anti-inflammatory, anticancer, antiviral, and antibacterial activities (Mlala et al., 2019). In agreement with the previous study, the ethanolic (80%) root extract of *A. officinalis* also reported to have two biologically active steroidal saponins (sarsasapogenin M and sarsasapogenin N) (X. Huang & Kong, 2006). These saponins compounds and their analogues have received a lot of attention due to its pharmacological and biological activities (Mustafa et al., 2022). These compounds have demonstrated a pronounced as anti-inflammatory activity in adipose tissue, anticancer agent, aids in AD complications improvement, and a protective agent used in end-stage renal disease as a result of hyperglycemia for a long time (Mustafa et al., 2022). Given all above evidences implicating saponins in roots as a biologically active metabolites, all ‘selected’ roots in this thesis confirmed the incidence of saponins in the roots of wild Palestinian plants. Accordingly, it is likely despite that the

‘selected’ roots in this thesis did not show antibacterial activity under our experimental conditions, may have some other pharmacological activities and yet to be explored.

Plant roots are considered a rich source of novel phytochemical compounds contributing in the management of type 2 diabetes (T2D) (Ardalani et al., 2021). For example, *Morinda citrifolia* (noni, Rubiaceae) roots has been reported to contain a new anthraquinone compound found in the chloroform root extract. The 2-rthoxy-1-hydroxyanthraquinone has been elucidated by (NMR), mass spectrometry (MS), and infrared (IR), and then isolated and purified using hexane-chloroform or petroleum ether-chloroform (Ee, Wen, Sukari, Go, & Lee, 2009). Zhao et al., (2017) also successfully isolated a wide range of bioactive compounds from the crude extract and fraction of the root *Polygonum cuspidatum* (Asian knotweed, Polygonaceae) which have a strong antidiabetic agent as α-glucosidase and protein-tyrosine phosphatase 1B (PTP1B) inhibitor, these compounds are procyanidin B2 3,3"-O-di-gallate, (-)-epicatechin gallate, stilbene analogues, trans)-emodin-physcion bianthrone, and (cis)-emodin-physcion bianthrone (Y. Zhao et al., 2017). Although this study has profiled the general groups of phytochemicals in ‘selected’ wild Palestinian plant roots. The pharmacological activity of the isolated compounds and their identity in these ‘selected’ roots remain to be tested and elucidated using appropriate protocols and advanced NMR techniques.

## 4.4 Antibacterial activity

The curing of diseases caused by microbes using medicinal plant (Phytotherapy) is a general practice worldwide. Medicinal plants have a lot of attention from developed and developing countries because they are a very wealthy sources of bioactive compounds which have antibacterial activities, safe, and effective remedies for different ailments (Shinwari et al., 2015). The excessive use of the antibiotic versus infections led to developing of multidrug-resistance bacteria (MDR). Therefore, the people toward to investigate the plants as another alternative medicine with therapeutic properties and little or no side effect (Zhai et al., 2023).

The antibacterial compounds isolated from plant parts usually dependent on the type of extraction and the solvent used (Khan et al., 2017). In this study, the results revealed that the methanolic extract of six WPPR have no effect on selected bacterial strains Figures 7, 8. However, the negative results do not specify the absence of bioactive compounds, nor that the plant is not active. Active compound/s may exist in insufficient amounts in the crude extracts to show antimicrobial activity with the dose levels used. Absence of activity can therefore only be confirmed by using large doses. Moreover, the antibacterial activity in the plant root affected by many factors including the type of solvent extraction used, crude extract concentration, and the nature of the environmental soil. This is confirmed by the study involved the evaluation of the effect of variable solvent (aqueous and alcohol) extract of *A. officinalis* root with different concentration (50, 100, 150 $mg.ml^{-1}$) on the inhibition of some strains of bacteria. The study furnish to that alcoholic extract with increasing extract concentration has more antibacterial activity than aqueous extract against *E. coli* and *S. aureus*. Whereas the aqueous extract has more

inhibitory effect against *P. aeruginosa* in all concentrations than alcoholic extract (Abbas & Al-Subaihawi, 2022). Accordingly, although we did not observe antibacterial activity in all 'selected' methanolic root extracts, from this point, we need further investigations that cover the research shortage regarding the plant roots, including the other factors that could affect the activities of the root extract as antibacterial, amongst using other different techniques like nanotechnology with different solvents and sample concentrations.

### 4.5 Volatile compounds in selected WPPR

Different methods have been used to analyze and identify different groups of compounds. GC-MS analysis method is a critical way to analyze compounds that have very low concentration without molecule destructive (Wang et al., 2018). Plant volatile compounds (VCs) are carbon metabolites ($C_6$, $C_{18}$) released by the plant in the spheres for multiple purposes for the plant itself or other parties. All plant parts release volatile compounds, the aerial parts emit VCs in the phyllosphere to warn from danger, defend from biotic and abiotic stress, signal, and attract pollinators (Baldwin, 2010). However, why roots emit volatile compounds in the rhizosphere?

Root exudate is a term called on any compounds or molecules that released by the root in the rhizosphere and may include amino acids, sugars, proteins, plant hormones, organic compounds, aromatic volatile compounds and others with low or high concentration in the soil (Dundek et al., 2011). Plant roots in their underground ecosystem emit volatile compounds for defense against pathogenic microbes and some insects. These compounds are also released to attract pests that can attack and feed on other harmful parasitical pests like pathogenic nematodes (Baetz & Martinoia, 2014). On the other hand, the compound could be toxic to other pathogenic

microorganisms, and so improve the health and maintenance of plants. The signaling within or with other neighbor plants, or plant language is an additional role to the root exudates but it is non-volatile compound, some of the aims of these compounds are to change the availability of the nutrients, microbial populations, and soil chemistry (Delory et al., 2016).

On the other hand, VCs have been reported to be emitted from all plant parts but in different amounts, because they consider the plant language. The emitted of VCs by the aerial parts is a results of many factors including photosynthesis and plant metabolism, whereas, the crucial understanding pathway of the VCs emissions is still unexplored (Vlot & Rosenkranz, 2022). One of the most recent explanations are that both of the above and below-ground parts are associated and dependent upon each other in VCs emissions, For example, the Arabidopsis thaliana leaves released a VCs for defense against pathogenic *Botrytis cinerea* fungi as a result of the high nitric oxide NO concentration released by the roots in the rhizophere motivated by *Trichoderma* fungi (Pescador et al., 2022).

This study is the first to provide evidence that roots of Palestinian wild plants express volatile compounds. The main volatile compounds detected in *A. officinalis*, *M. sylvestris*, *S. arvensis*, *C. spinosa*, *U. dioica*, *E. alata* were *D*-glucose, 4-O-alpha-d-glucopyranosyle, naphthalene-decahydro, vitamin A aldehyde, 11-hexadecynal, 1-heptatriacotanol, Rebitol, Aromadendrene oxide-2, and benzoic acid. (Tables 7,8,9,10, 11, and 12)

Irrespective of the number of volatile compounds detected in 'selected' methanolic root extracts, most of these compounds have been reported to have biological activity. For example,

Naphthalene-decahydro has been reported to use as insecticide (Vadivel & Gopalakrishnan, 2011)

Ribitol with another name adonitol (pentose alcohol) is a natural plant-derived compound formed by the reduction of the carbonyl group in ribose by the metabolism of some plant species like *Adonis vernalis* (Ranunculaceae) or by the fermentation of D-glucose found in the roots by some bacteria species to produce Ribitol (Okano, 2009). Ribitol is detected in the methanolic root extract of *C. spinosa* which acts as an antiviral medication in combination with pegylated-interferon (Ribavirin) for the management of hepatitis C by suppressing the multiplication of the RNA virus or by stimulating the host cells to secrete endogenous interferon (Sandokji et al., 2003).

The 1-Heptatriacotanol was detected in the methanolic root extract of *S. arvensis* and *A. officinalis* which is an alcoholic compound found naturally in plant parts that have been reported to possess an antimicrobial, anticancer, anti-inflammatory (Kalaimagal, 2019), and most important as antihypercholesterolemic effect by preventing the accumulation of lipids and inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzme A (HMG-CoA) reductase which is responsible for the activation of the pathway to produce cholesterol (Baskaran et al., 2015). The study investigated the methanolic (50%) leaf extract of *Basella alba* (Basellaceae) using GC-MS and HPLC analysis which revealed the presence number of hypocholesterolemic effect amongst 1-heptatriacotanol found in high percentage. Then the methanolic extract of *B. alba* was screened using an HMG-CoA reductase assay kit (Enzyme assay) (Baskaran et al., 2015)

Aromadendrene oxide-2 (AO-2) is a sesquiterpene of essential oil secondary compound that excretes naturally in many plant roots such as *Valeriana officinalis* and *Panax ginseng* (Ricigliano et al., 2016) (Qiu et al., 2008). In the present study, AO-2 was detected in the methanolic extract of *E. alata* roots, Aromadendrene oxide-2 has been reported to have anticancer, antifungal, antiviral, and antimicrobial activities (Njoku & ogugofor, 2019). Aromadendrene oxide-2 is an anticancer compound that could be used in chemotherapy or as a complementary treatment in skin carcinoma cases, Pavithra and Verma (2018) conducted a study to evaluate the cytotoxicity of AO-2 using human cells with epidermal skin cancer and HaCAT - human epidermal keratinocyte -cell line and furnish that AO-2 induce apoptosis in the epidermal cells cancer, prevent the multiplication of the cancer cells, increase ROS signaling, and lack of the mitochondria functions (Pavithra et al., 2018)

The bioactive compounds might be more concentrated in older plants than young and mature plants, (Cui., et al (2015), The bioactive compounds, and aroma profiles specifically in *Panax ginseng* (Araliaceae) roots were evaluated with different ages using GC-MS method and concluded that ginsenoside which is responsible for anticancer, anti-stress, and antioxidant have been increased with age (Cui et al., 2015). Furthermore, different VCs in different plant parts within the same plant species evidenced by, the present study revealed that the methanolic root extract of *M. sylvestris* contains 7 different VCs by GC-MS analysis, while, Dowek et al, (2020) showed the presence of at least 16 VCs in the methanolic leaf extract of *M. sylvestris* (Dowek et al., 2020).

*Panax ginseng, Curcuma longa L., Zingiber officinala* and many more are examples of starchy rhizome roots, which are very rich with bioactive compounds. These compounds have a potential place in pharmaceutical manufacturing industry (Priyanka et al., 2019). Thus, the underground part of the plant represented by the roots of all kinds needs additional insights and exploring.

# Chapter 5: Conclusion

## 5.1 Conclusion

This study was the first evidence confirmed that wild Palestinian plant roots namely *A. officinalis*, *M. sylvestris, S. arvensis, C. spinosa, U. dioica, E. alata* to be investigated for the presence of various bioactive compounds and their antioxidant, antibacterial, and phytochemical analysis. Based on the results obtained from our laboratories, this research revealed the following valuable points:

1. The methanolic extract of the selected WPPR displayed antioxidant activity due to high content of the bioactive compounds like: saponins, quinones and flavonoids present in these selected plants, these compounds may exhibit anticancer, antiviral, antioxidant, antibacterial, and anti-inflammatory activity.
2. Phytochemical screening of the selected WPPR expressed the presence of various plant secondary metabolites like: cardiac glycosides, flavonoids, glycosides, saponins, quinones, steroids, and terpenoids detected by different phytochemical screening tests and GC-MS analysis like *D*-glucose, 4-O-alpha-d-glucopyranosyle, naphthalene-decahydro, vitamin A aldehyde, 11-hexadecynal, 1-heptatriacotanol, Rebitol, Aromadendrene oxide-2, and benzoic acid.

3. This study verified the existence of bioactive compounds in the methanolic root extract of selected WPPR that have a promising use in pharmaceutical industries, like rebitol, Aromadendrene oxide-2, and 1-heptatriacotanol.

4. *Asparagus officinalis* and *Ephedra alata* may have an interesting value in the field of research that needs further investigation due to the presence of high antioxidant capacity and total phenol content.

5. The methanolic extract of selected WPPR had no antibacterial activity against both gram-negative and gram-positive bacterial strains

## 5.2 Recommendations

The following are some suggestions that may be taken into consideration for future investigations:

1. The current research used the solvent methanol 80% to extract the dried roots of selected WPP, further investigations are needed using different solvents to extract different stages of the plants.
2. It is recommended to expand reported tests to include antiviral, antifungal, and anticancer.
3. In the antibacterial test, it is recommended to use different solvents, other techniques like nanotechnological techniques, and concentrate the root extracts of selected WPPR.
4. 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).
5. Further studies are needed to investigate the saponins group and its pharmacological activities in roots of WPP.

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