An-Najah National University
Faculty of Graduate Studies

Characterization of Polyphenol-Containing Polar Extracts from *Stachys Palaestina* and *Stachys Viticina* and Evaluation of Their Pharmacological Properties

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This Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Chemistry, Faculty of Graduate Studies, at An-Najah National University, Nablus - Palestine.
2021

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Dedication

To my parents

To my sisters and brothers

To my friends

I dedicate this work

Acknowledgments

Praise to almighty Allah who has enabled me to finish this work. I would like to express my gratitude to my supervisors, Dr. Nawaf Al-Maharik and Dr. Nidal Jaradat, who always supported me with their knowledge and experiences. I would also like to thank Dr. Mohammed Al-Qadi, Ms. Fatima Hussen and Mr. Nafith Dweikat.

Specially thanks for my parents, sisters, brothers, and my friends who supported me during my study.

Finally, thanks to chemistry department at my University.

الإقرار

انا الموقعة ادناه مقدمة الرسالة التي تحمل العنوان:

Characterization of Polyphenol-Containing Polar Extracts from *Stachys Palaestina* and *Stachys Viticina* and Evaluation of Their Pharmacological Properties

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree of qualification.

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Date: **التاريخ:24/05/2021**

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Characterisation of Polyphenol-Containing Polar Extracts from *Stachys Palaestina* and *Stachys Viticina* and Evaluation of Their Pharmacological Properties

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Abstract

The aromatic plant *Stachys viticina* (Lamiaceae) is a perennial herb growing in the Mediterranean countries including Palestine. And Like other Stachys species, it's also used in folk therapy from ancient time. In view of this, the current work designed to isolate and characterize the chemical constituents and to assess the in-vitro, antioxidant, antimicrobial properties of the polar poly phenolic composition of *Stachys viticina*.

Methanol extract of *Stachys viticina* was subjected to a sequence of silica gel column chromatography using different eluents with various polarities. The purity of the isolated fractions was conducted by thin layer chromatography (TLC) and confirmed using high performance liquid chromatography (HPLC). Moreover, functional groups of the pure fractions were detected using infrared spectroscopy. In addition, the radical scavenging capacity of plant methanolic extracts was assessed by the DPPH assay, and antimicrobial properties against seven microbial strains using the microdilution method were also screened.

Seven extracts of *Stachys viticina* were separated (A-F and R). All of the extracts (A-D, F, and R) exhibited antioxidant activity with IC_{50}values of

85.88 µg/mL, 54.37 µg/mL, 77.58 µg/mL, 68.36 µg/mL, 58.62 µg/mL, and 18.58 µg/mL, respectively. Fraction C had the highest antibacterial activity at a minimum concentration (39 µg/mL) against methicillin-resistant staphylococcus aureus (MRSA) and *Staphylococcus aureus*. But all of the extracts had no activity against *candida albicans* fungi strain.

Chapter One

Introduction

1.1 Plants in Traditional Medicine

From ancient time plants and herbs have been an essential provenance for treatment of various diseases ranging from minor illness to the more severe ones like malaria, cancer, tuberculosis, and even HIV/AIDS [1].

The Sumerians were the first civilization to use medicinal plants for drug preparation over 5000 years ago. They used more than 250 different plants, such as poppy, mandrake, and henbane. The *Ebers Papyrus* written by the Ancient Egyptians from around 1500 BCE listed over 800 herbal medicines, many of these herbs are still used nowadays [2].

Traditional medicine system is deeply rooted in societal cultures that formed by people experiences and passed from generation to generation, for instance Chinese, Arabic and African folk medicine [1].

Seeds, flowers, leaves, fruits, roots, rhizomes, and oils are plant parts that are used for folk therapy in the form of powders, pills, creams, pastes, suppositories, and ointments, or sometimes combination of these [3].

Recently, the request for herbal products is raising all over the world including herbal medicines, herbal pharmaceuticals, herbal health products, herbal cosmetics, nutraceuticals, and food supplements, etc. The main reason for this trend is that herbal products exhibit less side effects, and are

available at reasonable prices [4]. The World Health Organization (WHO) Statistics stated that ” approximately 80% of the world inhabitants reckon on folk medicine for their primary health care” [5].

1.2 Use of Plants in Drug Development

Initial used of medicinal plants taken a form of crude drug as herbal mixtures, teas, poultices, powders and tinctures [6]. The isolation of morphine **1,** used as an analgesic, in1806 as a pure natural product from the opium poppy *Papaver somniferum* was the introductory for drug development from plants [7]. Since then several pure phytochemicals have been separated and used as drugs or analogues for the development of drugs. At the beginning of the 21st century, it was estimated that 11% of the 252 crucial drugs were of plant origin [8].

Several examples of essential drugs in use today derived from plant are shown in Figure (1.1). Camptothecin **2** isolated from *Camptotheca acuminata* tree growing in China is used as antitumor agent [9], and Galantamine **3** (trade name Reminyl®) isolated from *Galanthus woronowii Losinsk.* (Amaryllidaceae) is currently used for the treatment of Alzheimer’s disease. The antimalarial drug Arteether **4** (trade name Artemotil®) was isolated from *Artemisia annua* L. (Asteraceae) [10].

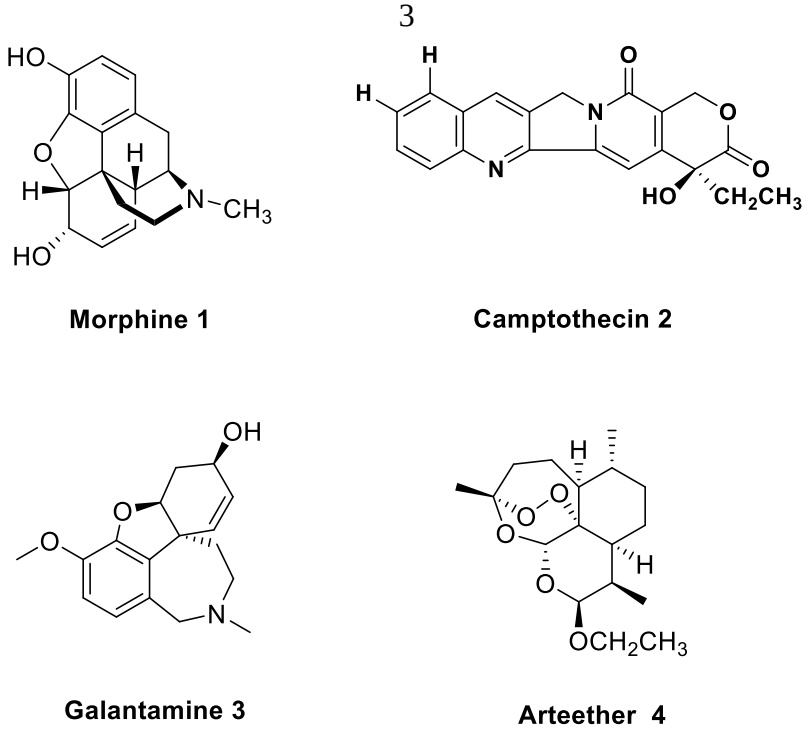


Figure (1.1): Examples of pharmaceutical drugs of plant origin [7, 9, 10].

Despite drug development process from plants tends to be a long, boring and expensive, its value exceeds other routes because plants have an immense potential. With an estimated 250000 plant species dispersed across the world, where each plant can produce up to thousands of structurally different phytochemicals, the ability of plants as provenance of novel compounds cannot be matched. The rapid development in chromatographic and spectroscopic procedures employed in the isolation and identification of phytochemicals further advances their pharmaceutical potential [11].

1.3 A Palestinian Perspective

Palestine with its special geographical location between three continents constitute of a wilderness in the south, a lot of mountains in the middle and the north in addition to the continental rift valley. Furthermore, it is

positioned at the coast of the Mediterranean. This geographical variation leads to the variety of soil and climate conditions and this in turns leads to biodiversity [12]. Therefore, Palestine is renowned for its high abundance of medicinal plants that are used since a long period of time [13]. However, the testing of pharmacologically active compounds in flora started in the late sixties [14].

More than 2600 plant species from different families vegetate across the Palestinian mountains, valleys and desert, of which more than 700 are eminent for their usages as medicinal herbs or as botanical pesticides [15].

1.4 Lamiaceae Family

The Lamiaceae family (Labiatae) is an important medicinal plant family with a cosmopolitan distribution. It contains approximately 236 genera and more than 6000 species, of which *Salvia* is the largest genus with 900 species followed by *Scutellaria*, *Stachys*, *Plectranthus*, *Hyptis*, *Teucrium*, *Vitex*, *Thymus,* and *Nepeta* [16]. Most of these species are aromatic and have essential oils that are valuable in different field, such as cosmetic, fragrance, flavouring, pesticide, and pharmaceutical industries [17, 18]. This family includes several plants that are widely find applications in traditional medicine as a remedy for a wide range of disease [17, 19].

1.4.1 *Stachys Viticina*

Stachys L. (Lamiaceae, Lamioideae) is a large genus of herbs and shrubs that embraces approximately 450 species disseminated in the warm temperate and tropical regions worldwide such as the Mediterranean and

Southwest Asia [20]. The common names of *Stachys* species are heal-all, self-heal, woundwort, betony, lamb's ears, and in numerous native areas of the world as 'mountain tea'. They are employed in folk medicine for medication of genital tumors, inflammatory diseases, sclerosis of the spleen, fevers, cough and ulcers, sore mouth and throat, internal bleeding and weaknesses of the liver and heart, and diarrhea [21, 22].

Recently, examinations of the different extracts and constituents of *Stachys* species displayed different pharmacological activities, including among others anti-anxiety [23, 24], antibacterial [25, 26], anti-inflammatory [27], anticancer [28], and antioxidant activities [26, 29]. They are used as antiaging and for curing other diseases related to radical scavenging mechanisms [30].

From reported works, the key classes of plant secondary metabolites which have been recognized from *Stachys* species are flavonoids, phenolic acids, iridoids and fatty acids [31].

Only 13 species of *Stachys* genus are growing in Palestine [21], of which *Stachys viticina* has been rather barely studied. Gören *et al*. reported the GC-MS analyses of the fatty acid composition of oil of *S. viticina* seeds in addition to 22 other *Stachys* species [32]. They found that linoleic acid (47.8%) and 6-octadecynoic acid (7.6%) are major acids isolated from *S. viticina* [32]. *N. Jaradat* and *N. Al-Maharik* reported the identification of fifty two compounds of *S. viticina* essential oil using microwave-ultrasonic and GC-MS techniques, of which endo-borneol **5** was the major

component, followed by eucalyptol **6** and epizonarene **7** [33] (figure 1.2). To the best of our knowledge there are no published reports neither on the polyphenolic composition of *Stachys viticina* nor on its biological properties till now.

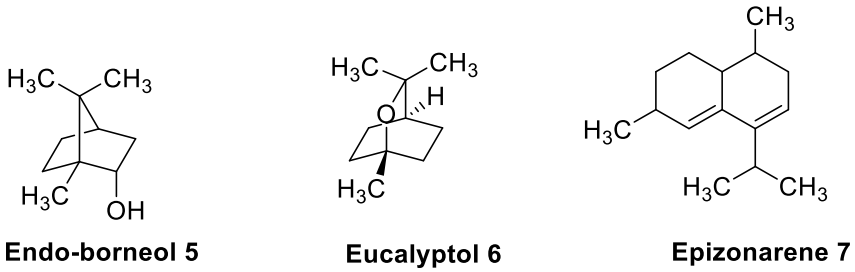


Figure (1.2): Major compounds of *Stachys viticina* essential oil [34-36].

Stachys viticina

1.5 Plant Natural Products

Plants are continuously synthesizing natural products often called phytochemicals. These compounds categorized into two broad groups based on their functions in plants [1]. Firstly, there are primary metabolites, they occur in all plants in sufficient amounts since they performing

essential metabolic roles in a plant, and they are directly engaged in plant growth and development [37]. Examples on primary metabolites include carbohydrates, amino acids, lipids and nucleotides [38]. Then there are secondary metabolites, which haven't direct primary role in the growth and development of a plant, but they are crucial for the plants' survival. Plants often synthesize secondary metabolites in response to attack by insects, microorganisms, herbivores and to suppress the growth of neighboring competitor plants. Some secondary metabolites are also produced in the form of aromas, flavors and colors to attract pollinators and seed dispersers [39].

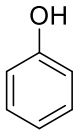
Secondary metabolites, which are the major active ingredients of medicinal plants, attract special interest due to their biological activity on other organisms especially animal cells [40]. Thus natural product chemistry field focuses mainly on isolation, structural elucidation, biological properties and preparation of secondary metabolites.

1.5.1 Classes of Secondary Metabolites

Classification of secondary metabolites into distinct groups is complicated, due to the wide structural diversity of them. Several classification approaches are used, of which the most use is the one based on the biosynthetic pathway of the compounds. Under this approach three major groups were isolated and identified: namely phenols, terpenoids, and alkaloids [41].

1.5.1.1 Phenols

Characterization of this group is referred to the occurrence of one or more hydroxy (OH) groups joined to an aromatic ring [42]. Phenol can be assumed to be the parent compound as shown in Figure (1.3). The structures of phenolic compounds exist either as simple skeleton with one aromatic ring or as sophisticated polymers (polyphenols) having various functional groups attached. Major groups of phenols include the flavonoids, phenolic acids and tannins. This classification is established based on the number of carbon atoms in the elementary skeleton. Phenolic compounds accumulate in plants and related with flavor and color characteristics of fruits and vegetables [43-45].



phenol 8

Figure (1.3): Phenol, parent compound of all phenolic compounds [44].

A) Flavonoids

Flavonoids are the prevalent and highest diversified group of polyphenolic compounds. Their general skeleton structure is formed of two benzene rings (A and B) joined through a three-carbon bridge oxygenated heterocycle (C), which form the C_6-C_3-C_6 structural backbone [45–48] (Figure (1.4)). Their main functions are protection against ultraviolet (UV) radiation and serving as signals to attract pollinators and seed dispersers [49].

Flavonoids have attracted special attention due to their pharmacological properties, which include antibacterial, anti-inflammatory, antimicrobial, estrogenic, anti-oxidant, cytotoxic and antitumor activity [50-53].

Classification of flavonoids is based on the differences in their chemical structure (oxidation or saturation of the intermediate C ring) [46], accordingly flavonoids can be categorized into six subclasses: flavanones **9**, flavonols **10**, flavones **11**, anthocyanins **12**, flavanols **13**, and isoflavonoides **14**, and each group has particular characteristics [53].

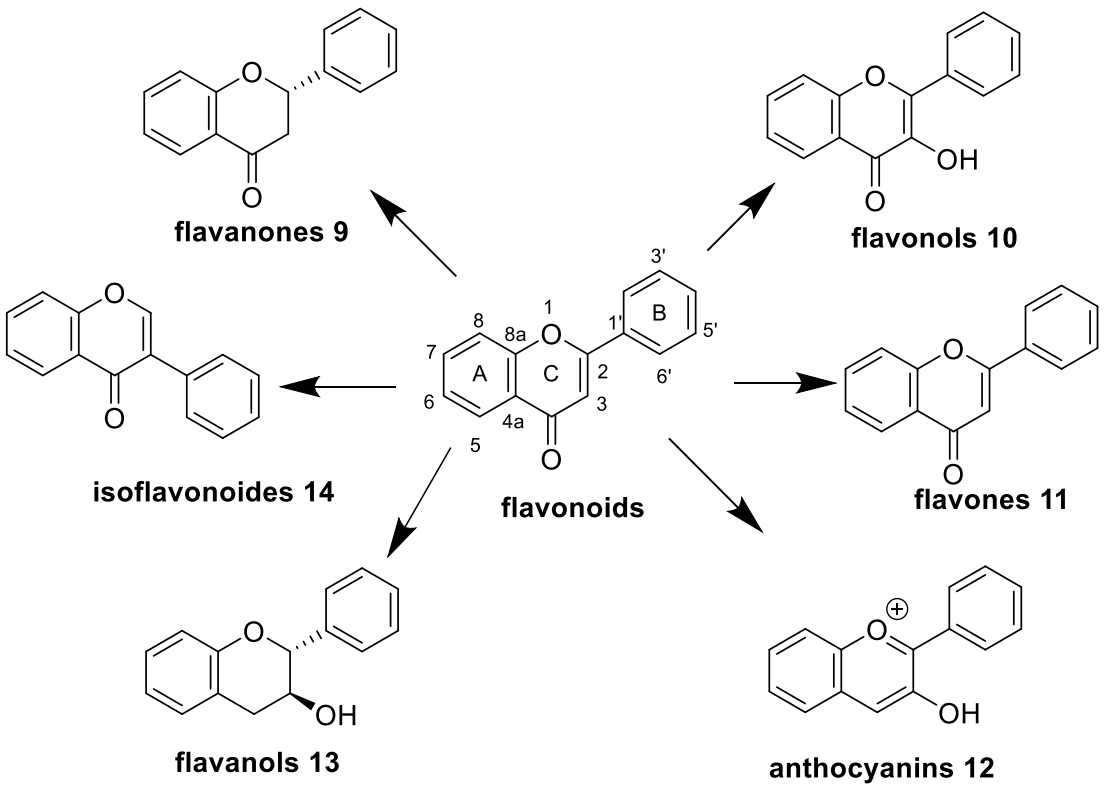


Figure (1.4): Elementary chemical structures of the flavonoids classes [53].

Flavanones 9

This type is found in aromatic plant (such as mint), citrus (especially grapefruit), and tomatoes [45]. Figure (1.5) represents the most abundant simple flavanones (naringenin **15**, eriodictyol **16**, hesperetin **17**). Flavanones were found to display a wide range of biological activities including among others radical scavenging, anticancer, anti-inflammatory, and antiviral activity [54].

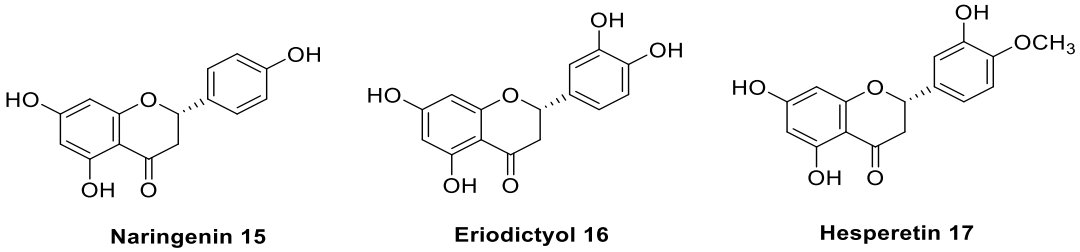


Figure (1.5): Structure of some flavanones [54].

Flavonols 10

Flavonols are the most abundant flavonoids' class in the plant kingdom [55]. Kaempferol **18,** quercetin **19**, and myricetin **20** (Figure (1.6)) are of the most important compounds that represent this group [56]. Flavonols possess antioxidant activity that may protect against oxidative damage to cells, lipids or DNA. Additionally, they have anti-inflammatory and neuroprotective properties [57].

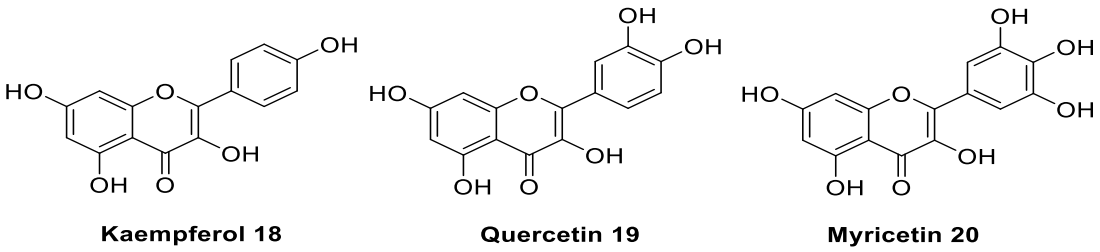


Figure (1.6) : Examples of some flavonols [57].

Flavones 11

Flavones exist in all major land-plant lineages, and can be found in all parts of the plants (seeds, fruits, flowers, leaves, stem, buds, bark, roots, rhizomes). flavones were found in plant species belonging to over 70 different families in the plant kingdom [58]. Apigenin **21** and luteolin **22** are the main representative of the simple flavones [57].

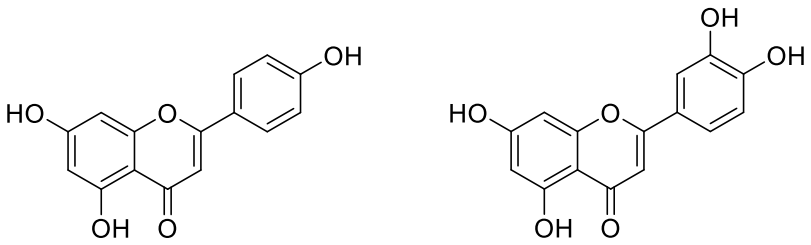


Figure (1.7): The major structures of flavones [57**].**

Anthocyanins 12

Anthocyanins are the chemicals that are accountable for plant tincture (such as blue, red, pink, and purple colour) [59]. The most representative compounds of this subclass are pelargonidin **23**, cyanidin **24**, and delphinidin **25** (Figure (1.8)) [45]. Anthocyanins play an essential role in preventing cardiovascular diseases, and have antioxidant properties [60].

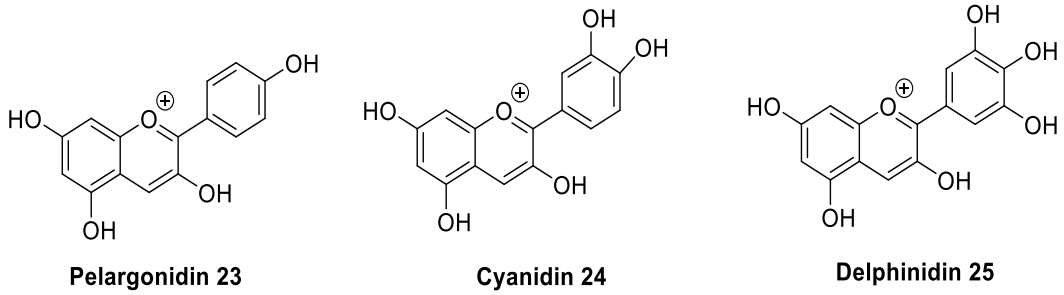


Figure (1.8): Structures of the most well-known anthocyanins in plant [57].

Flavanols 13

Flavanols (flavan-3-ols), also known as catechins, are categorized into free catechins and esterified catechins. The free catechins comprise catechin **10**, gallocatechin, epicatechin **26**, epigallocatechin **27**, while the esterified catechins contain of epicatechin gallate **28**, epigallocatechin gallate **29**, gallocatechin gallate **30,** and catechin gallate **31** (Figure (1.9)). The esterified catechins have bitter taste, while the free catechins have a slightly sweet taste. Compounds **26-29** are the four major catechins found in green tea [61]. Catechins represent the most complicated class of flavonoids due to their size, monomers (catechin), or polymeric forms (condensed tannins) [57].

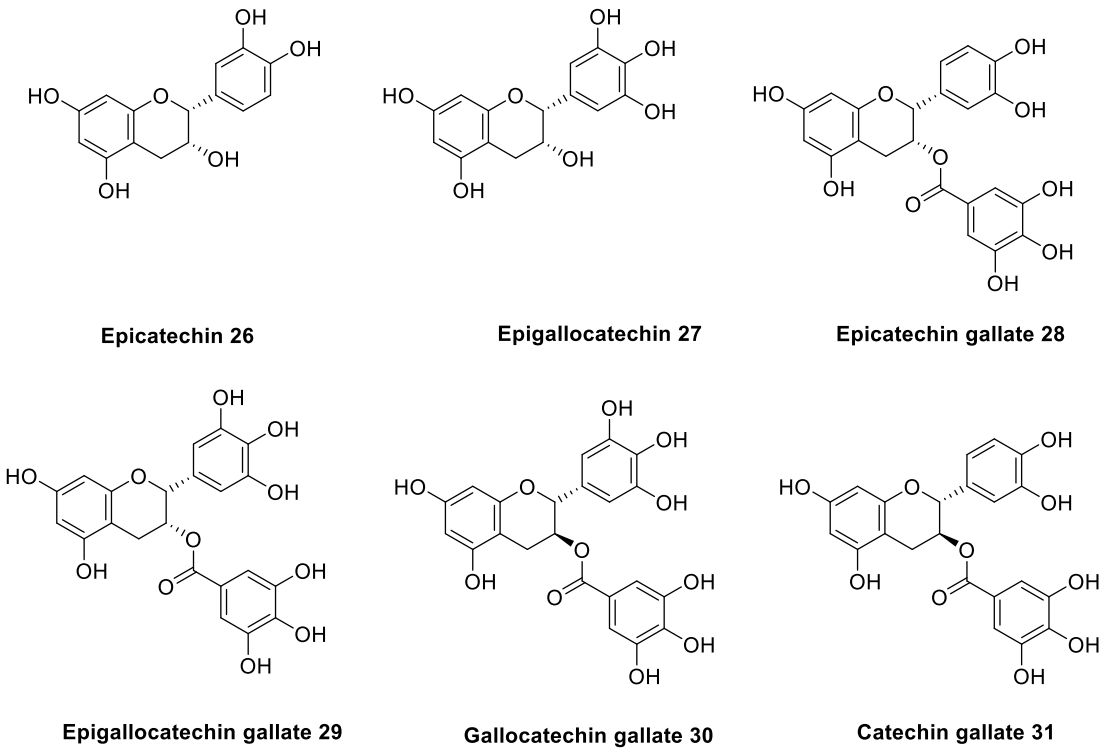


Figure (1.9): Chemical structures of some catechins [62].

Isoflavonoids 14

Isoflavonoids are another type of flavonoids, which have considerable estrogenic activity so they are referred to as phytoestrogens [63]. These compounds are found predominantly in the Leguminosae family plants including among others soybeans, alfalfa sprouts, and red clover leaves. Isoflavones are an essential group that find use in medicinal, cosmetically, and nutritionally industry [64]. The major isoflavonoids that found in human diet are daidzein **32** and genistein **33** and their methyl ether formononetin **34** and biochanin A **35** (Figure (1.10)) [57, 63].

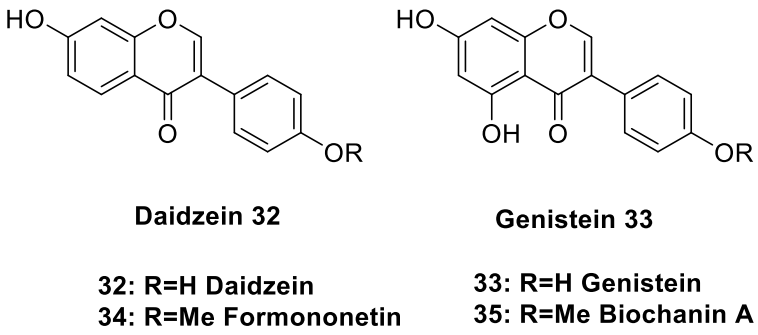
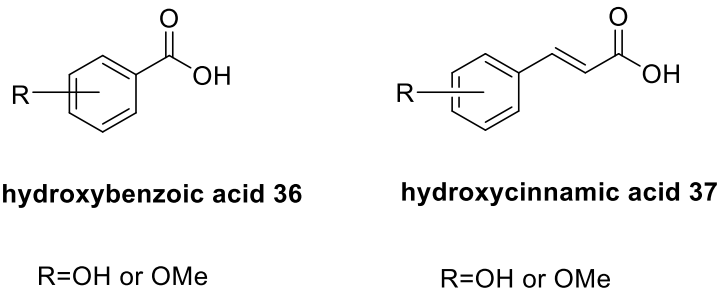


Figure (1.10): Chemical structures of major isoflavones [63].

B) Phenolic acids

Phenolic acids represent the most copious class of phenolic compounds extracted from plants. They are found predominantly in plant seeds, fruits skins and vegetable leaves. Phenolic acids could be classified into two major groups: hydroxybenzoic acid **36** and hydroxycinnamic acid **37** (Figure (1.11)) [65, 66]. Phenolic acids possess anticancer, antioxidant activity, and are used as remedy for diabetes, cardiovascular diseases, and retard the development of Alzheimer's disease [67].



hydroxybenzoic acid 36 **hydroxycinnamic acid 37**

R=OH or OMe R=OH or OMe

Figure (1.11): Chemical structures of the basic phenolic acids classes [66].

C) Tanins

Tannins are phenolic compounds having high molecular weight ranging from 500-3000 Da. They are found in many plants parts such as fruit, leaves, bark, wood and roots. They have been considered as plant defense mechanisms against herbivorous attacks because of their astringent taste. Based on their chemical structure and characteristics, tannins are categorized into two major groups: hydrolysable **38** and condensed tannins **39** (Figure (1.12)). Tannins are used in industry field specially by the leather industry, and have found other applications like adhesives for wood industry, mineral industry, wine production industry, animal nutrition, oil industry, and protecting metal from corrosion [68,69].

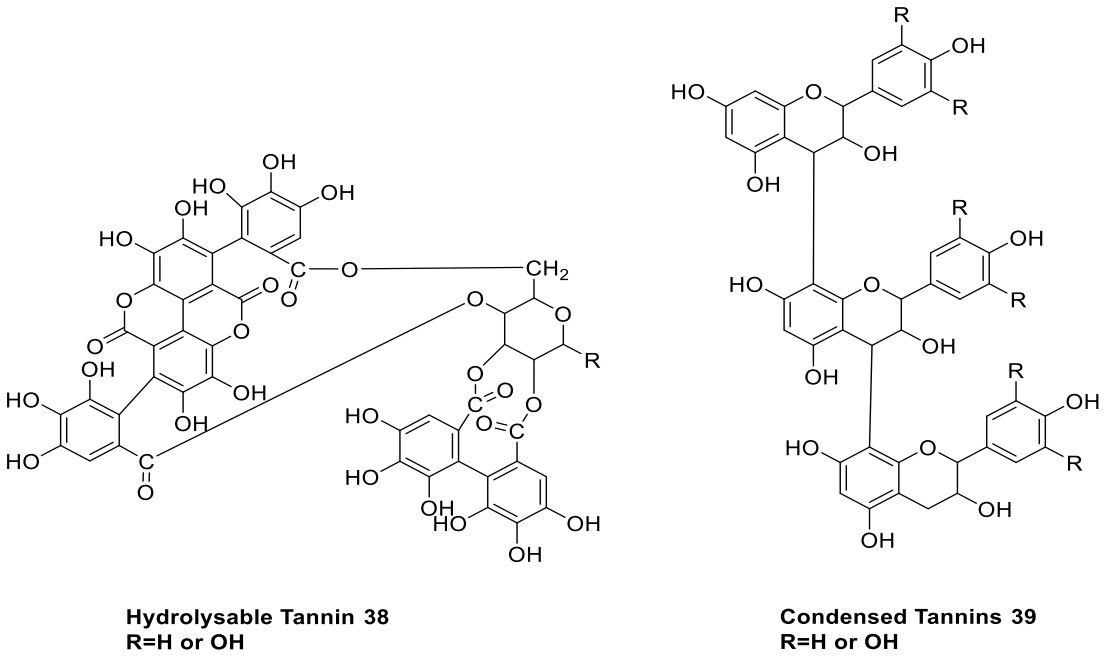


Figure (1.12): Hydrolysable and condensed tannins [69].

1.5.1.2 Terpenoids

Terpenoids are one of the most prevalent and diversified group of secondary metabolites. They are structurally characterized by a basic skeleton built from repeating isoprene building units, which are generally joined together in a head to tail fashion. Major groups of terpenoids are hemiterpenoid, monoterpenoid, sesquiterpenoid, diterpenoid, triterpenoid and tetraterpenoid [70, 71].

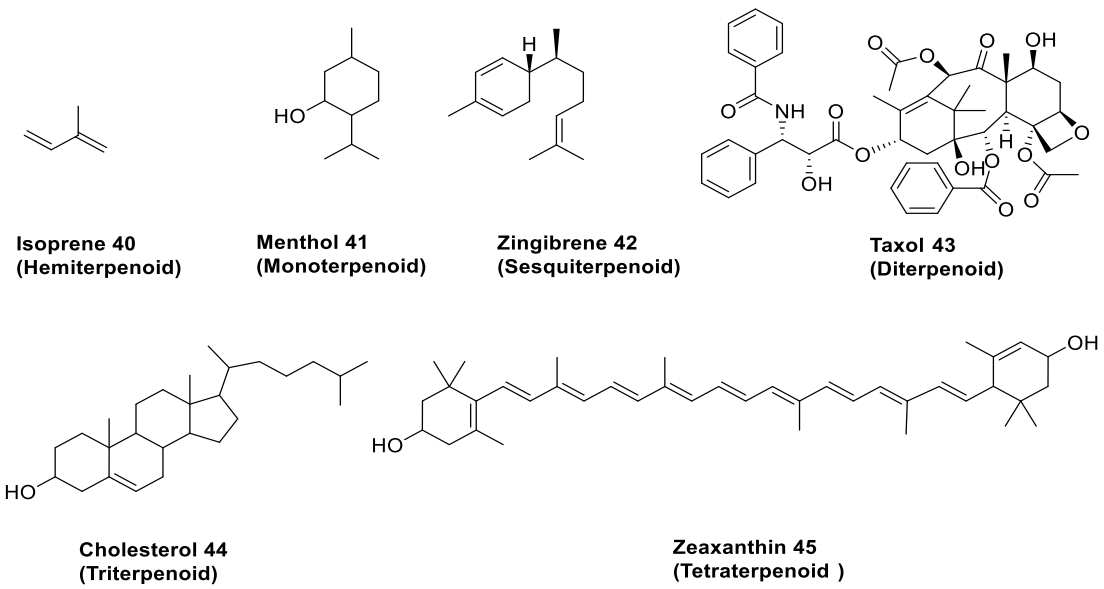


Figure (1.13): Examples of terpenoids classes [72].

1.5.1.3 Alkaloids

Alkaloids the third group of secondary metabolites. They contain at least one Nitrogen in their structure situated in some rings. About 20% of all flowering plants contain alkaloids [37]. The two stimulants caffeine **46** isolated from tea (*mostly Camellia sinensis*), coffee (*Coffea arabica*), and cacao (*Theobroma cacao*) plants, and nicotine **47** isolated from the tobacco plant *Nicotina tabacum* probably are two of the most common alkaloids. Alkaloids have been used for medication of illnesses related to the central nervous system (CNS), malaria and cancer [73]. However, their toxic, narcotic and addictive nature, regulated or restricted their use and most are rarely used in their pure form but semi-synthetic analogues are used [73]. Examples of some of the most common alkaloids (**46**, **47**, **48**) are shown in Figure (1.14).

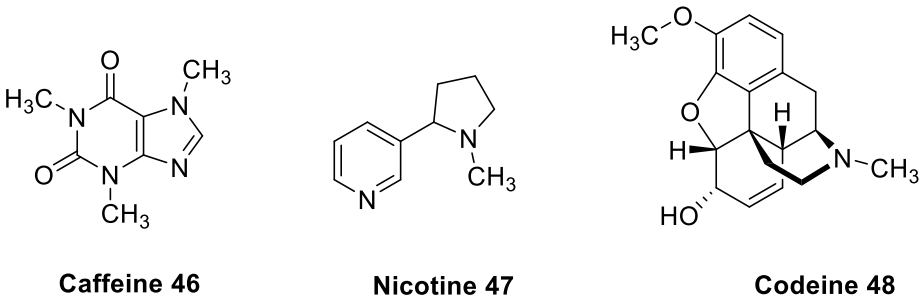


Figure (1.14): Examples of alkaloids [37].

1.6 An Overview of Techniques Used in Natural Products Chemistry

1.6.1 Extraction Techniques

Extraction, the first step in the examination of medicinal plants, involves the transfer of phytochemicals from plant parts into organic solvents in standard extraction procedures. The obtained extracts from plants are almost impure and need further separation and characterization [74]. Several extraction methods have been used for the extraction of phytochemicals from plants. The most widely used extraction techniques will be briefly discussed.

1.6.1.1 Conventional Extraction Methods

Conventional extraction methods are centered on solid–liquid extraction with various solvents. These methods have considerable drawbacks, like the relatively large amounts of organic solvents required and long extraction time [75].

1.6.1.1.1 Soxhlet Extraction

In this process, a soxhlet extractor is used to transfer the soluble constituents of a solid matrix to the liquid phase. This method has some

advantages. This technique is simple, cheap, and can extract more sample mass than other conventional methods. The main drawbacks are the long extraction time (at least 6 h), the bulky volume of solvent used, and the target compound may undergo thermal decomposition since the extraction usually occurs at the boiling point of the solvent for a long time [76].

1.6.1.1.2 Maceration

In this process, the coarse or powdered plant is submerged in the solvent in a sealed container, which was allowed to stir at room temperature for at least 3 days until most of the soluble matter has dissolved. Filtration or decantation after standing followed by removal of solvents gave a mixture of compounds. Maceration became popular extraction method for phenolic compounds because it is simple and inexpensive [77].

1.6.1.1.3 Digestion

Digestion is a maceration accompanied with gentle heat during the process. It's used when some of the plants' constituents can't be dissolved at room temperature, or when there are partially insoluble phytochemicals. [78].

1.6.1.2 Novel Extraction Methods

Recently, modern environmentally friendly extraction techniques that are quicker and more reliable than conventional extraction methods have been developed. Many advantages in terms of extraction time, solvent used, extraction yields, and reproducibility have been achieved by using these methods in the extraction of active ingredients from plant [79, 80]. These techniques include among others ultrasound assisted extraction

(UAE), microwave assisted extraction (MAE), and supercritical fluid extraction (SFE).

1.6.1.2.1 Ultrasound Assisted Extraction (UAE)

High ultrasound frequencies pulses ranging from 20 kHz to 2000 kHz are used in this technique. This causes cavitation, which destroys the plant's cell wall, allowing for better mass transfer [75]. UAE has a number of advantages. It takes less time and uses less solvent, and it improves extraction yield [81].

1.6.1.2.2 Microwave Assisted Extraction (MAE)

Microwave Assisted Extraction is a technique that involves heating the solvent containing a sample matrix with microwave energy ranging from 300 MHz to 300 GHz in order to extract analytes from the matrix into the solvent. The rapid heating of the solvent and sample matrix is the main advantage of this technique. Microwave-assisted extraction takes only 15–30 minutes and requires 10–30 mL of solvent. These volumes are roughly ten times smaller than those consumed by traditional extraction methods. Using MAE, the solute recovery and reproducibility are generally improved [75, 82].

1.6.1.2.3 Supercritical Fluid Extraction (SFE)

It's a technique for isolating a specific component from a matrix with the aid of a supercritical fluid extraction solvent. In the fine chemical industry, supercritical carbon dioxide (CO_2) is the most widely used extraction

procedure [83] . SFE under the right conditions, SFE yields purer extracts than traditional extraction with low or no organic solvents uses [75].

1.6.2 Chromatographic Techniques

Chromatography is a physical technique used for separating chemical mixtures into their individual components [84]. In all chromatographic processes, the mobile phase (in which the sample is dissolved) is pushed into the stationary phase (which is settled in a column or on a solid surface). To different degrees, the sample components distributed themselves between the two phases [85].

Based on the form of mobile phase used, chromatography can be classified in three typed namely Gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SCF) [85]. Liquid chromatography is often classified into two types depending on the form of stationary material used for separation: normal phase and reversed phase silica gel. Since the normal phase consists of a polar stationary phase and a non-polar mobile phase, non-polar ingredients are eluted first. Reversed process, on the other hand, includes a non-polar stationary phase and a polar mobile phase, resulting in polar compounds eluting first. In most cases, though, reverse phase is used because many of the chemical drugs are polar in nature [86].

1.6.2.1 Thin Layer Chromatography (TLC)

TLC is a form of chromatography in which a thin layer of adsorbent material (usually silica gel, aluminum oxide, or cellulose) is coated on a

solid support (aluminum sheet, glass, or plastic) and a liquid mobile phase is used [87].

After the sample spot has been applied on TLC plate, the solvent system (mobile phase) drawn up the plate by capillary force. Different analytes moved on plate at different rates depending on their solubility and retention by the stationary phase. Each spot on plate equivalent to one analyte and it is characterized by its retention factor (Rf) value. This is calculated by dividing the analyte's distance traveled by the solvent's distance traveled [87].

TLC, in combination with UV detection and spraying reagents, provides knowledge about the number of mixture components and can be used to distinguish a compound in a mixture by comparing its Rf to the Rf of a known compound [87].

1.6.2.2 Open Column Chromatography

It is a method of solid-liquid chromatography that is used to separate chemical compounds in a mixture. This technique utilizes a stationary phase, which is packed in a column, usually are silica gel (SiO_2) and alumina (Al_2O_3), and a mobile phase that passes through the column, commonly volatile organic solvents. A solvent is used to elute the sample mixture from the top of the column. Analytes disperse as they travel down the column due to polarity variations, forming bands that are collected in small fractions [88]. TLC is used to track these processes on a regular basis, and related fractions are pooled and concentrated [89].

Eluting the column can be achieved in one of two ways, namely isocratic and gradient. In isocratic elution, the column is eluted with a single solvent or solvent mixture, while in gradient elution, the column is eluted with a series of solvents of increasing polarity [88]. The best solvent flow rate for each column is determined by the form of mobile phase, analyte, and column size. The technique of open column chromatography is simple and inexpensive. However, it is time consuming, labor intensive, and boring. Since they spend too much time in the column, certain analytes are prone to decomposition [89, 90].

1.6.2.3 High Performance Liquid Chromatography (HPLC)

HPLC stands for high-performance liquid chromatography and is a type of column liquid chromatography. The HPLC instrument is an automated closed system consisting of a solvent delivery system containing a pump, a detector unit, injection port (manual or automated), data analysis unit, a column, and a reservoir for solution and waste deposit container [91, 92].

The column is the main part of the system. There are various HPLC column sizes. The standard analytical HPLC column has a diameter of 4.6 mm and a length of 10 to 25 cm. Larger columns are used in semi-preparative and preparative HPLC (10 mm and 20 mm in diameter). The size of the columns is determined by the separation's intent. Analytical columns are used to determine qualitative and quantitative information about a sample. Typically, once the components have been analyzed with an analytical column, they are discarded in a waste container. To separate

and collect sample fractions, larger columns such as semipreparative and preparative columns are used, and more purification can be performed [93].

Chemical partitioning with an HPLC system is possible because each compound migrates at a different rate in a certain mobile phase and column. The choice of stationary phase and mobile phase determines the degree of partitioning [94]. This technique is currently more common than other techniques because it is the first option for studying fingerprinting to monitor herb quality [95].

1.6.3 Structure Elucidation Techniques

Conventional spectroscopic techniques such as UV-visible, infrared (IR), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy are used in structure elucidation of organic compounds [96]. These techniques are always used to determine the number of atoms and bonds, type of bonds, as well as the structure and conformation of a pure compound. Improvements in technology have allowed the use of small quantities reached to milligram of sample, high resolution and minimized analysis times [97].

1.6.3.1 Infrared Spectroscopy (IR)

Infrared spectroscopy is a technique used for identifying the functional groups in a compound that are recorded in a spectrum by the IR instrument.[98].

1.6.3.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is an essential technique that provides detailed information concerning structural elucidation of organic compounds. Proton and Carbon -13 (^{1}H ,^{13}C) NMR are some of the most useful experiments for elucidation the compounds structures [99, 100].

1.6.3.3 Mass Spectrometry

The molecular weight of the compound and the fragmentation patterns of the origin compound are determined by analyzing the mass spectrum. Comprehensive study of the fragmentation patterns, in addition to information obtained from other techniques will lead to molecular structural elucidation of the studied compound [101].

1.7 Biological Activity

Biological activity refers to ability of a certain molecule to obtain a defined biological effect on a target living tissue. It is determined by using biological assay [102, 103].

1.7.1 Anti-oxidant Activity

Free radicals are molecules with an odd number of electrons, such as reactive oxygen and nitrogen species. A chain reaction can be started when these very active unstable radicals are formed. These free radicals could be generated both internally, as normal cells create them during metabolism, and externally, as a result of contaminants in the air, exposure to harmful radiation, and chemicals created by industrial processes [104].

Oxidative stress is caused by an imbalance between the antioxidant defense mechanism and the production of oxidants. Oxidative stress has been linked to Alzheimer's disease, cancer, heart disease, Parkinson's disease, and death [104, 105].

An antioxidant is a substance that inhibits or prevents the oxidation of a substrate. Antioxidants come in two forms: natural antioxidants like ascorbic acid, glutathione, flavonoids, uric acid, melatonin, and vitamin E, and synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole. However synthetic antioxidants have greater hazard of side effects; therefore, searches on determining the natural antioxidants have become very important issue. Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having antioxidant properties [106].

1.7.2 Anti-microbial Activity (Bacterial and Fungi)

Microorganisms that cause disease for its host are called pathogenic organisms. These pathogens include: bacteria, viruses, fungi, and parasites. They are infectious and transferred to human by animals, insects, and by taking contaminated water and food [107] .

Antibacterials are substances that inhibit the growth and reproduction of bacteria. All antibiotic drugs possess antibacterial properties [108].

Antifungi is one of the antibiotic groups which kill or stop the growth of fungi. Candidiasis are one of the most popular type of fungi [109].

However, some pathogens such as *Escherichia coli*, *Proteus sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, gained resistance to antimicrobials [110], so finding new antimicrobials drugs a major challenge in global health care.

Researchers have recently attempted to use medicinal plants as an alternative source of medicines, since medicinal plants are abundant in antimicrobial compounds such as flavonoids, terpenoids, and alkaloids [111, 112].

1.8 Aims of the Study

The objectives of this proposed study were:

1. Isolate and identify the active components shown by *Stachys viticina* using various techniques.
2. Exploring the antioxidant, antibacterial, and antifungal activities of *Stachys viticina* polar extracts.

Chapter Two

Experimental Part

2.1 Chemicals and Reagents

The materials used in this research were of analytical grade and used without further purification.

All solvents used (methanol, dichloromethane (DCM), ethyl acetate (EtOAc), hexane, and diethyl ether) were purchased from Sigma Aldrich (Germany). Silica gel (100–200 and 200–300 mesh) for column chromatography (CC), and GF254 silica gel for thin layer chromatography (TLC) were purchased from Sigma-Aldrich (USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and trolox were purchased from Sigma Aldrich (Germany). Finally, Mueller-hinton broth, Sabouraud dextrose agar, and dimethyl sulphoxide (DMSO) were obtained from Himedia (India).

2.2 Plant Material

Stachys vicitina plant was collected on the end of May 2018 from the mountains of Nablus by Dr Nidal Jaradat from Al Najah University. The plant was washed with water and dried in well ventilated room at ambient temperature in a shaded area until it is completely dry.

2.3 Extraction

The dried *Stachys viticina* leaves (585.53 g) were grinded, then macerated with sufficient amount of methanol (2L) and stirred using mechanical stirrer for 24 hours. The resulting extract was filtered, and the remaining

solid was subjected to extraction twice with methanol (0.5 L) under the same conditions. After filtration of the combined extract, methanol was removed under reduced pressure. Then concentrated extract was successively washed with pentane to remove the fats, waxes and the nonpolar compounds and with ethyl acetate to extract the flavonoids and other polyphenols present in the extract.

2.4 Isolation

Dried crude extract was dissolved in dichloromethane (DCM), the filtrate was concentrated under reduced pressure at 40 ^{o}C, afforded 3.42 g, and the residue precipitate (7 g) which didn't dissolve in DCM was called R and was stored.

The first step of separation of any extract started with determination of the best eluent that could be used in silica gel column chromatography, and which was achieved by TLC experiments.

The crude extract was dissolved in small amount of (DCM), and subjected to a first silica gel column chromatography using dichloromethane (DCM) and ethyl acetate (EtOAc) (9:1 v/v) as eluent. During the chromatographic run, the polarity of the eluent was increased in order to consent the elution of more polar compounds by passing to a solution composed of dichloromethane and ethyl acetate (8:2, 7:3 v/v). A total of 22 test tubes were collected (100 mL each). The content of each test tube was analyzed with TLC using DCM/EtOAc in (7:3) ratio as eluent. Compounds were visualized under UV light and similar tubes were pooled together to yield

four major fractions. Fraction 1 (F1) included tubes (1 and 2), Fraction 2 (F2) included tubes (3-12), Fraction 3 (F3) included tubes (13-16), and Fraction 4 (F4) included tubes (17-22). Then the solvent from each fraction was evaporated under reduced pressure at 40 ^{o}C and saved for further purification.

HPLC and TLC analysis of the isolated four fractions indicated the presence of a lot of compounds in F1, which proved to be very hard to separate. On the other hand, fractions 2 and 3 were subjected to further chromatographic separation techniques.

Fraction 2 was subjected to silica gel column chromatography using ether and hexane (7:3 v/v) as eluent. Five fractions namely, $F2_1$, $F2_2$, $F2_3$, $F2_4$ and $F2_5$ were isolated, from which the solvents were removed by rotary evaporator at 40 ^{0}C. From the five fractions $F2_3$, $F2_4$ and $F2_5$ have been subjected to further purification.

$F2_3$ fraction was subjected to the silica gel column chromatography using ether and hexane (8:2 v/v) as eluent gave three fractions. Based on results obtained from HPLC analysis two of the fractions were almost pure, and these fractions were called **A** (100.67mg, 97.82% purity) and **B** (85mg, 82.81% purity).

Further purification of $F2_4$ on the silica gel column chromatography employing ether and hexane (8:2 v/v) as eluent afforded two substances that were hard to separate by available simple techniques. They could be separated by preparative HPLC.

Fraction $F2_5$ was subjected to silica gel column chromatography using diethyl ether as eluent and resulted in the isolation of two fractions, among of them one was pure fraction **C** (102.7 mg, 90.82% purity) but the other one **D** (73.04 mg, 47.26% purity) need more purification. The purity of the two compounds was confirmed by HPLC analysis.

Fraction F3 was chromatographed on silica gel column eluted with dichloromethane and ethyl acetate (7:3 v/v) to obtain $F3_1$, $F3_2$, and $F3_3$. HPLC analysis indicated that $F3_1$ and $F3_2$ were pure fractions, they called **E** (45 mg, 96.8% purity) and **F** (91.4 mg, 94.13% purity).

2.5 Structure Elucidation and Purity Determination of the Compounds

2.5.1 HPLC Analysis

To detect the purity of the extracted fractions an analysis was conducted using HPLC-DAD Water 1525, with C18 column (5µm, 4.6×250 mm cartridge). The mobile phase consists of solvent A (water) and solvent B (methanol), HPLC separation was achieved using binary-solvent gradient elution that started with 100% of solvent A and 0% of solvent B till 0% of A and 100 % of B, with 0.7 ml/min flow rate. The detection of all extracts was at 254 nm and the injection volume was 20 µL.

2.5.2 IR Analysis

Functional groups of the extracted fractions were characterized using FT-IR spectrometer (NICOLIT iS5 from Thermo Fisher Scientific).

2.6 Pharmacological Screening

2.6.1 Antioxidant Activity 2, 2-diphenyl-1-picrylhydrazyl (DPPH assay)

Scavenging activity of *Stachys viticina* extracts were assessed using the method described in the literature [113, 114]. The anti-oxidant activities of the plants fractions (A, B, C, D, F, R) and Trolox (reference compound) were assessed by their ability to donate hydrogen atom or electron, which was recognized from the bleaching of deep violet color of the methanolic DPPH solution, as indicated in scheme 1.

Stock solution of plant fractions and Trolox were prepared in methanol, at a concentration of 1 mg/mL. Each of these stock solutions were diluted in methanol to prepare 12 working solutions with the following concentrations: 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 μg/mL.

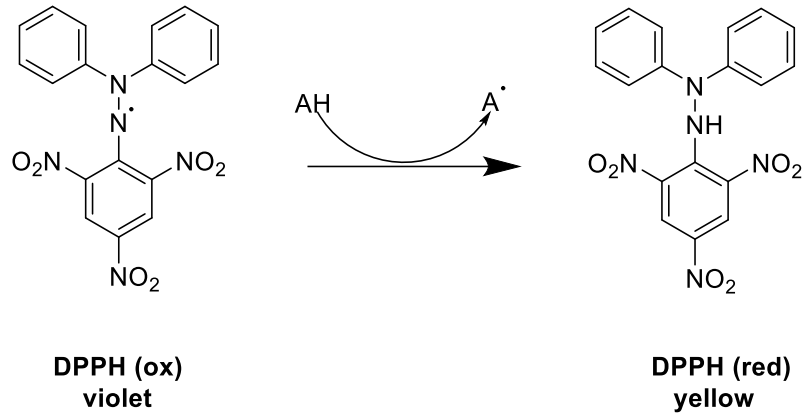
A freshly prepared DPPH solution (0.002% w/v) was mixed with both methanol and with each of the above-mentioned working solutions at 1:1:1 ratio. A negative control solution was prepared by mixing the DPPH solution with methanol in 1:1 ratio. Then, all of these solutions were incubated at room temperature in a dark cabinet for 30 min. By the end of the incubation period, the absorbance of these solutions was measured by UV-Vis spectrophotometer at a wave length of 517 nm. Methanol was used as the blank solution.

The antioxidant activity of the corresponding plant fractions and Trolox standard were determined in terms of inhibition percentage of DPPH activity using the following equation:

$$In\% = \frac{A\ blank - A\ sample}{A\ blank} \times 100 \qquad \textbf{Eq.1}$$

Equation (1): Inhibition% of antioxidant activity [115]

Where A_{blank} and A_{sample} represent the absorbance of the blank and the sample respectively.



Scheme (1): Principle of DPPH radical scavenging capacity assay [116].

The antioxidant half-maximal inhibitory concentration (IC_{50}) for the studied plant fractions and Trolox standard solution as well as their standard deviations, was calculated from the graph plotted of the inhibition percentage against fractions concentration, using Microsoft Office Excel 2010.

2.6.2 Antibacterial and Antifungal Activity Tests

2.6.2.1 Preparation of Plant Samples for Testing

10 mg of each of plant extracts (A, B, C, D, E, F, R) were dissolved in 0.5 mL sterile dimethyl sulfoxide (DMSO) then diluted with 0.5 mL of distilled water to obtain a concentration of 10 mg/ml. All extracts solutions were kept in UV disinfection chamber for 20 minutes.

2.6.2.2 Preparation of Growth Media

Mueller-Hinton Broth (MHB) media was prepared according to manufacturer's instructions labeled on the bottle. 8.4g of MHB powder was suspended in 400 ml distilled water, and heated with stirring to boiling until the medium was completely dissolved. The solution was autoclaved for 15 minutes at 121 ºC, and allowed to cool down to room temperature before use.

2.6.2.3 Test Microorganisms

a) **Bacterial strains:** Six bacterial strains were used in this study. These strains consist of five references bacterial strains obtained from the American Type Culture Collection (ATCC): four gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus vulgaris* (ATCC 8427), *klebsiella pneumonia* (ATCC 13883), and two gram-positive bacteria included *staphylococcus aureus* (ATCC 6538P). In addition to clinical isolate of methicillin resistant *staphylococcus aureus* (MRSA). All the bacterial strains were sub-cultured on Mueller-Hinton Agar.

b) **Fungal strain:** one fungal strain *Candida Albicans* (ATTC 90028) obtained from the American Type Culture Collection (ATCC) was used in this study. This strain was sub-cultured on Sabouraud Dextrose Agar.

2.6.2.4 Preparation of Bacterial and Fungal Suspension

All bacterial and fungal strains were cultured 24 hours before use. Then a sterile specimen was taken gently from the colony surface of each type of

cultured strains and transferred to a separable sterile tube containing 5 mL of sterile normal saline. The optical density of all solution was measured by spectrophotometer at ƛ = 620 nm, where normal saline was used as blank. The turbidity of the bacterial suspensions was adjusted into 0.5 McFarland turbidity standard (optical density 0.08 to 0.1), to obtain a bacterial suspension with a 1.5 x 10^8 colony forming units (CFU/mL). Also, the turbidity of the yeast *Candida Albicans* was adjusted to equal 0.5 McFarland solution (optical density of 0.12 to 0.15) with concentration of $1*10^6 – 5*10^6$ CFU/mL. Finally, the stock solutions of each bacterial and fungal strains were prepared by transferring 100 µL strains suspension into 10 mL of Mueller Hinton Broth media, these stock solutions were further used in experiment of the microdilution method.

2.6.2.5 Anti-microbial Assay

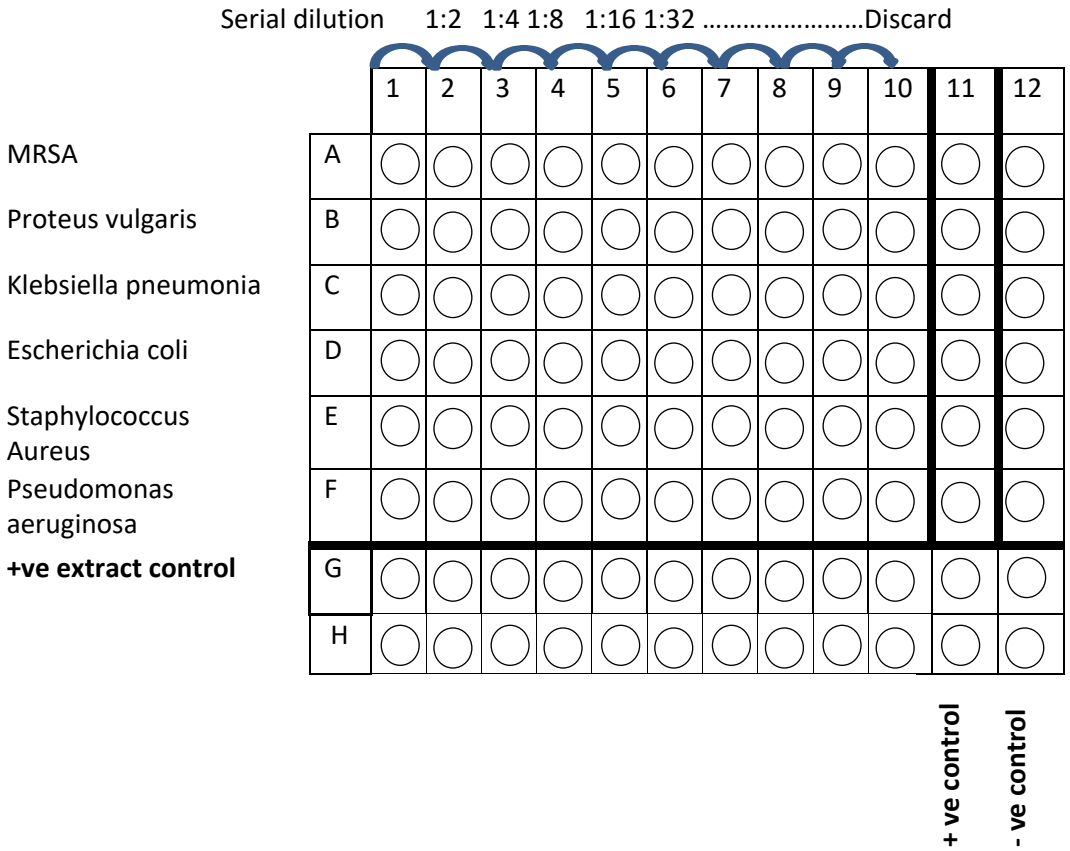
The antimicrobial activity of the plant samples was assayed employing broth micro-dilution method defined by procedure as described in the literature with some modifications [117, 118].

a. Anti-bacterial Assay

In the sterile 96 micro-wells plate 50 µL from the Mueller Hinton Broth media were filled in all microplate wells except the last raw (H) using multichannel pipette. Then 50 µL from the first prepared solution of plant extract (A) was added to the wells of first column with excluding H raw. Thereafter, 50 µL of the solution from the wells number 1 was transferred by multichannel pipette to wells number 2, which were mixed to obtain (2-

fold) serial dilution and so on till wells number 10. Then 50 µL from each type of the bacterial strain's suspension was filled in its specific row for the wells 1-11. The wells number 11 contain Mueller Hinton Broth media and bacteria suspension and didn't contain plant extract, served as positive growth control. Wells number 12 contain only the growth media and served as negative control. While G raw wells contain only the extract solution to ensure that there is no contamination and the resulted turbidity in the G raw wells was not due to the extract itself. Same steps were performed for all plants extracts (B-F, and R). Finally, all the inoculated plates were incubated at 35˚C for 24 hours. The resulting turbidity in the wells indicated the bacterial growth.

The lowest concentration of plant extracts, at which no visible bacterial growth in that microwell was observed, considered as the minimum inhibitory concentration (MIC) of the examined plant extracts. All the established experiments were performed in triplicate, to control the sensitivity of the tested microorganisms.



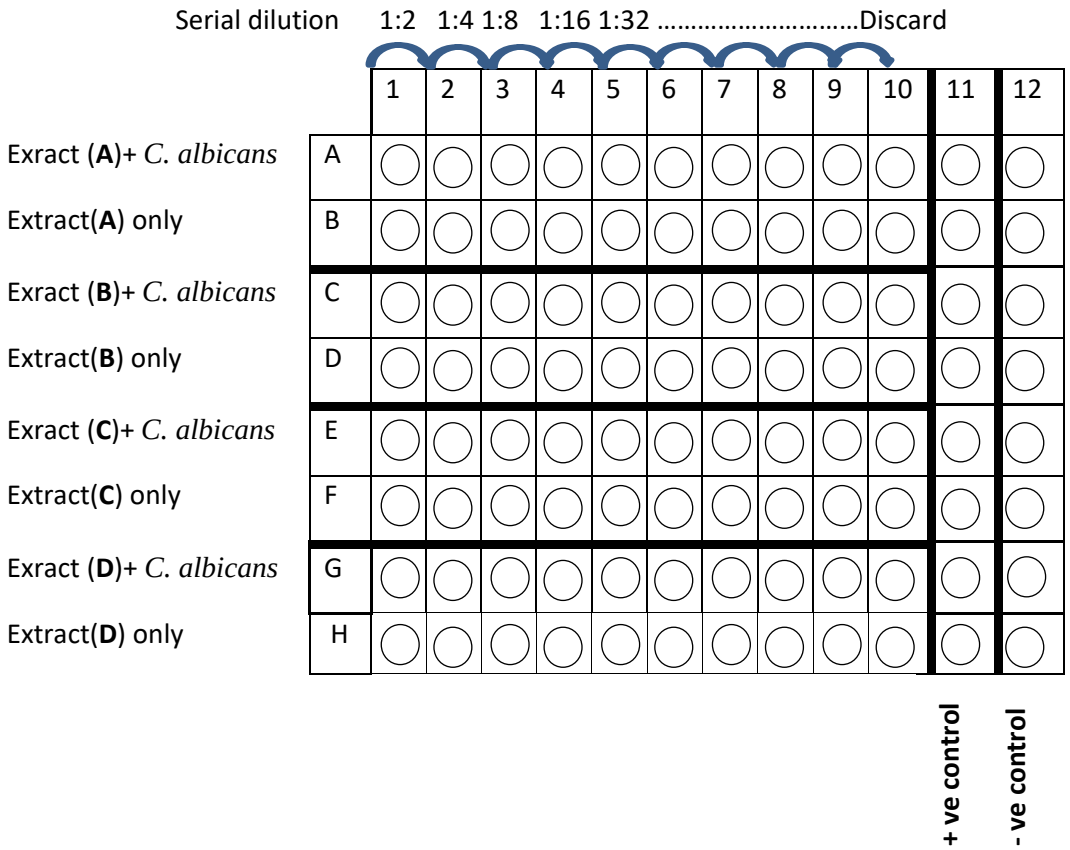
b. Anti-fungal Assay

The same procedure used for bacterial strains was used for the yeast *C. albicans* with some modifications. The plant extracts were added to microplate wells in duplicate; raw A and B for the first extract (A), C and D for the second extract (B), E and G for the third extract (C), G and H for the fourth extract (D). While the *C. albicans* suspension was added to raw A, C, E, and G. By the same steps experiment was carried out for the last three extracts (E, F, R). Finally, all the inoculated plates were incubated at 35˚C for 48 hours.

The lowest concentration of plant extracts, at which no visible *candidal* growth in that microwell was estimated as the minimum inhibitory

concentration (MIC) of the examined plant extracts. All the established experiments were performed in triplicate, to control the sensitivity of the tested *C. albicans.*

In parallel, a control experiment was run to study the impact of the solvent alone (without plant extracts) on growth of the seven test organisms. Dimethyl sulfoxide was diluted in a similar pattern with sterile MHB media followed by inoculation and incubation.



Chapter Three

Results

The prime target of this study was to isolate pure chemical compounds from phenolic extract of *Stachys viticina* plant and screen the potential pharmacological activities of these compounds.

3.1 Phytochemical of Polyphenolic Composition of *S. Viticina*

The isolation procedure was carried out using silica gel chromatography. Seven extracts were obtained. Among of them one was crude extract and was called R, and one with 47.26% of purity and was called (D), but others were almost pure (purity % > 80%) and were called (A, B, C, E, F). Purity of the obtained fractions was checked by TLC plates and confirmed using HPLC analysis. Unfortunately, due to the lack NMR techniques at An Najah National University as well as to the COVID-19 we were not able to send the fractions abroad in order to run ^{1}H- and C-NMR and 2D-NMR that will enable us to identify the purified fractions. The fractions are stored in the fridge and they will be sent abroad as soon as the restriction imposed due the spread of COVID-19 will be lifted.

Fraction **A**: fraction **A** was isolated as yellow solid, UV active, Rf value = 0.57 in eluent DCM and EtOAc (7:3 v/v), HPLC retention time = 4.234 minutes (fig.a.1, appendix), IR analysis showed that fraction **A** had a band in (2361 cm−1) which could be a cyanide group, carbonyl group (1702 cm−1), and carbon –carbon double bond group (1620 cm−1) (fig.a.7, appendix).

Fraction **B**: fraction **B** was isolated as green solid, UV active, Rf value = 0.65 in eluent DCM and EtOAc (7:3 v/v), HPLC retention time = 4.320 minutes (fig.a.2, appendix), IR analysis showed that fraction **B** had a band in (3200-3500cm−1) zoon which could be a hydroxyl group (OH), aromatic ring (2973 cm−1), conjugated carbonyl (1651 cm−1) and carbon –carbon double bond group (1596 cm−1) (fig.a.8, appendix).

Fraction **C**: fraction **C** was isolated as green solid, UV active, Rf value = 0.52 in ether, HPLC retention time = 4.323 minutes (fig.a.3, appendix), IR analysis showed that fraction **C** had aromatic ring (2900 cm−1), and carbonyl group (1651 cm−1), in addition to carbon –carbon double bond group (1600 cm−1) (fig.a.9, appendix).

Fraction **D**: fraction **D** was isolated as dark green solid, UV active, Rf value = 0.66 in ether and ethyl acetate (7:3 v/v), HPLC retention time = 4.259 minutes (fig.a.4, appendix), IR analysis showed that fraction **D** revealed just carbon –carbon double bond group (1600 cm−1) (fig.a.10, appendix).

Fraction **E**: fraction **E** was isolated as green solid, UV active, HPLC retention time = 4.251 minutes (fig.a.5, appendix), IR analysis showed that fraction **E** had an aromatic ring (2919 cm−1), carbonyl (1650 cm−1) and carbon –carbon double bond (1605 cm−1) groups (fig.a.11, appendix).

Fraction **F**: fraction **F** was isolated as green solid, UV active, Rf value =0.60 in ether, HPLC retention time = 4.246 minutes (fig.a.6, appendix), IR analysis showed that fraction **F** had a broad band in (3200-3500 cm−1) it seemed to be a hydroxyl (OH) group, and aromatic ring (2930 cm−1), and

carbonyl group (1710 cm−1), in addition to carbon –carbon double bond group (1603 cm−1) (fig.a.12, appendix).

3.2 Biological Activity

3.2.1 Anti-oxidant Inhibitory Activity

Antioxidant activities of the obtained extracts of *S.viticina* plant were investigated using the DPPH assay as *in vitro* approach and are expressed as DPPH % inhibition activity (Eq.1) and IC_{50} values (the amount of extract that giving 50% inhibition of DPPH radical). Table 3.1 and figure 3.1 present the DPPH inhibitory activity and the IC_{50} values of the extract R, the fractions A-D, F and Trolox. The higher % inhibition and Lower IC_{50} values reflect better anti-oxidant action of the extracts. The most powerful extract is the residue R with IC_{50} = 18.58 µg/mL, and % Inhibition = 93.55 % at concentration of 80 µg/mL.

Table 3.1: IC_{50} (µg/ml) values for different extracts.

	Trolox	**A**	**B**	**C**	**D**	**F**	**R**
IC_{50} (µg/mL) ± Standard deviation	2.70 ± 0.096	85.88 ± 1.61	54.37 ± 0.14	77.58 ± 0.304	68.36 ±. 0.94	58.62 ± 0.74	18.58 ± 0.99

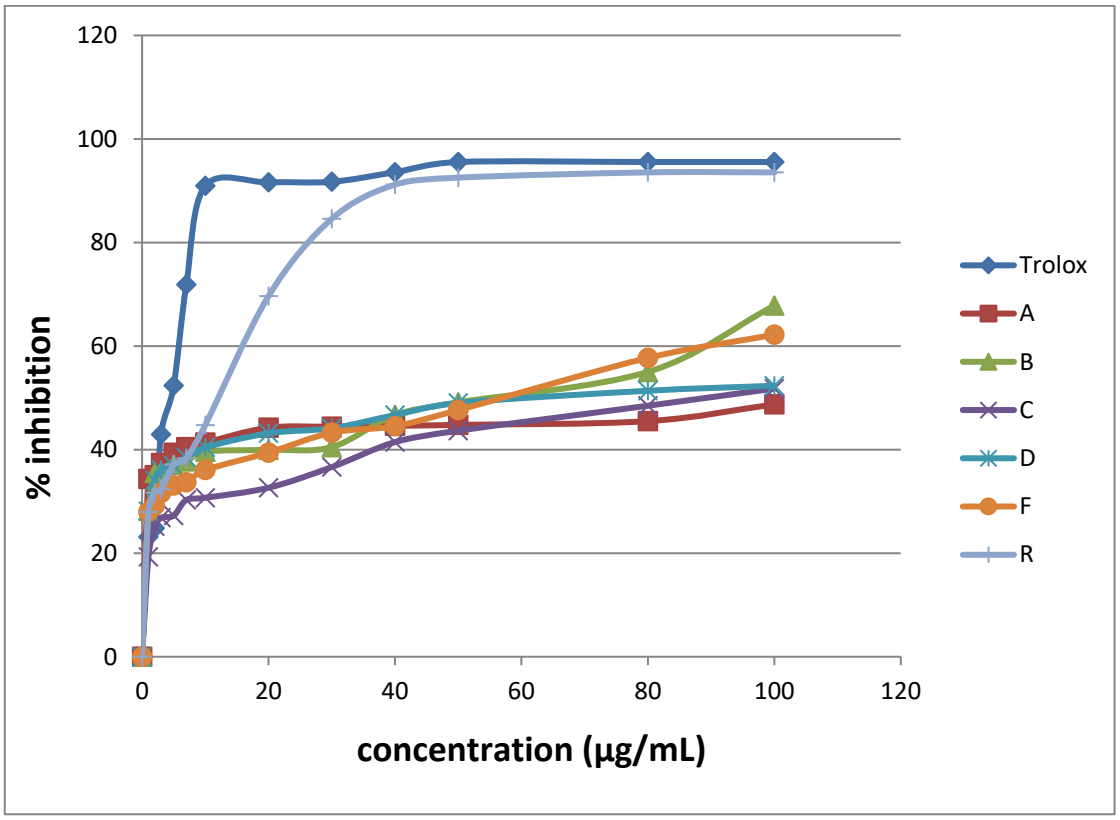


Figure (3.1): Anti-oxidant % Inhibition concentration (µg/ml) of different extracts.

3.2.2 Anti-microbial Activity

The microdilution assay was used to evaluate the antimicrobial activity of the R extract, and fractions A-F against six bacterial strains included *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *klebsiella pneumonia*, *staphylococcus aureus* and MRSA, inaddition to the yeast *C. albicans*. The results indicated in Table 3.2 revealed that only fractions C, B, and the extract R strongly inhibited the growth of MRSA and *S.aureus*. While, fractions D, E, F had moderate inhibitory effect against MRSA and *S.aureus*. And fraction A had poor inhibitory effect against all the microorganisms tested. However, *C. albicans* was not inhibited by the tested fractions.

Table (3.2): Minimum inhibitory concentration values (µg/ml) for different *Stachys viticina* extracts against selected pathogens

	Bacteria						**Fungus**
ATCC Number	**Clinical strain**	**ATCC 25923**	**ATCC 25922**	**ATCC 13883**	**ATCC 8427**	**ATCC 9027**	**ATCC 90028**
Microbe /extract MIC (µg/ml)	**MRSA**	***S.aureus***	***E. coli***	***Klebsiella pneumonia***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Candida albicans***
A	1250	1250	0	1250	1250	0	0
B	39	78.1	1250	1250	1250	1250	0
C	39	39	0	0	0	1250	0
D	156.3	156.3	0	1250	1250	0	0
E	312.5	156.3	1250	1250	1250	0	0
F	312.5	156.3	0	0	0	0	0
R	78.1	78.1	0	1250	625	625	0

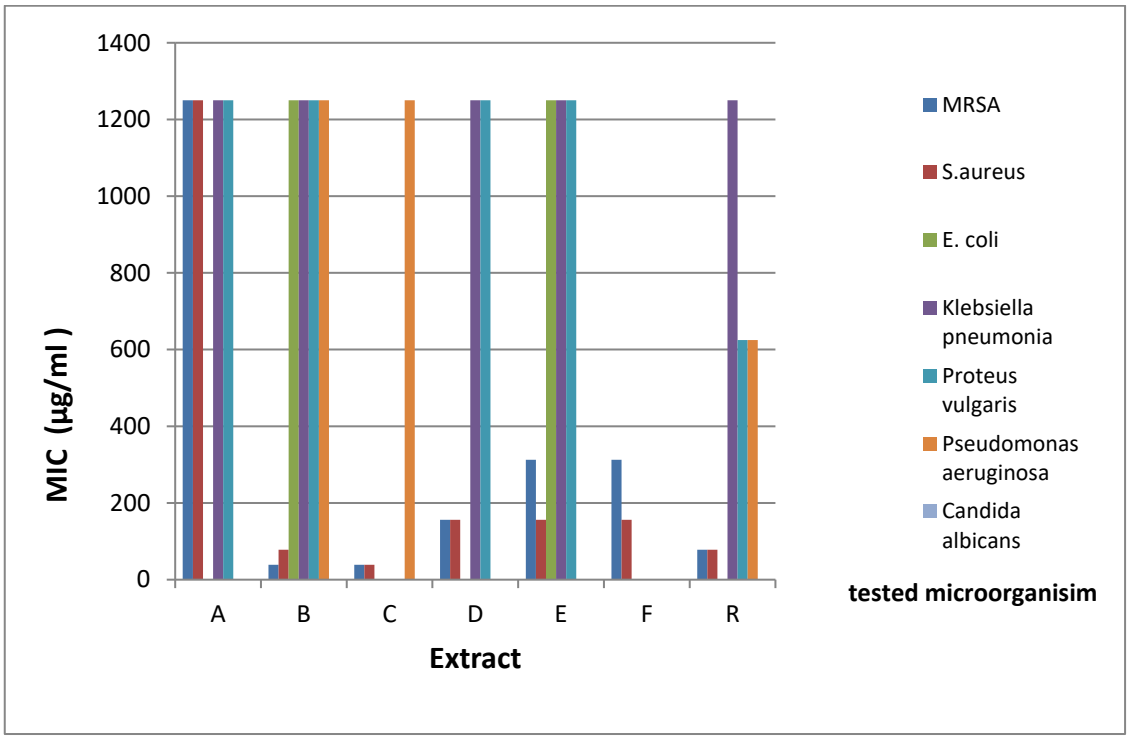


Figure (3.2): Minimum inhibitory concentration (µg/ml) of different plant extracts against different pathogens.

Chapter Four

Discussion and Conclusions

Scientific studies of plants secondary metabolites represent a global innovative strategy for the production of novel plant-derived pharmaceuticals drugs. Since plants contain an outstanding range of biological active compounds with intensive healing properties [119]. In this direction, characterization the profile of polyphenol-containing botanical extracts and their health-related properties have been a topic of the recent research. Since polyphenols appears as a prominent class of natural phytochemical compounds with enormous biological effects [120,121]. Thus, this investigation was designed in order to isolation and identification of the polar poly phenolic compositions of *S. viticina* plant, in addition to the antioxidant, and antimicrobial activity.

4.1 The Chemical Composition

In this study, seven extracts of *S. viticina* were obtained, one of them was crude R, another (D) was semipure fraction (47.26% purity), but the others (A, B, C, E, F) were almost pure fractions. Recently, *Venditti et al*. reported the identification of eight compounds from ethanolic extracts of *S. palustris* plant, of which verbascoside, echinacoside, and two of isoscutellarein derivatives were the major components [122].

4.2 Antioxidant Activity

The antioxidant capacity of the obtained extracts from *S. viticina* were measured using DPPH assay because it’s simple and highly sensitive method [123]. The results of the current work showed that the phenolic

extracts of *S. viticina* exhibited different antioxidant activity. R, B, F extracts had the highest antioxidant capacity with an IC_{50} value of 18.58 µg/mL, 54.37 µg/mL, 58.62 µg/mL, respectively, less than that of the positive control (Trolox) which has an IC_{50} value of 2.70 µg/mL. A previous study performed by *N.Jaradat* and *N.Al-Maharik* revealed that the essential oil of *S. viticina* exhibited antioxidant activity with an IC_{50} value of 19.95 µg/mL [33], higher than that of the present study extracts except R. Furthermore, a study conducted by *Kuki´c et al.* reported that *S. anisochila, S. beckeana,* S. *plumose,* and *S. 44lpine ssp. Dinarica* have antioxidant activity with IC_{50} values of 17.9, 20.9, 101.61, and 26.14 µg/mL, respectively [124].

4.3 Antimicrobial Capacity

The microdilution assay was used to screen the antimicrobial activity of phenolic S. *viticina* extracts against four gram-negative bacteria included *Escherichia coli*, *Pseudomonas aeruginosa, Proteus vulgaris*, *klebsiella pneumonia*, and two gram-positive bacteria included *Staphylococcus aureus*, and MRSA, in addition to the yeast *C. albicans*. Some of *S. viticina* extracts inhibited the growth of the screened bacterial strains. In particular, the extracts **B** and **C** exhibited strong antibacterial activity against MRSA with MIC value of 39 µg/mL. Which is equal to inhibition activity of extract **B** against *s.aureus* and two folds more potential than the inhibition activity of extract **R** against *s.aureus* (MIC = 78 µg /mL). That is mean the obtained extracts revealed stronger activity against gram-positive strains compared to the gram-negative ones. Typically, gram-negative bacteria are

less sensitive because their cell walls have an outer membrane which composed of proteins and lipids that prevents easy penetration of compounds]125]. However, all extract didn't have inhibition activity against the fungal strain *C. albicans*. Recent study conducted by *N.Jaradat* and *N.Al-Maharik* showed nearly matching result in the screening of the antibacterial activity of the *S. viticina* essential oil. And the essential oil of *S. viticina* inhibited the growth of the *C. albicans* with MIC value of 312.5 µg /mL [33]. While *C. albicans* strain was resistance against all the extracts in the present study.

4.4 Conclusions

The HPLC analysis confirmed the purity of five fractions in the phenolic S. *viticina* extract (A, B, C, E, F), and one fraction (D) with only 47.26 % purity, in addition to one crude extract (R). All extracts showed DPPH scavenging activity in the following order: R> B> F> D> C> A. Some extracts also showed very strong antibacterial effects, of which the strongest were extracts B and C against MRSA, with MIC value of 39 µg/mL. But all extracts had no antifungal activity against the yeast *C. albicans*. Briefly, polyphenol-containing polar extracts from *Stachys viticina* contain pharmacologically active fractions which could be further confirmed and examined clinically for their therapeutic potential and for the design of new natural medicinal preparations.

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Appendix

Appendix 1

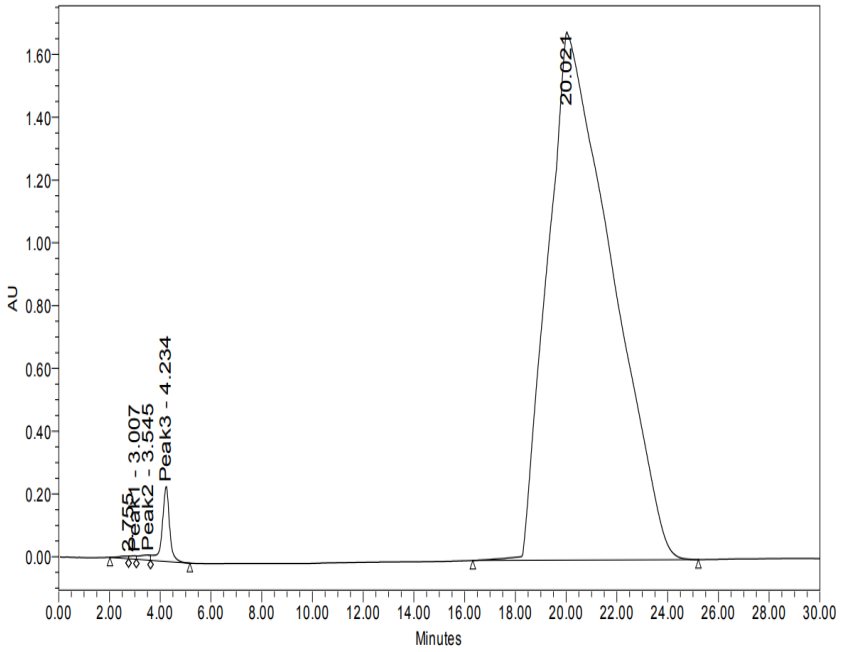

Fig.a.1: HPLC chromatogram for fraction A.

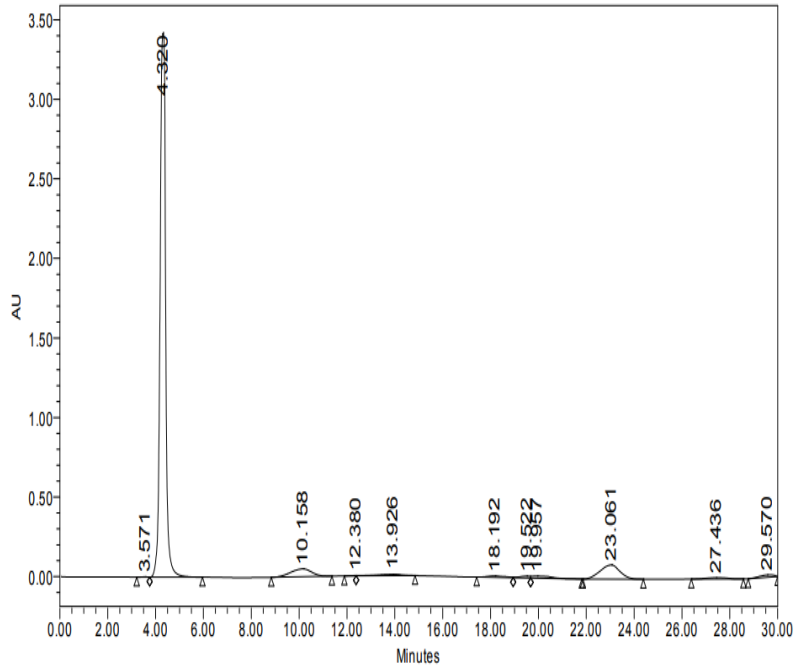

Fig.a.2: HPLC chromatogram for fraction B.

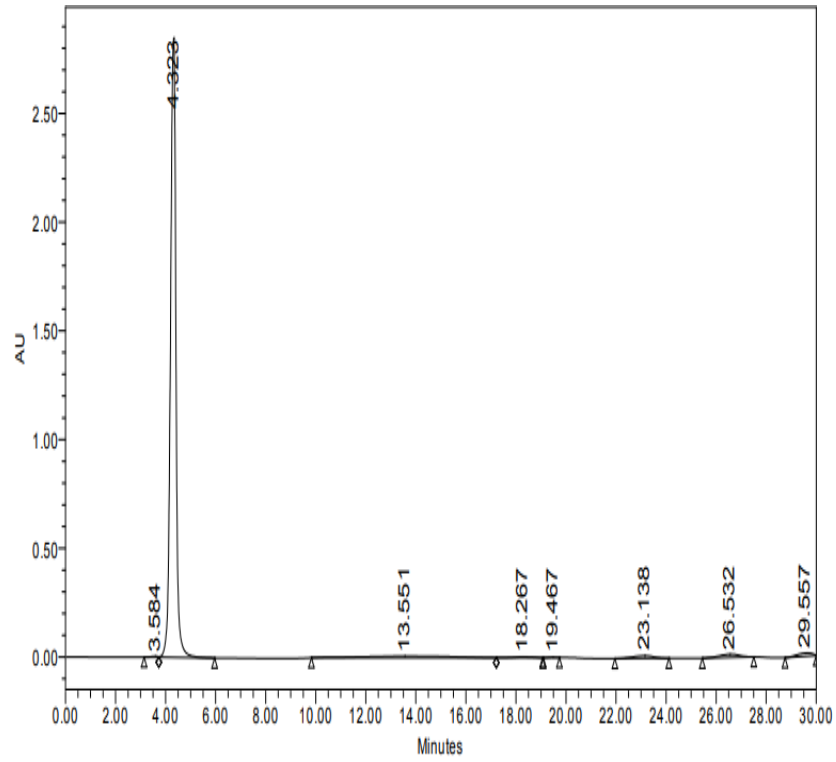

Fig.a.3: HPLC chromatogram for fraction C.

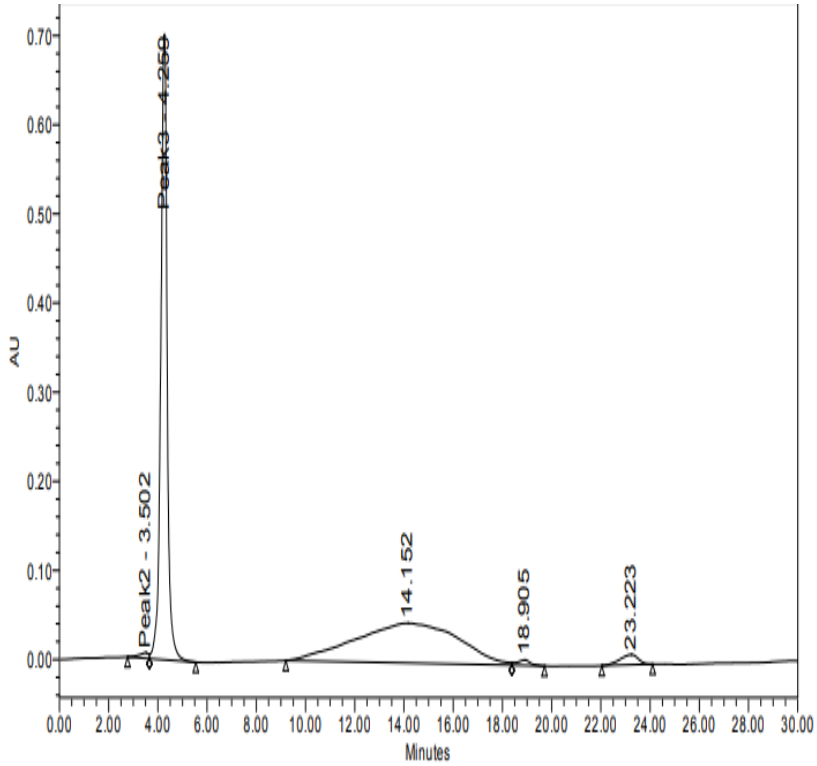

Fig.a.4: HPLC chromatogram for fraction D.

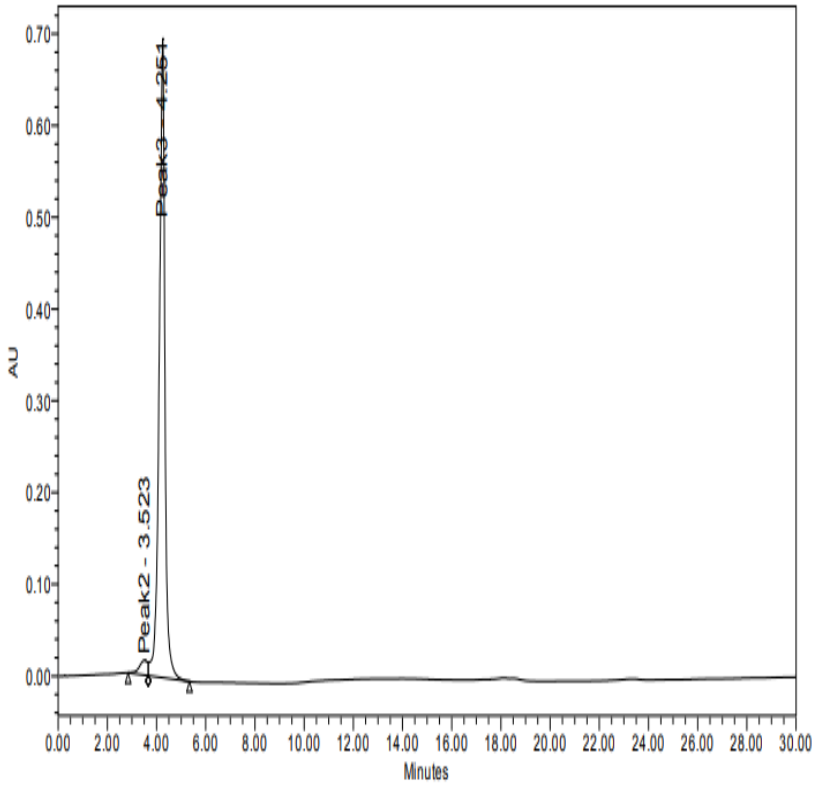

Fig.a.5: HPLC chromatogram for fraction E.

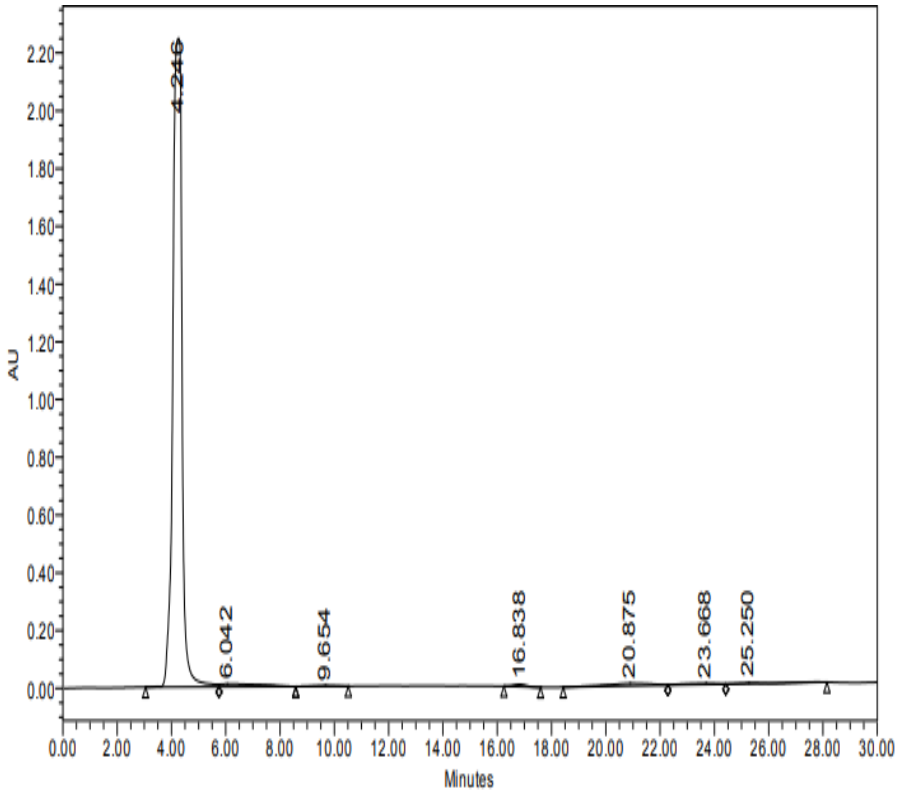

Fig.a.6: HPLC chromatogram for fraction F.

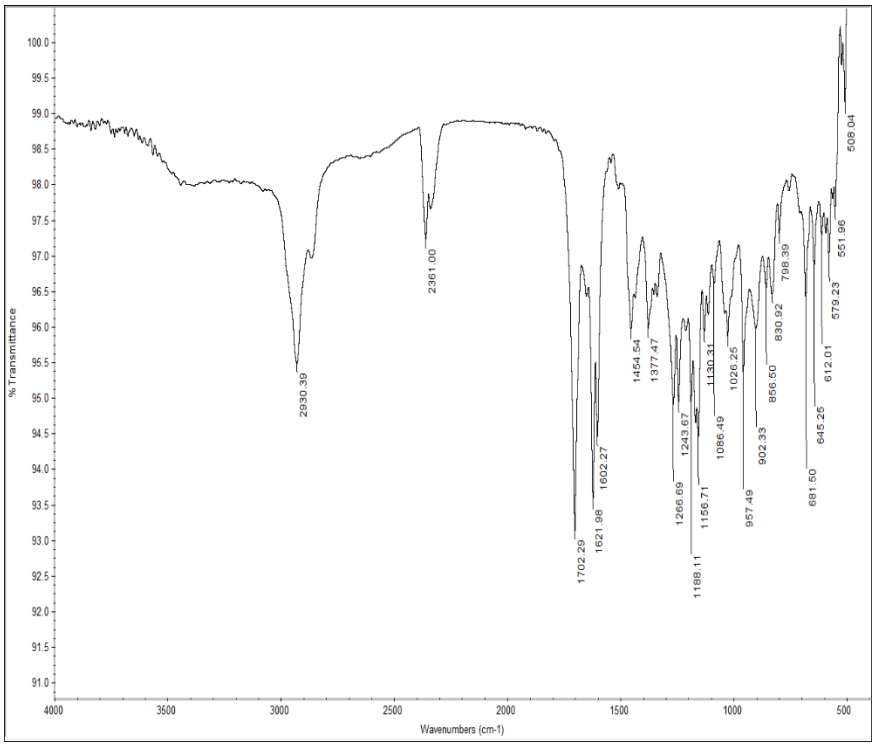

Fig.a.7: IR spectra for fraction A.

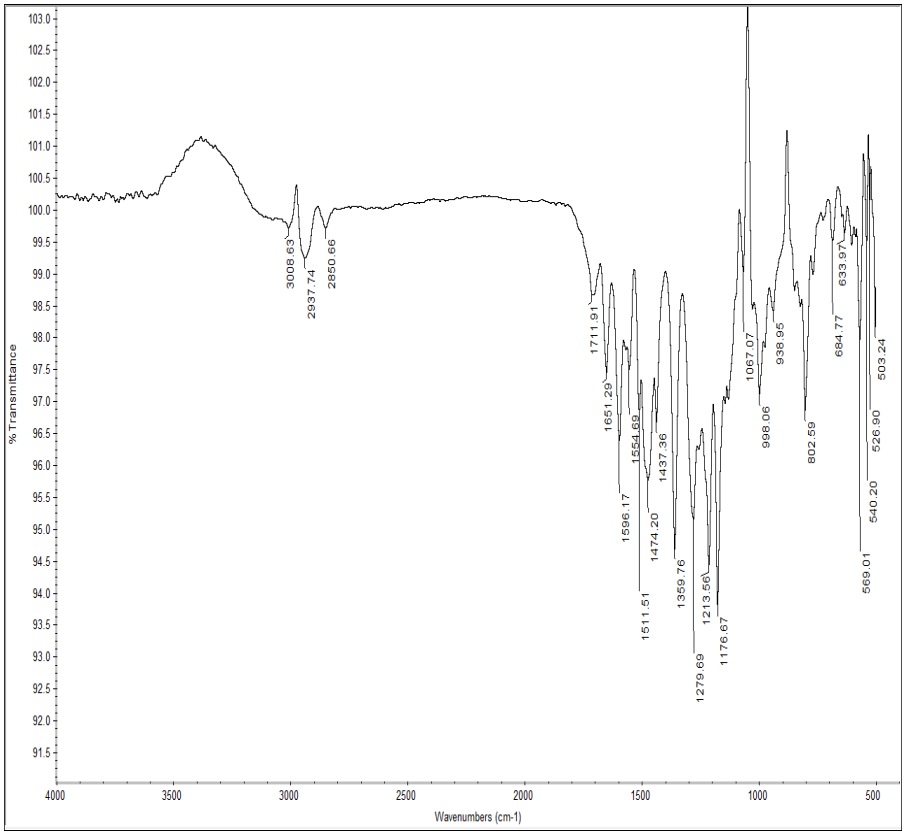

Fig.a.8: IR spectra for fraction B.

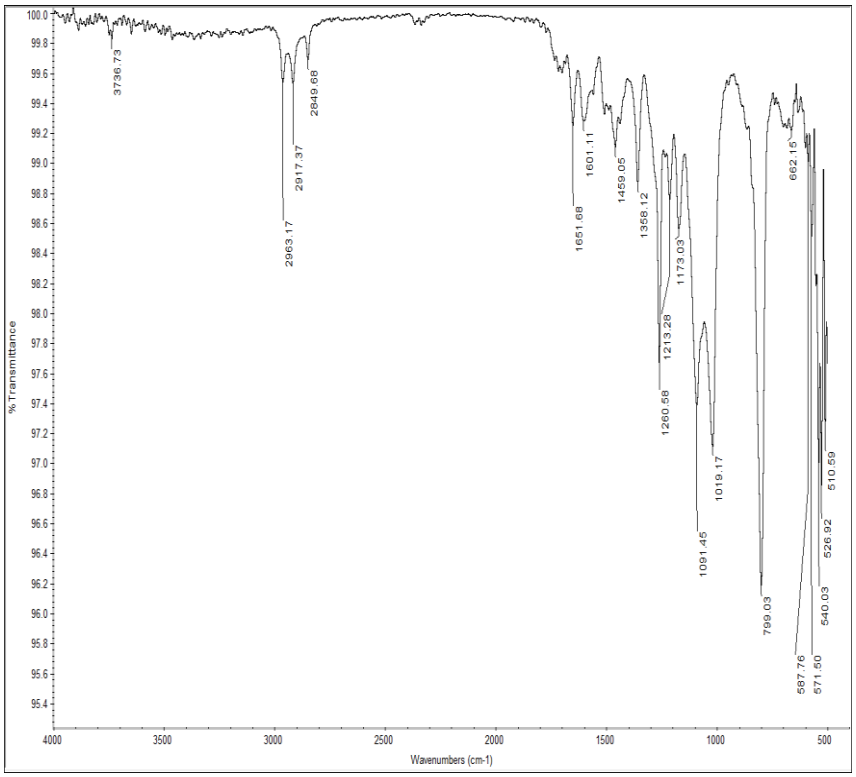

Fig.a.9: IR spectra fraction C.

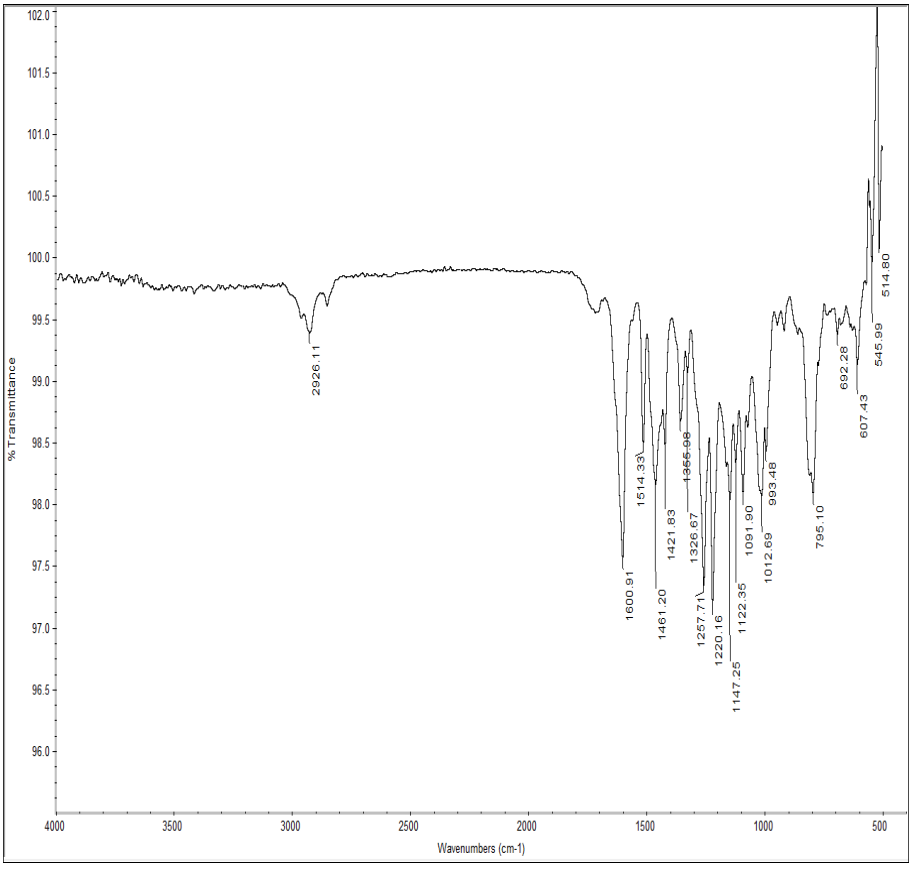

Fig.a.11: IR spectra for fraction D.

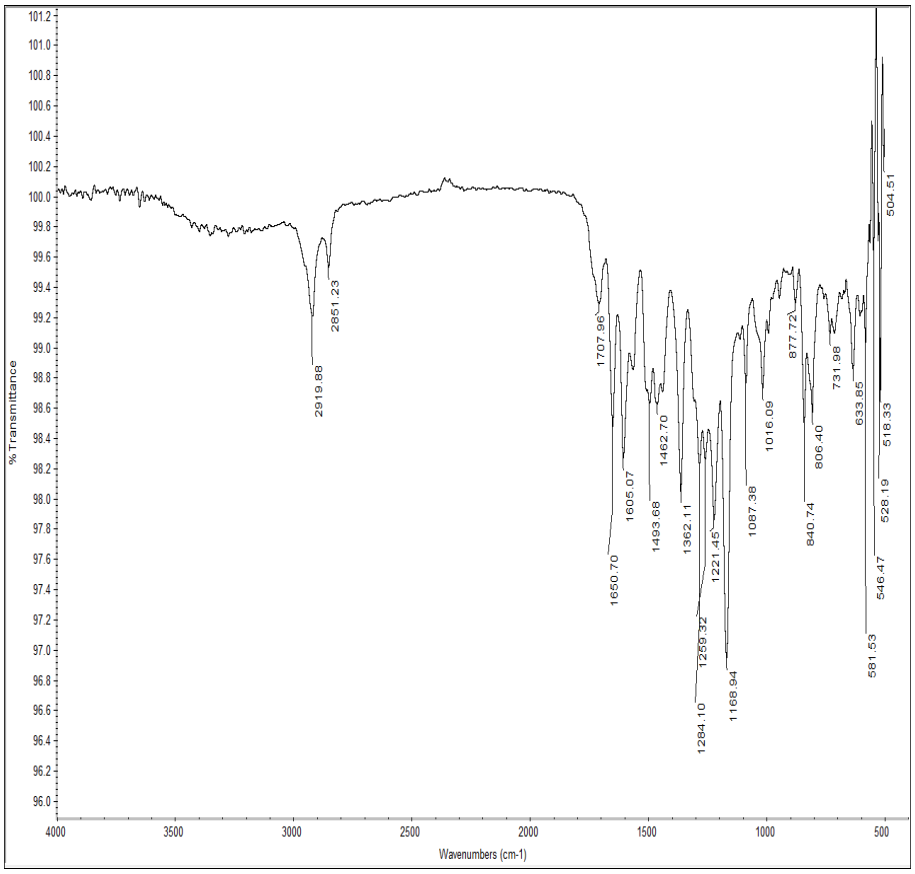

Fig.a.10: IR spectra for fraction E.

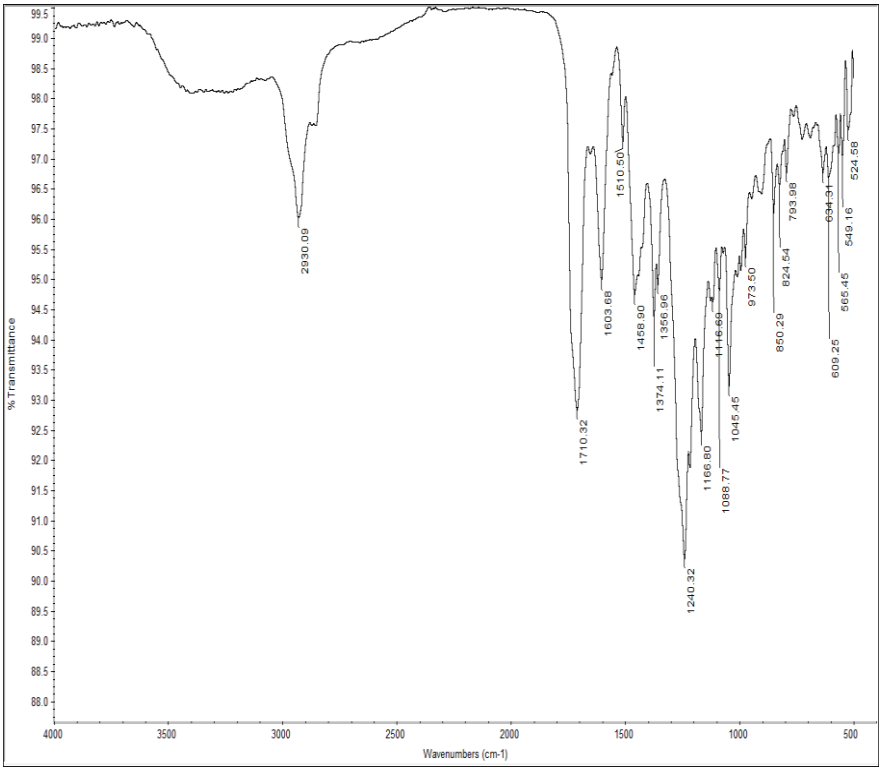

Fig.a.12: IR spectra for fraction F.

جامعة النّجاح الوطنية

كليّة الدّراسات العليا

الكشف عن محتويات نبتة البطنج الفلسطيني ونبتة البطنج الكرمي من المركبات متعددة الفينولات في مستخلصهما القطبي وتقيم تأثيرها الدوائي

إعداد

ليلى محمد عباس بدوان

إشراف

د. نواف المحاريق

د. نضال جرادات

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الكيمياء، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس – فلسطين.

2021

الكشف عن محتويات نبتة البطنج الفلسطيني ونبتة البطنج الكرمي
من المركبات متعددة الفينولات في مستخلصهما القطبي وتقيم تأثيرها الدوائي

إعداد
ليلى محمد عباس بدوان
إشراف
د. نواف المحاريق
د. نضال جرادات

الملخص

نبتة Stachys viticina نبتة عطرية تنتشر في دول البحر الأبيض المتوسط ومن بينها فلسطين, وتم استخدامها منذ القدم في الطب الشعبي. تهدف هذه الدراسة إلى الكشف عن المكونات الكيميائية والخصائص العلاجية للمركبات المتعددة الفينولات في المستخلص القطبي لنبتة *Stachys viticina*.

تم عزل سبعة مستخلصات من نبتة *Stachys viticina* باستخدام silica gel column chromatography وسميت المستخلصات كالتالي (A, B, C, D, E, F, R). تم التحقق من نقاوة المركبات باستخدام تحليل HPLC. وتم الكشف عن المجموعات الوظيفية في المستخلصات باستخدام تقنية IR spectroscopy.

تم اختبار فاعلية المستخلصات (A–D, F, R) كمضاد للأكسدة من خلال تثبيط free radical (2, 2–diphenyl–1–picrylhydrazyl) أظهرت هذه المستخلصات تراكيز IC_{50} كالتالي: 54.37 µg/mL,85.88 µg/mL ,77.58 µg/mL ,68.36 µg/mL ,58.62 µg/mL, 18.58 µg/mL, بالترتيب.

تم فحص فعالية المستخلصات كمضادات للميكروبات باستخدام طريقة (microdilution) وتم اختبار ستة سلالات من البكتيريا وسلالة واحدة من الفطريات. أظهر المركب (C) أعلى نشاط بحد أدنى من التركيز (MIC) يساوي 39 µg/mL, وذلك ضد نوعين من البكتيريا methicillin–resistant *staphylococcus aureus* (MRSA), *S. aureus*.