



Hebron University

College of Graduate Studies and Academic Research

**Antioxidant, phytochemical, nutritional composition, and biological activity of
selected fig genotypes (*Ficus carica* L.)**

By

Areej Kamel Ishnaiwer

Supervisor

Prof. Dr. Rezq Basheer-Salimia

Co-supervisor

Dr. Abdel Qader A. Qawasmeh

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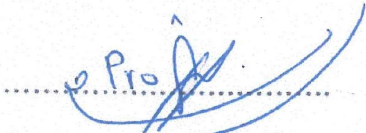
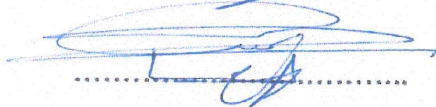
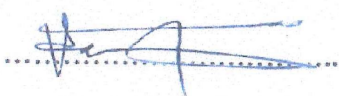

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Prepared by

Areej Kamel Ishnaiwer

This thesis was successfully defended and approved on Jan 19, 2023

By

Examination Committee	Signature
Prof. Dr. Rezaq Basheer-Salimia	Supervisor 
Dr. Abdel Qader Qawasmeh	Co-supervisor 
Dr. Hatem Amin Hejaz	External examiner 
Dr. Sami Makharza	Internal examiner 

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Dedication

I would like to dedicate this thesis to my father, my mother, my brothers, my sisters, and husband Mahmoud, and my kids Jude, Layla, Dima, and Obada.

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List of Abbreviations

°C	Celsius
µg	Microgram
µL	Microliter
ABTS ⁺	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
AOAC	Association of Official Analytical Chemists
AST, ALT	Aspartate transaminase, alanine transaminase
CAC	Codex Alimentarius Commission
Cm	Centimeter
CpHV-1	caprine herpesvirus-1
DNA	Deoxyribonucleic acid
DPPH [•]	2,2-diphenyl-1-picrylhydrazyl hydrate
DW	Distilled water
<i>E. coli</i>	<i>Escherichia coli</i>
F	Fruits
F.C	Folin ciocalteu reagent
FAO	Food and agriculture organization
FMD	fig mosaic disease
GC-FID	Gas chromatography equipped with flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GIT	Gastrointestinal tract
ICP-OES	Inductively coupled plasma-optical emission spectrometry
<i>K. pneumoniae</i>	<i>Klebsiella pneumoniae</i>
L	Leaves
LDL	Low density lipoprotein
M	Mwazi
MAP	Modified atmosphere packaging

mgGAE/g	milligrams of gallic acid equivalents per gram
Nm	Nanometer
NMR	Nuclear magnetic resonance
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
RBC	Red blood cell
RNA	Ribonucleic acid
ROS	reactive oxygen species
Rt	Retention time
S	Smari
S	Skins
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SD-GC-MS	Steam distillation Gas chromatography-mass spectrometry
Sh	Shhami
T2DM	Type 2 diabetes mellitus
TA	titratable acidity (TA
TPC	Total phenolic content
UNECE	United Nations Economic Commission for Europe
UV/Visible spectrophotometer	Ultraviolet-visible spectrophotometer
VOs	Volatile oils

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Abstract

Fig (*Ficus carica*) is one of the most important deciduous trees that are commonly used in the human diet as well as a traditional medicine to treat various illnesses such as leprosy, nose bleeding, antipyretic, emollient, laxative, liver disease, and others. Phytochemical characters, minerals, antioxidant capacity, and antibacterial properties of leaves, fruits and skins of the three fig genotypes (Shhami, Mwazi, and Smari) that commonly grown in Palestine were determined. Matured fruits and leaves of the three fig genotypes were collected from Hebron city, Palestine and accordingly dried. The volatile compounds were determined for the first time by GC-MS in the electron impact mode. The antioxidant activities were estimated by DPPH[•] and ABTS^{•+} methods while the antimicrobial activity was examined by agar well diffusion method for determining the inhibitory zone diameters. ICP-OES was used to determine the content of minerals in dried leaves, fruits and skins. Total phenolic content (TPC) was determined by using the Folin-Ciocalteu method.

The results revealed that fig leaves presented significantly higher fiber and ash contents over fig fruits and skins ($p < 0.05$) among the three examined genotypes. Accordingly, calcium (Ca), magnesium (Mg), Iron (Fe), and Boron (B) were also higher in the leaves of the three fig genotypes followed respectively by fig skins and fig fruits. The results indicated that the leaves of Shhami, Smari, and Mwazi fig genotypes exhibited higher antioxidant activities by DPPH[•] (85.2, 82.6, 83.4%) and ABTS^{•+} (75.1, 59.5, 80 %) respectively. Furthermore, similar trends also go with total phenolic content (TPC) in which they presented higher values with (1980.6 mgGAE/g, 1793.3 mgGAE/g, 2135.0 mgGAE/g), respectively compared with skins and fruits. Phytochemical analysis of fig samples including screening of saponins, steroids, tannins, terpenoids, phenolic groups, and other secondary metabolites revealed that fig fruit, skin, and leaves are a good source of saponins, steroids, and terpenoids. On the other hand, tannins and phenolic groups were only found in the leaves.

GC-MS analysis showed the presence of at least six volatile compounds, the major ones that were detected in the methanolic extract of fig samples were pyrazole benzotriazine (Rt=17,715), naphthaline dione (Rt=20,53) and phytol (Rt=20,806). Other minor volatile compounds identified include but are not limited to caryophyllene, 1-H indole ethylamine, and 4-acetyl isocoumarin.

Indeed, the major volatile compounds are found in a high quantity in the leaves, followed by fruits, and at traces amount in fig skin.

Our results demonstrate a significant linear correlation between the obtained antioxidant activity and phenolic and volatile contents.

Methanolic extracts of fig samples were investigated for their antimicrobial activity against five bacterial strains, one gram-positive (*Staphylococcus aureus*) and four gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus*). The results revealed that only the methanolic extract of Smari and Shhami leaves displayed antibacterial activity against the gram-negative pseudomonas bacteria with a zone of inhibition of 36.86 % and 32.16% respectively, compared with the positive control (meropenem).

From our results, it is clear that the leaves of figs have advantages over the skin and the fruits as it contains higher components, have more minerals and have higher antioxidants and antimicrobial activities. However, for any further investigations on figs; the leaves should be taken instead of skin or fruits. Thus, the fig leaves could be a potential antioxidant natural source that could have a great importance as therapeutic agents to prevent or slowing the progresses of oxidative stress related diseases. Our results revealed a considerable variation in the phytochemical, antioxidant activity, fibers, ash, total proteins and antibacterial activity for the fig different parts as well as the three genotypes studied.

Keywords: *Ficus carica*, nutritional values, antioxidant activity, phytochemical, antimicrobial, phenolic contents.

Chapter 1: Introduction

1. Introduction

Ficus carica also known as 'fig', a deciduous tree related to the Moraceae family, is one of the earliest cultivated fruit trees worldwide which is mainly used for dry and fresh consumption (Çalışkan & Polat, 2011). The world fig exchanges are common as dried (77%) which are mostly exported from Turkey, the USA, and Spain (Nuri & Uddin, 2021). Statistically, yearly fig production reaches over one million metric tons around the world (FAO, 2009). The Mediterranean region is the most important fig production region presenting more than 82% of the total world annual production (Basheer-Salimia *et al.*, 2012 c). Palestine is considered one of the original countries of figs due to its wide range of environmental conditions and rich natural biodiversity in which the fig trees are mainly grown in marginal land (with grape and/or olive), around the orchards, and in-home gardens (Radwan *et al.*, 2020). Recent studies showed that twelve fig genotypes exist in the southern regions of West Bank, Palestine (Radwan *et al.*, 2020), having different traditional names depending principally on the country of origin, maturity date, skin ground color, and internal color (Basheer-Salimia *et al.*, 2012 a).

This crop is considered one of the most important constituents of the Mediterranean diet mainly due to its therapeutic and pharmacological benefits as antioxidant, anti-mutagenic or anti-carcinogenic, anti-inflammatory, and antimicrobial activities (Çalışkan & Polat, 2011; Radwan *et al.*, 2020). Compared with other fruits, *F. carica* is a rich source of vitamins, carbohydrates, minerals, sugars, phenolic compounds (mainly anthocyanin), organic acids, and a high amount of crude fiber (Mawa *et al.*, 2013; Basheer-Salimia *et al.*, 2012 b).

Traditionally, people used figs as a traditional medicine for centuries with all of their different parts including roots, latex, seeds, leaves, fruit, and bark (Shahinuzzaman *et al.*, 2020), to treat various illnesses (Salma *et al.*, 2020) such as leprosy, nose bleeding, antipyretic, emollient, laxative, liver disease and in the treatment of various inflammation. In addition, fig roots were used as a tonic in the treatment of ringworm infection. Latex is used as a diuretic, expectorant, and anthelmintic and is applied locally to treat the wart. Seeds are used as edible oil and lubricant. Leaves are used in the treatment of contact dermatitis, vermifuge, and as antidiabetic agents (Mawa *et al.*, 2013; Badgujar *et al.*, 2014).

In Palestine, very rare studies have been oriented toward evaluating the antioxidant, phytochemical and antibacterial activities of fig fruit extracts involving different genotypes. Therefore, the main goal of the present research is to compare the nutrient values, antioxidant, phytochemical, and antibacterial properties of three different Palestinian fig genotypes.

Chapter 2: Literature Review

Chapter Two: Literature Review

2.2. General aspects

2.2.1. Fig classification (Salma *et al.*, 2020)

Family: Moraceae

Genus: *Ficus*

Species: *carica*

2.2.2. Origin and distribution

Despite its cultivation and spread in various places worldwide, however, it mainly originated from Asia Minor and spread to Persia, Syria, and the Mediterranean region by humans and currently is considered an imperative world crop (Mawa *et al.*, 2020)

2.2.3. Production areas and levels

Turkey and Egypt are considered the most important countries with the highest fig production representing approximately 43% of the world production, followed by Algeria, Morocco, and Iran. These five countries represent 71% of the total fig production. Moreover, Turkey is the major fig exporter followed by Austria, Spain, and Italy. In addition, France, Germany, Austria, and United Kingdom are the main importing countries of fig (Isa *et al.*, 2020).

2.2.4. Description of the plant

The fig tree is generally a 4.57-6.1 meter-tall, large deciduous tree or large shrub with many spreading branches from a trunk more than 2 meters in diameter. The tree has grey bark, smooth or dull or delicately hairy. The leaves are long with multi-lobes, 10-20 blades, petiole 5-7.5 cm, rough above and juvenile below. Unisexual flowers, that closely crowded on the inner surface of the large, hollow receptacle. Male flowers are few, found on the lower part of receptacles, and female flowers are found on the upper part of receptacles. The fruit is a fleshy hollow receptacle of a pear shape form (Salma *et al.*, 2020).

2.2.5. Fig genotypes and cultivars

Fig is considered one of the largest genera of angiosperm that mainly grow in tropical and subtropical regions worldwide (Badgujar *et al.*, 2014), and more precisely in Turkey (Çalışkan, & Polat 2008), Iran (Fatahi *et al.*, 2017) and Egypt (Mon *et al.*, 2020). For example, many cultivated and wild forms of fig can be found in Turkey, such as Sarilop, Bursa Siyah, Yesilgu, Morguz, and others, that have a great diversity of color, shape, and flavor mainly for fresh consumption (Çalışkan, & Polat .2008).

In Palestine, due to the wide range of environmental conditions many cultivars are existing and are currently grown all over the country and more precisely in marginal lands, home gardens, and at the periphery of orchards (Radwan *et al.*, 2020). However, fig landraces and cultivars are either misidentified or have different names in different areas, this is due to both, the exchange and spread of figs into other growing regions around the world, and the different factors that determined fig names such as fig skin ground color, internal color, country of origin and maturity date (Basheer-Salimia *et al.*, 2012a). Scientifically, the identification of different fig plant cultivars could be achieved by morphological (visible) markers, molecular markers, and biochemical markers (Basheer-Salimia *et al.*, 2012b). Indeed, morphological markers are still useful tools that have been used for many years for the identification and discrimination of plant genotypes (Basheer-Salimia *et al.*, 2012b). In general, many pomological and morphological markers were used to distinguish between fig genotypes such as fruit ripening, fruit color, and shape, ostiole characteristics, pulp texture and flavor, bud break, leaf and petiole descriptions (**Table 2.1**) (Basheer-Salimia *et al.*, 2012a).

Table (2.1): Descriptors of some common Palestinian fig genotypes

Descriptor	Genotypes		
	Shhami	Mwazi	Smari
Beginning of ripening	1-15 August	1-15 August	15-31 August
Harvested period.	More than 60 days	21-40 days	21-40 days
Fruit External Color	Brown green	Green purple	Black- Purple
Skin cracks	Cracked skin	Minute	Minute
Fruit shape	Ovoid	Pyriformed	Pyriformed
Fruit weight	20-39 g	20-39 g	20-39 g
Fruit firmness	Soft	firm	Medium
Fruit Neck length	5-15 mm	Less than 5 mm	5-15 mm
Fruit Stalk length	4-8 mm	4-8 mm	Less than 4 mm
Ostiole type	Open	Open	Closed
Skin peeling	Easy	Easy	Difficult
Internal color	Pink	Amber	Amber
Flesh thickness	25-35mm	25-35 mm	25-35 mm
Bud break	Mar 15-30	Mar 15-30	Mar 15-30
Leaf color	Dark green	Dark green	Light green
Lobes number	Five	Five	Five
Leaf venation	Apparent	Apparent	Apparent
Apex shape	Obtuse	Triangle-obtuse	Triangle-obtuse
Leaf roughness	Fairy rough-rough	Fairy rough	Smooth-fairy rough
Leaf area	Less than 160cm ²	Less than 160cm ²	Less than 160cm ²
Petiole length	50-80 mm	Less than 50 mm	Less than 50mm
Beginning of the leaf drop	November	December	November

2.2.6. Environmental requirements

2.2.6.1. Climacteric requirements

In general, fig trees tolerate drier conditions more than other fruit trees, but it still requires enough water, which depends on soil type, rainfall, tree size, and vigor (Isa *et al.*, 2020). The optimal conditions for fig production are temperate and rainy winters with a summer temperature range between 30-40 °C, and relative humidity of about 45-50% (Arpaci *et al.*, 2018). Indeed, a dry climate with moderate temperature is preferable to produce large fig fruits (Khan, 2018).

2.2.6.2. Soil requirements

Fig trees grow well in most soil types (Azmi *et al.*, 2020). But it performs best in deep, well-drained black and sandy loams, that could tolerate a high level of chloride salts but not sodium salts which are present in the soil (Khan, 2018). Additionally, red loams are the most suitable for fig cultivation (Khan, 2018), and the ideal pH should be between 6 to 6.5 (Isa *et al.*, 2020).

2.3. Cultivation practices

2.3.1. Plant propagation

Generally, figs are propagated by vegetative means *via* rooting-cutting indoors, and rooting-cutting outdoors, less common by layering (Badgujar *et al.*, 2014; Khan, 2018), and very rare through tissue culture. However, seed propagation also existed but at breeding levels.

2.3.2. Planting

However, fig trees that are raised through cuttings, layering, tissue culture, or seeds can be planted at any time during the year, but the spring season is preferred for planting.

2.3.3. Irrigation and fertilization

Despite its high tolerance to drier conditions, the plant still required enough water for optimal production and performance (Isa *et al.*, 2020). In fact, [fig trees should be irrigated immediately as soon as possible after planting. Irrigation at 10-20 days' intervals is required during the summer months. Moreover, excessive irrigation during the time of fruit ripening

and development causes fruit splitting and inferior quality (Khan, 2018). Also, a proper fertilizer should be applied to enhance growth with improved fruit yield and quality, because any deficient or excess nutrient can lead to a reduction in crop yield and fruit quality (Isa *et al.*, 2020). The use of organic fertilizer that is highly soluble in water or soil solution is effective and has no harmful effect on the plant; moreover, it increases nutrient absorption and improves the vegetative and root traits of the plant if it is added to the soil or spraying on the plant (Rzouki *et al.*, 2019).

2.3.4. Pests and diseases control

Pests and pathogens attack surely reduce fig growth, development, and yield production. Indeed, the fig is very sensitive to rain which mainly increases the risk of pests and pathogens attacking and causing fruit rot and leaves necrosis (Shamin-Shazwan *et al.*, 2019). Additionally, fig mosaic disease (FMD) is the most common disease that attacks fig trees and causes many disorders in fig growth that finally leads to poor fruit quality, shortening of the tree life, and low yield production (Shamin-Shazwan *et al.*, 2019). Therefore, pesticides such as insecticides, fungicides, herbicides, acaricides, and nematicides are approved for use on figs to minimize the damage that might be caused by insects, fungal infections, and other pests (CAC, 2008).

2.3.5. Harvesting

Figs become fruitage from the first year, especially for the tissue culture-raised plants. The fruit mostly should be harvested when they are ripe and mature (Khan, 2018).

2.4. Post-harvesting handling

2.4.1. Field heat removal

The field containers should be put under shade in the interval between harvest and transport to the packinghouse because direct exposure of fig fruits to the sun will increase the internal temperature and speed up the chemical process in fruits such as rancidity in fruits, deterioration of chlorophyll pigments and flavor changes. Other chemical changes that could occur are lipid oxidation and non-enzymatic browning. This led to a significant defect in sensory quality and

changes in the color and flavor of fruits. Therefore, the elaboration in temperature management requires rapid removal of heat that could be reached through different techniques such as hydro-cooling, packaging in iced containers, evaporating cooling, room cooling vacuum cooling, and others. This cold chain system is an important need to be sure that the value chain of fruits is being kept from the farm gate to the consumer (Adewoyin *et al.*, 2022).

2.4.2. Grading

The grading system establishment and enforcement are mainly used to prevent fruit losses during postharvest marketing and to protect the consumer. However, the current grading systems have strict limitations that mostly led to a high incidence of fruit wastage, but the grading systems for minor crops such as fresh figs are not well defined to non-existence (Kong *et al.*, 2013).

The United Nations Economic Commission for Europe (UNECE) categorized the fresh fig fruits into two classes, and considered the fruits of the fresh fig that might have slight longitudinal skin cracks and split of the ostiole that not exceed 30mm to be of good quality (class I), while figs with more skin damage with the total length of the split and the ostiole not exceed 40mm is class II (UNECE, 2010; Kong *et al.*, 2013).

2.4.3. Packaging and labeling

Modified atmosphere packaging (MAP) is considered one of the most important methods that extend the shelf life of fresh fruits and vegetables, which depends on modifying the level of oxygen (O₂) and carbon dioxide (CO₂) within the polymeric film packing atmosphere. Moreover, the desirable levels of O₂ and CO₂ in the film could affect the product metabolism or the activity of organisms that can increase the product shelf life by reducing decay, respiration, and ethylene production of fruit, and preserving firmness (Tuai *et al.*, 2020).

Cantín *et al.* (2020) demonstrated the benefits of using MAP technology as one of the optimal postharvest strategies to control the storage potential of fresh fig fruits because it can reduce decay and maintain the fresh fig fruit quality for up to 2 weeks, due to reduced respiratory and ethylene production rate in fig fruits, also delay the decrease of titratable acidity (TA), firmness and ripening of the fresh fruit.

2.4.4. Storage

Some scientific sources considered the storage at (0-6 °C with 90- 95% relative humidity (low temperature and high relative humidity) to be the optimal conditions for fresh fig storage to maintain its quality and control spoilage (Tuai *et al.*, 2020), but still there is a confusion among growers and retailers about the best storage conditions that could be used for different fig cultivars, and the information about the postharvest tools that tend to extend the shelf life of fresh fig is rare (Cantín *et al.*, 2020).

2.4.5. Chemical and physical treatments

Despite the highly perishable fig fruits are difficult to be stored for long periods, the coating is one of the main methods that extending the shelf life of fresh fig fruits due to its ability to thicken and harden the fruit skin without losing its nutritional content (Irfan *et al.* 2013). The coating could reduce the metabolic processes and water loss by providing a barrier to moisture, oxygen, and solute movement from such fruits (Nor and Ding, 2020). Moreover, this coating material must be edible and able to function in retarding the ripening, preventing enzymatic browning, or holding the fruit's fresh flavor and aroma.

Calcium chloride is considered one of the major coating materials that can be used as a post-harvest treatment of fresh fig fruits and other fruits and vegetables because it can both, strength the fruit cell wall and protect it from pectin degrading enzymes that lead to fruits softening and reducing the pathogen germination (Irfan *et al.* 2013). Irfan *et al.*, (2013), revealed that treated fig fruits with 4% of CaCl provide a positive effect on fruit quality, in which the fruit texture, color, titratable acidity, and ascorbic acid content were maintained for 14 days of storage as initial quality, compared to untreated control fig fruit that only recorded 7 days of storage life under the same conditions. Also, Villalobos *et al.* (2017, 2016) showed that the phenolic and isoflavones compounds extracted from soybean meal are a suitable coating material to treat fig fruits, as it has an antioxidant and anti-fungal activity that reduces fruit spoilage (del Carmen Villalobos *et al.*, 2016).

Marpudi *et al.* (2013) reported that Aloe vera gel which is mainly composed of polysaccharides, soluble sugars, proteins, vitamins, and minerals can preserve the physio-

chemical qualities of fig fruits, such as color, firmness, total acidity, and reduced respiration rate and weight loss (Marpudi *et al.*, 2013).

2.5. Food, medicinal and therapeutic

2.5.1. Fig as food

Fig is considered one of the most important constituents of a Mediterranean diet, as a high-energy food for all the social strata during the entire year due to its easy preservation process (Falistocco, 2020). The importance of figs is also demonstrated in Qur'an with olives, dates, and grapes as gifts and heavenly fruits of God (Nuri & Uddin, 2021). Fig fruits could be consumed fresh or dried and it is highly nutritious that provided a rich source of minerals, vitamins, carbohydrates, and dietary fibers (Khan, 2018).

2.5.2. Fig as a cosmetic ingredient

Psychological stress can increase the oxidation and inflammation processes in the human body, mainly the skin, through collagen and elastic fiber degradation and finally wrinkle formation (Dini *et al.*, 2021). Research on new cosmetic materials revealed that natural ingredients are generally safer than synthetic material to relieve the psychological stress diseases affecting the skin. Interestingly, Fig fruits and leaves are one of the most important natural sources that mainly act as an anti-oxidant because it has a high level of anti-oxidant compounds such as vitamin C, phenolic compounds anthocyanins, and carotenoids (Khan *et al.*, 2014). These natural components have been used in cosmetic industries as co-emulsifiers, emollients, creams, and suspensions for skin care mainly by inhibiting protein oxidation, the main threat to skin functionality and integrity, also able to moisturize and stop excessive water loss of the skin, protect the epidermal lipid barrier against the microbial attack and physical injuries to support skin integrity (Dini *et al.*, 2021; Khan *et al.*, 2014; Ivanov *et al.*, 2018).

2.5.3. Medicinal uses

2.5.3.1. Traditional uses as medicine

Fig with all its different parts like roots, bark, leaves, fruit, latex, and seeds has been used for the treatment of various illnesses for centuries. Also, it was used in combination with some natural food such as honey and milk. In Mediterranean countries, the fig is called "the poor man's food", and is regularly used dried or fresh (Badgujar *et al.*, 2014).

Fig fruits were used in leprosy, nose bleeding, antipyretic, emollient, laxative, liver disease, and in the treatment of various inflammation (Badgujar *et al.*, 2014). In addition, fig roots were used as a tonic in the treatment of ringworm infection. Latex is used as a diuretic, expectorant, and anthelmintic and it could be applied locally to treat the wart. Seeds are used as edible oil and lubricant. Leaves are used in the treatment of contact dermatitis, as a vermifuge, and as an antidiabetic agent (Mawa *et al.*, 2013).

2.5.3.2. Nutritional value of fig

Nutrition is an applied medical science that studies the action of biologically active components of food with their role in maintaining human health. The main human nutritional needs are energy and raw materials for the various activities and processes that occur in the body. In addition to water, humans need five types of nutrients from food, which are carbohydrates, proteins, and fat which are relatively required in large amounts and known as macronutrients. While the other two types, vitamins, and minerals, are required in small amounts and are called micronutrients (Kumar *et al.*, 2017).

Figs with their edible fruits, which can be eaten fresh or dried or used as jam, are considered one of the most important natural nutrients sources that are very rich in minerals (with the highest content of calcium, potassium, and phosphorus, while other minerals are in moderate amounts); vitamins (mainly vitamin K, vitamin C, and B9); carbohydrates, sugars and dietary fiber content (Slatnar *et al.*, 2011). Also, dried figs are richer in fiber, calcium, copper, manganese, magnesium, potassium, and vitamin K. (Badgujar *et al.*, 2014), mainly due to the concentrated nutrients in solids when the water is removed (Khan *et al.*, 2011). In contrast, figs are approximately sodium, cholesterol, and fat-free (Silva *et al.*, 2009).

Generally, the nutritional value increases during fruit development. However, fruit composition is mainly affected by the genetic makeup/cultivars (Khan *et al.*, 2011), physiological and environmental factors such as cultivation mode, dehydration methods, soil chemistry, fertilizers, and climatic conditions.

(Khan *et al.*, 2008) revealed that dried fig fruits have a high calorific value, which makes the fruit more nutritious, therefore is recommended for people who are joined in vesical activities and children who need a lot of calories during their continuous activity. Also, it is an important source of minerals, mainly potassium, calcium, and magnesium in high amounts, and iron (Fe), copper (Cu), zinc (Zn), and phosphorus (P) in small amounts (Khan *et al.*, 2011).

Concerning fig leaves; it is well documented that it's an excellent source of minerals (mainly iron, potassium, manganese, and calcium); vitamins (such as vitamin A, vitamin B1, and B2) and have a high fiber content (Radwan *et al.*, 2020).

2.5.3.2.1. Macro-nutrients

2.5.3.2.1.1. Carbohydrates

Carbohydrates play a major role in promoting health and fitness, constitute a major part of food, and maintain the building body's strength because they are considered an excellent energy provider after the biological breakdown of it. Carbohydrates are also considered one of the main three components of DNA and RNA, and serve as a structural material (cellulose), a part of the energy transport compound ATP, and a recognition site on cell surfaces (Khowala *et al.*, 2008).

Additionally, dietary fibers are a plant constituent that is considered a complex carbohydrate, that can't break down (digested) by human GIT enzymes. The main health benefits of dietary fibers are preventing stomach and intestinal problems, such as constipation due to their ability to increase the motility of the small intestine, decrease the transient time, and hold water to increase stool softening, they also it could help in control the cholesterol level and blood sugar, therefore, decrease the risk cardiovascular diseases and diabetes (Kumar *et al.*, 2017; Lunn & Buttriss, 2007).

2.5.3.2.1.2. Proteins

Proteins are organic nitrogenous compounds that constitute the most numerous components of the body (about 20% of the adult body weight) and are made up of smaller units known as amino acids. Proteins are essential for the structure, function, and regulation of the body tissues and organs, in which they exert an important function for body building, repair, and maintenance of body tissues, and control the tissue acid-base balance when they act as a buffer. Also, they play an important role in the synthesis of certain substances such as antibodies, plasma proteins, enzymes, hormones, and others (Kumar *et al.*, 2017).

2.5.3.2.2. Micro-nutrients

2.5.3.2.2.1. Vitamins

Vitamins are a group of organic nutrients that are needed in small amounts for multiple biochemical reactions for normal cell function, growth, survival, and development, and mainly, they are grouped into two categories: fat-soluble vitamins (ADEK), and water-soluble vitamins (vitamin C and all B vitamins) (Al-gharer, 2019)

Vitamins, mostly cannot be synthesized by the body, therefore, must be supplied by the diet (vitamin B3 is an exception). A deficiency of vitamins leads to a certain disease(hypovitaminosis) that mainly affected the skin, blood cells, and nervous system. On the other hand, an overdose of vitamins causes hypervitaminosis that could be toxic mainly in the case of vitamins A and D (Al-gharer, 2019)

Vitamins provide health benefits through different crucial functions, for example, vitamin B9 (folic acid), is needed in the production of DNA that maintains tissue growth and cell function, and also contributes to forming the RBC. Vitamin K works as an anticoagulant and supports bone health. Vit. C mainly works as an antioxidant, enhances the body to absorb iron, and contributes to collagen, catecholamine, and steroid hormone synthesis. Vit. A (plant sources are called provitamin A carotenoids) is important to vision and maintaining healthy teeth, bone, and soft tissue, also it acts as a powerful antioxidant that supports immunity. Vit.B1 (thiamin), is the central gate in energy metabolism, and its deficiency could affect the nervous system, heart, and brain (Maqbool *et al.*, 2017).

2.5.3.2.2. Minerals

Minerals are the constituents that mainly remain as ash after burning of the plant tissues, which are divided into two categories; main elements, also known as macro-elements (Ca, P, K, Cl, Na, Mg), needed in large amounts, and trace (micro) elements (Fe, Zn, Cu, Mn, I, etc.) needed in a small amount comparing to the main one. However, the mineral content can be fluctuating in the same raw material according to genetic and climatic factors. Minerals can be received from different plants (vegetables and fruits) and animal sources consumption and apply for a key role in the body to make a necessary function for a healthy life. For example, Ca, P, and F are found mainly in teeth structure, and bone (Ca, Mg, Mn, P, B, and F), while (Cu, Fe, Mn, Mg, Se, and Zn) are a significant part in many enzymes structure. macro-elements (Ca, Mg, P, Na, and K) have a crucial function in nerve cells (transmission and signaling), although, Ca and K contribute to controlling blood pressure. Micro-elements have a different key role in the formation of erythrocyte cells (I, Fe), control the glucose levels (Cr), activation of antioxidant enzymes (Mg), and others (Gharibzahedi & Jafari, 2017).

2.5.3.3. Phytochemistry

Phytochemicals a naturally occurring compounds that are mainly found in fruits, vegetables, legumes, beans, nuts, and whole grains, and include many compounds such as phytosterols, saponins, flavonoids, terpenes, and others. which is responsible for the protective health benefits of these plant-based foods and beverages (Brindha, 2016), beyond their vitamins and minerals contents. Phytochemicals, as a part of a large and varied group of chemical compounds, are responsible for the color, flavor, and odor of plant foods, such as garlic's pungent odor and blueberries' color. Also, it is considered a multifunctional component of food due to its important biological properties and antioxidant activity.

Phytochemical research revealed that *F. carica* with all its different parts has phytosterols, anthocyanins, amino acids, organic acids, fatty acids, phenolic compounds, hydrocarbons, aliphatic alcohols, volatile components, and some other classes of secondary metabolites. These phytochemicals are mainly found in latex followed by leaves, fruit, and roots. Also, it supports figs with remarkable pharmacological properties such as anticancer, antioxidant, anti-inflammatory effects, and others (Badgujar *et al.*, 2014).

2.5.3.3.1. Phenolic compounds

Phenolic compounds are the most important secondary metabolites in plants, mainly produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization. Also, it contains many compounds, such as simple flavonoids, phenolic acids, complex flavonoids, and anthocyanins. That is usually responsible for the color and flavor properties in addition to the protecting defense in the plants.

Phenolic compounds that are mainly found in fruits and vegetables have different biological benefits, such as improving β -cells and relieving oxidative stress and inflammatory response. (Lin *et al.*, 2016).

Fig leaves are a significant source of phenolic compounds, that are mainly reported to have seven phenolic compounds, namely 3CQA, 5-CQA Q-3-Glu, Q-3-rut, ferulic acid psoralen, and bergapten. They provide health-promoting benefits of figs mainly for their antioxidant properties (Badgujar *et al.*, 2014)

2.5.3.3.2. Flavonoids

Flavonoids, a group of natural products that have a polyphenolic structure, are mainly found in fruits, vegetables, and beverages. flavonoids are potent inhibitors of several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase, and lipoxygenase, and they have strong antioxidant, anti-inflammatory, anti –mutagenic and anti–carcinogenic properties, therefore, they are mainly having special health-promoting effects and consider as one of the most important compositions of nutraceutical, pharmaceutical, medicinal, and cosmetic application (Panche *et al.*, 2016).

F. carica leaf has the potential to play a significant health-promoting role, due to its anti-inflammatory, anti-microbial, and antioxidant effect, that mainly related to luteolin as a major free flavonoid found in Ficus leaf with slightly higher flavonoid content than quercetin. And biochanin –A in a small amount.

Quercetin is mainly used in allergic conditions, such as asthma, eczema, and hay fever. Also, in the treatment of gout, pancreatitis, and prostatitis as a part of inflammatory conditions, due

to their ability to facilitate the production and manufacturing of pro-inflammatory compounds (Badgujar *et al.*, 2014).

2.5.3.3.3. Anthocyanins

Anthocyanins are naturally red, purple, and blue pigments found in plants mainly fruits, vegetables, and flowers. Anthocyanins belong to phenolic phytochemicals, that have a wide range of usage. Traditionally used as a natural dye and food colorant, on the other hands, it has been used as a medicine for preventing several diseases such as cardiovascular diseases, cancer, and diabetes as well as, improving visual and neurological health, through different mechanisms and pathways including free radicals scavenging pathway that reducing oxidative stress, and the indirect pathways that include down-regulation of cell proliferation and apoptosis through reduction of lipid peroxidation and oxidative stress. cyanidin-3glucoside is the major anthocyanin that found in the plants among the anthocyanin pigments. However, the most common types of anthocyanidins distributed in plants are cyanidin, delphinidin, pelargonidin, malvidin, peonidin, and petunidin (Khoo *et al.*, 2017).

Consumption of fresh fig fruits provides a health benefit due to the antioxidant potential that is mainly related to the anthocyanins found in fig fruits, especially cyanide-3- rhamnoglucoside (C3R), which can inhibit lipid peroxidation by producing peroxy radicals and malonaldehyde. Also, scavenging on reactive oxygen species (Badgujar *et al.*, 2014).

2.5.3.3.4. Phytosterols

Phytosterols are cholesterol-like bioactive compounds that are naturally found in foods of plant origin and are mainly divided into plant sterols and plant stanols. The most common plant sterols found in the diet are campesterol, beta-sitosterol, and stigmasterol. Phytosterols food sources have the highest concentration in vegetable oils mainly corn, sunflower, soybean, and olive; in addition to oleaginous fruits such as almond and wheat bran, also, fruits and vegetables with a lower concentration.

Phytosterol daily consumptions are associated with a significant reduction in LDL cholesterol, due to its ability to reduce the intestinal absorption of cholesterol (30-50%), decreased the rate of cholesterol esterification in the enterocyte, and increase cholesterol removal by the

transintestinal cholesterol excretion (TICE). Therefore, phytosterols are considered an adjunct to pharmacological therapy in individuals with hypercholesterolemia to reduce the CVD risk factors (Cabral & Klein, 2017).

Shiraishi and others 1996, identified seven phytosterols from *Ficus latex* extract via the GCITMS, namely: butelol, lupeol, lanosterol, lupeol acetate, b-amyirin, b-sisterol, and a-amyirin. B-sisterol has the highest quantities and a-amyirin was the minor one (Shiraishi *et al.*, 1996).

2.5.3.3.5. Organic acids

Organic acids are primary metabolites that could be found in a great amount in all plants, mainly in fruits, and are considered an important protective agent against various diseases due to their antioxidant activity, also exerts a crucial role in pH regulation. Additionally, the type and content of organic acids are different between species, development stages, and tissue types, and it affects the organoleptic characteristics of fruits and vegetables, mainly flavor, and contributes to their acidity (Sweetman *et al.*, 2009).

(Oliveira *et al.*, 2010) showed that the organic acids profile of *Ficus latex* mainly contains six organic acids, namely: oxalic, citric, malic, quinic, shikimic, and furamic acids. However, these compounds are already revealed before in *Ficus* leaves, pulp, and peels aqueous extract. The most abundant organic acids contents are malic and shikimic followed by quinic acid, while furamic acid was a minor one (Oliveira *et al.*, 2010).

2.5.3.3.6. Fatty acids

Fatty acids are the building blocks of the fat in our bodies and in the food that we eat, in which the blood could be absorbed after breaking down of the fat during digestion and considered the main sources of cell fuel if glucose is not available for energy. Fatty acids consist of a long hydrocarbon chain as the main precursor with a carboxyl group at the terminus of the molecules, and different from each other in both, the number of carbon atoms that mainly consist of 12C to 24C, and the number of the double bonds in the chain (from none to one, two, or three). Accordingly, the fatty acids found in nature are saturated or unsaturated compounds.

However, the most common fatty acids are, palmitic, stearic, oleic, linoleic, and linolenic (Lund and Rustan, 2020).

Ficus latex is one of the main natural sources of fatty acids, that determined through the (GCITMS) by (Oliveira *et al.*, 2010), who revealed the presence of 14 major detectable fatty acids, identified as, palmitic, behenic, and arachidic as the major three fatty acids of the latex fatty acids content, in addition to myristic, pentadecyclic, margaric cis-10heptadecenoic, stearic, oleic, elaidic, linoleic, heneicosylic, tricosylic, and lignoceric in a smaller amount. Also, (Jeong & Lachance, 2001) showed that dried fig fruit mainly contains linolenic as the most predominant fatty acid in dried fig fruit, followed by linoleic acid, palmitic acid, and oleic acid (Jeong & Lachance, 2001).

2.4.3.3.7. Volatile compounds

Plants produce an important variety of metabolites, that could involve in the primary metabolic pathway which is common to all organisms, and others indicate secondary metabolites that are considered a characteristic of a smaller plant group (Vivaldo *et al.*, 2017).

Volatile organic compounds (VOCs) are amazing secondary metabolites that are produced as a response to forthcoming stressful situations. Volatile compounds play an important role among the secondary metabolites and could be released by almost any kind of tissue and type of vegetation (trees, shrubs, etc.) as green leaf volatile s, N-containing compounds, and aromatic compounds that mainly induced from the leaves following abiotic and biotic stresses, to apply an earlier, stronger, and faster response to prevent farther stress occurrence, thereby increased plants resistance and tolerance. And linked to the unique taste and flavor of the fruits (Brilli *et al.*,2019).

Oliveira *et al* have identified 34 volatile compounds distributed in the latex, fruits, and leaves of *F. carica* through (GCIT-MS). That mainly includes 5 aldehydes; 7 alcohols; 1 ketone; 9 monoterpenes; 9 sesquiterpenes and 3 other miscellaneous compounds (Oliveira *et al.*, 2010a).

2.4.3.3.8. Amino acids

Amino acids can be defined as the main building blocks responsible for protein molecule formation (Baqir *et al.*, 2019). They are mainly categorized as essential and non-essential types related to the basis of their synthesis in humans. The essential amino acids (leucine, isoleucine, methionine, phenylalanine, arginine, histidine, tryptophan, valine, threonine, and lysine) are synthesized only by plants (Kumar *et al.*, 2017), and it should be taken from outside in the form of animal and vegetable foods (Yang *et al.*, 2020), however, the non-essential amino acids (alanine, b-alanine, asparagine, cysteine, glutamine, aspartic acid, glycine, proline, serine, and tyrosine) could be synthesized by both plants and human (Kumar *et al.*, 2017).

Various human disorders such as diabetes, insomnia, obesity, and arthritis are mainly caused by metabolic disturbances that could be able to repair by correcting the composition of the amino acids because it's necessary for the metabolic process and the transport and storage of all nutrients such as water, protein, carbohydrate, fat, vitamins, and minerals. Also, the amino acids profile of plants is considered an essential parameter in the assessment of their growth potential, and the nutritional quality of proteins in food and feed materials (Dahl-Lassen *et al.*, 2018).

Oliveira and others (2010) revealed that the amino acids profile of *F. carica* latex contains 13 compounds, comprising five essential amino acids and eight non-essential amino acids, in which tryptophan, cysteine, and tyrosine have higher quantities related to other amino acids.

2.4.3.4. Role of Ficus in disease prevention (biological activity)

Fig with all its parts and ingredients has therapeutic and pharmacological benefits such as antioxidant, anti-inflammatory, antimicrobial effects, and others.

The health-beneficial effect of fig has been mentioned in Al-Quran, Surat al-al-Teen (sura no.95 verso no.1). although, its health-promoting effect has been mentioned as Hazrat Abu Darda (Radiallaho Anho) relates Prophet Mohammad (Peace Be Upon Him), said: "Eat fig, it cures the piles and is useful in rheumatism" (Rahmani & Aldebasi, 2017).

2.4.3.4.1. Antioxidant activity

Fig has many phenolic compounds which apply to many physiological roles in plants, and also have a beneficial effect on human health, due to its ability to act as an antioxidant in different ways: hydrogen donors, free radical scavengers reducing agents, singlet oxygen quenchers, and else.

Six commercial types of fig fruits with different colors (black, red, yellow, and green) were studied for total polyphenols, total flavonoids, antioxidant capacity, and anthocyanins profile.

And the ferric-reducing antioxidant method was used to determine the anti-oxidant properties, which show that the fig fruit has a high antioxidant capacity because it contains a high level of polyphenols, flavonoids, and anthocyanins (Çalışkan & Polat, 2011). Another study confirms that the main anthocyanin in all fig fruits is cyanidin-3-O-rutinoside (C3R), which gives 92% of the total antioxidant capacity of the anthocyanin fraction, according to the NMR data. Also, the fig extract color appearance mainly correlated with total polyphenols, flavonoids, anthocyanins, and antioxidant capacity (Solomon *et al.*, 2006).

2.4.3.4.2. Anti-inflammatory activity

Fig is one of these medicinal plants, and its leaves were examined through experiments to check the anti-inflammatory activity through the different types of extract. The result of this study revealed that the ethanolic extract of fig leaves gives the maximum anti-inflammatory effect, and it was 75.90% in acute inflammation and 71.90% reduction of granuloma weight in the chronic study (Patil & Patil, 2011).

2.4.3.4.3. Anti-microbial effect

Multi-drug-resistant bacteria which significantly cause the failure of treatment is a big problem that limits the effectiveness of drugs worldwide. Therefore, investigating an alternative source with antimicrobial activity is an urgent need to overcome this problem (Hancock, 2005).

2.4.3.4.3.1. Antibacterial: To reveal the anti-bacterial activity of figs, an experiment was carried out using methanol extract from fig leaves, which show strong antibacterial activity

against oral bacteria. Also, the combination of fig leaves methanol extract with gentamicin or ampicillin gives a synergistic effect against oral bacteria, which improves that fig could act as a natural antibacterial agent (Jeong *et al.*, 2009).

2.4.3.4.3.2. Anti-viral: Camer *et al.*, 2014 performed an *in vitro* study to investigate the ability of fig latex to interfere with the infection of caprine herpesvirus-1. The result revealed that the simultaneous addition of latex and caprine herpesvirus-1 (CpHV-1) to monolayer kidney cells shows a remarkable reduction of CpHV-1, which is comparable to that induced by acyclovir (Camero *et al.*, 2014).

2.4.3.4.3.3. Anti-fungal activity

Another experiment was carried out using different extracts of fig latex (hexane, chloroform, ethyl acetate, and methanol) to investigate their antimicrobial activities against five bacterial species and seven strains of fungi using the disk diffusion method, the methanol fraction minimal inhibition concentration (MIC) gives a total inhibition (100%) against candida Albicans at a concentration of 500 micros. g /ml, and negative effect against Cryptococcus neoformans. Furthermore, both methanolic and ethyl acetate extract strongly inhibit *Microsporum canis* (Aref *et al.*, 2010).

2.4.3.4.4. Anti-cancer activity

Cancer is a condition in which the cells in a specific part of the body uncontrollably grow and reproduce, which can invade and destroy the surrounding healthy tissue, including organs (Hassan, 2019). *F. carica* is one of some medicinal plants that have a role as an anti-tumor due to its ability to inhibit the proliferation of various cancer cell lines. A study was performed to explain the anti-cancer effect of *F. carica* latex in a different concentration, the result revealed that the 5mg/ml concentration gives the maximum inhibition effect on stomach cancer cell line growth (Hashemi *et al.*, 2011). The main cytotoxic agent in *F. carica* latex which applies the inhibition effect on the proliferation of various cancer cell lines is 6-O-Acyl-Beta-D-glucosylbeta sitsterols (Rubnov *et al.*, 2001).

Another study examined the antiproliferative activity of *F. carica* latex (FCL) in a combination with temozolamide (TMZ) in some cell lines such as T98G, U-138 MG, and U87 MG

glioblastoma multiforme (GBM), the results revealed that the FCL causes cell death in GBM cells, also, these combination gives a synergistic effect (Tezcan *et al.*, 2015).

2.4.3.4.5. Anti-obesity and fatty liver action

Liver-related diseases are one of the major health problems worldwide that are associated with morbidity and mortality. Natural products are popular in the treatment of some liver diseases.

Mohan *et al.*, (2007) showed that the methanol extract of fig leaves has a potential hepatoprotective activity in the CCl₄-induced liver damage rat model. An oral dose of 500mg/kg of the extract provided a significant protective effect by lowering the serum level of AST, ALT, total serum bilirubin, and Malonaldehyde equivalent as an index of liver lipid peroxidation.

2.4.3.4.6. Anti-constipation effect

Constipation is one of the most common gastrointestinal problems worldwide, mainly illustrated by a hard and painful bowel movement, due to a low fiber diet, stress, depression, low water intake, and other reasons. Fig is one of the most common medicinal plants that has been mainly used to treat constipation since ancient times. (Lee *et al.*, 2012) examined the activity of fig paste in the treatment of loperamide-induced constipation in a rat model. The result of this study demonstrated that in the fig-treated group, both the fecal pellet number, weight, and water content were increased, also, a reduction in body weight and increased intestinal transient length were observed. Exercise and ileum tension increased, in contrast, the fecal pellet number was reduced in the distal colons of the fig-treated rat. Therefore, fig fruit has proven its ability to decrease constipation (Lee *et al.*, 2012).

2.4.3.4.7. Anti-diabetic activity

Type 2 diabetes (T2DM) or non-insulin-dependent diabetes is an impairment of the body's regulation and uses of sugar (glucose) as a fuel, which leads to a high level of blood sugar that eventually causes a disorder of the circulatory, nervous, and immune system. T2DM accounts for 90-95 % of the world's human diabetic population, and by 2045 the total number of diabetics is predicted to reach 629 million (Berbudi *et al.*, 2020). Fig is one of the medicinal

plants that are traditionally used to treat diabetes mellitus due to its activity as a hypoglycemic agent, and safety (nontoxic, with little or no side effects) (Irudayaraj, *et al.*, 2012).

A study was performed to reveal the hypoglycemic effect of fig leaf extract, finding of the study showed that the ethyl acetate extract of *F. carica* leaves possesses a clear hypoglycemic activity in treated versus non-treated rats, due to its ability to stimulate insulin production and secretion from the pancreas, that positively affects the carbohydrate metabolism in the diabetic rat (decreasing gluconeogenesis and increasing glycolysis), that finally decreased hyperglycemia. (Stephen *et al.*, 2017)

2.4.3.4.8. Anti-pyretic activity

Patil Vikas *et al.* showed that the ethanol extract of *ficus carica* leaves, at doses of 100, 200, and 300mg/kg have a significant dose–dependent reduction in a normal body temperature. As it compares with a standard antipyretic agent, paracetamol 150mg/kg. Furthermore, the effect of the extract extended up to five hours after administration (Patil Vikas *et al.*, 2010).

2.5. Problem statement and motivation of the study

A few studies about Palestinian herbs are available, but their composition, efficacy, and safety are still unexplored. In addition, due to the undesirable side effects of orthodox synthetic medications such as toxicity and carcinogenicity and the emerging microbial resistance to available antimicrobial agents, attention has considerably increased to find out naturally occurring antioxidant and antimicrobial compounds suitable for use in food and/or medicine.

Palestinian Fig Genotypes (*Ficus carica* L.) was chosen as a model for this study because of its long medicinal reputation among Palestinians. The lack of phytochemical composition of its volatiles, semi-volatiles, and minerals and the scarcity of pharmacological studies on indigenous figs in Palestine motivated this research.

2.6. Aim of the study

This study aims to screen fig genotypes (Shhami, Smari, and Mwazi) commonly grown in Palestine, secondary metabolites, and minerals by using SD-GC-MS and ICP-OES and to

examine some of their claimed pharmacological activities. The test will include antioxidant and anti-bacterial biological activities.

2.7. Objectives of the study

1. To extract the major crude volatile compounds present in *Ficus carica* L. genotypes (Shhami, Smari, and Mwazi) by methanol (80%) and to analyze the extract by GC-MS technology at the electron impact (EI) mode.
2. To compare the type and composition of the volatile compound of the *Ficus carica* L. genotypes fruits, skin, and leaves collected from Hebron city.
3. To compare the biological activities, total ash, fibers, fat, and minerals content of the *Ficus carica* L. genotypes fruits, skin, and leaves collected from Hebron city.
4. To assess the relation between *Ficus carica* L. genotypes fruits, skin, and leaves concerning the contents and biological activities.

Chapter 3: Materials and Methods

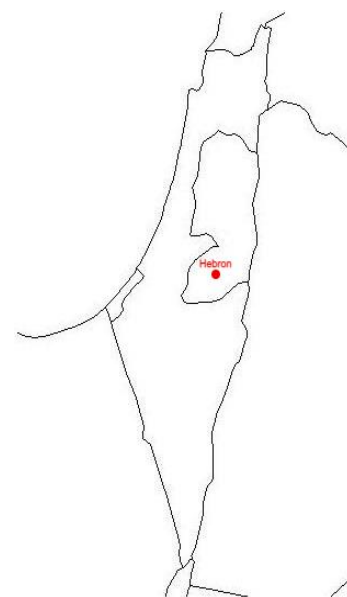
Materials and methods

3.1. Experimental site and sampling

During the growing season of 2021, mature fruits, and leaves of three fig genotypes namely (Shami, Mwazi, and Smari) were collected (1 kg each) from Hebron city that located at the southern region of West-Bank, Palestine (**Map 1, Table 3.1**).

All samples were directly transferred to the laboratories of Hebron University. Fruits were peeled and leaves were cleaned properly with distilled water. Using Forced Air-Drying Oven at 40°C, fruits and skins were dried for three days. Leaves were dried for only one day (**Table 3.2**).

Fruits and skins were then shifted into a nearby greenhouse under shading for another three days for further drying purposes. Obtained dried fruits and skins were stored in a normal refrigerator at 4°C until they were used. However, the leaves were directly ground and gained powders were stored in tightly-glass jars until they were used.



Map 1. Location of the study site

Table (3.1): Study sites description

Item	Hebron
Location	36 Kilometers south of Jerusalem
Elevation	1000m
Latitude	31°33'31.65"N
Longitude	35°5'43.02"E
Topography	Mountainous
Rain-full	550-600 mm

Table (3.2): Steps of samples preparation



Harvesting



Fruit peeling



Forced Air-Drying Oven



Greenhouse drying.

3.2. Measured and evaluated parameters.

Total ash, fibers, fat, and minerals content were evaluated according to the Association of Official Analytical Chemists (AOAC, 2000) procedure as the following:

3.2.1. Ash estimation

Ash content was evaluated using a Muffle Furnace oven (GALLENKAMP, UK) at 550 °C for three hours. Crucible was weighed without and with 1g sample and accordingly placed in the oven obtained ash was calculated according to the following formula:

$$\text{Ash \%} = \{(\text{weight of crucible with ash} - \text{weight of crucible}) / (\text{weight of sample})\} * 100.$$

3.2.2. Fat estimation

The crude fat of fig samples was determined using Soxtherm (Gerhardt Germany) with petroleum ether as solvent.

Crude fat (%) = $\{(\text{Weight of flask with fat} - \text{weight of empty flask}) / (\text{Weight of original sample})\} \times 100$

3.2.3. Fiber estimation

The crude fiber of 1 g from each sample was estimated using a fiber analyzer (Ankom 2000, UK), through digestion with H₂SO₄ (1.25% v/v) at 100°C for 30 min, then with KOH (1.25%, v/v) at 100°C for 30 min. Fig samples were then dried at 100°C for 3 hours and their weight was recorded.

3.2.4. Proximate analysis

3.2.4.1. Na, Ca, K, Fe, Mg, and Zn estimation

5 mL of HCl (2N) were added to the ash samples and accordingly filtrated in a volumetric flask, then distilled water was added up to 100 mL. Finally, the absorbance of all samples was taken about the standard of each mineral using atomic absorption (PERKIN ELMER, AAnalyst100, country).

3.2.4.2. Phosphorous (P) estimation

10 mL of ammonium vanadomolybdate was added to 10 mL of previous extract ash, then diluted with distilled water up to 100 mL. Finally, the absorbance of all samples was taken using a spectrophotometer at 410nm. The vandomolybdate reagents produce a stable yellow color with phosphates.

3.2.4.3. Boron (B) estimation

10 mL of H₂SO₄ (0.36 M) were added to the new ash samples for an hour. Then, 1ml of the acidic ash suspension was mixed with 2mL of a buffer solution (ammonium acetate + EDTA + acetic acid + 2mL of azomethine (H)), and incubated for 30 min. The absorbances of the

samples were recorded using a spectrophotometer (Cat number, company, country) at 420nm.

3.2.5. Data analysis

Data were statically analyzed using one-way analysis of variance (ANOVA) and means were separated using the Tukey's pairwise comparison at a significance level of $p \leq 0.05$ using the SPSS package system.

3.2.6. Antioxidant

The antioxidant capacity of Fig samples (leaves and fruits) from Shhami, Smari, and Mowazi genotypes were assessed *in vitro* using the DPPH[•] and ABTS^{•+} Free radical scavenging assays as described in (Dowek *et al.*, 2020).

3.2.6.1. Extract preparation

Fig samples were extracted as described in (Qawasmeh *et al.*, 2012), where 1g of each sample was extracted with 10 ml methanol 80% for 24h at 25 °C in a shaking incubator. Then the extracts were filtered through a filter paper and used to estimate the antioxidant capacity.

3.2.6.2. 2, 2'-diphenyl-1-picrylhydrazyl stable radical (DPPH[•]) assay

A stock solution of DPPH[•] was prepared by dissolving 6.8 mg of DPPH[•] with 17 ml of methanol 80%. Then, 200 μ l of DPPH[•] of the stock solution was mixed with 30 μ l of extract or methanol 80%(control) in a plastic cuvette, continuously all cuvettes were mixed and kept in dark for 1 h at room temperature. Finally, the absorbance of the samples and the control were measured at 517 nm using a UV-visible spectrophotometer.

The radical scavenging activity was calculated as a percentage of DPPH[•] discoloration using the following equation:

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

3.2.6.3. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid radical (ABTS^{•+}) assay

The ABTS solution was prepared by mixing 3ml of ABTS^{•+} stock solution (7mmol, prepared by dissolving 6.9mg in 3 ml DW (each 18 mg of ABTS^{•+} need 5ml D.W), with 3 ml of

potassium persulfate solution (2.45 mmol, prepared by dissolving 10.5mg of $K_2S_2O_8$ with 15.9 ml D.W). Then keep in the dark for 24h at room temperature.

The working solution of $ABTS^{•+}$ was prepared by diluting the $ABTS^{•+}$ solution with D.W. to get the final absorbance of 0.7000 ± 0.02 at 734 nm. A 15 μ l of extracts solutions was mixed with 1 ml $ABTS^{•+}$ working solution in micro cuvettes. For control, 15 μ l methanol (80%) was mixed. All cuvettes were mixed and kept in dark for 1h at room temperature. The absorbance of plant extracts (A sample) and the methanol (A control) were measured at 734 nm using the UV/Visible spectrophotometer.

3.2.7. Total phenols

3.2.7.1. Preparing the extract

Fig samples were extracted as described in (Dowek *et al.*, 2020), where 1g of each sample was extracted with 10 ml methanol 80% for 24h at 25 °C in a shaking incubator. Then the extracts were filtered through a filter paper and used to estimate the total phenols as described in (Qawasmeh *et al.*, 2012).

3.2.7.2. Preparing Gallic acid as a stock solution

Gallic acid was prepared by dissolving 250 mg of G.A. in 5 ml of 80% methanol (because the G.A. dose did not dissolve in water), then diluted with distilled water up to 50 ml.

Different concentration of G.A was prepared by adding a sex different volumes of G.A (50, 100, 200, 300, 500 and 1000 μ l) in a cuvette, then added 80% methanol up to 10ml.

3.2.7.3. Preparing Na_2CO_3

Na_2CO_3 was prepared by dissolving 5g of Na_2CO_3 in 20 ml D.W in a beaker, heating it to boil, then Cooling and filtering the solution, and finally, adding D.W up to 25 ml.

3.2.7.4. Total phenols procedure

Fig extracts (20 µl) were mixed with 1.58 ml D.W, 150 µl F.C (folin ciocalteu reagent), and 30 µl of Na₂CO₃ in plastic macro-cuvettes. All cuvettes were mixed and keep in the dark for 1 h. the absorbance of the resulting solution was measured at 760 nm,

For the G.A stock solution, 20 microliters of G.A were added (from each concentration in 10 cuvettes) instead of extract. Data were expressed as milligrams of gallic acid per gram of dried plant samples (mg GAE/g).

Blank is water and the assay was done in triplicate

3.2.8. Phytochemicals

Phytochemicals tests of the fig samples were examined according to the procedure described by (Velavan, 2015).

3.2.8.1. Extract preparation

Fig samples (3g) were extracted in 60ml methanol 80% for 24h at 25 C in a shaking incubator. Then the extracts were filtered through a filter paper and used to screen the phytochemicals content in each sample as the following:

- **Test for Coumarin:** in a test tube, 1ml of NaOH was added to 1ml extract and kept in a boiling water bath for a few minutes, the appearance of yellow color indicates positive coumarins.
- **Test for Saponins:** in a test tube, 5ml of distilled water was shaken with 2ml of extract, and the formation of foam indicates positive Saponins.
- **Test for anthraquinones:** in a test tube, 1ml of 10% NH₃ solution was added to 2ml extract, which was mixed with benzene, the presence of red, pink, or violet color indicates positive for anthraquinones.
- **Test for Quinone:** in a test tube, 1ml of conc. H₂SO₄ was added to 1ml of extract. The presence of red color indicates a positive for quinines.

- **Test for steroids**, in a test tube, 2ml of CHCl_3 , and 1ml of H_2SO_4 were added to 1ml extract, the appearance of a reddish-brown ring indicates positive for steroids.
- **Test for Tannins**, in a test tube, 1 ml of distilled water and 1-2 drops of FeCl_3 , were added to 2 ml extract, the presence of green or bluish color indicates positive for tannins.
- **Test for terpenoids**, in a test tube, 2 ml of CHCl_3 , and 3 ml conc. H_2SO_4 were mixed with 2 ml extract. The formation of a reddish-brown layer indicates a positive for terpenoids.
- **Test for flavonoids**, in a test tube, a few drops of 1% NH_3 solution was mixed with 2ml extract. The presence of yellow color indicates a positive for flavonoids.
- **Test for glycosides**, in a test tube, 2ml of 50% H_2SO_4 was added to the 2 ml of extract. After 5 min of heating a mixture in the water bath, 10ml of Fehling's solution was added and boiled. The presence of red brick precipitate indicates a positive for glycosides.
- **Test for Phlobatannins**, in a test tube, 1 ml of 10% NaOH was added to 2 ml extract. The formation of yellow color indicates a positive for phlobatannins.
- **Test for phenolic groups**, in a test tube, 2 ml of distilled water and a few drops of 10% FeCl_3 were added to 1 ml extract. The formation of blue or black color indicates positive for phenolic groups.
- **Test for alkaloids**, in a test tube, 1ml of 1% HCl was added to 2ml extract then a few drops of Meyers reagent were added to the mixture. The presence of white precipitate indicates a positive for alkaloids.
- **Test for anthocyanin**, in a test tube, 1ml of 1N NaOH was added to 1ml extract and heated for 5 min. the formation of bluish-green color indicates a positive for anthocyanin.

- **Test for cardiac glycosides**, in a test tube, 2ml of glacial acetic acid, 1ml of conc. H₂SO₄ and a few drops of FeCl₃ were added to the 2ml extract. The formation of a brown ring indicates a positive for C. glycosides.

3.2.9. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The volatile compounds in 1g of fig fruits, leaves, and skin were extracted in methanol 80% (10ml) overnight and analyzed using GC-MS (Clarus SQ 8S, Perkin Elmer, USA), fitted with a BD-5ms capillary column (30m, 0.25 μm film thickness, 0.25μm capillary diameter) as described by (Qwasmeh *et al.*, 2011) with minor modification. The injection volume was 1 μL. The oven temperature was maintained at 80 C for 2 min and raised to 280 C at the rate of 6 C /min. the temperature of the injector was set at 280 C. helium was used as the carrier gas, and the total gas flow and velocity were maintained at 134.3ml/min and 43.1 cm/s, respectively. MS scan speed was 1000amu/s. the molecular mass of the compounds between 50-500 in which *M/Z* were acquired at 70mv. The analysis for each sample was repeated 2 times.

The compounds were identified using the NIST05 mass spectral library, and finally, their mass spectra were compared with those published in the literature.

3.2.10. Antibacterial

The antibacterial activity of the fig samples was examined according to the procedure described by (Dowek *et al.*, 2020)

3.2.10.1. Extract preparation

The methanolic extract of fig samples was performed according to the method described by (Qawasmeh *et al.*, 2012), in which 1 gr of each fig sample was extracted in 10 ml of 80% methanol for 24hrs in the shaking incubator at 25c. The next day the extracts were filtered and subjected to antibacterial analysis.

3.2.10.2. Media preparation

Differential media Muller Hinton agar (MHA), Eosin methylene blue (EMB), Mannitol Salt agar (MSA), nutrient agar, and MacConky agar were prepared based on manufacturers'

recommendations. All prepared media were autoclaved at 121 °C for 1.5 hr. The sterile media were poured into sterile Petri dishes (90 × 16 mm) and stored in the refrigerator at 2–4 °C for later use.

3.2.10.2.1. Muller Hinton agar (MHA) preparation

Three bottles of Muller Hinton were prepared (28.5g/750ml), using the following procedure for each bottle:

- Weighted 28.5g of M.H powder.
- Added 750ml of distilled water.
- Heated it on the hot plate until it was become clear (use magnetic stirrer) • Waited 20min to cool and then close the bottle and cover it with aluminum foil.
- Autoclaved the sample for 1.5 hr.
- Poured in sterile Petri dishes.
- Kept in the refrigerator.

3.2.10.2.2. Preparation of the other four media

To prepare 250ml of each nutrient media, the following procedure was used:

- **EMB:** 8,99g were used (35,96g/ 1000ml).
- **MSA:** 27,755g (111,2/1000ml).
- **Nutrient agar:** 7g (28g/1000ml).
- **MacConky agar:** 12.883g (51,53g/1000ml).
- **3.2.10.3. Bacterial samples preparation**

Five pathogenic bacterial strains were grown. A gram-positive bacterium (*Staphylococcus aureus*), and four strains of gram-negative bacteria including *Escherichia coli*, *Pseudomonas*

aeruginosa, *Proteus*, and *Klebsiella pneumonia* were cultured on suitable media and incubated at 37 °C for 24 h. Cultured plates from all bacterial strains were preserved in a refrigerator at 2–4 °C until further use.

3.2.10.4. Bacterial culture and subculture

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

3.2.10.5. Sensitivity testing

Sensitivity testing was carried out for all bacteria strains using well diffusion (Kokkaiah *et al.*, 2017) and disk method on Muller Hinton agar plates. Bacterial suspensions were prepared related to the density of 0.5 McFarland units equivalent to 1.5×10^8 CFUs/ml using a sterile cotton swab. Then, made five holes in each plate (for well diffusion method), in which 10 μ l extract was added onto each of the first three holes, where the negative control (methanol), and the positive control disks (vancomycin for *S. aureus* bacteria and meropenem for all other types of bacterial strain) were added onto the other holes. After 24h incubation at 37°C, the zone of inhibition of the positive controls and fig extract were measured and expressed as a percentage (%).

Chapter 4: Results

4.1. Chemical composition and quality parameters for fig samples

Among the three examined fig genotypes comprising Smari, Shhami, and Mwazi; leaves presented significantly higher fiber and ash contents over fig fruits and skins ($p=0.00$). In addition, calcium (Ca), magnesium (Mg), Iron (Fe), and Boron (B) were higher in the leaves of the three fig genotypes followed by fig skins, and fig fruits.

No significant differences were observed with Na, Zn, and P among the evaluated parts of the three examined fig genotypes.

Leaves of Shhami genotype exhibited significantly higher fiber percentage (49.97%), K (8.77), and P (0.08) compared to Smari and Mwazi fig genotypes. Additionally, Shhami genotype showed higher fiber contents in the three fig parts including leaves, fruits, and skins over the other genotypes (**Table 4.1**).

Table (4.1): Chemical composition and quality parameters of the three selected fig genotypes

Fig genotypes	Parts	Conducted parameters									
		Fiber (%)	Ash (%)	Na	Zn	Ca	Mg	K	Fe	B	P
Smari	Leaves	39.19± 0.966	19.88±0.123	0.90±0.005	*0.02±0.001	17.72±0.012	3.15± 0.003	4.41±0.002	0.06± 0.002	0.61±0.004	0.06±0.001
Shhami		49.97± 0.201	15.07±0.061	1.43±0.005	*0.02±0.001	13.31±0.002	2.92± 0.003	8.77±0.004	0.04± 0.001	0.74±0.005	0.08±0.001
Mwazi		35.18± 0.978	14.35±0.129	2.01±0.002	*0.03±0.001	12.23±0.004	2.98± 0.005	6.76±0.005	0.04± 0.001	0.76±0.002	0.06±0.001
Smari	Fruits	6.84± 0.584	2.21± 0.110	2.2± 0.005	0.04±0.001	2.44± 0.006	2.22± 0.001	4.69±0.002	0.01± 0.001	0.31±0.001	0.03±0.001
Shhami		9.80± 0.218	3.31± 0.193	2.24±0.003	0.04±0.001	2.73± 0.001	2.11± 0.001	5.77±0.001	0.004± 0.001	0.29±0.002	0.06±0.002
Mwazi		8.28± 0.775	2.99± 0.037	1.91±0.001	0.03±0.001	2.06± 0.003	2.00± 0.002	5.12±0.004	0.01± 0.001	0.28±0.003	0.09±0.001
Smari	Skins	6.99± 0.167	2.47± 0.010	1.81±0.005	0.03±0.001	3.28± 0.71	2.27± 0.002	4.15±0.004	0.01± 0.001	0.34±0.001	0.03±0.001
Shhami		8.03± 0.089	3.11± 0.111	1.73±0.003	0.03±0.001	3.65± 0.004	2.05± 0.003	4.23±0.005	0.004± 0.001	0.36±0.003	0.03±0.001
Mwazi		4.54± 0.699	3.43± 0.126	1.58±0.001	0.03±0.002	4.49± 0.007	1.70± 0.001	5.26±0.009	0.01± 0.001	0.19±0.001	0.03±0.001

Values expressed as means (mg/g) ± standard deviations. Significant difference at 0.05 level, n=3.

4.2. Antioxidant

4.2.1. ABTS^{•+} scavenging capacity

The methanolic extract of the different fig samples displayed antioxidant capacity using the ABTS[•] free radical scavenging assays, the average percentage of scavenging is shown in (Figure 4.1). Leaves of shhami, mwazi, and smari genotypes exhibited the highest levels of antioxidant capacity, with ABTS[•] scavenging assay 75.1%, 80.0%, and 59.5% respectively. However, shhami, mwazi, and smari fruits revealed the lowest level of antioxidant capacity, with ABTS scavenging assay 37.3%, 31.3%, and 26.3% respectively.

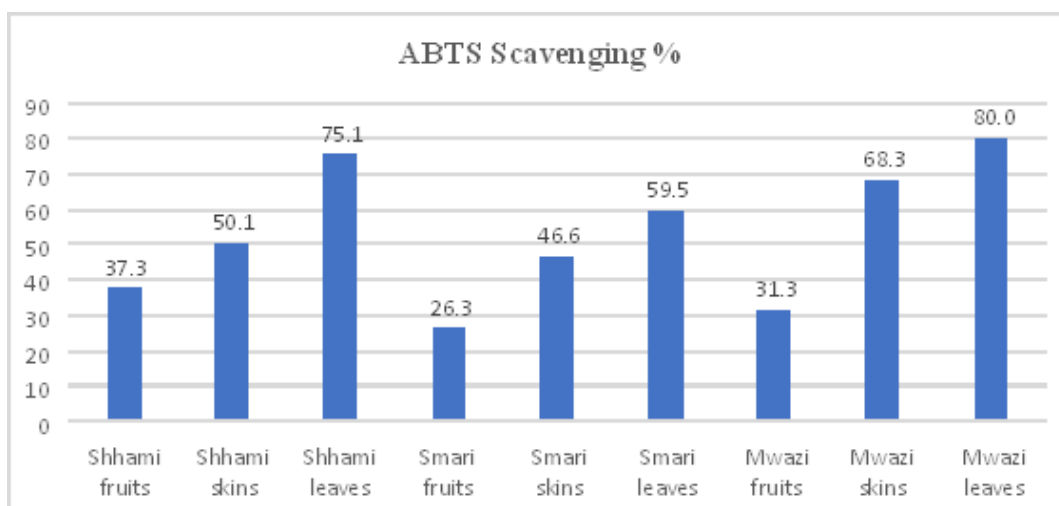


Figure (4.1): Mean radical scavenging effect of three fig genotypes assayed by ABTS^{•+} radical scavenging method using methanolic extracts, n=3.

4.2.2. DPPH[•] scavenging capacity.

The methanolic extract of the different fig samples displayed antioxidant capacity using the DPPH[•] free radical scavenging assays, the average percentage of scavenging is shown in (Figure 4.2). Leaves of shhami, mwazi, and smari genotypes exhibited the highest levels of antioxidant capacity, with ABTS^{•+} scavenging assay 85.2%, 83.4%, and 82.6% respectively. However, shhami, mwazi, and smari fruits revealed the lowest level of antioxidant capacity, with DPPH[•] scavenging assay 5.9%, 13.4%, and 3.3% respectively.

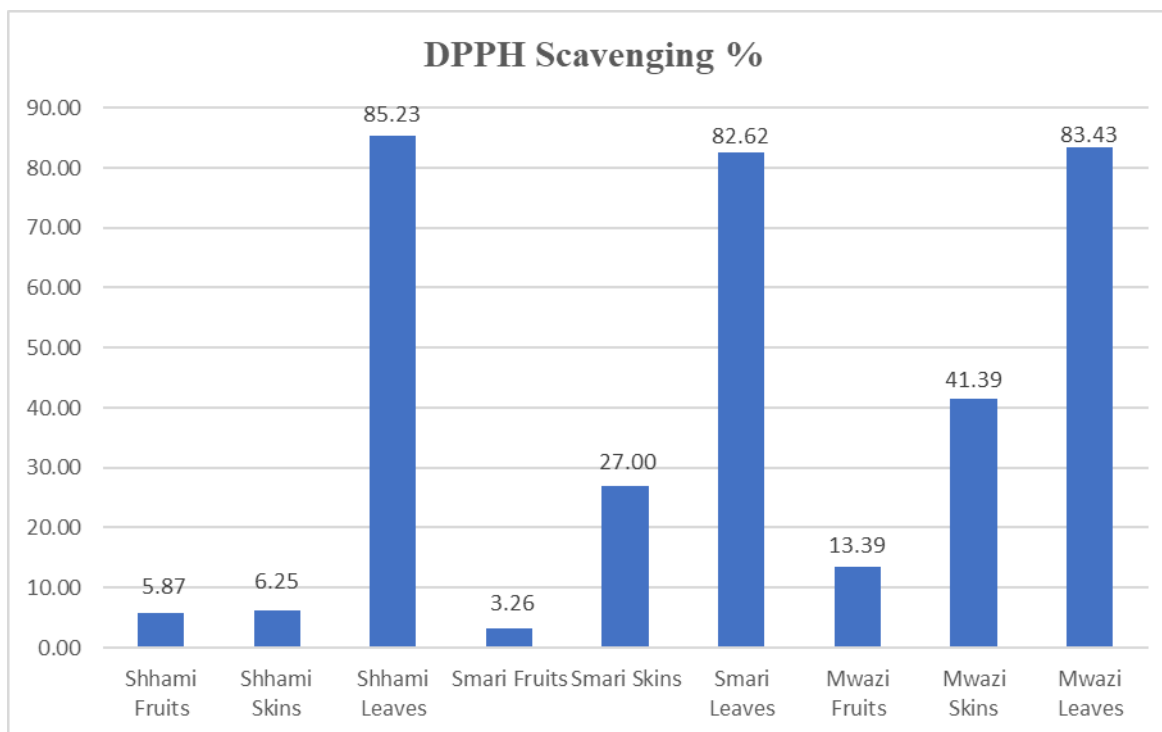


Figure (4.2): Mean radical scavenging effect of three fig genotypes, assayed by DPPH radical scavenging method using methanolic extracts. n=3

4.3. Total phenol

The total phenols in the methanolic extract of different fig samples were quantitatively estimated as mg of Gallic acid equivalent (GAE mg /g) (n=3) as shown in **(Figure 4.3)**. Significant higher total phenolic contents ($p < 0.05$) were found in leaves of shhami, smari and mwazi with 1980 mg GAE /g, 1793 mg GAE /g, and 2135 mg GAE /g, respectively; compared with fruits and skins. However, among all tested fig samples, Shhami, Smari, and Mwazi fruits revealed the lowest total phenolic contents, with values of 506.7 mg GAE /g, 668.9 mg GAE /l, and 691.1 mg GAE /g respectively **(Figure 4.4)**.

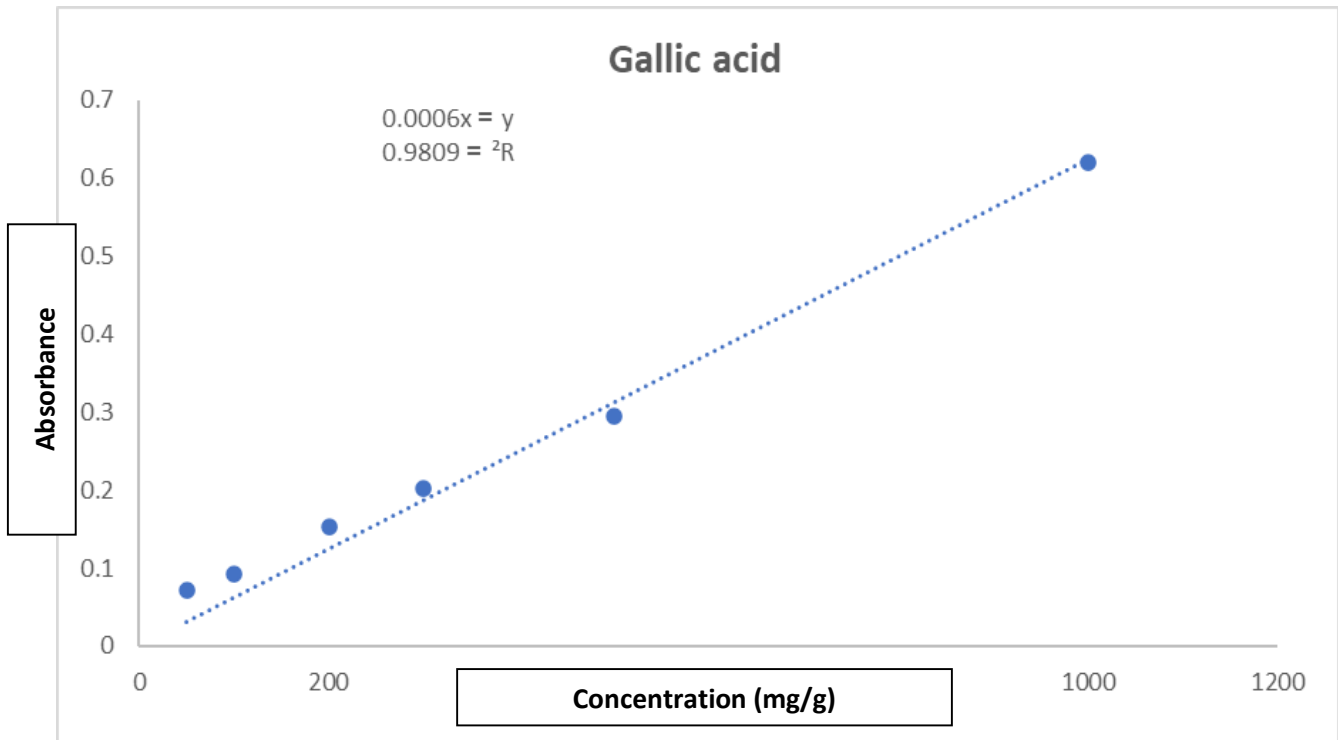


Figure (4.3): Calibration curve of gallic acid. Each point represents the mean of triplicates.

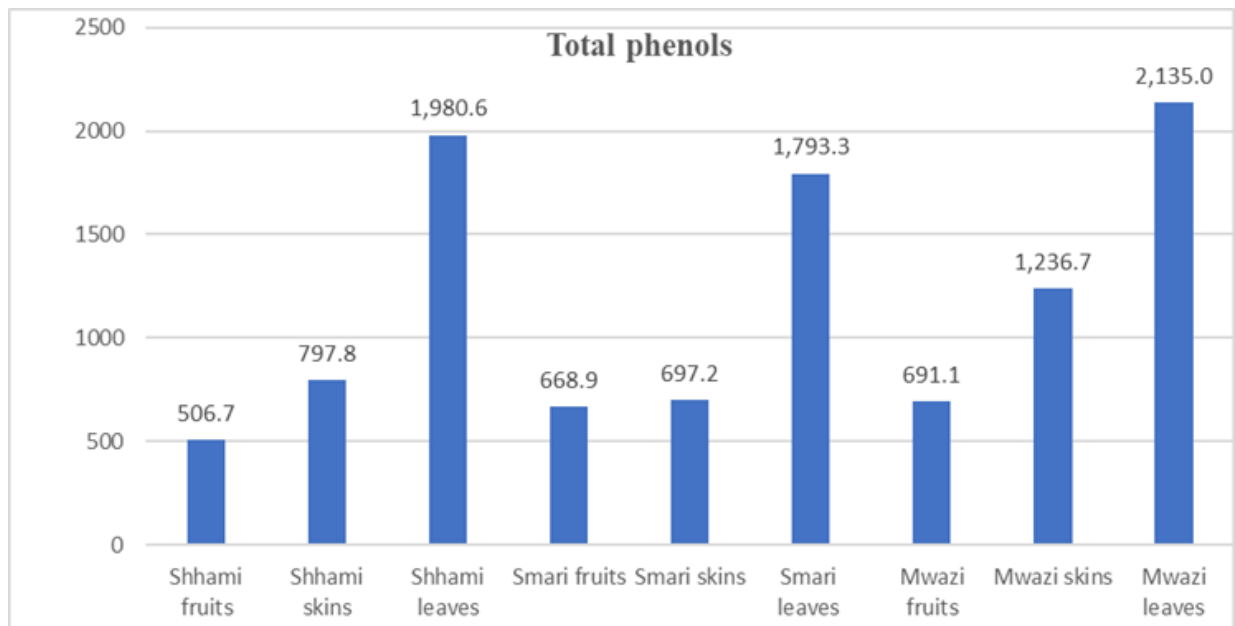


Figure (4.4): Total phenols contents of three fig genotypes methanolic extracts produced by Folin -Ciocalteu method, n=3

4.4. Phytochemical screening

The phytochemical screening assays for the methanolic extracts of fig samples revealed the presence of a wide range of phytochemical groups including saponins, steroids, tannins, terpenoids, and phenolic groups. Other groups such as alkaloids, anthocyanins, and others were not detected as summarized in **(Table 4.2)**. Interestingly, tannins and phenolic groups were found only in the leaves of the three examined fig genotypes compared with the other parts.

Table (4.2): Phytochemical screening for the methanolic extracts of fig samples, n=3

Fig genotypes	Parts	Phytochemical screening tests													
		Cardiac glycosides	Phenolic groups	Alkaloids	Anthocyanin	Coumarins	Saponins	Anthraquinone	Quinones	Steroids	Tannins	Terpenoids	Flavonoids	Glycosides	Phlobatnins
Smari	Leaves	-	+	-	-	-	+	-	-	+	+	-	-	-	
Shhami		-	+	-	-	-	+	-	-	+	+	-	-	-	
Mwazi		-	+	-	-	-	+	-	-	+	+	-	-	-	
Smari	Fruits	-	-	-	-	-	+	-	-	+	-	+	-	-	
Shhami		-	-	-	-	-	+	-	-	+	-	+	-	-	
Mwazi		-	-	-	-	-	+	-	-	+	-	+	-	-	
Smari	Skins	-	-	-	-	-	+	-	-	+	-	+	-	-	
Shhami		-	-	-	-	-	+	-	-	+	-	+	-	-	
Mwazi		-	-	-	-	-	+	-	-	+	-	+	-	-	

4.5. GC-MS analysis

The GC-MS analysis showed the presence of at least 6 volatile compounds in each fig sample with different values (**Figures 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, 4.12, and 4.13**). Major volatile compounds detected in the methanolic extract of fig samples were *pyrazole benzotriazine* ($Rt=17,715$), *naphthaline dione* ($Rt=20,53$), and *phytol* ($Rt=20,806$). Other minor volatile compounds identified include but are not limited to *caryophyllene*, *1-H indole ethylamine*, and *4-acetyl isocoumarin* (**Table 4.3**). From all tested samples, fig leaves showed a high level of volatile compounds followed by fruits and traces amount in the skin.

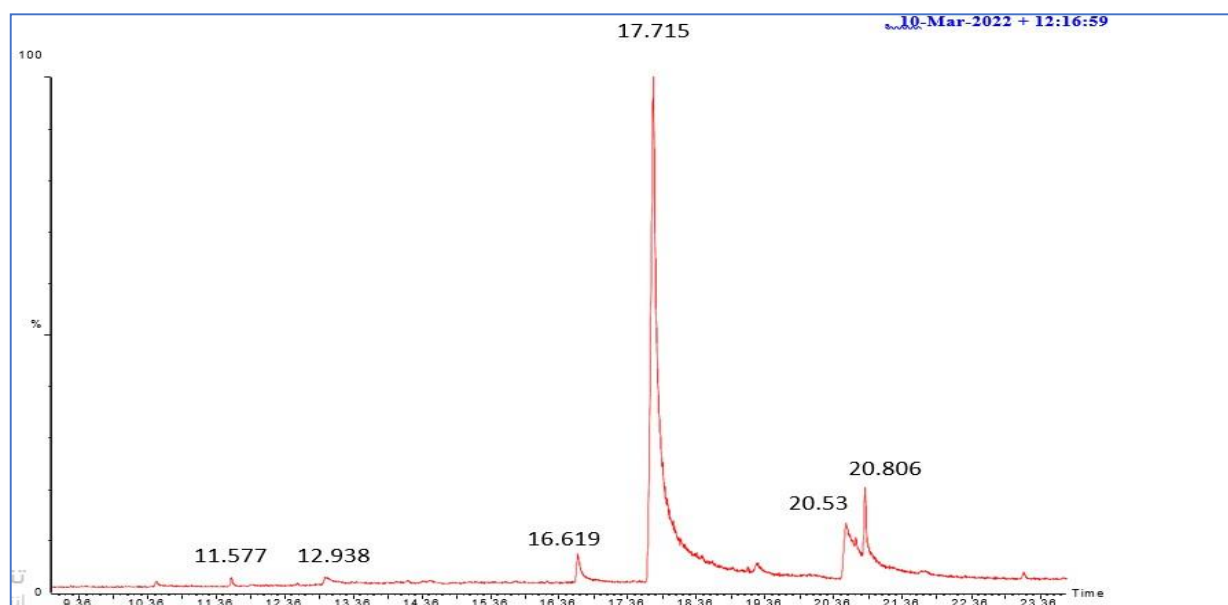


Figure (4.5): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Smari leaves. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

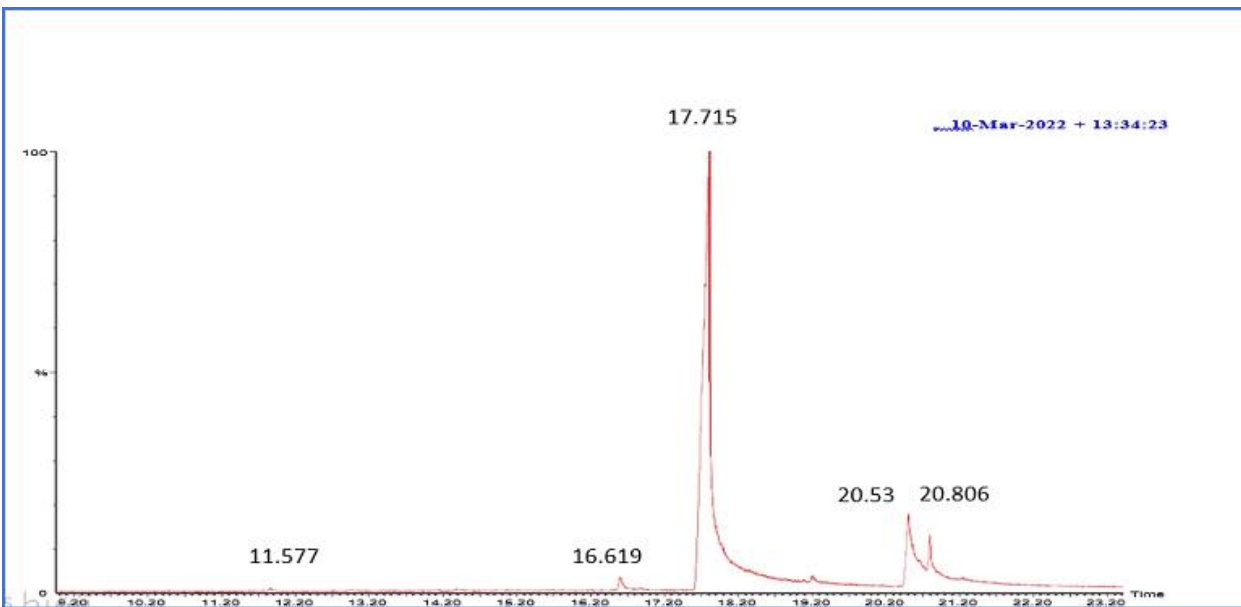


Figure (4.6): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Shami leaves. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

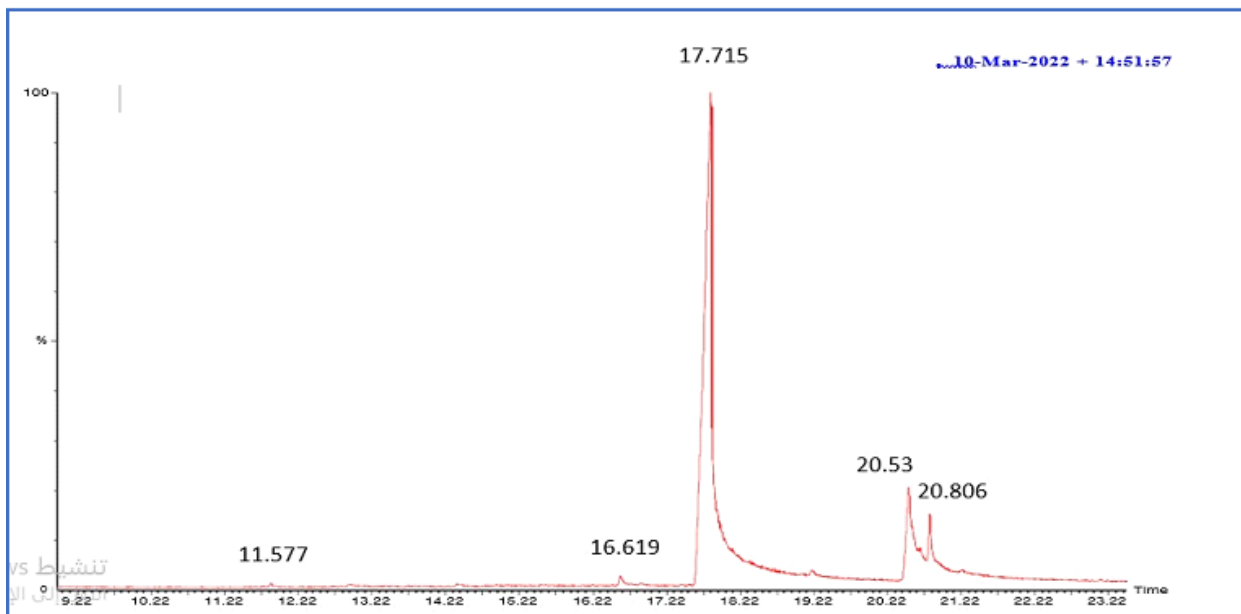


Figure (4.7): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Mwazi leaves. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

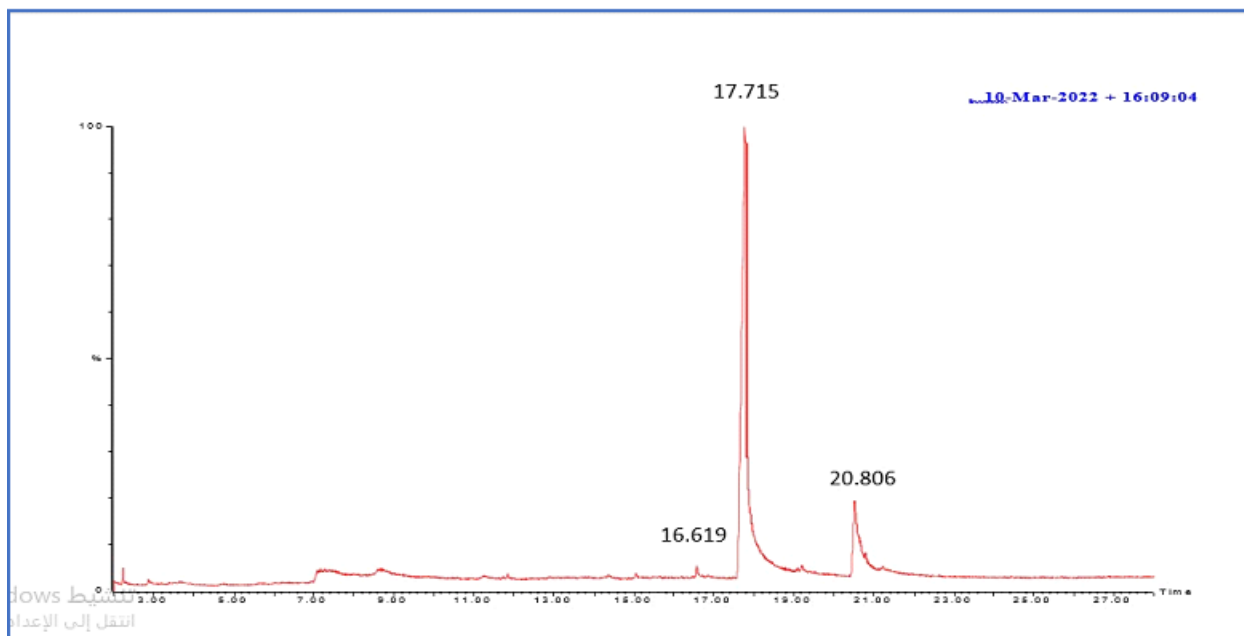


Figure (4.8): Representative GC -MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Smari fruit. Numbers on peaks represent the retention time (Rt) in minutes for each peak

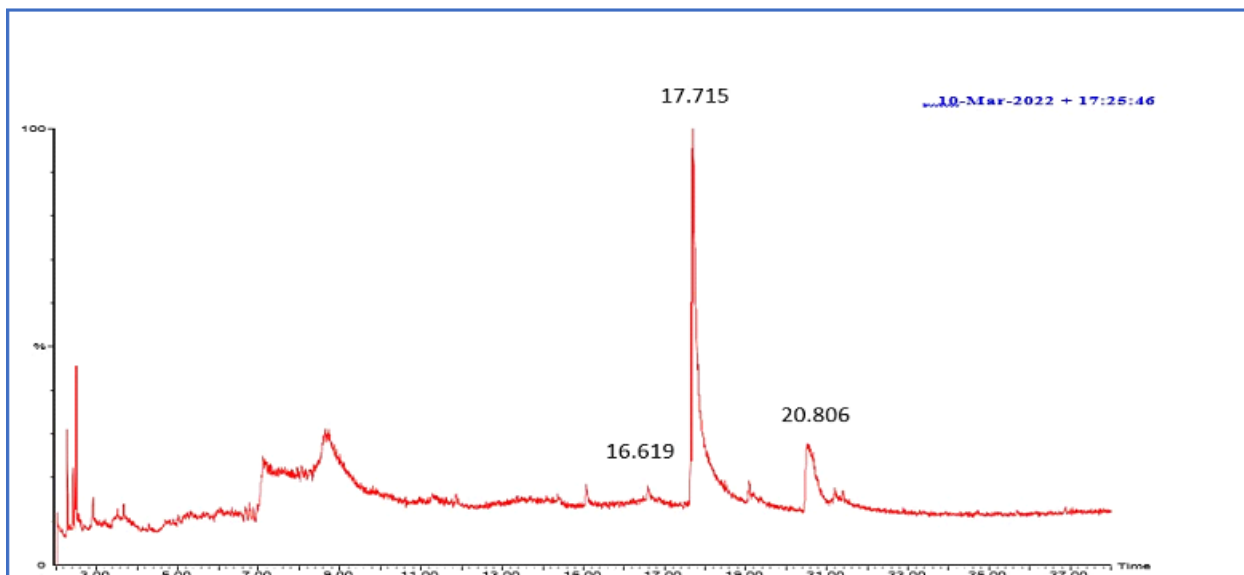


Figure (4.9): Representative GC -MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Shami fruit. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

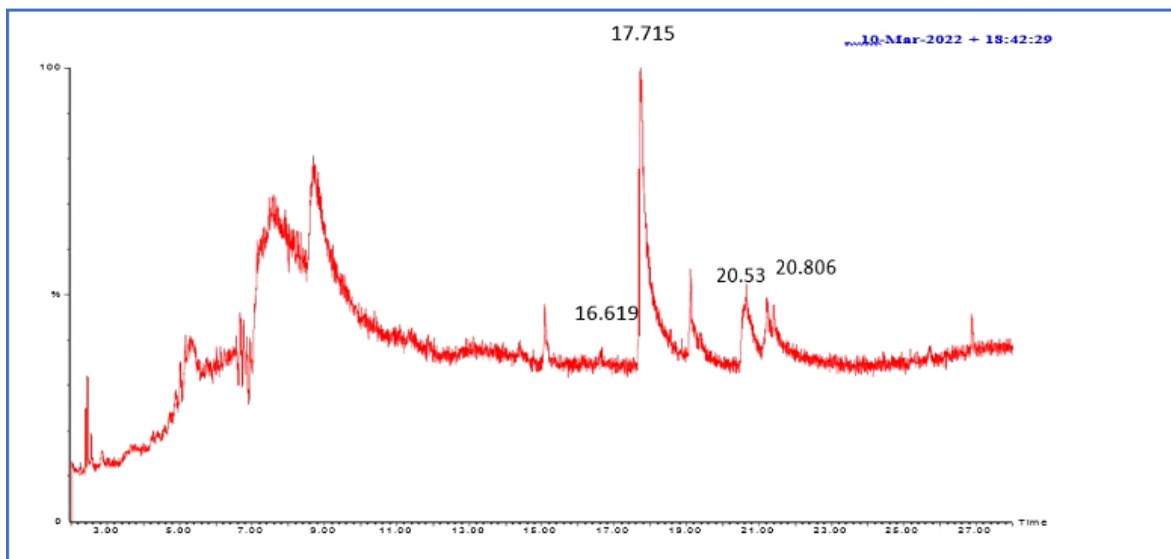


Figure (4.10): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Mwazi fruit. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

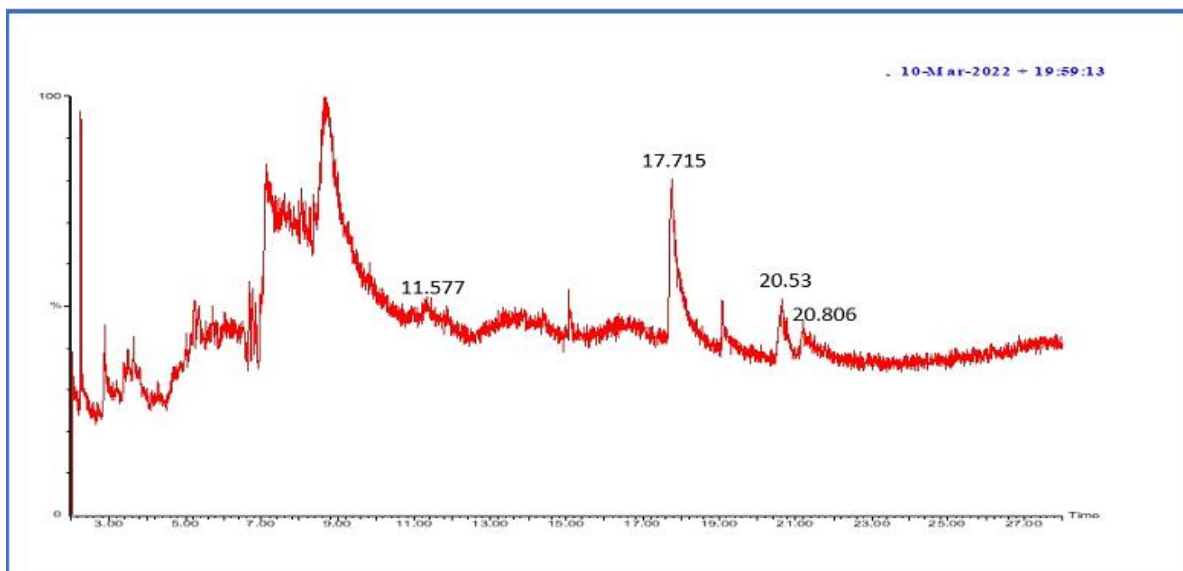


Figure (4.11): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Smari skin. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

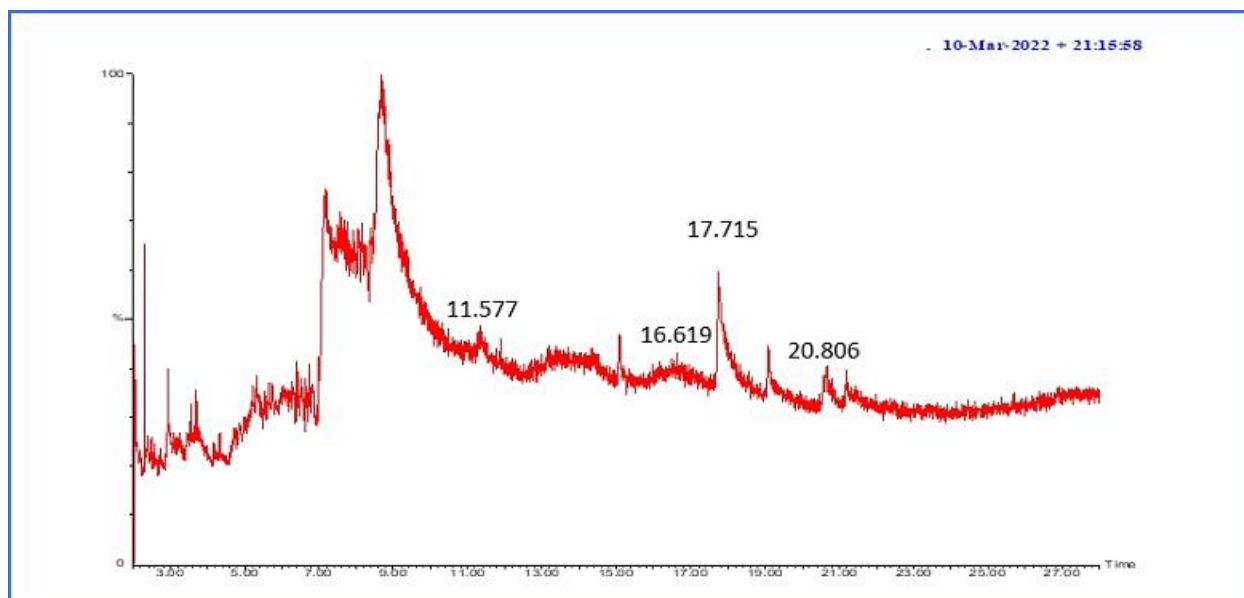


Figure (4.12): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Shhami skin. Numbers on peaks represent the retention time (Rt) in minutes for each peak

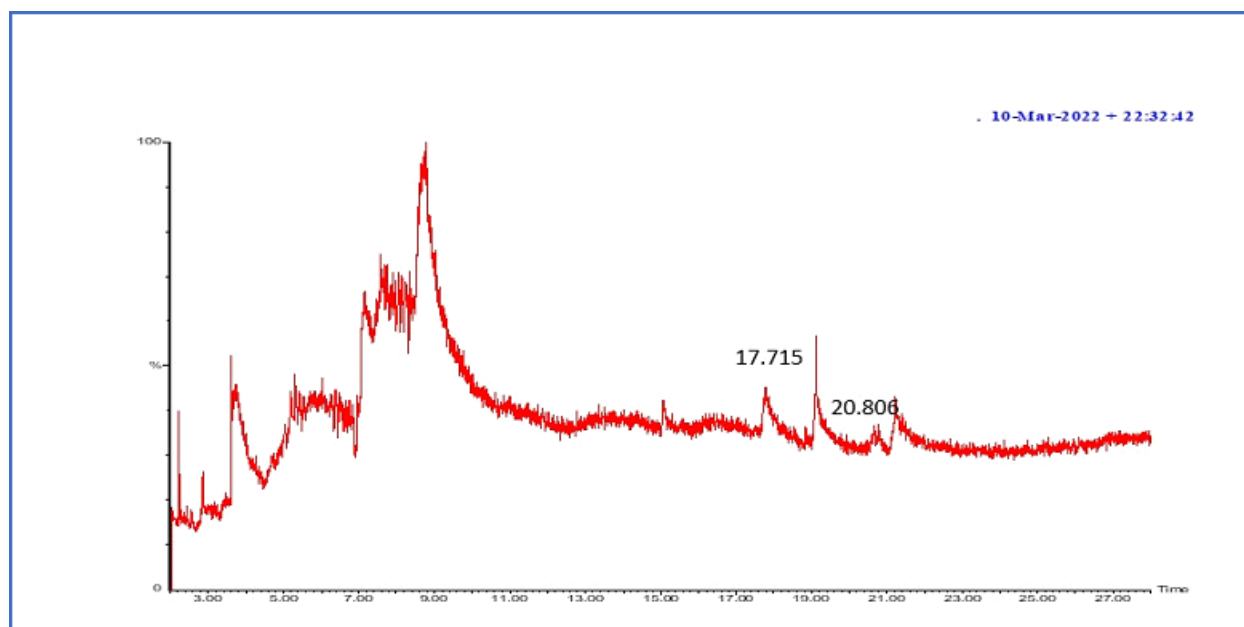


Figure (4.13): Representative GC-MS total ion mass chromatograms of the volatile compounds detected in the methanolic extracts of Mwazi skin. Numbers on peaks represent the retention time (Rt) in minutes for each peak.

Table (4.3): Major compounds detected in three fig genotypes extracts with their retention time (*Rt*) and molecular weight (MW), and molecular mass (*M/Z*).

No	<i>T</i>	<i>M/Z</i>	Compound identification	MW
1	11.577	69,93,105	Caryophyllene	204
2	12.938	131,160,173	1-H.indole ethylamine	160
3	16.619	118,160,188	4-acetyl isocoumarin	188
4	17.715	102,185,186	Pyrazole benzotriazine	186
5	20.53	216,173,201	Naphthaline dione	216
6	20.806	71,123,216	Phytol	296

4.6. Antibacterial activity

Among the three examined fig genotypes and their parts, Methanolic extract of Smari and Shhami leaves displayed antibacterial activity against the gram-negative pseudomonas bacteria with 36.86 % and 32.16% respectively, compared with the positive control (**Figure 4.14**). Other bacterial species were not affected by any type of fig sample methanolic extract.

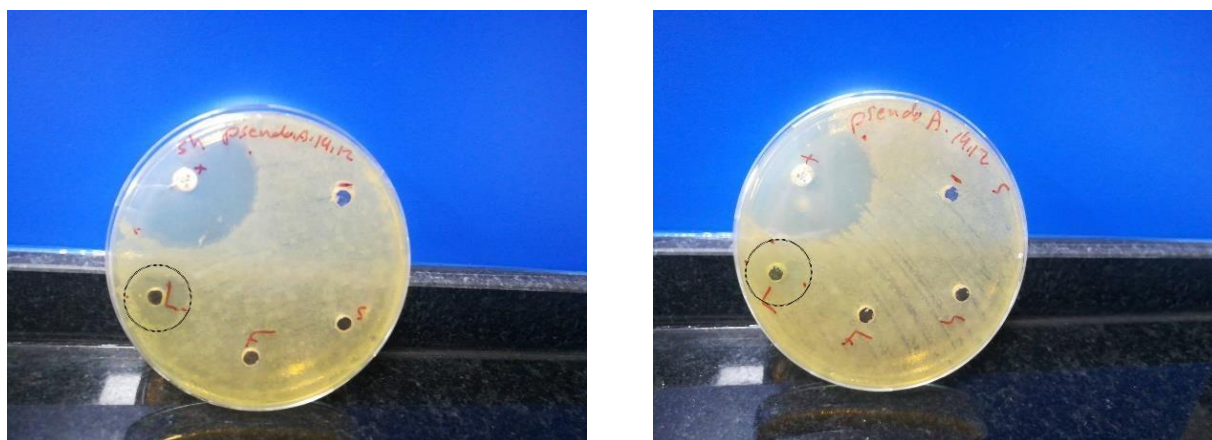


Figure (4.14): The zone of inhibition against pseudomonas bacteria for Shhami leaves (left side) and Smari leaves (right side).

Chapter 5: Discussion

5. Discussion

Plants are essential for providing energy and nutritional requirements to human beings. Fig and its different parts have a very high safety and healthy nutritional profile. Fig is a deciduous tree belonging to the Moraceae family. Fig fruits are one of the highest consumed for their richness of nutrients and accordingly health promotion as they have been used for the treatment of various ailments (constipation, diabetes, high cholesterol, eczema, psoriasis, vitiligo, cancer, inflammation, etc.). Palestine is one of the countries in the region for fig growing and production because it is characterized by a wide range of environmental conditions and rich natural biodiversity. There are hundreds of species of the tree, but *Ficus carica* Linn is the most common one in Palestine. Based mainly on skin ground color, internal color, country of origin, and maturity date, the Fig name is given. The genotypes of the fig identified in Palestine are twelve in number which includes; Shhami, Ghzali, Bladi, Khdari, Swadi, Smari, Aswad, Ruzzi, Hmari, Moozi, Mouze, and Mwazi which grow mainly in the southern regions of West Bank [Radwan *et al.*, 2020]. Thus, chemical composition, quality parameters, and the pharmacological properties of the three targeted fig genotypes (Shami, Mwazi, and Smari; which commonly grown in Palestine) including their different parts (leaves, fruits, and skin) were investigated.

The fiber and ash values in our samples for the leaves, fruits, and skins of the three genotypes studied were different compared to the other studies (Aljane and Ferchichi, 2009, Ghazi *et al.*, 2012, Pereira *et al.*, 2017, and Mahmoudi *et al.*, 2018). Leaves presented significantly higher fiber and ash contents over fig fruits and skins (**Table 4.1**). The crude fiber content of fig fruits was below those obtained by Sadia and others (2014) who demonstrated 14.2% crude fiber content in *ficus carica* species, on the other hand, Tanwar and others (2014) showed lower values (1.2%) than those obtained in our study. Moreover, Chauhan and others (2015) achieved higher ash values (4.4%) for Indian fig fruits, than our fig samples values. The ash content of fig leaves in this study is higher than those reported by Ghazi and others (2012) who reported 4% ash content in Indian *ficus carica* leaves. The differences might be and probably due to different environmental conditions and analytical methods.

The exhibited higher amount of fiber and ash in fig leaves compared with fig fruits and skins might be related to the age of the leaves because generally, the trees' leaves at their early stages of growth

contain a high percentage of proteins and low fiber content. After flowering the leaves became mature and protein content decreased and fiber content increased with the increasing age of tree leaves. For the same reason also, mature leaves contain high content of minerals and ash, as the main composition of the ash is minerals [Singh, 2019]. Dietary fiber applies for an important role in human health and is mainly required for efficient digestion and waste removal. It is also able to lower both blood cholesterol and the risk of coronary heart disease (Houghton, 2007). Continuously, ash content is known to be responsible for determining the consistency of the material, identifying it as carbon-free, as well as showing the organic, inorganic, and impurity content found in the samples (Ilodibia, *et al.*, 2016). Moreover, the soluble and insoluble minerals in plant samples are mainly predicted by the total ash content.

Minerals are elements that originate in the soil and can't be created by living creatures. But all creatures need minerals in certain amounts to maintain proper health. Plants absorb minerals from the soil then animals get their minerals from the plants or other animals they eat. Human beings obtain minerals either directly from plants or indirectly from animal sources. Ours presented significantly higher values of Ca, K, and Mg agreeing with the findings of Khan and others (2011) who stated that Ca, K and Mg as major minerals of fresh figs comparing with other minerals. On contrary, the significantly lower values of P, Na, and Zn might be attributed to differences in plant genotypes, climatic conditions, soil abundance (Khan, *et al.*, 2001; Bengtsson, *et al.*, 2003), and maturity stages (Choudhury, 2007)). Generally, mineral elements exist in a small amount compared with the total composition of most plant materials and thereby total body weight; however, they are having extremely physiological importance, and help to improve overall health and disease prevention (Singare, 2010). The medicinal necessity of minerals was observed in several studies compromising Ca essentiality for strong teeth and bones, improving the structural rigidity of the body, and helping in blood clotting (Indrayan, *et al.*, 2005); Mg necessity for energy metabolism, enzymatic action, blood pressure as well as cardiac excitability (Volpe, 2013); Zn mainly acts a stimulator to improve the activity of beta cells in the pancreas to release insulin and maintains the normal glucose rate, also its necessary for tissue repair and growth (Prashanth, *et al.*, 2015); Fe as the main precursor for hemoglobin production, O₂ transport, and help to improve the body's immunity (Kruczek, 2005). However, daily intake of Sodium (Na) and potassium (K) should be in a small amount, but generally, they play a vital role in human physiology and pathology. In addition, K is one of the main precursors for cells, tissues, and organs to exhibit their

function well. Also, it is important to manage the heart function and the contraction of both skeletal and smooth muscle to get normal digestive and muscular function. Furthermore, the constant movement of Na and K between cells is crucial to maintain the electrolyte balance, regulating renal acid-base balance, and generating the electrical potential that finally conducted the nerve impulse (Pohl *et al.*, 2013).

Free radicals are highly unstable chemical species that mainly contain one or more unpaired electrons that are responsible to cause damage to other molecules by extracting electrons from them to attain stability. The human body continuously produces free radicals, which are essential for energy supply, detoxification, chemical signaling, immune function, and else. However, free radicals are necessary for the body but at the same time, they could be harmful. The production of reactive oxygen species (ROS) could be induced by ionizing radiation, ultraviolet light, chemical reactions, and metabolic processes (Zhou *et al.*, 2016). The imbalance between antioxidants and reactive oxygen species results in oxidative stress, that considered the main cause initiating the oxidation of biomolecules, such as lipids, amino acids, proteins, and DNA that finally lead to cell injury and induce several diseases (cancer, Parkinson's disease, and others) (Li *et al.*, 2015). Several enzymes like superoxide dismutase, catalase, glutathione reductase, and others, could help to repair the free radicals-induced damage. Additionally, antioxidants (polyphenols, vitamin A, vitamin C, and else) play a key role in reducing oxidative stress in cells due to their ability to neutralize or scavenging the ROS by hydrogen donation, therefore it is essential in the treatment of several human diseases such as cancer, cardiovascular diseases, and inflammatory diseases (Baiano *et al.*, 2016). Medicinal plants, which are mainly used as spices and food plants are the main sources of organic compounds such as tannins, alkaloids, carbohydrates, steroids, terpenoids, and others that make definite physiological actions on the human body. Phenolics groups that mainly exist in leaves, flowering tissues, and woody parts, such as stems and barks, provide a major capacity to scavenge free radicals, due to their ability to act as reducing agents, and hydrogen donors. Therefore, they could play an important preventive role in the development of some diseases (Li *et al.*, 2014).

The antioxidant activity of fig samples was determined using DPPH[•] and ABTS^{•+} assays, as a stable free radical method, which is an easy, rapid, and sensitive way to examine the antioxidant activity of a specific compound or plant extracts (Baliyan *et al.*, 2022). Interestingly, DPPH and

ABTS⁺ scavenging activity concurrently increased with the increase of phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. Among the three examined fig genotypes and their parts, Shhami, Mwazi, and Smari leaves revealed the highest antioxidant activity respectively, with a high percentage of scavenging activity above 80% for DPPH[•] (**Figure 4.2**) and less %age for ABTS^{•+} (**Figure 4.1**). Our DPPH results are consonant with Akhtar, and others (2019) who obtained that the DPPH[•] scavenging activity of *Ficus carica* leaves extract ranged from 66.19 to 87.06%, but the ABTS assays (96-99) % were higher than those obtained in our study. However, the fruit showed the lowest antioxidant capacity, with a percentage of scavenging activity ranging between 3.3-13.4% and 26.3-37.3% for DPPH[•] and ABTS^{•+} respectively. El-Sayed and Abdel-Hamed (2009) also revealed that the leaves methanolic extracts of eleven *Ficus carica* species including *Ficus carica* grown in Egypt had mainly phenolic compounds as one of the major components with significant radical scavenging activity. Moreover, Mahmoudi and others (2018) obtained that the fig fruit peels exhibited the highest antioxidant activity compared with fig fruit pulps because they contained the highest concentrations of phytochemicals.

The total phenolic contents of fig samples methanolic extracts were determined using the FolinCiocalteu method. The highest TPC was found in Shhami, Mwazi and Smari leave respectively (**Figure 4.3**). The values are higher than those obtained by El-Khateeb and others (2013) who possessed that the fig leaves TPC is 122.52 mg GAE/g. However, fruit revealed the lowest total phenolic contents. Which supported by Wojdyło and others (2016) who showed that the TPC of different cultivars of *ficus carica* fruits ranged less than our range. Generally, these results also agree with Mahmoudi and others (2016) who obtained that the TPC among the three vegetal materials was significantly different, following the order: leaves > peels > pulps.

During the present work, the leaves of the three fig cultivars possessed the strongest antioxidants activity and the fruits had the weakest activity, these results agree with Ammar and others (2015) who obtained that the leaves of two different fig genotypes have the strongest antioxidant activity comparing with its fruits. This may be explained by the occurrence of the highest amounts of phenolic compounds in leaves. Interestingly, our results indicate an excellent correlation between the total phenolic contents and antioxidant activity. So, these findings are from previous studies that also revealed the linear relation between antioxidant activity and total phenolic contents (Alali *et al.*, 2007). Therefore, it can be suggested that the phenolic compounds significantly contributed

to the antioxidant potential of fig, and this is the main reason that always applies the leaves as the most effective part. Moreover, the results of the work indicated that phytochemicals were responsible for the medicinal effects of fig, and this finding agrees with (Akinmoladun *et al.*, 2007) study. The finding of this study suggests that fig leaves could be a potential antioxidant natural source that could have great importance as therapeutic agents to prevent or slow the progress of oxidative stress-related diseases.

Phytochemicals naturally occurring compounds mainly found in fruits, vegetables, legumes, beans, nuts, and whole grain, and include many compounds such as phytosterols, saponins, flavonoids, terpenes, and else, which are responsible for the protective health benefits of these plant-based foods and beverages (Brindha, 2016). Phytochemicals, as a part of a large and varied group of chemical compounds, are responsible for the color, flavor, and odor of plant food, also, considered multifunctional components of food due to their important biological properties and antioxidant activity. Phytochemical research revealed that *F. carica* with all its different parts have phytosterols, anthocyanins, amino acids, organic acid, fatty acids, phenolic compounds, hydrocarbons, aliphatic alcohols, volatile components, and some other classes of secondary metabolite. These phytochemicals are mainly found in latex followed by leaves, fruit, and roots. However, these naturally occurring secondary metabolites get widespread attention due to their ability to have remarkable pharmacological properties such as anticancer, antioxidant, and anti-inflammatory effects and others. (Badgujar *et al.*, 2014). Phytochemical analysis of fig samples included a screening of saponins, steroids, tannins, terpenoids, phenolic groups, and other secondary metabolites, revealed that fig fruit, skin, and leaves are a good source of saponins, steroids, and terpenoids. On the other hand, tannins and phenolic groups were only found in the leaves (**Table 4.2**), these results were supported by Farag and others (2020) who revealed that fig leaves are a rich source of tannins and phenolic groups which improve their antioxidant activity. As known, saponins mainly help to reduce the risk of heart disease, due to their ability to lower the cholesterol level (Soni *et al.*, 2014). Phenolic compounds, including tannins, exhibited therapeutic uses due to their anti-inflammatory, anti-fungal, antioxidant, and healing properties. Which, some groups of tannins that mainly act on arachidonic acid metabolism in leucocytes, apply an important role in reversing inflammations, treatment in fostering wound healing, and else (Okuda, 2005). Among natural products, phytosterols as promising compounds that have diverse

pharmacological activities, such as the treatment of inflammatory diseases, multiple sclerosis, asthma, and cardiovascular diseases (Marahatha *et al.*, 2021).

The volatile components of methanolic extract for fig samples were analyzed by GC-MS in the Electron Impact (EI) mode and identified by comparing with the NIST library and by calculating Kovats Index (KI). The GC-MS analysis of fig samples revealed the presence of at least six volatile compounds. The bioactive compounds identified with their retention time and molecular weight are shown in **(Table 4.3)**. The major volatile compounds detected in the methanolic extract of fig samples were pyrazole benzotriazine ($R_t=17,715$), naphthaline dione ($R_t=20,53$), and phytol ($R_t=20,806$). Other minor volatile compounds identified include but are not limited to caryophyllene, 1-H indole ethylamine, and 4-acetyl isocoumarin. However, the major compounds are found in a high quantity in the leaves, followed by fruits, and at traces amount in fig skin. These results are also compatible with the results obtained by Ivanov and others (2018); Soniand and others (2014); Sertkaya and others (2021); LazregAref and others (2012), who showed that the GC-MS analysis of different parts of *figus carica* has phytol, naphthaline dione, caryophyllene, coumarin, indole, and other volatile compounds. Phytol is a compound abundantly found in nature, which is a part of the chlorophyll molecules, and is considered the main cyclic isoprenoid present in the biosphere of plants. However, phytol is being used as a fragrance constituent; its significant biological properties have recently gotten the attention of other possible applications in the pharmaceutical and biotechnological fields. Interestingly, phytol as one of the main constituents of plants derived essential oils, could reveal its antimicrobial and cytotoxic activity against *Escherichia coli* (Ghaneian *et al.*, 2015) and *Pseudomonas aeruginosa* (Pejin *et al.*, 2015), but there's no specific cellular target against *Staphylococcus aureus* (Ghaneian *et al.*, 2015). Moreover, phytol possesses an essential antioxidant activity, as it was able to scavenge reactive oxygen and /or nitrogen species (ROS/RNS) through direct and indirect mechanisms (Bakkali *et al.*, 2008). Therefore, phytol with its antioxidant activity could apply anticancer effects (Chikati, 2013), anti-teratogenic activity (Arnhold *et al.*, 2002), and anti-tumor and tumor promoter activity (Líška *et al.*, 2011). Additionally, phytol provides other pharmacological activities such as anxiolytic, anticonvulsant effects, anti-inflammatory effects, and others, which makes it highly recommended in the pharmaceutical industry (Islam *et al.*, 2018). Selestino *et al.* revealed that β -caryophyllene exhibited moderate antibacterial activity and strong antifungal activity and apply a major effect against hepatoma cancer cells (Selestino *et al.*, 2017).

Infectious diseases are one of the main causes of morbidity and mortality worldwide, which approximately accounted 50% of all deaths in tropical countries. The excessive use of antibiotics has contributed to multidrug-resistant bacteria which significantly cause the failure of treatment, and that is considered a big problem that limits the effectiveness of drugs worldwide. Therefore, investigating a new strategy for the prevention and treatment of infectious diseases is an urgent need to overcome these problems. In the last decade, chemical compounds were isolated from medicinal plants have applied as a model for many clinically proven drugs, which now being reassessed as antimicrobial agents, this is related to the reduction in the new antibacterial drugs, increase antimicrobial drug resistance, and the need for new emerging pathogens treatment (Mahady, 2005). Medicinal plants can exhibit antibacterial activities through multiple mechanisms, such as cytoplasmic membrane disruption, inhibition of nucleic acid synthesis, energy metabolism, cell wall synthesis cell membrane synthesis, and else (Al-Snai, 2019). Besides, Antibacterial resistance is a serious global problem, and the discovery of natural antimicrobials may provide valuable solutions to overcome this problem. Thus, it is important to identify new sources of natural antioxidants and antimicrobials. The present study investigated the antioxidant as well as antibacterial activities of fig genotypes, which are traditional Palestinian fruits. However, Methanolic extract of Shhami, Smari, and Mwazi fig genotypes were investigated for their antimicrobial activity against five bacterial strains, one gram-positive (*Staphylococcus aureus*), and four gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus*) using agar well diffusion method for determining the inhibitory zone diameters. Interestingly, our results revealed that only the methanolic extract of Smari and Shhami leaves displayed antibacterial activity against the gram-negative *pseudomonas* bacteria with a reasonable zone of inhibition, compared with the positive control (meropenem) (**Figure 4.14**). These findings align with Rashid and others (2014) who showed that the *Ficus carica* leaves extract was able to inhibit the growth of *pseudomonas aeruginosa*. However, Other bacterial species were not affected by any type of fig samples of methanolic extract. The fig of Mwazi leaves lacks antibacterial activities against *Pseudomonas aeruginosa*. It was suggested that the antimicrobial activity of figs was probably due to its constituents, as the detection of methanolic extract of fig leaves revealed the presence of the steroids, tannins, alkaloids, saponins, and phenolic groups which possess diverse biological effects like antioxidant, anti-inflammatory, and antibacterial activities (Solomon *et al.*, 2006). Specifically, tannins and phenolic groups which were only detected in fig leaves

possessed remarkable toxic activity against bacteria and provide the main pharmacological importance (Doss *et al.*, 2009; Mahmoudi *et al.*, 2016). Tannins are considered a toxic compound for bacteria, due to their ability to cause growth inhibition and bacterial death by reacting with phospholipids contained in the bacterial cell wall, triggering membrane damage and leakage of a metabolite that finally inactivate the bacterial enzyme system and killing the bacteria (Mailoa *et al.*, 2014). Further quantitative analysis of volatile compounds is needed to explain the lack of antibacterial activities against *Pseudomonas aeruginosa* in fig of Mwazi leaves. Further studies also are needed to estimate the minimum inhibitory concentration (MIC) and the safety of the fig methanolic extracts.

Chapter 6: Conclusions

6. Conclusions

We examined the chemical constituents of leaves and fruits of the three fig genotypes (Shhami, Mwazi, and Smari) commonly grown in Palestine from Hebron city and some of their pharmacological activities. This study proved variability among the genotypes studied. The methanolic extract was analyzed by GC-MS technology. Particularly the leaves contain a higher number of components than the fruits and skin of *Ficus carica* L. The GC-MS technique was utilized and found to be precise, accurate, and reliable in the separation and identification of the components of *Ficus carica* L. complex volatile mixtures.

Six volatile and semi volatile components were detected and identified in *Ficus carica* L. leaves, fruits, and skin. The major volatile compounds detected in the methanolic extract of fig samples were pyrazole benzotriazine, naphthaline dione, and phytol. Other minor volatile compounds identified include but are not limited to caryophyllene, 1-H indole ethylamine, and 4-acetyl isocoumarin. However, the major compounds are found in a high quantity in the leaves, followed by fruits, and at traces amount in fig skin. The usage of leaves is recommended for medicinal use. In other words, it is advisable to use leaves in the preparation of pharmaceutical dosage forms for the treatment of diseases,

Ficus carica L. has potential metabolites which can be used as an alternative to antibacterial and antimicrobial drugs. Thus, *Ficus carica* leaves extracts also could be considered original ingredients with potential applications in the formulation of antibacterial drugs.

The results showed that fig leaves are a good and valuable source of natural antioxidants that can be used in the food and medical sectors. The important antioxidant capacity of methanolic extracts of fig is probably due to their richness in phenolic compounds. Such bioactive molecules react as natural antioxidants and then, they are well known to display a positive impact on human health and can be considered for future uses as antioxidant components in agro-food industries.

We have estimated that these data will be useful for studies to improve the nutritional and medicinal content of fig genotypes and their parts. This work is important to assess the significance of *Ficus carica* L. use by Palestinians.

Our results revealed a considerable variation in the phytochemical, antioxidant activity, fibers, ash, total proteins, and antibacterial activity for the fig different parts as well as the three genotypes studied.

Further research is required to identify active compounds and their mechanisms of action against pathogens. More research is required to isolate the secondary metabolites to establish clinical trials for human benefits.

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المخلص

تعتبر اشجار التين واحده من اهم الاشجار المتساقطة الأوراق، والتي كانت وما زالت تستخدم كغذاء وعلاج شعبي للكثير من الامراض مثل الجذام، نزيف الانف، ارتفاع الحرارة، امراض الكبد وغيرها.

تم في هذه الدراسة قياس المركبات الكيميائية، المعادن، مضادات الأكسدة، ومضادات البكتيريا لكل من الاوراق والثمار والقشور لثلاثة اصناف مشهورة من التين في فلسطين هي الشحامي، الموازي، السماري. قطفت ثمار واوراق التين الناضجة تماما من مدينة الخليل الفلسطينية ثم جففت. تم تحديد المواد المتطايرة عن طريق جهاز GCMS. تم قياس مضادات الاكسدة من خلال طريقتي DPPH و ABTS، بينما اختبرت مضادات البكتيريا عن طريق agar well diffusion method. واستخدمت تقنية ICP-OES لتحديد محتوى العناصر في جميع العينات.

بشكل عام اظهرت النتائج بأن اوراق التين تحتوي على نسبة الياف ورماد أعلى من الثمار والقشور. كما وتحتوي على نسب أعلى نسبة من الكالسيوم والمغنيسيوم والحديد والبورون، تليها على التوالي القشور ومن ثم الثمار. بالإضافة الى ذلك تحتوي اوراق التين لجميع الاصناف المذكورة على أعلى نسبة مضادات اكسدة لكلا الطريقتين (٨٥,٢، ٨٣,٤، ٨٢,٦) DPPH٪ و (٧٥,١، ٥٩,٥، ٨٠) ABTS٪ للتين الشحامي، السماري والموازي على التوالي. وكانت هذه النتائج تتماشى مع نتائج ال total phenolic content، التي كانت اعلاها بالأوراق مقارنةً بالثمار والقشور. تضمن التحليل للمواد الكيميائية لعينات التين اربعة عشر مركب كيميائي اتضح من خلالها ان جميع العينات تحتوي على saponins, steroids and terpenoids، في حين تواجدت مركبات tannins و phenolic groups في الأوراق فقط. وباستخدام جهاز GC-MS تم تحديد هوية ستة مركبات اساسية متطايرة تواجدت تنازلياً في الأوراق، تليها الثمار ثم القشور وبكميات مختلفة وهي Phytol, Caryophyllene, 1-H. هذا واطهرت نتائج المستخلص الميثانولي لكل عينات التين بان اوراق التين الشحامي والسماري فقط لهم قدرة على تثبيط النشاط البكتيري ولنوع واحد فقط من البكتيريا وهي pseudomonas .

يتضح من النتائج السابقة وجود تبايناً كبيراً في النشاط الكيميائي النباتي، ومضادات الأكسدة، والألياف، والرماد، والمعادن والنشاط المضاد للبكتيريا سواء بين أصناف التين أو بين الأجزاء المستهدفة في الدراسة. كما بينت الدراسة وجود مزايا عديدة للأوراق على حساب الثمار والقشور من حيث نسبة المعادن، ومضادات الأكسدة، وقدرة أكبر على تثبيط نمو البكتيريا. توصي الدراسة ضرورة التوسع في دراسة أوراق التين كونها مصدرًا طبيعيًا مهماً لمضادًا للأكسدة يساعد على العلاج من الامراض المرتبطة بعمليات الأكسدة داخل الجسم.