

**Deanship of Graduate Studies  
Al-Quds University**



**Antioxidant activity, Phenolic Content, and Flavonoid  
Content of Palestinian Ziziphus Spina-Christi**

**Shawqi M. A. Harahsheh**

**M.Sc. Thesis**

**Jerusalem – Palestine**

**2017/1438**

**Antioxidant activity, Phenolic Content, and Flavonoid  
content of Palestinian Ziziphus Spina-Christi**

**Prepared by:**

**Shawqi M. A. Harahsheh**

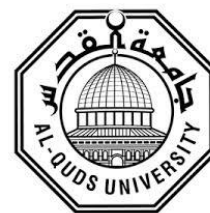
**B.Sc. Chemistry. Alquds University. Palestine**

**Supervisor: Dr. Fuad Al-Rimawi**

**Co- Supervisor: Dr. Imad Odeh**

**A thesis Submitted in Partial fulfillment of  
requirement for the degree of the Master of Applied  
and Industrial Technology, Al-Quds University**

**2017/1438**



## Thesis Approval

### Antioxidant activity, Phenolic Content, and Flavonoid content of Palestinian Ziziphus Spina-Christi

Student name: Shawqi M. A. Harahsheh

Registration No:21210132

Supervisor: Dr. Fuad Al- Rimawi.

Co supervisor: Dr.Imad Odeh.

Master thesis submitted and accepted, Date: 13/06/2017

The names and signatures of the examining committee members are as follows:

1- Head of Committee/ Dr. Fuad Al-Rimawi Signature:

2- Co supervisor/ Dr. Imad Odeh Signature:

3- Internal Examiner/ Dr. Ibrahim Kayali Signature:

4- External Examiner/ Dr. Sameh Jarrar Signature:

Jerusalem-Palestine

2017/1438

## ***Dedication***

*To my beloved parents, Mahmoud and Moyassar, to my beloved wife Suzan, to my beloved Mohammad, to my beloved daughters Thkra, Lara, Mallak, Lareen, to my supervisor Dr. Fuad Al- Rimawi, to my home land Palestine.*

*Special thanks for Dr. Jehad Abbadi for his continuous help and supervision during my research.*

## **Declaration**

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

Signed:

Shawqi Mahmoud Ali Harahsheh

Date: 13/06/2017

## **Acknowledgements:**

I am very pleased to express my gratitude to all people who assisted me to go forward in my studies, and to all people who offered me help and support.

I would like to thank my supervisors Dr. Fuad Al-Rimawi, Dr. Imad Odeh and Dr. Jehad Abbadi for their continuous help and supervision during my research. Also, I would like to thank the staff of the chemistry lab

Finally, I am glad to thank my family: my father, my mother, my sisters and my brothers for their help and encouragement.

## List of Abbreviations

AA	Antioxidants activity.
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid.
Nc	Neocuproine.
FRAP	Ferric Ion Reducing Antioxidant Power Assay.
DPPH	2,2-Diphenyl-1-picrylhydrazyl.
CUPRAC	Cupric reducing antioxidant capacity.
HPLC	High-performance liquid chromatography.
SD	Standard deviation.
TFC	Total phenolic content.
TPC	Total flavonoid content.
RSA	radical scavenging activity.
TPTZ	2,4,6-tri(2-pyridyl)-1,3,5-triazine.
ROS	Radical Oxygen Species.
CE	Catechin.
GAE	Gallic acid equivalent.
DM	Dry matter.
DW	Dry weight.

## **Abstract**

In this study, in vitro evaluation of antioxidant activity, total phenolic content, and total flavonoids content of three different extracts of leaves and fruits (99% ethanol, 80% ethanol, and distilled water) of *Ziziphus Spina-Christi*, from three different geographical regions of West Bank (Al-Zbedat in the north, Jericho in the middle, and Bani Naeem in the south) were the leaves collected in April and the fruits were collected in May.

The extracts of leaves and fruits were evaluated for their total phenolic content (TPC) using Folin-Ciocalteu method, total flavonoids content (TFC) using Aluminium chloride method, and antioxidant activity which was determined by four different methods: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity, Ferric reducing antioxidant power (FRAP), Cupric reducing antioxidant power (CUPRAC), and free radical scavenging activity using ABTS. All the analyzes were made with the use of UV-Visible spectrophotometer.

Overall the *Ziziphus Spina-Christi* leaves and fruits showed strong antioxidant ability and high phenolic content. The total phenolic content (TPC) in leaves collected in April in two seasons were ranged between 273.4 (North, D.water) to 94.1 (South, 99% Ethanol) mg of GAE/g DW, and 448 (North, 80% Ethanol) to 301.5 (Middle, 99% Ethanol) mg of GAE/g DW, respectively. Total phenolic content in fruits collected in May in two seasons were ranged between 421.7 (middle, 99% Ethanol) to 58.5 (North, 99% Ethanol) mg of GAE/g DW, and 339.8 (North, 99% Ethanol) to 90.6 (North, D.water) mg of GAE/g DW, respectively.

The total flavonoids content (TFC) in leaves collected in April in two seasons were ranged between 118.7 (Middle, 80% Ethanol) to 9.1 (Middle, 99% Ethanol) mg CE/g DW, and 39.6 (North, 80% Ethanol) to 10.5 (Middle, 99% Ethanol) mg CE/g DW, respectively. Total flavonoids content in fruits collected in May in two seasons were ranged between 11.2 (Middle, D.water) to 2.5 (South, 99% Ethanol) mg CA/g DW, and 16.7 (North, 80% Ethanol) to 4.5 (South, 99% Ethanol) mg CE/g DW, respectively.

Antioxidant activity using FRAP assay in leaves collected in April in two seasons were ranged between 11.9 to 2.6 and 13.6 to 7.4 mg Trolox/g DW, respectively, and



for fruits were ranged between 11.2 to 1.3 and 21.1 to 3.1 mg Trolox/g DW, respectively. CUPRAC assay in leaves collected in April were ranged between 124.5 to 35.6 and 139.8 to 91.1 mg Trolox/g DW, and for fruits collected in May in two reasons were ranged between 155.2 to 12.7 and 261.7 to 27.6 mg Trolox/g DW, respectively. DPPH assay in leaves collected in April were ranged between 84.5 to 32.4 and 134 to 67.4 mg Trolox/g DW, respectively, and for fruits were ranged between 87.3 to 28.1 and 141.7 to 29.3 mg Trolox/g DW, respectively. APTS assay in leaves collected in April in two seasons were ranged between 2.89 to 1.3 and 2.7 to 2.07 mg Trolox/g DW, respectively, and for fruits were ranged between 2.9 to 1.6 and 2.27 to 1.53 mg Trolox /g DW, respectively.

This study indicates that the leaves and fruits of *Ziziphus Spina-Christi* is a good natural source of phenolic content, flavonoids content, and antioxidant content. It seems that the antioxidant content and phenolic content in leaves is higher than that in fruits.

## Table of contents

### Content

#### Chapter One: Introduction

1.1	Background and Rationale.....	2
1.2	Names and Geographic regions of Ziziphus Spina Christi.....	2
1.3	Ziziphus species uses as foods and medication.....	2
1.4	Common bioactive compounds found in Ziziphus species.....	3
1.5	Polyphenolic Compounds.....	3
1.5.1	Phenolic Acids.....	4
1.5.2	Flavonoids.....	5
1.6	Bioactive compounds in plants.....	6
1.7	Antioxidant Activity.....	6
1.7.1	Antioxidant Activity Concept.....	6
1.7.2	Antioxidant Activity Assays.....	7
1.7.2.1	FRAP assay (Ferric reducing-antioxidant power).....	8
1.7.2.2	Cupric ion reducing antioxidant capacity (CUPRAC) method....	8
1.7.2.3	Free radical scavenging activity using DPPH.....	8
1.7.2.4	Free radical scavenging activity using ABTS.....	9
1.7.2.5	Total Phenolic Content (TPC) assay by Folin-Ciocalteu Reagent.....	9
1.7.2.6	Total Flavonoid Content (TFC).....	10

#### Chapter two: Literature Review and Objectives

2.1	Previous Studies.....	12
2.2	Hypotheses and research questions.....	16
2.3	Objectives and Aims.....	16

## **Chapter Three: Experimental**

3.1 Plant material.....	18
3.2 Chemicals.....	18
3.3 HPLC Chemicals and Reagents.....	18
3.4 Reagent.....	18
3.4.1 Total Phenolic Contents (TPC).....	19
3.4.2. Total flavonoid contents (TFC).....	19
3.4.3 FRAP reagent.....	19
3.4.4 CUPRAC method reagent.....	19
3.4.5. Free radical scavenging activity using DPPH.....	20
3.4.6. Free radical scavenging activity using ABTS.....	20
3.5 Instrumentations.....	20
3.6 HPLC Instrumentation systems.....	20
3.7 Methodology.....	20
3.7.1 Extractions.....	20
3.7.2 Determination of total Phenolic Contents (TPC).....	21
3.7.3 Determination of Total flavonoid content (TFC).....	21
3.7.4 Determination of antioxidant activity (AA).....	21
3.7.5 Cupric reducing antioxidant power (CUPRAC).....	22
3.7.6 Free radical scavenging activity using DPPH.....	22

3.7.7 Free radical scavenging activity using ABTS.....	22
3.7.8 Chromatographic conditions.....	23
3.7.9 Sample preparation for HPLC analysis.....	23
3.8 Effect of soaking time on the studied parameters (TPC, TFC, and antioxidant activities).....	23
3.9 Statistical analysis.....	24

## **Chapter Four: Results and Discussions**

4.1 Total phenolic content (TPC).....	26
4.2 Total flavonoid contents (TFC).....	28
4.3 Antioxidant activity (AA).....	30
4.4 Cupric reducing antioxidant power (CUPRAC).....	32
4.5 Free radical scavenging activity using DPPH.....	35
4.6 Free radical scavenging activity using ABTS.....	38
4.7 HPLC results and discussion.....	40
4.7.1 Ziziphus Spina-Christi fruit extracts.....	40
4.7.1.1 Water extract.....	40
4.7.1.2 Ethanol extract.....	42
4.7.1.3 Ethanol 80% extract.....	43
4.7.2 Ziziphus Spina- Christi leaves extract.....	43
4.7.2.1 Water extract.....	43

4.7.2.2 Ethanol extract.....	45
4.7.2.3 Ethanol 80% extract.....	46
4.8 Pearson correlations.....	47
4.8.1 Pearson correlations between antioxidant contents and their activities of leaves collected from Northern West Bank (2014/2015)..	47
4.8.2 Pearson correlations between antioxidant contents and their activities of fruits collected from Northern West Bank (2014/2015)...	48
4.8.3 Pearson correlations between antioxidant contents and their activities of leaves collected from Middle West Bank (2014/2015)...	49
4.8.4 Pearson correlations between antioxidant contents and their activities of Fruits collected from Middle West Bank (2014/2015)....	50
4.8.5 Pearson correlations between antioxidant contents and their activities of leaves collected from South West Bank (2014/2015)....	51
4.8.6 Pearson correlations between antioxidant contents and their activities of fruits collected from Middle West Bank (2014/2015)...	52
4.9 Effect of soaking times on TPC, TFC, and Antioxidant activities..	53
<b>Chapter Five: conclusions</b>	
Conclusions.....	55
<b>References.....</b>	<b>56</b>
<b>Appendices.....</b>	<b>62</b>
<b>Arabic abstract.....</b>	<b>78</b>

## List of Tables

Table	Name	Page
Table 4.1	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the northern region of the West Bank in 2014/2015	47
Table 4.2	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the northern region of the West Bank in 2014/2015.	48
Table 4.3	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the Middle regions of the West Bank in 2014/2015.	49
Table 4.4	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the middle region of the West Bank in 2014/2015	50
Table 4.5	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the southern region of the West Bank in 2014/2015	51
Table 4.6	Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the southern region of the West Bank in 2014/2015	52

## List of Figures

Figure	Name	Page
Figure 1.1	Chemical structures of the different classes of polyphenols.	4
Figure 1.2	Chemical structures of the different classes of flavonoids.	6
Figure 1.3	Chemical structures of reaction of yellow Fe <sup>3+</sup> TPTZ complex (2,4,6-tri (2- pyridyl)-1,3,5-triazine) with antioxidants.	7
Figure 1.4	Mechanism of DPPH• free radical.	9
Figure 1.5	ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid).	9
Figure 1.6	Basic structure of flavonoid.	10
Figure 4.1 (A)	Total phenolic content (TPC, mg Gallic acid/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	27
Figure 4.1 (B)	Total phenolic content (TPC, mg Gallic acid/g DW) of Ziziphus plant parts (Fruits) in two years (2014 and 2015).	27
Figure4.2 (A)	Total flavonoid content (TFC, mg catechin /g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	29
Figure4.2 (B)	Total flavonoid content (TFC, mg catechin /g DW) of Ziziphus plant parts (Fruits) in two years (2014 and 2015).	30
Figure 4.3 (A)	FRAP antioxidant activity (mg Trolox/ g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	34
Figure 4.3 (B)	FRAP antioxidant activity (mg Trolox/ g DW) of Ziziphus plant parts (fruits) in two years (2014 and 2015).	34
Figure 4.4 (A)	Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	34
Figure 4.4 (B)	Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (fruits) in two years (2014 and 2015).	35
Figure 4.5A	Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	37
Figure 4.5 (B)	Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (fruits) in two years (2014 and 2015).	37
Figure 4.6 (A)	Free radical scavenging activity (ABTS, mg Trolox/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015).	39

Figure 4.6 (B)	Free radical scavenging activity (ABTS, mg Trolox/g DW of Ziziphus plant parts (fruits) in two years (2014 and 2015).	40
Figure 4.7	HPLC-PDA chromatograms of crude water of Ziziphus Spina-Christi fruits extract at 325 nm.	40
Figure 4.8	HPLC-PDA chromatograms of ethanol of Ziziphus Spina-Christi fruits extract at 325 nm.	42
Figure 4.9	HPLC-PDA chromatograms of 80% ethanol of Ziziphus Spina-Christi fruits extract at 325 nm.	43
Figure 4.10	HPLC-PDA chromatograms of crude water of Ziziphus Spina-Christi leaves extracts at 280 nm.	44
Figure 4.11	HPLC-PDA chromatograms of crude ethanol of Ziziphus Spina-Christi leaves extracts at 500 nm.	45
Figure 4.12	HPLC-PDA chromatograms of crude ethanol 80% of Ziziphus Spina-Christi leaves extracts at 400 nm.	46



## List of Appendices

Appendix	Name	Page
Appendix A	Total phenolic absorbance and calibration curve.	63
Appendix B	FRAP absorbance (2 – 5 mM) FeSO <sub>4</sub> .7H <sub>2</sub> O), Fe (II) and calibration curve.	64
Appendix C	Total Flavonoid absorbance and calibration curve.	65
Appendix D	CUBRAC absorbance and calibration curve.	66
Appendix E	DPPH absorbance and calibration curve.	67
Appendix F	ABTS absorbance and calibration curve.	68
Appendix G	Table results for total phenolic content.	69
Appendix H	Table results for total flavonoid content.	70
Appendix I	Table results for FRAP assay.	71
Appendix J	Table results for CUPRAC assay.	72
Appendix K	Table results for DPPH assay.	73
Appendix L	Table results for ABTS assay.	74
Appendix M	Table results for TPC assay with different soaking time	75
Appendix N	Table results for TFC assay with different soaking time	75
Appendix O	Table results for FRAP assay with different soaking time	76
Appendix P	Table results for CUPRAC assay with different soaking time	76
Appendix Q	Table results for DPPH assay with different soaking time	77
Appendix R	Table results for APTS assay with different soaking time	77

# **Chapter: One**

## **Introduction**

## **INTRODUCTION**

### **1.1 Background and Rationale**

Plants have a great importance in our lives because they fulfill our basic needs for food, shelter, clothing, fuel, ornamentals, flavoring and medicine. Medicinal plants have been known for millennia and are considered as potential sources of pharmaceutical agents and/or as sources of lead compounds in drug development (Lulekal et al. 2013). Also, medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs (Bukar et al. 2015). Herbal medicine has gained much importance in recent years due to the good efficacy and cost effectiveness worldwide (Dahiru et al. 2006). The beneficial effects of plant materials are due to the presence of secondary plant metabolites (Fürstenberg-Hägg et al. 2013).

### **1.2 Names and Geographical regions of *Ziziphus Spina-Christi*.**

Nature has bestowed Palestine with an enormous wealth of medicinal plants, one of most important plant is *Ziziphus Spina-Christi* (L) Willd locally known as Sidr, is a multipurpose tree species belonging to the botanical family *Rhamnaceae*. It derived its name from kinnara, a sweet edible fruit produced from Kists' thorn tree, one of the prickly/thorny shrubs found in Palestine believed to have been used for Christ's crown of thorns (Adzu et al. 2007). *Ziziphus* species comprises of about 40 species of deciduous or evergreen trees and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East (Yossef et al. 2011).

### **1.3 *Ziziphus* species uses as foods and medication.**

For a long time, in folklore medicine, sidr has been used for the treatment of some diseases, such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anemia, diarrhea, and insomnia (Kirikar et al. 1984, Han et al. 1986). *Ziziphus Spina-Christi* has also been reported to have activity against bacterial and fungal pathogens that are normally quite resistant to modern medications (Bukar et al. 2015).

Z. Spina-Chirsti has very nutritious fruits that are usually eaten fresh. The flowers are important source for honey bee. The winter honey (i.e Napek honey) collected from the flowers of the sidr is in high demand by citizens for its medicinal qualities in addition to its excellent taste and fragrant smell (Adzu et al 2001). The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, and for dysentery (Abalaka et al 2010). The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu et al 2001). Also, the leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Mechel et al. 2002). The seeds are sedative and are taken some time with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Kaaria 1988). The root bark infusion is used traditionally in Africa as a remedy for stomach pain and other gastrointestinal tract ailments. It has been used in folk medicine as a demulcent, a stomachic, as astringent for toothaches and as mouth wash (Ghafour et al 2012).

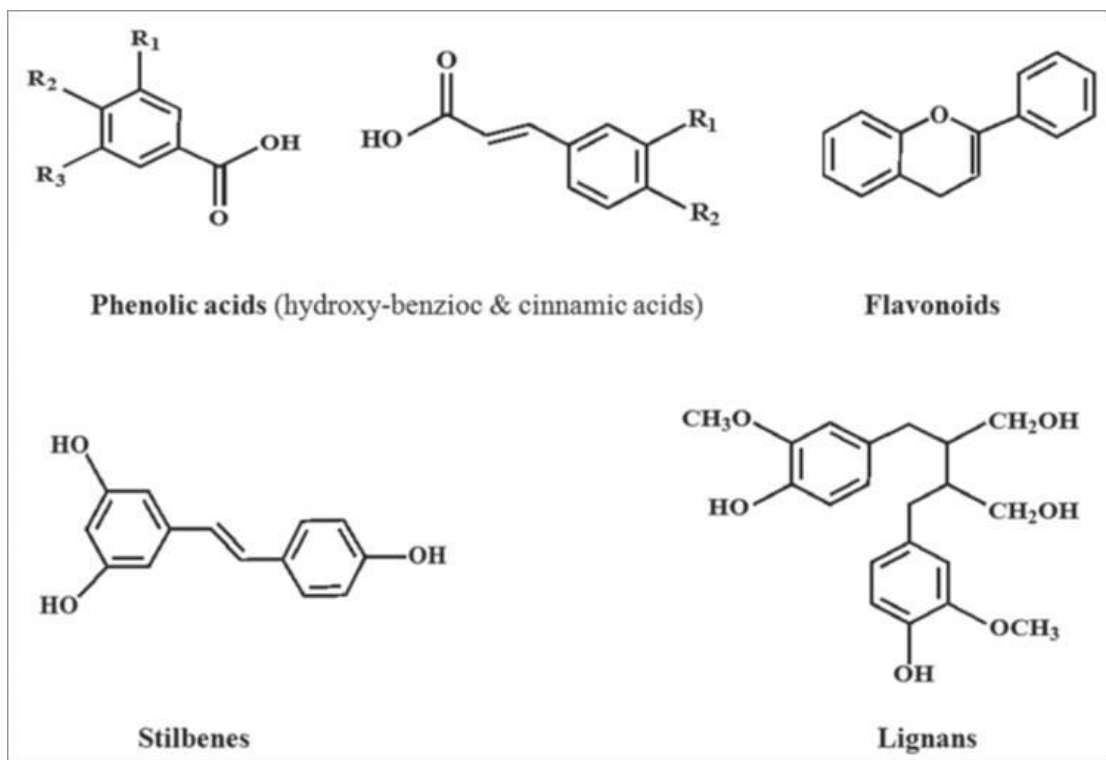
#### **1.4 Common bioactive compounds found in Ziziphus species.**

Flavonoids, alkaloids, triterpenoids, saponins, lipids, proteins, free sugar and mucilage are the main important compounds characterized in this plant (Adzu t al 2003). The leaves of these plants contain betulinic and ceanothic acids, various flavonoids, saponins, erols tannins and triterpenes (Asgarpanah et al 2012).

#### **1.5 Polyphenolic Compounds**

Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Scalbert et al 2005, Beckman 2000). In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Towards the end of 20th century, epidemiological studies and associated meta-analysis strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Graf et al 2005, Arts et al 2005).

**Figure:1.1** illustrates the different groups of polyphenols and their chemical structures. Polyphenols are broadly divided in four classes; Phenolic acids, flavonoids, stilbenes and lignans.



**Fig.1.1** Chemical structures of the different classes of polyphenols, where R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are H, OH or OCH<sub>3</sub>.

### 1.5.1 Phenolic Acids.

Many studies have shown that phenolic compounds display antioxidant activity as a result of their capacity to scavenge free radicals (Seyoun et al 2006). Antioxidants are chemical substances that reduce or prevent oxidation. They have the ability to counteract the damaging effects of free radicals in tissues and thus are believed to protect against cancer, arteriosclerosis, heart disease and several other diseases (Bandyopodhyay et al 2007). Phenolic compounds can also act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Ammar et al 2009). These compounds are known to act as antioxidant not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates (Al-Marzooq et al 2014).

Phenolic acids are found abundantly in foods and divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxybenzoic acid content of edible plants is generally low, except for certain red fruits, black radish and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight. The

hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic and sinapic acids (Pandey et al 2009).

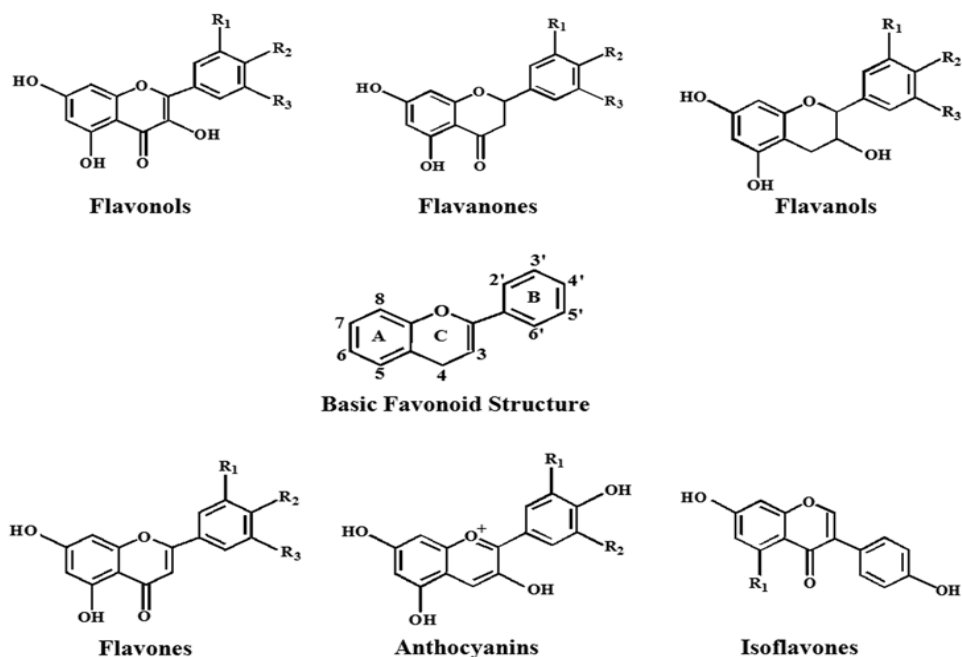
### **1.5.2 Flavonoids**

Probably the most important natural phenolic are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen et al 1999). In fact, flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation (William et al 2002). These compounds which are widely distributed across the plant kingdom represent the most abundant antioxidants in the diet and they have gained tremendous interest as potential therapeutic agents against a wide variety of diseases, most of which involve oxidant damage (Ross et al 2004 ).

Flavonoids have also been shown to be highly effective scavenging of most types of oxidizing molecules, including singlet oxygen and other various free radicals that are probably involved in several diseases. On the other hand, numerous studies have shown structure-activity relationships governing antioxidant capacities of flavonoids (Bors et al 2001, and Cai et al 2006).

This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle (Fig. 1.2). More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of the flowers, fruits and leaves (Groot et al 1998).

Pharmacological studies have demonstrated that ZSC were known to possess hypoglycemic, hypotensive, antimicrobial, hepatoprotective, antioxidant, antitumor and immune stimulatory activities (Avizeh et al 2012, and singh et al 2012). These biological activities could be attributed to the presence of secondary plant metabolites present in *Ziziphus Spina-Christi* (Alhakmani et al 2014).



**Fig. 1.2** Chemical structures of the different classes of flavonoids, where R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are H, OH or OCH<sub>3</sub>.

## 1.6 Bioactive compounds in plants

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals. Although nutrients elicit pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Thus, a definition of bioactive compounds in plants is: secondary plant metabolites eliciting pharmacological or toxicological effects in man and animals (Bernhoft et al 2008).

## 1.7 Antioxidant activity

### 1.7.1 Antioxidant activity concept

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer (Kinnula and Crapo, 2004), cardiovascular disease (Singh and Jialal, 2006), neural disorders (Sas et al, 2007), Alzheimer's disease (Smith et al, 2000), mild cognitive impairment (Guidi et al, 2006), Parkinson's disease (Bolton et al., 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna et al, 1997), aging (Hyun et al, 2006) and atherosclerosis (Upston et al., 2003). Protection against free radicals can be enhanced by ample intake of dietary antioxidants (Alam et al, 2013).

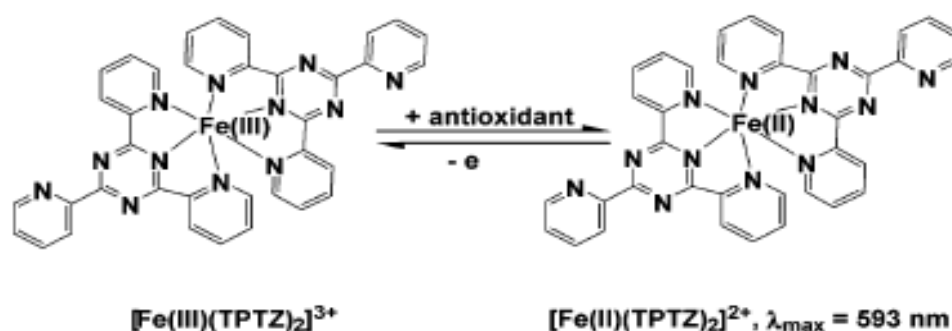
Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery (Alam et al, 2013).

### 1.7.2 Antioxidant Activity Assays

Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.). Six assays were used in this research, it is as follow:

#### 1.7.2.1 FRAP assay (Ferric reducing-antioxidant power).

This method measures the ability of antioxidants to reduce ferric iron. It is based on the reduction of the yellow complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the blue ferrous form by electron-donating substances (such as phenolic compounds) at low pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer. Antioxidant assay can be conducted by the method developed by (Benzie and Strain 1999). FRAP values can be obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of (Fe<sup>3+</sup>). Results were expressed in terms of μmol. of Trolox/g.

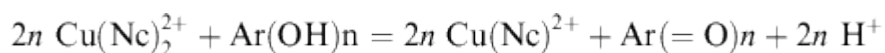


**Figure:1.3** Chemical structures of reaction of yellow Fe<sup>3+</sup> TPTZ complex (2,4,6-tri (2-pyridyl)-1,3,5-triazine) with antioxidants is reduced to the blue Fe<sup>2+</sup> TPTZ complex by electron-donating substances (Prior et al 2005).



### 1.7.2.2 Cupric ion reducing antioxidant capacity (CUPRAC) method

The chromogenic oxidizing reagent of the developed CUPRAC method, that is, bis(neocuproine)copper(II) chloride [Cu(II)-Nc], reacts with polyphenols [Ar(OH)<sub>n</sub>] in the manner.

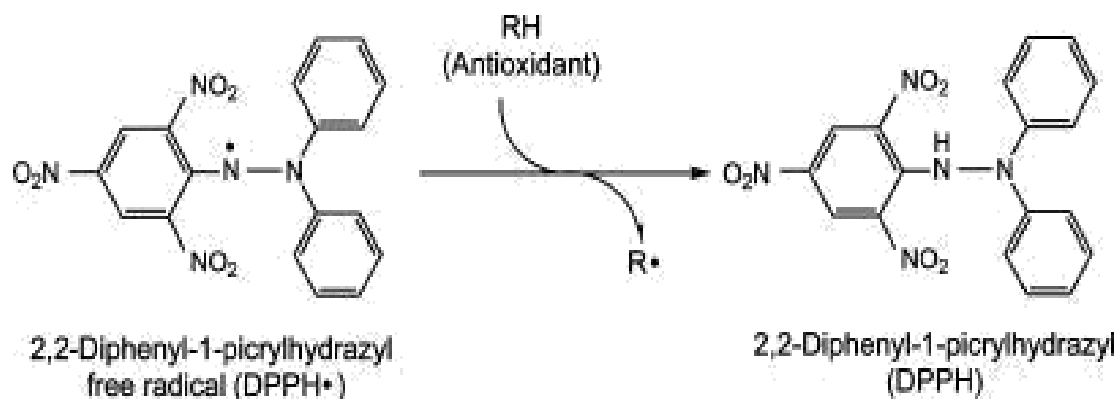


where the liberated protons may be buffered with the relatively concentrated ammonium acetate buffer solution. In this reaction, the reactive Ar-OH groups of polyphenols are oxidized to the corresponding quinones and Cu (II)-Nc is reduced to the highly-colored Cu (I)-Nc chelate showing maximum absorption at 450 nm. (Alam et al 2013). The cupric ion reducing antioxidant capacity of Ziziphus species was determined according to the method of (Apak et al. 2008).

### 1.7.2.3 Free radical scavenging activity using DPPH

The molecule 1, 1-diphenyl-2-picrylhydrazyl ( $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (Alam et al 2013).

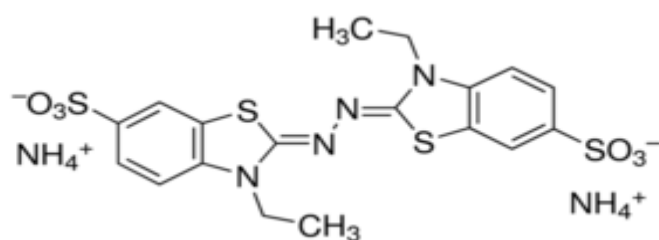
In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in absorbance of DPPH radicals is monitored. According to (Manzocco et al. 1998) the sample extract (0.2 mL) is diluted with methanol and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm.



**Figure.1.4:** Mechanism of DPPH $\cdot$  free radical (Moon et al, 2009).

#### 1.7.2.4 Free radical scavenging activity using ABTS.

A modified procedure using ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)) as described by (Re et al. 1999) was used. The ABTS $^{+\cdot}$  stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent. The working solution of ABTS $^{+\cdot}$  was obtained by diluting the stock solution in ethanol to give an absorption of  $0.70 \pm 0.02$  at  $\lambda = 734$  nm. Sample extract (100  $\mu$ l) was added to 900  $\mu$ l of ABTS $^{+\cdot}$  solution and absorbance readings at 734 nm were taken at 30  $^\circ$ C exactly 10 min after initial mixing.



**Figure:1.5** ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid))

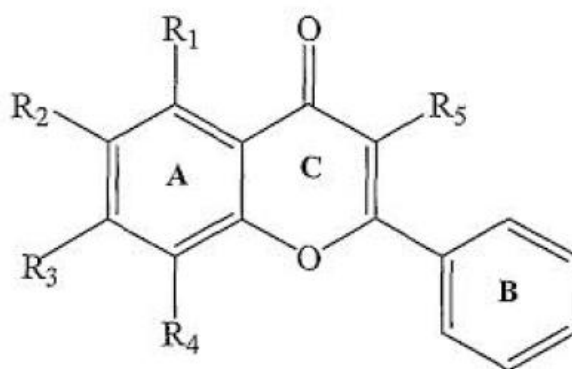
#### 1.7.2.5 Total Phenolic Content (TPC) assay by Folin-Ciocalteu Reagent

The Folin-Ciocalteu FC Assay was developed in 1927 for the measurement of tyrosine (Foloin 1927). The reagent consists of a mixture of sodium molybdate, sodium tungstate and other reagents, works on the mechanism of oxidation-reduction reaction. Upon reaction with phenols, it produces a blue color which absorbs at 765 nm. It is believed that the blue color is due to a complexed Mo(V) species (Singleton et al 1965). The assay has been used

for many years by the food and agricultural industries for determining phenolic content of plant products (Prior et al 2005).

### 1.7.2.6 Total Flavonoid Content (TFC)

TFC was analyzed using the Aluminium chloride method (Zhishen et al, 1999). The principle of this method based on Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. The results were expressed as catechin equivalent (mg CA/g sample).



**Figure 1.6:** Basic structure of flavonoid

**Chapter: two**  
**Literature Review**  
**And Objectives**

## 2.1 Previous Studies

Motamedi et al. (2009) reported that the antibacterial activity of *Ziziphus spina-christi* leaves ethanolic and methanolic extracts were examined using agar disc diffusion method against eight bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica* and *Pseudomonas aeruginosa*). These extracts had inhibitory effect at various concentrations (0.05, 0.1, 0.2, 0.3 and 0.4 g/ml) against tested bacteria. The ethanolic extract had the highest activity (20 mm) against *B. bronchiseptica* while the lowest activity (7 mm) was demonstrated by the methanolic extract on *K. pneumoniae*. Studies on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic extract on two selected bacteria showed that the *S. dysenteriae* had the highest MIC (18 mg/ml) and MBC (64 mg/ml) values.

Koley et al. (2011) reported that twelve commercial cultivars of *Z. mauritiana* were evaluated for their ascorbic acid (AA), total phenolics (TPH), flavonoids (TF), and total antioxidant activity (AOX). Results indicate that Indian jujube is a good source of ascorbic acid and total phenolics ranging from 19.54 to 99.49 mg/100 g and 172 to 328.6 mg GAE/100 g, respectively. Total AOX ranged from 7.41 to 13.93 and 8.01 to 15.13 μmol Trolox/g in FRAP and CUPRAC, respectively.

Memon et al. (2012) reported that total phenolic acids (free, soluble-bound and insoluble-bound) were analyzed from Ber fruit extracts by applying a pressurized liquid base hydrolysis extraction (PLBHE) using Dionium cells. Nine phenolic acids (protocatechuic, p-hydroxybenzoic, ferulic, chlorogenic, vanillic, caffeic, vanillin, o- and p-coumaric acids) were extracted, separated, and quantified by HPLC-DAD. Identification of phenolic acids was achieved by comparison of retention times, ultraviolet, and mass spectral data with authentic commercial standards. Results showed that p-coumaric acid ( $3719 \pm 22 \mu\text{g/g}$ ) was the predominant phenolic acid extracted from Ber samples. In addition, four phenolic acids, namely p-hydroxybenzoic ( $2187 \pm 71 \mu\text{g/g}$ ), vanillin ( $2128 \pm 20 \mu\text{g/g}$ ), ferulic ( $2629 \pm 96 \mu\text{g/g}$ ), and o-coumaric acids ( $2569 \pm 41 \mu\text{g/g}$ ) were obtained in intermediate amounts from dried *Ziziphus mauritiana* L. fruit. The total phenolic acids content was determined as  $18231 \pm 306 \mu\text{g/g}$  dry matter.

Ghafoor et al. (2012) have described isolation, qualification and determination of some of these phenolic acids using reversed phase HPLC after the purification of the compounds by

passing the plant extract through sephadex LH-20 column. P-Coumaric acid, rutin, apigenin, quercetin, chlorogenic acid and syringic acid were found and isolated in the methanolic extract of the stem, in which rutin content was found at higher concentration (325.0 mg /100g) and apigenin (122.90 mg/100g). Ferulic acid, rutin, p-hydroxybenzoic acid and chlorogenic acid were found and isolated in the extracts of fruit of *Ziziphus* with maximum rutin content (15.88 mg/100 g). It seems that the phenolic acid contents in stem are higher than that in fruits.

Nuru et al. (2012) reported that the nectar secretion of *Ziziphus* flowers was studied by removing and measuring the nectar every four hours, for two consecutive days, from 88 flowers of four trees ('repeated sampling'). In another 120 flowers from the same trees, the accumulated sugar was measured at the end of the flowering stage. According to this study, one *Ziziphus* tree is estimated to produce 3.6 kg of honey (range 2.2 - 5.2 kg), equivalent to about 900 kg of honey/ha (range 550 -1300 kg). This indicate the high potential value of the plant for honey production. Nectar secretion was positively correlated with temperature, indicating the adaptation of the tree to hot climates.

A study of Wu et al. (2013) was conducted to compare fruit yields, phenolic profiles and antioxidant activity of jujube in response to different fertilizers. Application of organic fertilizer (Biogas residue fertilizer BRF , Decomposed soybean meal DSM) and inorganic fertilizer (supplemental potassium as an individual nutrient ) appeared to enhanced the phenolics and antioxidant activity accumulation of jujubes .So the combination of organic fertilizers and inorganic fertilizers such as more supplemental individual potassium, and less supplemental individual nitrogen and phosphorus, might be the best management combination for achieving higher phenolic concentration, stronger antioxidant activity and a good harvest.

Al-Jassabi et al. (2013) studied in vitro antioxidant activity, total phenolic content and concentration of flavonoids of five different extracts from the *Ziziphus Spina- Christi* fruits were determined using spectrophotometric methods. Antioxidant activity of extracts were expressed as percentage of DPPH radical inhibition and values were ranged from 31.76% - 90.23% which indicated that *ziziphus* manifested the strongest capacity for neutralization of DPPH radicals. The total phenolic content ranged from 11.04 - 56.44 mg/g expressed as quercetin equivalent. The concentration of flavonoids in the *ziziphus* extracts varied from 16.66 - 58.32 mg/g expressed in terms of rutin equivalent (mg of RU/g extract).

Methanolic extract of ziziphus showed the highest phenolic and flavonoid concentration and strong antioxidant activity. The significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of plant extracts.

Alhakmani et al. (2014) performed phytochemical screening and evaluated in-vitro antioxidant and anti-inflammatory activities of ethanolic extract of seeds and fruits of *Ziziphus spina-christi* (ZSC) growing in Oman. Phytochemical analysis of both the extracts revealed the presence of major classes of phytochemicals such as tannins, alkaloids, flavonoids, cardiac glycosides etc. ZSC seeds were found to contain the highest total phenolics but ZSC fruits exhibited the maximum antioxidant activity. The anti-inflammatory activity of both parts of the plant extract was significant and comparable with the standard anti-inflammatory drug, diclofenac. It can be concluded that ZSC is a good source of natural antioxidants which can be used to prevent progression of many chronic diseases.

AL-Marzooq et al (2014) studied Crude juices of Sidr (*Ziziphus Spina-Christi* L.) which are obtained from leaves by hydraulic press. The levels of polyphenolic compounds in the (leaves) juice were 510.00 and 722.00ppm. Aliquots of the concentrated sidr juice (leaves), represent 200, 400, 800 and 1600ppm and butylated hydroxyl toluene (BHT, 200ppm) were investigated by Rancimat method at 100°C and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. These compounds were administered to rats daily for 6 weeks by stomach tube. The liver (Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities) and kidney (bilirubin, uric acid and creatinine) function tests and serum contents (total lipids, total cholesterol and low and high-density lipoproteins) were measured to assess the safety limits of the phenolic compounds in the sidr juice (fruits and leaves). The data of the aforementioned measurements indicates that the administration of sidr juice (leaves) did not cause any changes in liver and kidney functions. On the contrary, BHT at 200ppm induced significant increases in the enzyme activities and the serum levels of total lipids, uric acid and creatinine.

Bukar et al. (2015) investigated phytochemical and antibacterial activity of the seed oil extracts of *Ziziphus spina-christi* L. which was found to have potential antibacterial activity against four medically important bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Shigella* and *Pseudomonas aeruginosa*). The antibacterial activity of seed oil extracts

of *Ziziphus spina-christi* was examined using agar well diffusion method. The result obtained showed that the essential oil was active against Gram-positive more than Gram-negative bacteria, the essential oil had strong antibacterial activity against *Staphylococcus aureus* (zone of inhibition of growth is 11 mm), *E. coli* (zone of inhibition of growth is 10 mm), *Shigella* spp (zone of inhibition of growth is 8mm) and *P. aeruginosa* (zone of inhibition of growth is 8 mm). Physicochemical screening of the oil extract of *Ziziphus spina-christi* revealed presence of different types of secondary metabolites such as glycosides, tannins and alkaloids. The seed oil extract of *Ziziphus spina-christi* showed significant activity against *Staphylococcus aureus* and *Escherichia coli* that suggest that the chemical components that exist in the extract such as glycosides, alkaloids and tannins have the powerful antibacterial effects on the bacterial cell wall and DNA. The results of this study have provided scientific validity for the use of this seed oil in the treatment of bacteria-related infections in herbal medicine.

Khaleel et al. (2016) determined the total phenolic content and in vitro antioxidant activity of three different leaves extracts (methanolic, ethanolic and aqueous) of *Ziziphus spina-christi* grown in Jordan. The total phenolic content was ranged between 11.8 to 52.5 mg/g expressed in terms of Gallic acid equivalent (mg of GAE/g extract). In vitro antioxidant activity of the plant extracts revealed that all the extracts showed good antioxidant power with IC<sub>50</sub> values of 21.4, 24.2 and 54.3 µg/mL for methanolic, aqueous and ethanolic extracts, respectively. The reducing power of the extracts was found to be concentration dependent. The results of this study revealed that, the methanolic extract of leaves showed the highest phenolic concentration and largest antioxidant activity.

The overall objectives of this study are to evaluate the total antioxidant capacities and the total phenolic and flavonoid content of *Ziziphus Spina-Christi* Species (leaves and fruits) from three different geographic regions in West bank. And to investigate the relationship between the total antioxidant activity and total phenolic and Flavonoid content in the samples tested. HPLC also used to determine the phenolic and flavonoid compounds in *Ziziphus* plant extracts.

The data obtained will be helpful in comparison of the total antioxidant activity, total phenolic, and flavonoid content in different regions (north, middle, and south of West bank) and different *Ziziphus* plant parts (Leaves and Fruits). Also, it useful for understanding their chemical constituent and functionality.



## **2.2 Hypotheses and research questions**

There have been several studies on the antioxidant activities of various plants, fruits, herbs in many countries. Therefore, Hypotheses of this study declares the existence of the antioxidant activity, phenolic and flavonoid content in variable quantities in the leaves and fruits of *Ziziphus Spina-Christi* in different geographical regions in the Palestine territories.

The Specific questions for discussions

1. Do leaves extracts of *Ziziphus Spina-Christi* have phenolics and flavonoids?
2. Do leaves extracts of *Ziziphus Spina-Christi* have antioxidant activity?
3. Do Fruits extracts of *Ziziphus Spina-Christi* have phenolics and flavonoids?
4. Do Fruits extracts of *Ziziphus Spina-Christi* have antioxidant activity?
5. Is there a correlation between TPC or TFC and antioxidant activities?
6. Do the geographical regions affected the TPC, TFC, and AA of the plant extracts?
7. Do the extraction solution affected the TPC, TFC, and AA of plants extracts?

## **2.3 Objectives and Aims**

1. To evaluate antioxidant activity, total phenolic content, and flavonoid content of ethanolic and aqueous extracts of leaves and fruits species of *Ziziphus Spina-Christi* from three different geographical regions (North, Middle, and South) of West bank, using Folin-Ciocalteu method for total phenolic content, Aluminium chloride colorimetric assays for flavonoid content, and FRAP, CUPRAC, DPPH, ABTS for Antioxidant capacity.
2. To determine the phenolic and flavonoid compounds in leaves and fruits of *Ziziphus Spina-Christi* using HPLC with PDA detector.
3. To investigate a possible relationship between phenolic contents and antioxidant activity and also between total flavonoids content and antioxidant activity.
4. To investigate which extraction solvent used are the optimum among distilled water, 80% ethanol or 99% ethanol.

## **Chapter: Three**

### **Experimental**

### **3.1 Plant materials**

Leaves and fruits of Palestinian *Ziziphus Spina-Christi* were collected from three different regions in Palestine (Al-Zbedate in the North, Jericho in the Middle, and Bani Naeem in the South). The leaves were harvested in April 2014 and in April 2015. Fruits were collected in May 2014 and May 2015.

### **3.2 Chemicals**

The chemicals and reagents were used for analyzing the antioxidant compounds (TPC, AA, and TFC) of *Ziziphus Spina-Christi* are: 2,4,6-tripyridyl- S-triazine (TPTZ), ferric chloride hexahydrate, catechin, gallic acid, sodium hydroxide, hydrochloric acid, acetic acid, sodium nitrite, aluminum chloride, iron (II) sulfate hexahydrate, sodium bicarbonate, ethyl alcohol (80%), ethyl alcohol (99%), Folin–Ciocalteu Reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Neocuproine, Ammonium Acetate, methanol (95%), Cupper (II)Chloride, Potassium Persulphate ( $K_2S_2O_8$ ), ABTS (2,2-azino-di- (3-ethyl-benzothialozine-sulphonic acid)), DPPH (2,2-diphenyl-1- (2,4,6-trinitrophenyl)hydrazyl). Chemicals were obtained from Sigma-Aldrin.

### **3.3 HPLC Chemicals and Reagents**

The acetonitrile and water were of an HPLC grade from Sigma. Phenolic and flavonoids standards: Vanillic acid, Ferulic acid, Syringic acid, trans-cinnamic acid, Catechin, p-coumaric acid, Sinapic acid, 4-Hydroxyphenylacetic acid, Rutin hydrate, Caffeic acid, Quercetin, Gallic acid, 3,4-dihydroxyphenylacetic acid, chlorogenic acid, Taxifolin, Luteolin 7-glucoside, Apigenin 7-glucoside, Luteolin, Quercetin 3-D-galactose were from Sigma.

### **3.4 Reagents**

All reagents were prepared according standard procedures.

### **3.4.1 Total Phenolic Contents (TPC)**

Folin–Ciocalteu reagent (10 folds diluted with distilled water). 7.5% NaHCO<sub>3</sub>, (18.75 g of NaHCO<sub>3</sub> were put in a 250 volumetric flask and dissolved in distilled water, then the volume was up to the mark).

### **3.4.2. Total flavonoid contents (TFC)**

10% AlCl<sub>3</sub>.6H<sub>2</sub>O was prepared by dissolving 10g of AlCl<sub>3</sub>.6H<sub>2</sub>O in 100ml of water.

5% NaNO<sub>2</sub> was prepared by dissolving 5g of NaNO<sub>2</sub> in 100ml of water.

1M NaOH, was prepared by dissolving 4 g of NaOH in 100 ml of water.

### **3.4.3 FRAP reagent**

FRAP reagent was prepared according to Benzie and Strain (1999) by the addition of 25 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20mM FeCl<sub>3</sub>.6H<sub>2</sub>O and 25 ml of 0.3 M acetate buffer at pH 3.6.

Acetate buffer (0.3 M) at pH 3.6 was prepared according to British Pharmacopeia by dissolving 16.8g of acetic acid and 0.8g of sodium hydroxide in 1000 ml of water.

10 mM TPTZ (M.wt = 312.34 g/mol) was prepared by dissolving 0.321g TPTZ in 100ml HCl.

40 mM HCl was prepared by diluting 3.77ml of stock HCl solution (10.6M) to 1000ml with water.

20mM FeCl<sub>3</sub>.6H<sub>2</sub>O was prepared by dissolving 0.54 g of FeCl<sub>3</sub>.6H<sub>2</sub>O in 100 ml of water.

### **3.4.4 CUPRAC method reagent**

CuCl<sub>2</sub> (1×10<sup>-2</sup> mol/L) was prepared by dissolving 0.134g of anhydrous CuCl<sub>2</sub> in 100 ml of water.

neocuproine alcoholic solution (7.5×10<sup>-3</sup> mol/L) was prepared by dissolving 0.152g in 100 ml of ethanol (96%).

Ammonium acetate (1mol/L, pH7.0) was prepared by dissolving 7.708g in 100 ml of water.

### **3.4.5. Free radical scavenging activity using DPPH.**

0.0634 mM of (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) (DPPH) solution was prepared by dissolving 0.0064 g of DPPH in 250 ml ethanol (96%).

### **3.4.6. Free radical scavenging activity using ABTS**

7 mM ABTS (2,2-azino-di-(3-ethyl-benzothialozine-sulphonic acid)) was prepared by dissolving 0.384g of ABTS in 100 ml of water.

## **3.5 Instrumentations**

UV visible spectrophotometer (helios, Model No. UVA 9446, Great Britain), Hot plate with magnetic stirrer, Heating mantel, Water bath, Incubator, Analytical balance: (metler Toledo), Quartz cuvetts, Ordinary laboratory glassware, Jenway pH meter (3310) with a combination glass electrode and a tolerance of  $\pm 0.01$  pH units, Vortex.

## **3.6 HPLC Instrumentation systems**

The analytical HPLC is Waters Alliance (e2695 separations module), quipped with 2998 Photo diode Array (PDA). Data acquisition and control were carried out using Empower 3 chromatography data software (Waters, Germany).

## **3.7 Methodology**

### **3.7.1 Extractions**

The fruits and leaves were rinsed with distilled water to remove any dust and particulate matter, then left to dry at room temperature for 20 days. The fruits from each region were homogenized in a domestic blender to produce small fine granules. Also, the leaves from each region were grinded with domestic blender to produce dry powder. Five grams of the powder of each cultivar of fruits and leaves were macerated with 50 ml of absolute ethanol, 80% ethanol, and distilled water (1:10 w/v) in water bath at 37°C for different period of time (2, 5, 10, and 72 hours). The extracts were then filtered. Then the crude extracts were stored in Refrigerator at 4 °C until analysis.

### **3.7.2 Determination of Total Phenolic Contents (TPC)**

Total phenolic was determined spectrophotometrically using Folin–Ciocalteu reagent (Singleton et al. 1999). 40µl of the sample extract were mixed with 1.8 ml of Folin–Ciocalteu reagent (pre-diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 minutes, then 1.2 ml of sodium bicarbonate 7.5% was added to the mixture. The mixture was allowed to stand for 90 min and absorption was measured at 765 nm against a reagent blank. Aqueous solutions of known gallic acid concentrations in the range (100-500 ppm) were used for calibration. Results were expressed as gallic acid equivalent (mg GAE/g sample).

### **3.7.3 Determination of Total flavonoid content (TFC)**

Total flavonoids were analyzed using the Aluminum chloride method (Zhishen et al., 1999). An aliquot (1 ml) of Ziziphus extract in 10 ml of volumetric flask containing 4 ml of distilled water, 0.3 ml portion of 5% sodium nitrite followed by 0.3 ml portion of 10% hydrated aluminum chloride. The mixture was allowed to stand for 6 min at room temperature, then 2 ml of 1M sodium hydroxide was added, and the solution was diluted to 10 ml with distilled water. The absorbance of the pink solution versus a blank at 510 nm was measured immediately. Aqueous solutions of known Catechin concentrations in the range of (30 – 200 ppm) were used for calibration. The results were expressed as catechin equivalent (mg CE/g sample).

### **3.7.4 Determination of antioxidant activity(AA)**

Ferric reducing antioxidant power (FRAP) will be performed according to the procedure described by Benzie and Strain (1999). Freshly prepared FRAP reagent included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl<sub>3</sub> in the ratio 10:1:1 (v/v/v). Freshly prepared FRAP reagent was warmed at 37°C, then 3ml of warmed FRAP reagent was mixed with 40µl of the sample extract in a test tube. The mixture vortexed and incubated at 37°C for 30 min. Reduction of ferric-tripyridyltriazine(TPTZ) to the ferrous complex will form an intense blue color was measured at 593 nm at the end of 4 min. Reagent blank containing distilled water was also incubated at 37°C for up to 1 hour instead of 4 minutes which was the original time applied in FRAP assay. Results were

expressed in terms of  $\mu\text{mol Trolox/g}$ . Aqueous solutions of known Fe (II) concentrations in the range of (0.1 – 1.2 mM) ( $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ ) will be used for calibration.

### **3.7.5 Cupric reducing antioxidant power (CUPRAC)**

The cupric ion reducing antioxidant capacity of *Ziziphus* species were determined according to the method of Apak et al. (2008). 100  $\mu\text{l}$  of sample extract was mixed with 1ml each of 10 mM of copper chloride solution, 7.5 mM of neocuproine alcoholic solution (99.9% ethanol), and 1 M (pH 7.0) of ammonium acetate buffer solution, and 1ml of distilled water to make final volume 4.1ml. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentrations of Trolox. The results were expressed as mg Trolox/g.

### **3.7.6 Free radical scavenging activity using DPPH**

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical (Brand-Williams et al., 1995). A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%) was added to 100  $\mu\text{l}$  of each extract. The mixture was vortexed 5-10 sec. Change in the absorbance of the sample extract was measured at 515 nm for 30 min till the absorbance reached a steady state. Methanol (95%) was used as a blank. Results were expressed as mg Trolox/g DW sample.

### **3.7.7 Free radical scavenging activity using ABTS**

A modified procedure using ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)) as described by Re et al. (1999) was used. The ABTS stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent. The working solution of  $\text{ABTS}^{\cdot+}$  was obtained by diluting the stock solution in 99.9% ethanol to give an absorption of  $0.70 \pm 0.02$  at 734 nm. (200 $\mu\text{l}$ ) Sample extract was added to 1800 $\mu\text{l}$  of  $\text{ABTS}^{\cdot+}$  solution and absorbance readings at 734 nm were taken at 30°C exactly 10 min after initial mixing (A). The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC) mg Trolox/g DW.

### **3.7.8 Chromatographic conditions**

The HPLC analytical experiments of the crude water, 80% ethanol and 100% ethanol extracts were run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5  $\mu$ m) with guard column of Xbridge ODS, 20 mm x 4.6mm ID, 5  $\mu$ m. The mobile phase is a mixture of 0.5% acetic acid solution (A) and acetonitrile (B) ran in a linear gradient mode. The start was a 100% (A) that descended to 70% (A) in 40 minutes. Then to 40% (A) in 20 minutes and finally to 10% (A) in 2 minutes and stayed there for 6 minutes and then back to the initial conditions in 2 minutes. The HPLC system was equilibrated for 5 minutes with the initial acidic water mobile phase (100 % A) before injecting next sample. All the samples were filtered with a 0.45  $\mu$ m PTFE filter. The PDA wavelengths range was from 210-500. The flow rate was 1 ml/min. Injection volume was 20  $\mu$ l and the column temperature was set at 25°C. The HPLC system was then equilibrated for 5 minutes with the initial mobile phase composition prior injecting the next sample. All the samples were filtered via 0.45  $\mu$ m micro porous disposable filter.

### **3.7.9 Sample preparation for HPLC analysis**

The plant extracts were filtered using suction filtration, and then the solvents were evaporated under reduced pressure at 40 C using Rotary evaporator. The resulting crude extracts were dissolved in the respective solvents (water, ethanol, and 80% ethanol) at a concentration of 5 mg/mL, and 20  $\mu$ L were injected into the HPLC chromatograph, and analyzed for their phenolic and flavonoids. Seventeen phenolic and flavonoid standards were injected and separated simultaneously to identify the presence of any of these compounds in the crude extracts. Calibration curve of each individual standard was also prepared at three concentration levels namely 50, 100 and 250 ppm.

### **3.8 Effect of soaking time on the studied parameters (TPC, TFC, FRAP, CUPRAC, DPPH, and APTS)**

Leaves and fruits powders of Ziziphus plant were macerated in three different solvent (D.Water, 80% Ethanol, and 99% Ethanol) at 37°C at various times included (2, 5, 10, and 72 hours). Afterwards, the extracts of leaves and fruits were analyzed for their TPC, TFC, antioxidant activities.



### **3.9 Statistical Analysis**

All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of solvent (distilled water, 80% ethanol and absolute ethanol), geographical region (north, middle and south) and plant part (leaves and fruits) were carried out using the GLM procedure considering a fully randomized design, treating years separately. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferroni procedure was employed with multiple t tests in order to maintain an experiment wise  $\alpha$  of 5%.

Initially Pearson correlations were calculated to test the relation between different antioxidants (TPC and TFC) and their activities (FRAP, CUPRAC, DPPH and ABTS).

**Chapter: Four**  
**Results**  
**And Discussions**

#### **4.1 Total phenolic content (TPC)**

The results in figure 4.1A and figure 4.1B reveals that in 2014 total phenolic contents (TPC) increased significantly when the extraction contained more contribution of distilled water in the extraction solution (Distilled water > 80% Ethanol > 99% Ethanol) in all studied geographical regions (North, Middle, and South) in both studied plant parts (Leaves and Fruits) except that of fruits collected from the middle part of the West Bank where the response was the opposite.

TPC of the leaves collected in 2015 yielded higher amounts significantly in 80% ethanol extraction as compared to the extraction with distilled water or absolute ethanol in samples collected from the northern and southern part of the West Bank. 80% ethanol yielded statistically similar amounts of TPC as found in distilled water extracted leaves in the southern part of the West Bank. The Leaves collected from the middle part of the West Bank didn't differ significantly in terms of TPC in different extraction methods.

Among the three studied extraction methods, results reveal that distilled water can extract high TPC from biological materials higher or at least similar to ethanolic extraction methods.

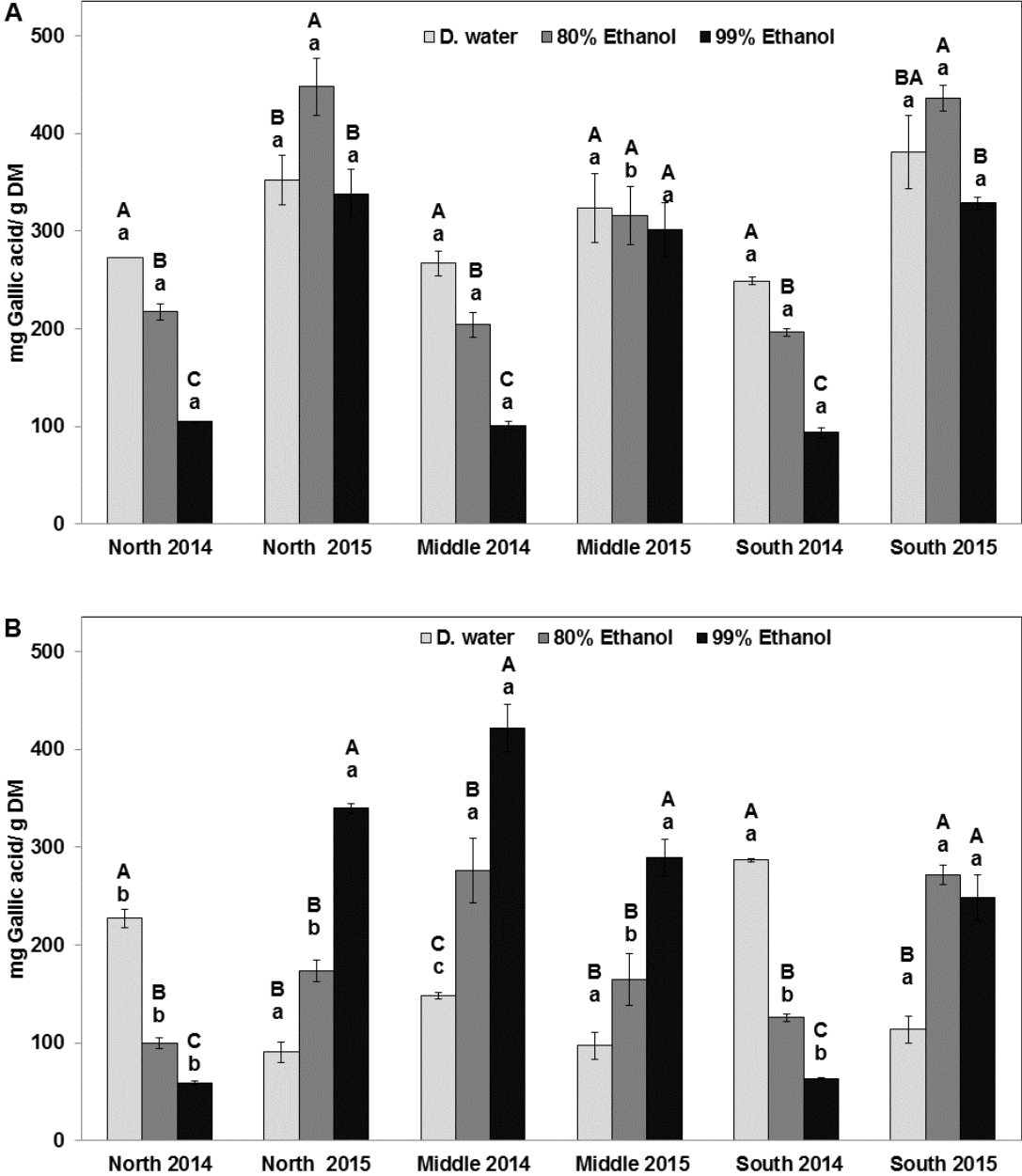
Water extraction of the leaves collected in 2014 from all geographical regions contained more than two folds of the TPC as compared to that extracted by absolute ethanol. Furthermore, the difference in the content of the extracted TPC in fruits collected from both northern and southern part of the country in 2014 was four folds in water extraction as compared to that in absolute ethanol, while the data concerning the middle part of the West Bank was exception (figure 4.1B).

In 2015, the three extraction methods yielded TPC in leaves collected from the three geographical regions in the range of 301 (in absolute ethanol) and 448 (in 80% ethanol) (figure 4.1A). While the fruits collected in 2015 in all geographical regions gave 2-3 folds higher in absolute ethanol as compared to distilled water (figure 4.1B).

Comparing the total phenolic contents in the leaves as affected by geographical regions in both years in each extraction method separately, we can see that all geographical regions had similar TPC amounts significantly.

In term of fruits, the TPC contents increase significantly as we go to south of the West Bank. Generally speaking, leaves contained more TPC than fruits in most equivalent treatments.

(Khaleel et al, 2016) reported that the total phenolic content in leaves of *Ziziphus Spina-Christi* in ethanloc and distilled water extracts were  $34.0 \pm 0.23$  and  $11.8 \pm 0.51$  mg GAE/g extract.



**Figure 4.1(A):** Total phenolic content (TPC, mg Gallic acid/g DW) of *Ziziphus* plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** Total phenolic content (TPC, mg Gallic acid/g DW) of *Ziziphus* plant parts (Fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

## 4.2 Total flavonoid contents (TFC)

Total flavonoids content (TFC) in leaves collected in 2014 increased significantly using 80% ethanol as extraction method in all studied geographical regions, while the extraction with the distilled water and 99% ethanol have almost statistically similar amounts in all studied geographical regions (North, Middle and South) in the West Bank (Figure 4.2A).

TFC of the leaves in 2014 yielded high amounts (118.7 mg catechin/g DW) significantly in 80% ethanol extraction in the middle then north parts of the West bank, while the amounts of TFC of the leaves in south region was the lowest amount (16.6 mg catechin/g DW) in the same method of extraction.

TFC of the fruits in the year 2014 yielded high amounts significantly when they were extracted by distilled water in all studied geographical regions of the West bank, compared to those extracted by absolute and 80% ethanol, especially in the middle region which recorded higher amount of TFC (11.2 mg catechin/g DW) in distilled water extraction method (figure 4.1B).

Among the studied extraction methods, the 80% ethanol extracted high amount of TFC in leaves collected in 2014 (figure 4.2A), while in fruits, the distilled water extraction yielded high amounts of TFC in the same year (figure 4.2B).

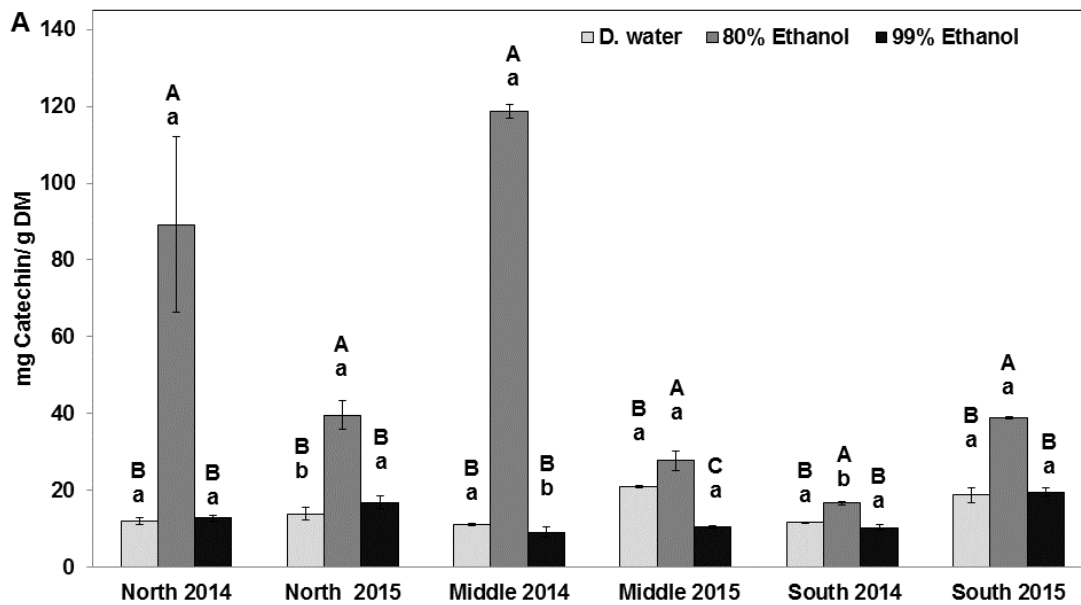
As the results of in 2014, TFC of the leaves collected in 2015 yielded high amount significantly in 80% ethanol method in all studied geographical part in the West Bank compared to distilled water and 99% ethanol extraction method, while the TFC of the leaves extracted with both 99% ethanol and distilled water had statistically similar amounts (figure 4.2A).

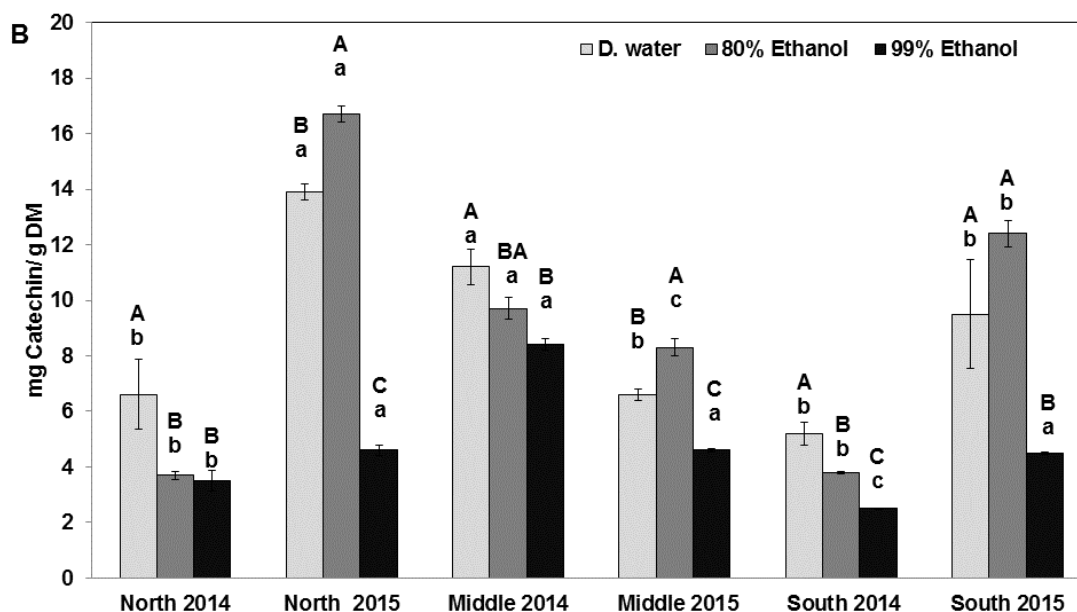
Also, the fruits collected in 2015 yielded high amounts of TFC significantly in 80% ethanol extraction method in all studied geographical regions. Statistically the higher yield of TFC in the fruits was found in 80% ethanol, then distilled water, then absolute ethanol extraction methods for samples collected from north and middle parts of the West Bank, while the yield of TFC in fruits collected from the south part of West Bank extracted with both distilled water and 80% ethanol was statistically similar (figure 4.2B).

Comparing the TFC in the leaves as affected by geographical regions studied in 2014 in each extraction methods separately, we can see that the yield of TFC was statistically high in the middle and north regions in 80% ethanol extraction method (figure 4.2A). TFC yield was statistically similar in all geographical regions when they were extracted with distilled

water. In 2015, leaves collected from all geographical regions had statistically similar amounts in both ethanol extraction methods separately, while in distilled water extraction method, leaves collected from the north region of the West Bank yielded lower amount of TFC as compared to the middle and the south regions (figure 4.2A).

Amounts of TFC in fruits collected in 2014 as affected by geographical regions in each extraction method separately reveals that TFC yielded high amount in middle part of West Bank as compared with the other studied regions which yielded statistically similar amounts. In year of 2015 the TFC in fruits yielded high amount in the north regions compared to the middle and south in both 80% ethanol and distilled water extraction methods, while the TFC in fruits was statistically similar in all studied geographical regions in absolute ethanol extraction.





**Figure 4.2 (A):** Total flavonoid content (TFC, mg catechin /g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** Total flavonoid content (TFC, mg catechin /g DW) of Ziziphus plant parts (Fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

### 4.3 Antioxidant activity (AA)

The results in figure 4.3A reveals that the antioxidant activity interpreted as FRAP in leaves collected in 2014 from the middle and south part of West Bank increased significantly when the extraction solution contained more contribution of distilled water, while in north regions, FRAP in leaves in both distilled water and 80% ethanol extraction contained statistically similar amounts but significantly higher than those extracted by absolute ethanol.

FRAP in fruits collected in 2014 from north and south regions yielded higher amounts significantly in distilled water extraction solution, while that in middle regions was significantly higher in 80% ethanol extraction. The yield of FRAP in fruits collected in 2014 in both distilled water and 80% ethanol extraction in south part of West Bank had significantly similar amount (figure 4.3B).

FRAP contents collected in 2014 from the three different geographical regions ranged from 11.9 to 2.6 and from 11.2 to 1.3 mg Trolox/g DW in leaves and fruits respectively, and in 2015 ranged from 13.6 to 7.4 and from 21.1 to 3.1 mg Trolox/g DW respectively.

As the results in 2014, FRAP of the leaves collected in 2015 yielded higher amount significantly in 80% ethanol extraction in all geographical regions of the West Bank as compared to the other extraction methods (distilled water and 99% ethanol). Furthermore, FRAP in leaves in both distilled water and 99% ethanol extraction had statistically similar amounts (figure 4.3A).

FRAP of the fruits collected in 2015 from north and south regions of the West Bank yielded higher amount significantly in 80% ethanol extract, while the fruits collected from the middle part of the country yielded the higher amount in distilled water extract as compared to that in 99% ethanol extract (figure 4.3B).

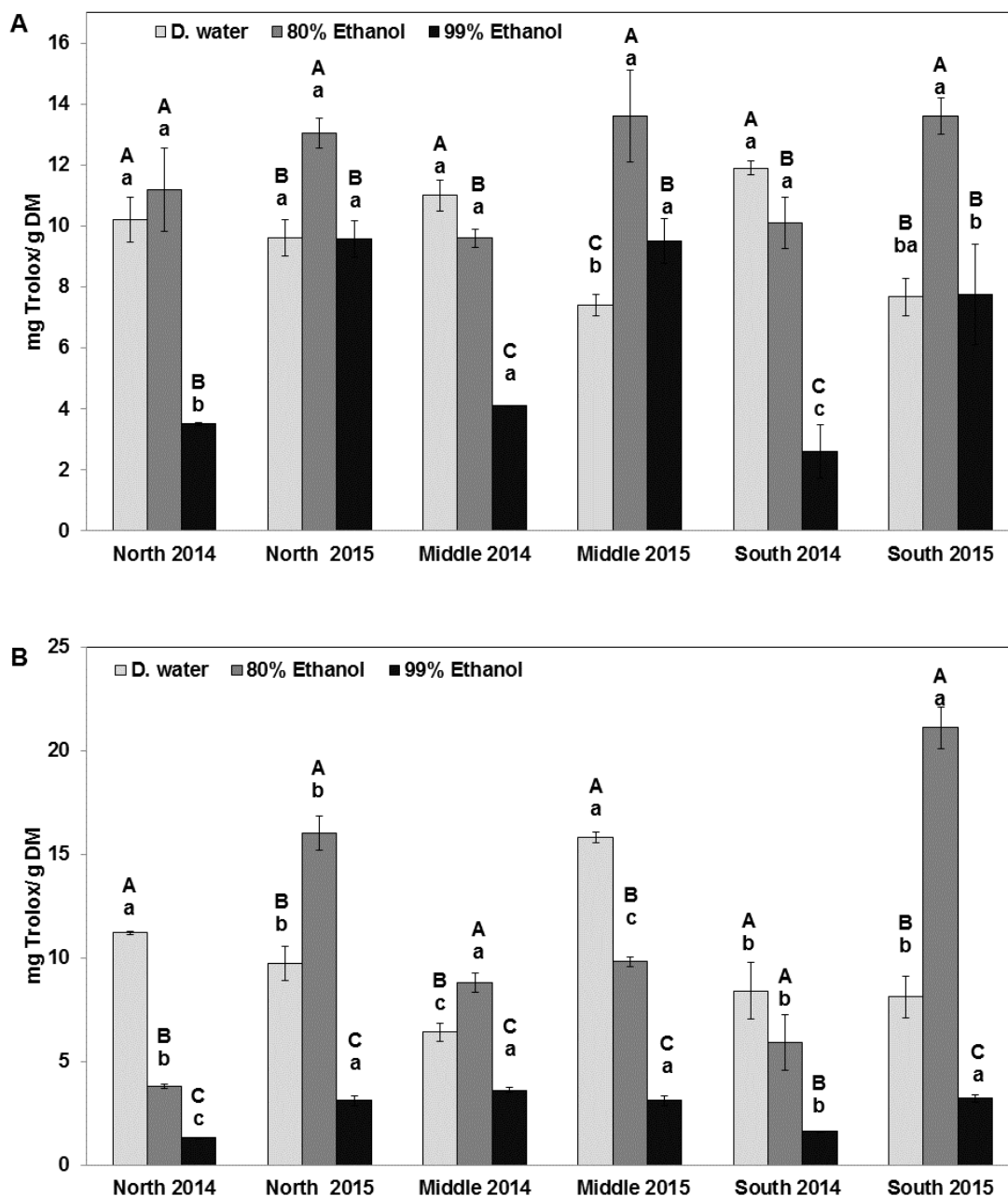
Comparing FRAP antioxidant activity in leaves as affected by geographical regions in 2014 in each extraction method separately, we can see that all geographical regions had statistically similar amounts in both distilled water and 80% ethanol extraction methods, while this antioxidant activity in leaves yielded higher amount (4.1 mg) in the middle when used absolute ethanol, then in the north (3.5 mg) then in the south (2.6 mg) of the country (figure 4.3A).

FRAP in fruits collected in 2014 from the middle region yielded high amount significantly in 80% ethanol and distilled water extraction, while the significantly highest amount of FRAP was recorded in north (11.2 mg Trolox/g DW) in distilled water extraction (figure 4.3B).

FRAP in leaves collected in 2015 yielded high amount significantly in 80% ethanol extraction method in all studied regions. Northern region gave higher amount significantly in all extraction methods, while the southern part of the country, both distilled water and 99% ethanol extraction methods had statistically similar amounts (figure 4.3A).

FRAP in fruits collected in 2015 in all geographical part of country had higher amounts significantly in 80% ethanol extraction method as compared to that distilled water and absolute ethanol extraction (figure 4.3B).





**Figure 4.3 (A):** FRAP antioxidant activity (mg Trolox/ g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** FRAP antioxidant activity (mg Trolox/ g DW) of Ziziphus plant parts (Fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

#### 4.4: Cupric reducing antioxidant power (CUPRAC)

The results in figure 4.4A reveals that the antioxidant activity interpreted as CUPRAC in leaves collected in 2014 was significantly higher in the north region of the West Bank,

when the extraction solution contained more contribution of distilled water, while the CUPRAC in the middle and south regions in leaves yielded high amount significantly in 80% ethanol extraction method compared to that in distilled water and 99% ethanol extraction method.

CUPRAC in fruits collected in 2014 yielded high amount significantly in distilled water extraction in all studied geographical regions of the West Bank as compared to the absolute ethanol extraction method. Also 80% ethanol extraction has higher amounts of CUPRAC significantly, but statistically similar amount in the north and south regions of the country (figure 4.4B).

In 2015, CUPRAC in leaves yielded high amount significantly in the absolute ethanol extraction in the samples collected from the middle and south part of the West Bank, while the CUPRAC in leaves collected from the north extracted with distilled water yielded high amount significantly as compared to that extracted with 80% ethanol. Also, CUPRAC in leaves collected from the southern region extracted with both distilled water and 80% ethanol had statistically similar amounts (figure 4.4A).

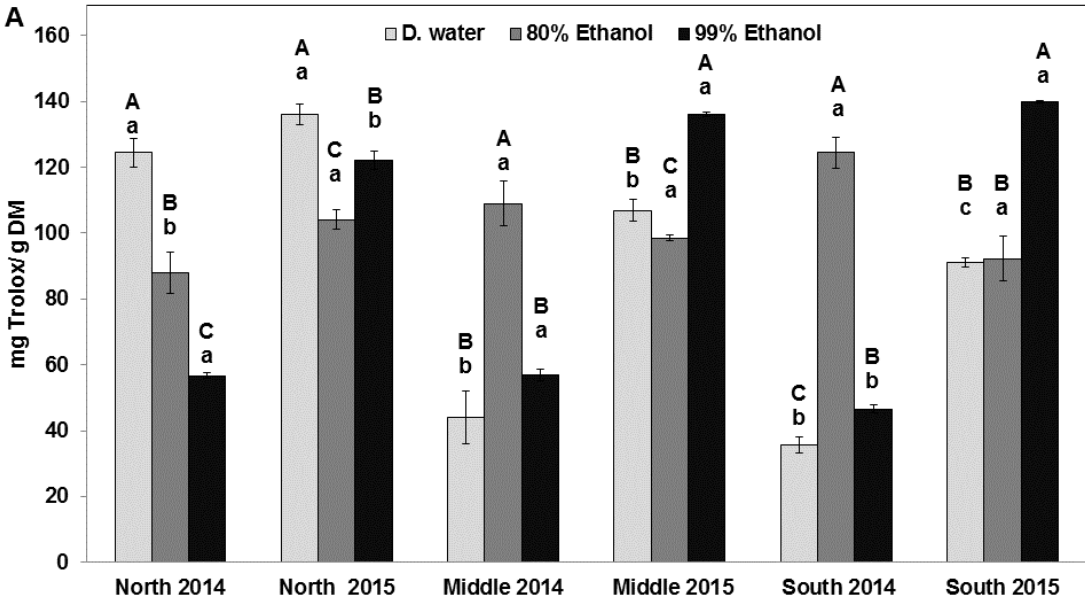
Fruits collected in 2015, yielded higher CUPRAC significantly when the extraction contained more contribution of distilled water (distilled water > 80% ethanol > 99% ethanol) in all studied geographical regions of the West Bank (figure 4.4B).

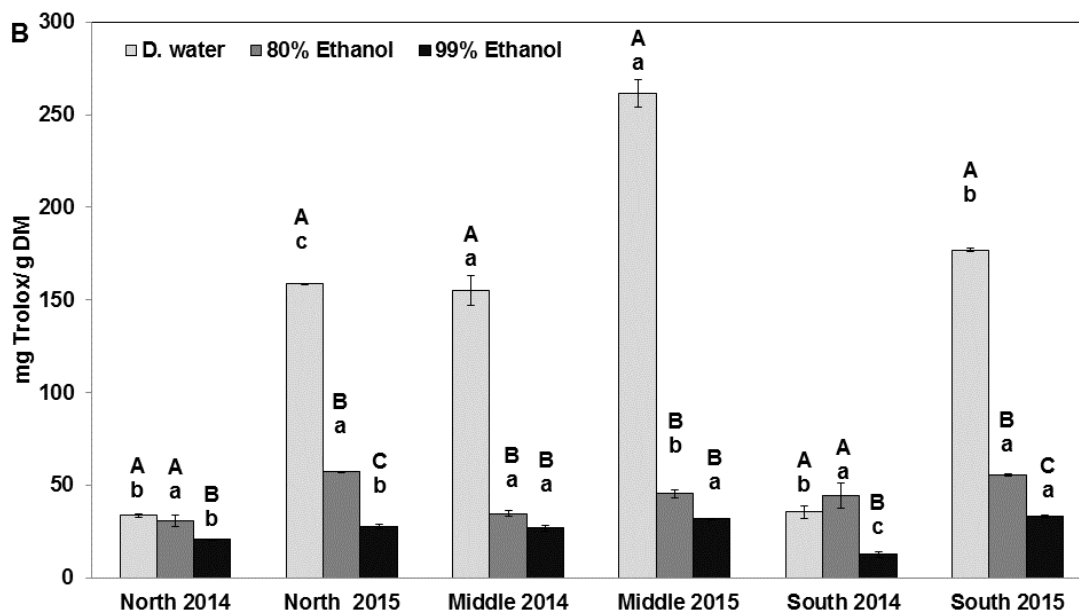
Comparing CUPRAC as affected by geographical regions in both years and in each extraction method separately. we can see that CUPRAC in leaves collected in 2014 yielded higher amount significantly in the northern part of the West Bank when was extracted by the distilled water as compared to that in the middle and south regions using the same extraction method. Also, CUPRAC in leaves had higher amount significantly in the middle and south regions of West Bank when they were extracted by 80% ethanol, while the CUPRAC in leaves had higher amount significantly in samples collected from the northern and middle regions when they were extracted by absolute ethanol (figure 4.4A).

CUPRAC in fruits collected in 2014 had statistically similar amount when they were extracted with 80% ethanol in all studied geographical regions of country. Furthermore, CUPRAC in fruits collected in 2014 yielded higher amounts significantly in the middle part of West Bank when they were extracted with distilled water (figure 4.4B).

CUPRAC amounts in leaves collected in 2015 were statistically similar in all studied geographical regions of West Bank when they were extracted with 80% ethanol, while the CUPRAC in leaf in 2015 yielded higher amounts significantly in the northern region when was extracted with distilled water. Also, CUPRAC has high amount significantly in the middle and southern regions of West Bank when they were extracted with absolute ethanol (figure 4.4A).

CUPRAC in fruits collected in 2015 yielded higher amounts significantly in the middle regions of the West Bank when they were extracted with distilled water. Additionally, CUPRAC in the northern and southern part of the country extracted with 80% ethanol had statistically similar amount (figure 4.4B)





**Figure 4.4 (A):** Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (Fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

#### 4.5: Free radical scavenging activity using DPPH

The results in figure 4.5A reveals that DPPH in leaves collected in 2014 yielded higher amount significantly when they were extracted with 99% ethanol in all studied geographical regions of the West Bank. Also, it yielded high amounts significantly in 80% ethanol extraction method in both middle and southern parts of the country as compared to that in the northern region. Furthermore, DPPH in leaves in both 80% ethanol and 99% ethanol extraction methods had statistically similar amounts.

DPPH in fruits collected in 2014 yielded high amount significantly in 80% ethanol extraction method in all studied geographical regions of the West Bank as compared to that in 99% extraction method. Also, it yielded high amount significantly in distilled water extraction in both north and south parts as compared to those in the middle region of the country (figure 4.45B).

In 2015, DPPH in leaves collected in this year yielded high amount significantly in 80% ethanol extraction method as compared to that extracted by distilled water, while the DPPH

in leaves in 2015 in both ethanolic extraction (80% ethanol and 99% ethanol) methods had statistically similar amounts (figure 4.5A).

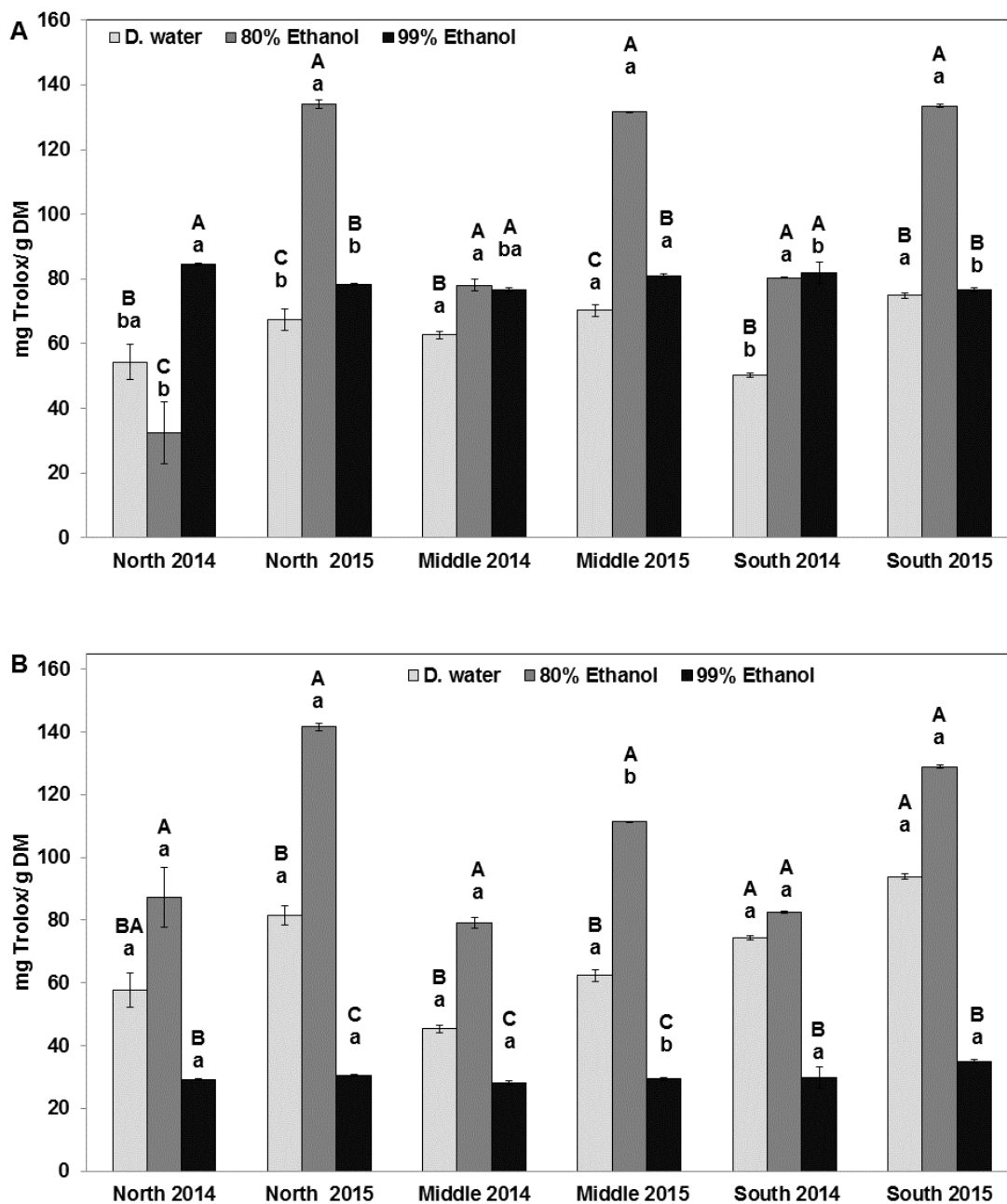
DPPH in fruits collected in 2015 yielded high amount significantly in 80% ethanol extraction method in all studied geographical regions of the country as compared to that in 99% ethanol extraction method. Also, DPPH collected in 2015 yielded high amount significantly in distilled water extraction method in the samples collected from the southern regions, and DPPH in fruits in both distilled water and 80% ethanol extraction methods in the samples collected from the southern region had statistically similar amount (figure 4.5B).

Comparing DPPH as affected by geographical regions in both years and in each extraction method separately, We can see that DPPH in leaves collected in 2014 yielded higher amount significantly in samples collected from the north and middle parts of the country in both distilled water and 99% ethanol extraction method and it has statistically similar amounts, while the DPPH in leaves in 2014 yielded higher amount significantly in the samples collected from the middle and south regions of West Bank in 80% ethanol extraction method as compared to that in north regions (figure 4.5A).

Comparing DPPH in fruits collected in 2014 as affected by geographical regions, all geographical regions studied in the West Bank had statistically similar amount with all extraction methods used (figure 4.5B).

In 2015, DPPH in leaves yielded higher amount significantly in all geographical regions extracted by 80% ethanol, but in distilled water extraction method it was significantly higher in samples collected from both middle and southern parts of the country, while in 99% ethanol extraction method the higher significant values were just in the samples collected from the middle region. Otherwise DPPH in leaves in samples collected from both middle and southern geographical parts extracted by both distilled water and 80% ethanol had statistically similar amount (figure 4.5A).

DPPH in fruits collected in 2015 as affected by geographical regions, indicate that the DPPH in fruits yielded higher amount significantly in all geographical regions when they were extracted by distilled water, but those were extracted with 80% ethanol and 99% ethanol were higher in samples collected from the northern and southern regions of the West Bank as compared to those collected from the middle regions (figure 4.5B)



**Figure 4.5 (A):** Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

#### **4.6: Free radical scavenging activity using ABTS**

The results in figure 4.6A reveals that ABTS in leaves collected in 2014 yielded higher amounts significantly in 99% ethanol extraction method in all geographical regions of the West Bank, and it also yielded higher amount significantly in 80% ethanol extraction method in samples collected from the northern and southern parts of the country as compared to that in the middle region.

ABTS in fruits collected in 2014 yielded high amount significantly in distilled water and 99% ethanol extraction methods in samples collected from the northern region of the West Bank, and it yielded higher amounts significantly with all extraction methods in samples collected from the middle and southern parts of the West Bank, so ABTS in fruits in samples collected from these two regions (middle and south) with all extraction method had statistically similar values (figure 4.6B).

In 2015, ABTS in leaves yielded higher amounts significantly in samples collected from the northern regions when were extracted by distilled water and 80% ethanol, and in the middle regions in distilled water extraction method, while in samples collected from the southern regions, it yielded higher amounts significantly with all extraction method. So ABTS in leaves in samples collected from the south regions with all extraction methods had statistically similar amount and ABTS in samples collected from the northern region in both water and 80% ethanol extraction method had statistically similar values, while ABTS in leaves in 2015 in samples collected from the middle part of the country increased significantly when the extraction solution contains more contribution of distilled water (figure 4.5A).

ABTS in fruits collected in 2015, yielded high amounts significantly in samples collected from the northern regions in 80% ethanol extraction method, and in the middle regions in 99% ethanol extraction method, and in the south in both 80% ethanol and 99% ethanol extraction methods, furthermore in the samples collected from the southern regions, ABTS in fruits had statistically similar amounts with both extraction method mentioned (figure 4.6B).

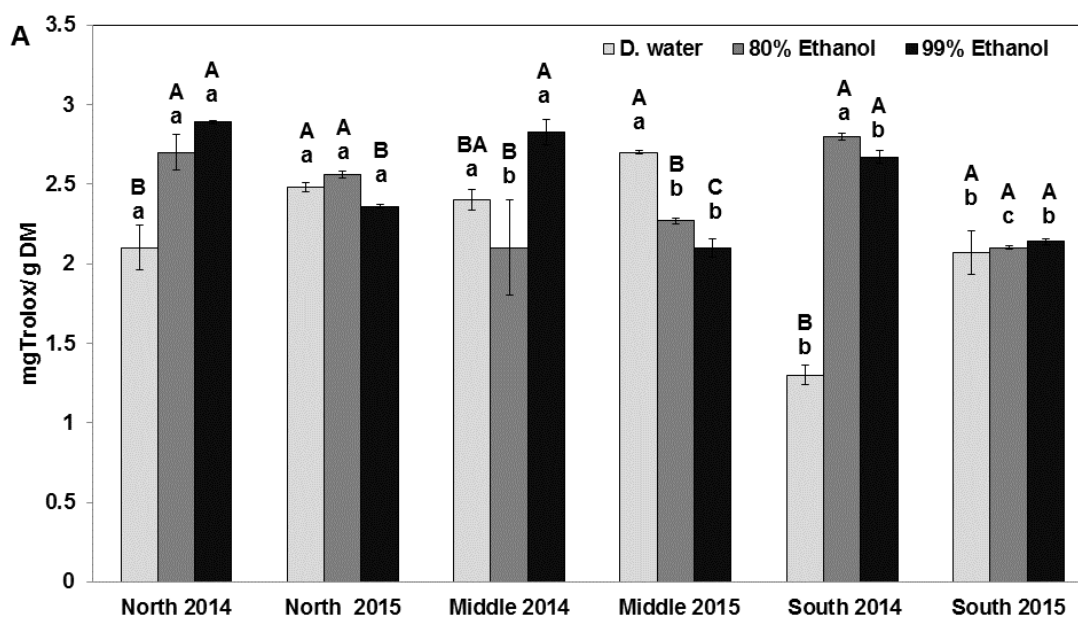
Comparing ABTS as affected by geographical regions in both years and in each extraction method separately, ABTS in leaves collected in 2014 yielded higher amounts significantly

in the northern and middle part of the country in both distilled water and 99% ethanol extraction methods. Also, it yielded high amount significantly in northern and southern regions of the West Bank in 80% ethanol extraction method (figure 4.6A).

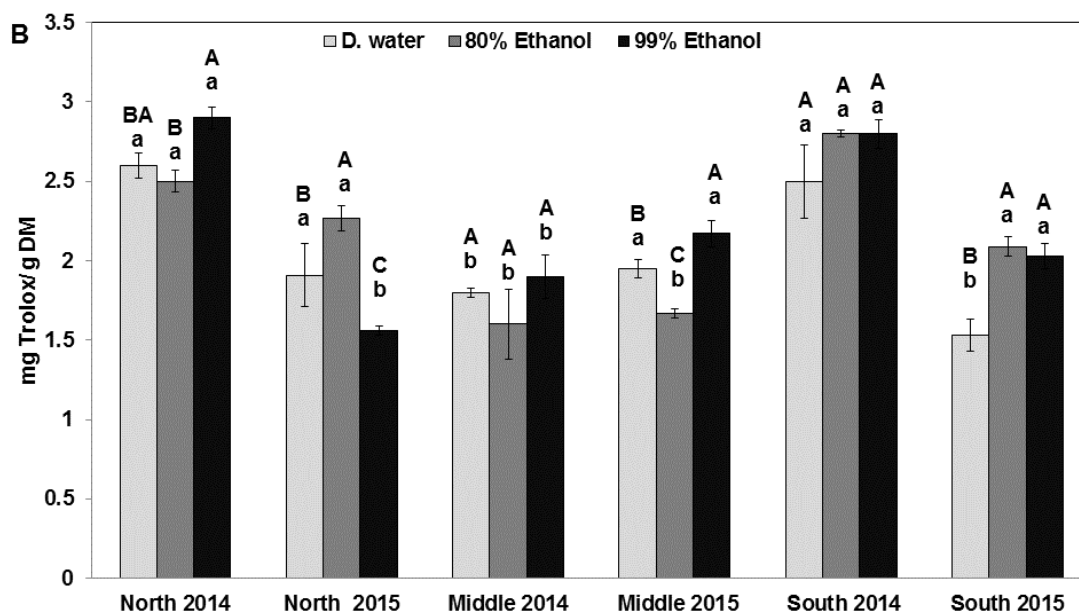
Fruits collected in 2014 as affected by geographical regions, indicate that ABTS in fruits yielded higher amounts significantly in samples collected from the northern and southern regions of the West Bank with all extraction methods as compared to that in the middle regions (figure 4.6B).

ABTS in leaves collected in 2015 yielded higher amounts significantly in samples collected from the northern and middle regions of the West Bank when they were extracted by distilled water, but in 80% ethanol extraction method ABTS increase significantly as we move from the south to the north regions of the West Bank, while in 99% ethanol extraction method ABTS yielded higher amount significantly in samples collected from the northern regions, but the other regions had statistically similar amounts (figure 4.6A).

ABTS in fruits collected in 2015 as affected by geographical region, yielded high amount significantly in samples collected from the northern and middle regions of the West Bank in distilled water extraction method, and in samples collected from the northern and southern regions, it yielded higher amounts when they were extracted with 80% ethanol, and in samples collected from the middle and southern regions it yielded higher amounts when they were extracted with 99% ethanol (figure 4.6B).







**Figure 4.6 (A):** Free radical scavenging activity (ABTS, mg Trolox/g DW of Ziziphus plant parts (leaves) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). **(B)** Free radical scavenging activity (ABTS, mg Trolox/g DW of Ziziphus plant parts (fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south).

## 4.7 HPLC Results and discussion

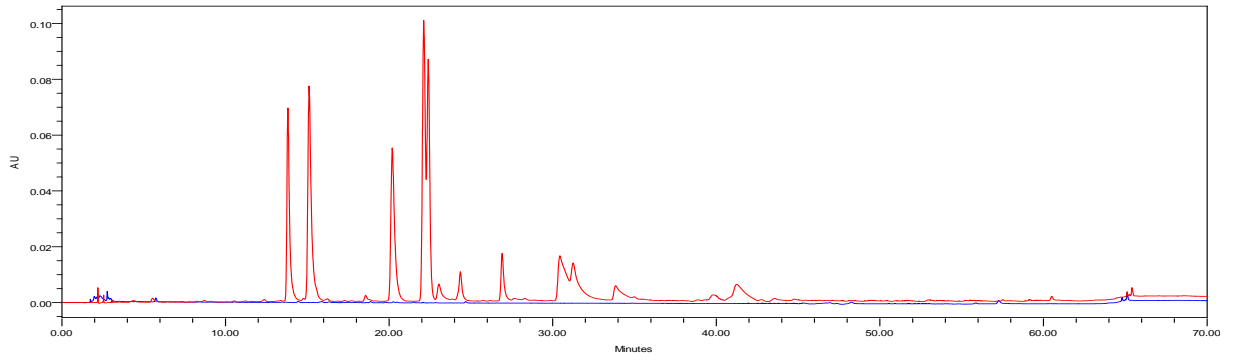
### HPLC-PDA profiles of the extracts

#### 4.7.1 Ziziphus Spina-Christi fruit extracts

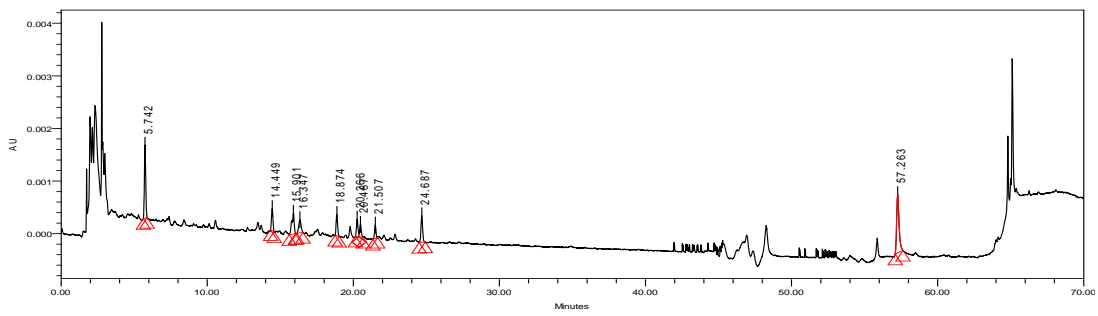
##### 4.7.1.1 Water extract

Figure 4.7 shows an overlaid chromatogram of the crude extracts at 325 nm. This wavelength was selected since the main peaks showed a maximum absorption close to it. As seen from Figure 4.7A, different phenolic compounds were detected in the range of 5-25 minutes. Unfortunately, these compounds are not part of the standards injected as per their retention and UV-Vis spectra tells.

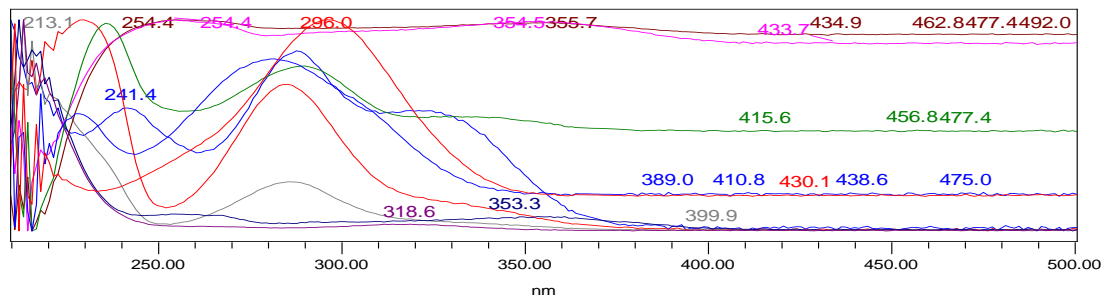
(A)



(B)



(C)



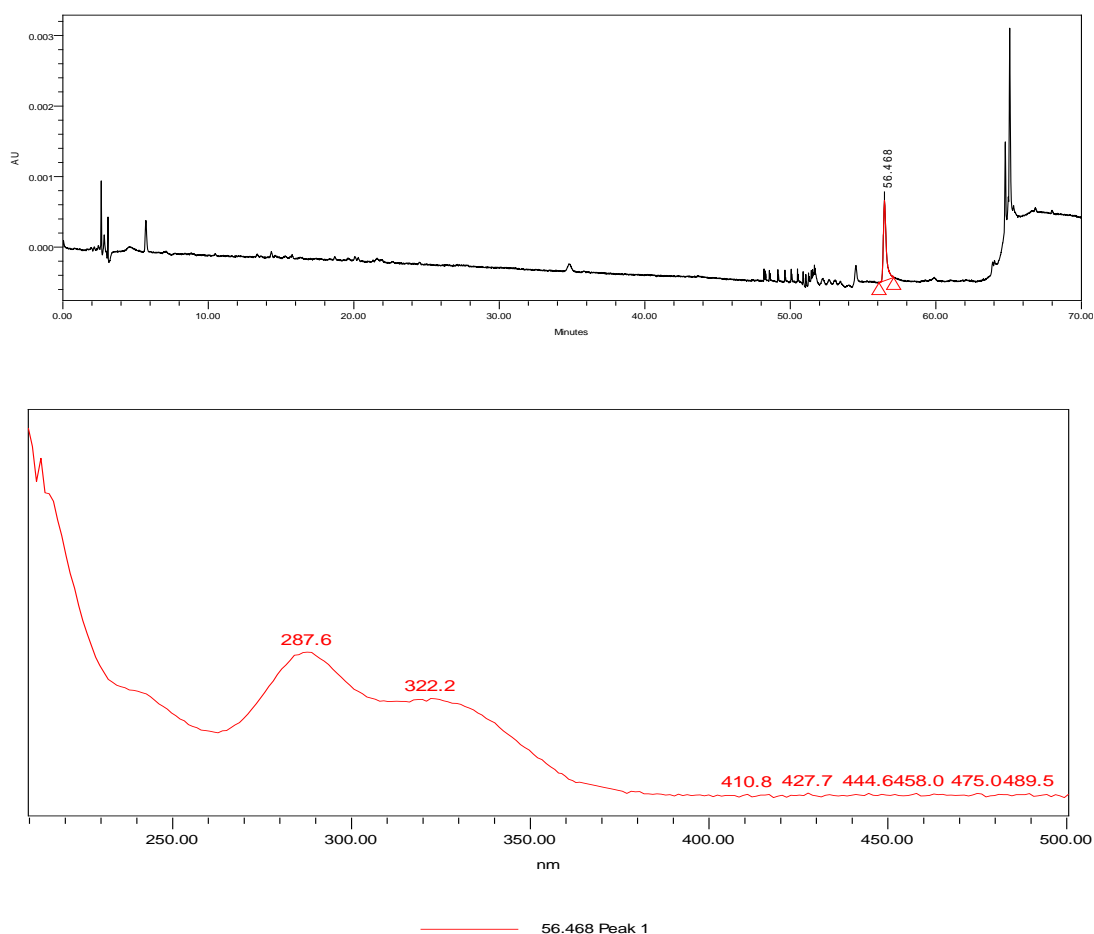
- 5.741 Peak 1
- 14.449 Peak 2
- 15.900 Peak 3
- 16.346 Peak 4
- 18.875 Peak 5
- 20.266 Peak 6
- 20.486 Peak 7
- 21.506 Peak 8
- 24.686 Peak 9
- 57.262 Peak 10

**Figure 4.7:** Overlaid HPLC-PDA chromatograms of crude water extract (blue) and standard (red) at 325 nm (A). (B) depict zoomed chromatogram of A. The overlaid UV-Vis spectra of the main peaks are depicted at the right corner of chromatogram (C).

#### 4.7.1.2 ethanol extract

Figure 4.8 shows chromatograms of ethanol Ziziphus Spina-Christi extract at 325 nm. One peak appeared at 56.4 minutes which may correspond to a lipophilic compound.

(A)

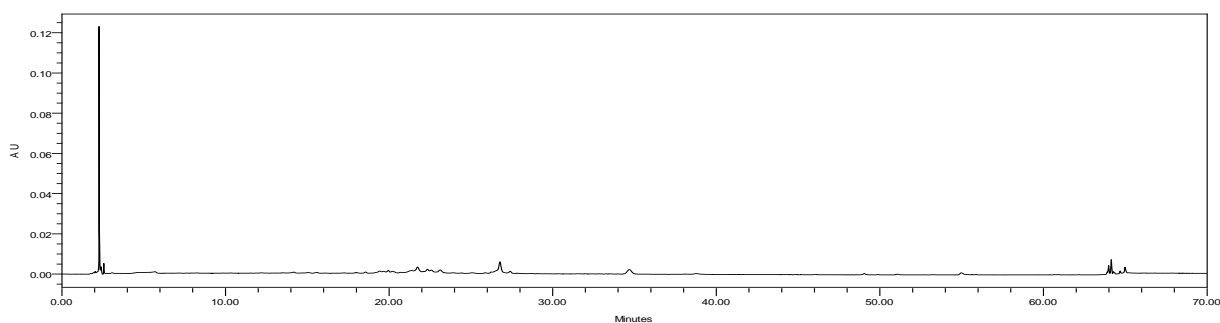


(B)

**Figure 4.8:** HPLC-PDA chromatograms of ethanol extract at 325 nm (A). The UV-Vis spectra of the main peak are depicted in Figure (B).

### 4.7.1.3 Ethanol 80% extract

Figure 4.9 shows chromatograms of the crude extracts at 325 nm. As it is seen in this Figure, no phenolic compounds were detected in this extract

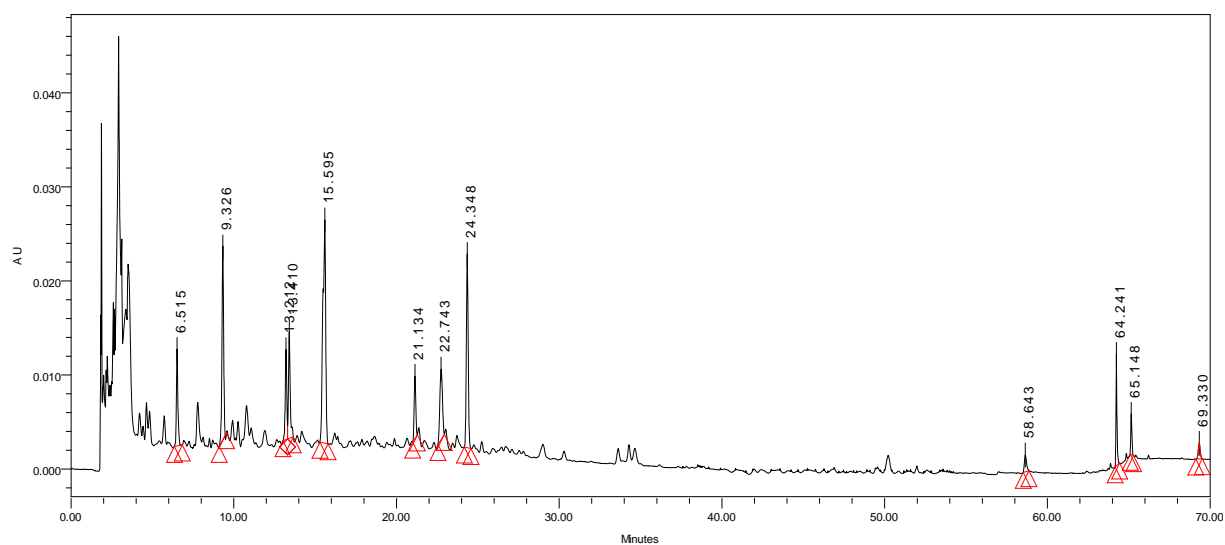


**Figure 4.9:** HPLC-PDA chromatograms of 80% ethanol extract at 325 nm.

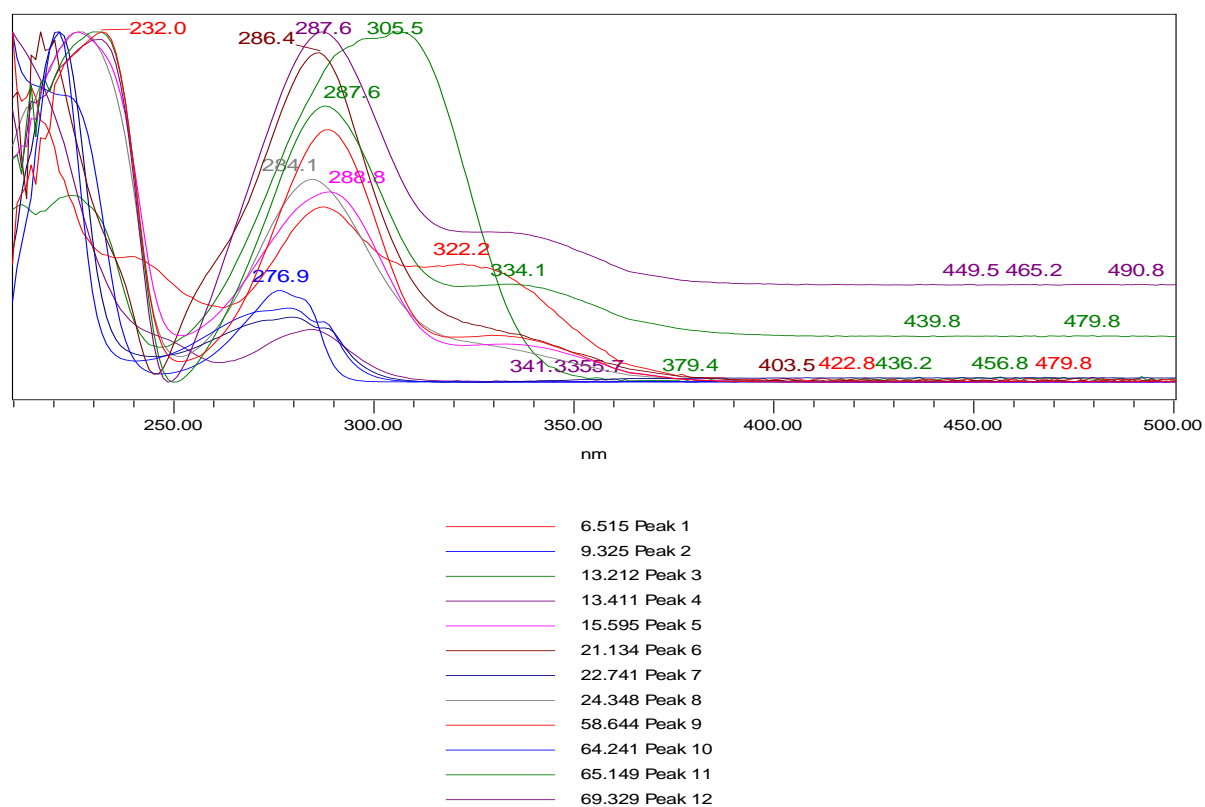
## 4.7.2 Ziziphus Spina- Christi leaves extract

### 4.7.2.1 water extract

Figure 4.10 shows chromatogram of the crude water extract at 280 nm. This wavelength was selected since the main peaks showed a maximum absorption close to it. As it is clear from this figure, many phenolic compounds were detected in the range of 5-25 minutes; peaks at retention times of: 6.5, 9.3, 13.3, 13.4, 15.5, 21.1, 22.7, 24.3, 58.6, 64.2, and 66.1 minutes were detected. These compounds are not part of the standards injected as per their retention and UV-Vis spectra tells. Additionally, four lipophilic compounds were detected in the range of 55-70 minutes.



(A)

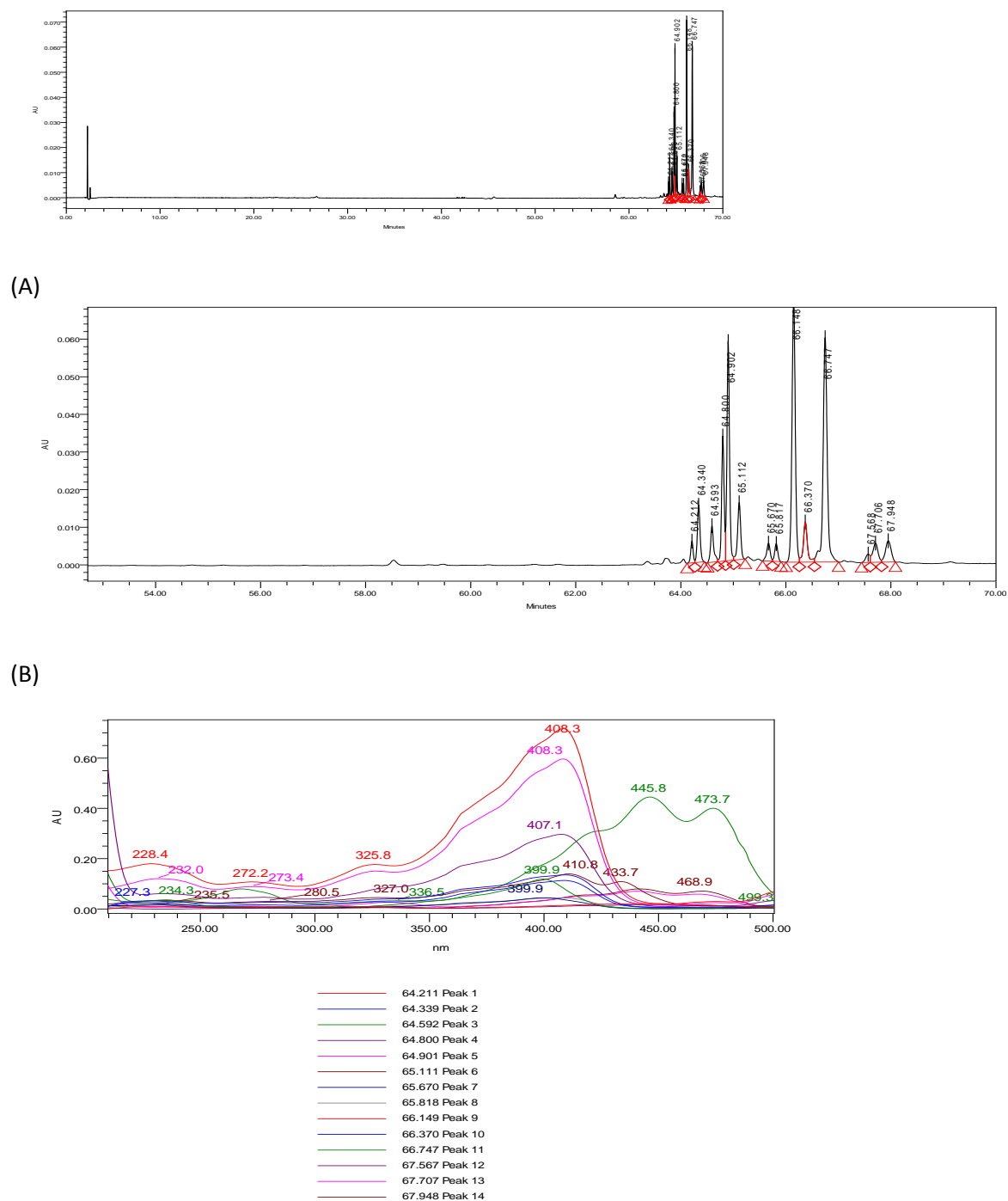


(B)

**Figure 4.10:** HPLC-PDA chromatograms of crude water extracts at 280 nm. The overlaid UV-Vis spectra of the main peaks are depicted at Figure 4.10B.

### 4.7.2.2 Ethanol extract

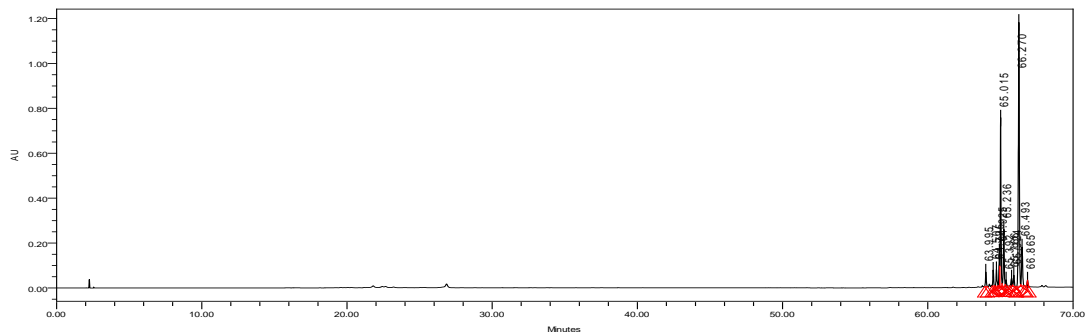
Figure 4.11 shows chromatogram of the crude ethanol extract at 500 nm. As it is clear from this figure, many phenolic compounds (lipophilic) were detected in the range of 64 to 68 minutes.



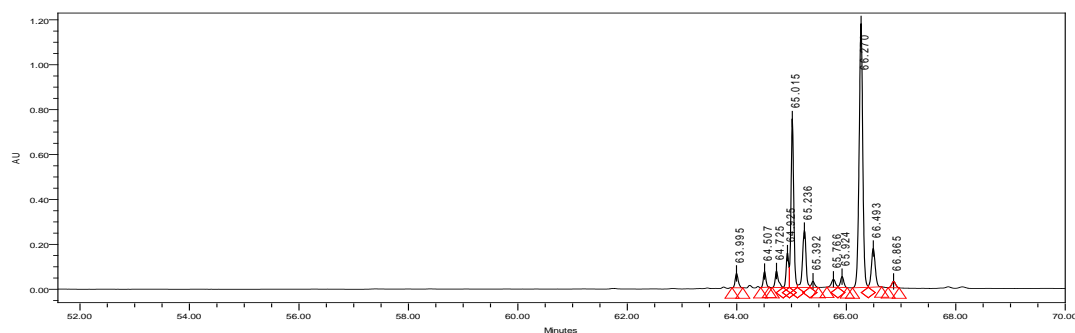
(C) **Figure 4.11:** HPLC-PDA chromatograms of crude ethanol extracts at 500 nm (A). Figure 4.11B is the zoomed region from 64-68 minutes. The overlaid UV-Vis spectra of the main peaks are depicted at Figure 4.11C.

### 4.7.2.3 ethanol 80% extracts

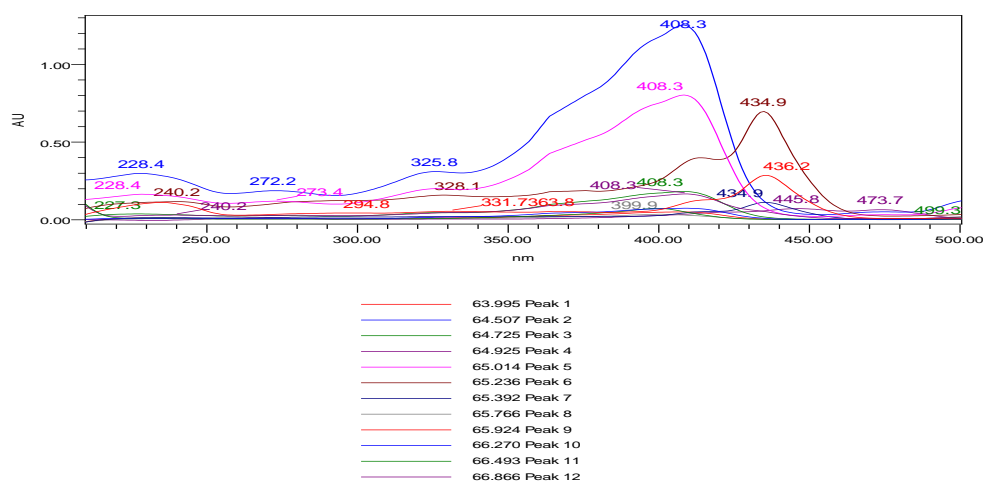
Figure 4.12 shows chromatogram of the crude ethanol 80% extract at 400 nm. As it is clear from this figure, many lipopholic compounds were detected in the range of 64 to 67 minutes.



(A)



(B)



(C)

**Figure 4.12:** HPLC-PDA chromatograms of crude ethanol 80% extracts at 400 nm (A).

Figure 4.12B is the zoomed region from 64–68 minutes. The overlaid UV-Vis spectra of the main peaks are depicted at Figure 4.12C.

## 4.8 Pearson correlations

**4.8.1** Pearson correlations between antioxidant contents and their activities of leaves collected from Northern West Bank extracted by all solvents in both years (2014/2015).

Table 4.1 shows that, in 2014, all antioxidant activities under study were found significantly correlated with TPC. It was highly and positively correlated with both FRAP and CUPRAC, while the significant correlation with both DPPH and ABTS was found negative. TFC was significantly and negatively correlated with DPPH only, while the correlation with the other antioxidant activities were not significant.

FRAP was positively and significantly correlated with CUPRAC and was negatively significantly correlated with DPPH while its negative correlation with ABTS was not significant. Both CUPRAC and ABTS were highly significant correlated with each other in negative manner.

In 2015 both TPC and TFC was positively and significant correlated with FRAP, DPPH, and ABTS, and significantly negatively correlated with CUPRAC, but the correlation between TFC with all the antioxidant activities (except APTS) was higher than with TPC (Table 4.1).

FRAP showed high significant positive correlation with both DPPH and APTS, while negative correlation with CUPRAC. CUPRAC was found negatively and significant correlation with DPPH, while it was not correlated with the ABTS. DPPH and ABTS were positively and significantly correlated with each other. TPC and TFC were found positively correlated with each other.

**Table 4.1:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the northern region of the West Bank in 2014 (above diagonal) and 2015 (below diagonal).

2014/2015	TPC	TFC	FRAP	CUPRAC	DPPH	ABTS
<b>TPC</b>	-----	0.158	0.877**	0.951***	-0.702*	-0.835**
<b>TFC</b>	0.802***	-----	0.497	-0.011	-0.646*	0.203
<b>FRAP</b>	0.743*	0.887**	-----	0.696*	-0.908***	-0.526
<b>CUPRAC</b>	-0.752*	-0.925***	-0.761*	-----	-0.490	-0.921***
<b>DPPH</b>	0.848**	0.967***	0.943***	-0.923***	-----	0.240
<b>ABTS</b>	0.770*	0.715*	0.705*	-0.477	0.679*	-----



**4.8.2** Pearson correlations between antioxidant contents and their activities of fruits collected from Northern West Bank extracted by all solvents in both years.

In 2014, TPC was found positively and significantly correlated with the TFC (Table 4.2). It had also positive and significant correlation with both FRAP and CUPRAC, while it was not correlated with both DPPH and ABTS. TFC was found significantly and positively correlated with the FRAP, while the other antioxidant activities (CUPRAC, DPPH, and ABTS) were not correlated.

FRAP was found positively correlated with the CUPRAC, while it was not correlated with both DPPH and ABTS.

CUPRAC was found negatively and significantly correlated with the ABTS, while it was not correlated with DPPH. Both DPPH and ABTS were found positively correlated with each other.

In 2015, TPC was found significantly correlated and negatively with the TFC, it was negatively with all antioxidant activities, but only significantly correlated with the CUPRAC.

TFC was found highly significantly and positively correlated with all antioxidant activities except CUPRAC.

FRAP was found highly significantly correlated and positively with DPPH and ABTS, while was not correlated with the CUPRAC.

CUPRAC was found not correlated with both DPPH and ABTS.

DPPH and ABTS were high significantly and positively correlated with each other.

**Table 4.2:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the northern region of the West Bank in 2014 (above diagonal) and 2015 (below diagonal).

2014/2015	TPC	TFC	FRAP	CUPRIC	DPPH	ABTS
TPC	-----	0.863**	0.998***	0.770*	0.207	-0.432
TFC	-0.817**	-----	0.869**	0.470	0.087	-0.085
FRAP	-0.641	0.950***	-----	0.778*	0.238	-0.436
CUPRIC	-0.842**	0.496	0.227	-----	0.611	-0.701*
DPPH	-0.593	0.937***	0.989***	0.167	-----	0.667*
ABTS	-0.620	0.933***	0.969***	0.203	0.984***	-----

**4.8.3** Pearson correlations between antioxidant contents and their activities of leaves collected from Middle West Bank extracted by all solvents in both years.

In 2014, TPC and TFC were found not correlated, but TPC was found highly significantly correlated in positive manner with FRAP, and significantly correlated with DPPH in negative manner, while it was not correlated with another antioxidant (CUPRAC and ABTS) (Table 4.3).

TFC was found highly significantly and positively correlated with CUPRAC, while it was negatively and significantly correlated with ABTS, but it was not correlated with both FRAP and DPPH.

FRAP was found significantly correlated with ABTS but in negative manner while it was not correlated with neither CUPRAC nor DPPH.

CUPRAC was found significantly and positively correlated with DPPH, while was not significantly correlated with ABTS. DPPH and ABTS were not correlated.

In 2015, TPC was found not correlated with TFC, and with any of the antioxidant (FRAP, CUPRAC, DPPH, and ABTS).

TFC was found highly significantly and negatively correlated with CUPRAC, and positively correlated with DPPH, while it was not correlated with both FRAP and ABTS.

FRAP had high significant and positive correlation with DPPH, while it was not correlated with neither CUPRAC nor ABTS.

CUPRAC was found not correlated with both DPPH and ABTS. Also, DPPH and ABTS were found not correlated with each other.

**Table 4.3:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the Middle regions of the West Bank in 2014 (above diagonal) and 2015 (below diagonal).

2014/2015	TPC	TFC	FRAP	CUPRAC	DPPH	ABTS
TPC	-----	0.152	0.959***	-0.048	-0.728*	-0.564
TFC	0.117	-----	0.340	0.952***	0.548	-0.675*
FRAP	-0.163	0.530	-----	0.154	-0.570	-0.735*
CUPRAC	-0.218	-0.948***	-0.342	-----	0.694*	-0.600
DPPH	0.015	0.677*	0.919***	-0.531	-----	0.015
ABTS	0.214	0.353	-0.513	-0.538	-0.403	-----

**4.8.4** Pearson correlations between antioxidant contents and their activities of fruits collected from Middle West Bank extracted by all solvents in both years.

In (2014), TPC was found highly significantly and negatively correlated with both TFC and CUPRAC, while it is not correlated with FRAP, DPPH, and ABTS (Table 4.4).

TFC was found only significantly and positive correlated with CUPRAC, while it was found not correlated with other antioxidants.

FRAP had high significant correlation with DPPH, while it was not correlated with both CUPRAC and ABTS.

CUPRAC was found not correlated with DPPH and ABTS. Also, DPPH and ABTS were not correlated to each other.

In (2015), TPC was found significantly and negatively correlated with TFC, CUPRAC, and highly significantly correlated with FRAP.

TFC was found highly significantly correlated with DPPH in positive manner and highly significantly correlated with ABTS in negative manner, while another antioxidant was not correlated.

FRAP was found significantly and positively correlated with CUPRAC, while another antioxidant (DPPH and ABTS) were not correlated.

CUPRAC was found not correlated with both DPPH and ABTS.

DPPH was found highly significantly correlated with ABTS in negative manner.

**Table 4.4:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the middle region of the West Bank in 2014 (above diagonal) and 2015 (below diagonal).

2014/2015	TPC	TFC	FRAP	CUPRIC	DPPH	ABTS
TPC	-----	-0.888**	-0.532	-0.849**	-0.357	0.247
TFC	-0.647*	-----	0.529	0.836**	0.295	-0.032
FRAP	-0.931***	0.551	-----	0.112	0.946***	-0.526
CUPRIC	-0.746*	0.085	0.873**	-----	-0.129	0.137
DPPH	-0.507	0.968***	0.428	-0.057	-----	-0.636
ABTS	0.550	-0.954***	-0.456	0.003	-0.923***	-----

**4.8.5** Pearson correlations between antioxidant contents and their activities of leaves collected from Southern West Bank extracted by all solvents in both years.

In 2014, TPC was found not correlated with neither TFC nor CUPRAC, while it was highly significantly correlated with FRAP in positive manner and significantly correlated with both DPPH and ABTS in negative manner (Table 4.5).

TFC was found highly significantly correlated and positively with CUPRAC, while it was not correlated with all other antioxidant.

FRAP was found significantly correlated and negatively with DPPH, while it was not correlated both CUPRAC and ABTS. CUPRAC was found significant correlated with ABTS, while not correlated with DPPH. DPPH and ABTS were found highly significant correlated to each other in positive manner.

In 2015, TPC was found significantly correlated with TFC, FRAP, and DPPH in positive manner and negatively correlated with CUPRAC, while it was not correlated with ABTS. TFC was found highly significantly correlated with FRAP and DPPH, while it was not significant with both CUPRAC and ABTS.

FRAP was found high significant correlated and positively with DPPH, while it was not correlated with both CUPRAC and ABTS. There was no correlation with CUPRAC and both DPPH and ABTS, also there was no correlation between DPPH and ABTS.

**Table 4.5:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus leaves extracts collected from the southern region of the West Bank in 2014 (above diagonal) and 2015 (below diagonal).

2014/2015	TPC	TFC	FRAP	CUPRIC	DPPH	ABTS
TPC	-----	0.393	0.981***	0.074	-0.775*	-0.699*
TFC	0.710*	-----	0.527	0.935***	0.267	0.376
FRAP	0.671*	0.915***	-----	0.228	-0.674*	-0.581
CUPRIC	-0.706*	-0.446	-0.414	-----	0.551	0.657*
DPPH	0.752*	0.991***	0.929***	-0.451	-----	0.986***
ABTS	-0.215	0.029	0.086	0.297	-0.041	-----

**4.8.6** Pearson correlations between antioxidant contents and their activities of fruits collected from Southern West Bank extracted by all solvents in both years.

In 2014, TPC was found highly significantly correlated and positively with TFC and FRAP, and negatively correlated with ABTS, while not correlated with DPPH (2014). TFC was found highly significantly correlated and positively with FRAP, and significantly correlated with DPPH, while not correlated with both CUPRAC and ABTS (Table 4.6).

FRAP was found significantly correlated and positively with both CUPRAC and DPPH while not correlated with ABTS. CUPRAC was found highly significantly correlated and positively with DPPH while not correlated with ABTS.

DPPH and ABTS were found not correlated.

In 2015, TPC was found not correlated with TFC, FRAP, and DPPH, while it was highly significantly correlated with CUPRAC in negative manner and with ABTS in positive manner.

TFC was found highly significantly and positively correlated with both FRAP and DPPH, while not correlated with both CUPRAC and ABTS.

FRAP was found significantly correlated and positively with DPPH, while not correlated with both CUPRAC and ABTS. CUPRAC was found highly significantly correlated and negatively with ABTS, while not correlated with DPPH. DPPH and ABTS were not correlated to each other.

**Table 4.6:** Pearson coefficients between Antioxidant contents (TPC and TFC) and activity (FRAP, CUPRAC, DPPH, and ABTS) of ziziphus fruits extracts collected from the southern region of the West Bank in 2014 (above diagonal) and 2015 (below diagonal)

.2014/2015	TPC	TFC	FRAP	CUPRIC	DPPH	ABTS
TPC	-----	0.955***	0.887**	0.473	0.599	-0.697*
TFC	-0.035	-----	0.913***	0.645	0.734*	-0.544
FRAP	0.369	0.864**	-----	0.780*	0.819**	-0.574
CUPRIC	-0.932***	0.279	-0.110	-----	0.918***	-0.087
DPPH	-0.025	0.996***	0.872**	0.272	-----	-0.229
ABTS	0.966***	-0.099	0.343	-0.920***	-0.093	-----

#### **4.9 Effect of soaking times on TPC, TFC, and Antioxidant activities**

From the experimental that done on the leaves and fruits samples which was soaked in different solvents (D.Water, 80% Ethanol, and 99% Ethanol) and in different periods of times (2, 5, 10, 72 hours) in water bath at 37°C, the results indicates that the phenolic compounds are more abundant after 5 hrs. soaking in ethanol extraction solvent comparing with the D.water solvent where the phenolic compounds have no significant difference. TPC in fruits in all studied extraction solvent and in all periods of times studied had no significant deference.

Flavonoids abundance in leaves increased after 5 hrs. of soaking in 80% Ethanol extraction solvent, while, the content of flavonoids in other extraction solvent used (D.Water, 99% Ethanol) and in all period of times studied had no significant difference. TFC in fruits in all studied extraction solvent and in all periods of times studied had no significant deference.

Antioxidant activities (FRAP, CUPRAC, DPPH, ABTS) in leaves and fruits in all extraction solvent and in all studied period of times had no significant difference.

**Chapter: Five**  
**Conclusions**

## Conclusions

Antioxidant properties of plant extracts have become of great interest due to their possible uses as natural additives to replace synthetic ones. To our knowledge, this is the first study dealing with the in vitro antioxidant activity of *Ziziphus Spina-Christi* grown in Palestine. This study revealed that tested plant extracts have moderate to significant phenolic contents and presented a good DPPH and APTS radical scavenging activities. *Ziziphus Spina-Christi* leaves could have potential source of antioxidants for pharmaceutical drug preparations. The plant part (leaves and fruits) extracts have also, good reducing abilities represented by FRAP and CUPRAC.

It is noticed that the highest concentration of phenolic and flavonoid compounds in the extracts were obtained using 80% ethanol solvents in the leaves, while in the fruits the highest concentration of phenolic compounds in the extracts were obtained using 99% ethanol solvent.

The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity.

Further studies on this plant species should be directed at a detailed qualitative analysis of all its parts and carried out in vivo evaluation of its antioxidant properties.



## REFERENCES

- Abalaka M E, Daniyan S Y and Mann A (2010). Evaluation of the antimicrobial activities of two Ziziphus species (*Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.) on some microbial pathogens. *Afr. J. Pharm. Pharmacol.* 4(4):135-139.
- Adzu, B., & Haruna, A. K. (2007). Studies on the use of *Zizyphus spina-christi* against pain in rats and mice. *African Journal of Biotechnology*, 6(11).
- Adzu, B., Amos, S., Amizan, M. B., & Gamaniel, K. (2003). Evaluation of the antidiarrhoeal effects of *Zizyphus spina-christi* stem bark in rats. *Acta tropica*, 87(2), 245-250.
- Adzu, B., Amos, S., Wambebe, C., & Gamaniel, K. (2001). Antinociceptive activity of *Zizyphus spina-christi* root bark extract. *Fitoterapia*, 72(4), 344-350.
- Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152.
- Alhakmani, F., Khan, S. A., & Ahmad, A. (2014). Determination of total phenol, in-vitro antioxidant and anti-inflammatory activity of seeds and fruits of *Zizyphus spina-christi* grown in Oman. *Asian Pacific Journal of Tropical Biomedicine*, 4, S656-S660.
- Alhakmani, F., Khan, S. A., & Ahmad, A. (2014). Determination of total phenol, in-vitro antioxidant and anti-inflammatory activity of seeds and fruits of *Zizyphus spina-christi* grown in Oman. *Asian Pacific Journal of Tropical Biomedicine*, 4, S656-S660.
- Al-marzooq, M. A. (2014). Phenolic compounds of Napek leave (*Zizyphus spina-christi* L.) as natural antioxidants. *Journal of Food and Nutrition Sciences*, 2(5), 207-214.
- Ammar, R. B., Bhourri, W., Sghaier, M. B., Boubaker, J., Skandrani, I., Neffati, A., ... & Dijoux-Franca, M. G. (2009). Antioxidant and free radical-scavenging properties of three flavonoids isolated from the leaves of *Rhamnus alaternus* L.(Rhamnaceae): A structure-activity relationship study. *Food Chemistry*, 116(1), 258-264.
- Apak, R., Güçlü, K., Özyürek, M., & Celik, S. E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*, 160(4), 413-419.

- Arteel, G. E. (2003). Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology*, *124*(3), 778-790.
- Arts, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies. *The American journal of clinical nutrition*, *81*(1), 317S-325S.
- Asgarpanah, J., & Haghghat, E. (2012). Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. *African Journal of Pharmacy and Pharmacology*, *6*(31), 2332-2339.
- Avizeh, R., Najafzadeh, H., Pourmahdi, M., & Mirzaee, M. (2010). Effect of glibenclamide and fruit extract of *zizyphus spina-christi* on alloxan-induced diabetic dogs. *The Journal of Applied Research in Veterinary Medicine*, *8*(2), 109.
- Bandyopadhyay, M., Chakraborty, R., & Raychaudhuri, U. (2007). A process for preparing a natural antioxidant enriched dairy product (Sandesh). *LWT-Food Science and Technology*, *40*(5), 842-851.
- Beckman, C. H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants?. *Physiological and Molecular Plant Pathology*, *57*(3), 101-110.
- Benzie, I. F., & Strain, J. J. (1999). [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology*, *299*, 15-27.
- Bernhoft, A., Siem, H., Bjertness, E., Meltzer, M., Flaten, T., & Holmsen, E. (2010). Bioactive compounds in plants—benefits and risks for man and animals. In Proceedings from a Symposium Held at The Norwegian Academy of Science and Letters, Novus forlag, Oslo.
- Bolton, J. L., Trush, M. A., Penning, T. M., Dryhurst, G., & Monks, T. J. (2000). Role of quinones in toxicology. *Chemical research in toxicology*, *13*(3), 135-160.
- Bors, W., Michel, C., & Stettmaier, K. (2001). Structure-activity relationships governing antioxidant capacities of plant polyphenols. *Methods in enzymology*, *335*, 166.

- Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), 25-30.
- Bukar, A. M., Kyari, M. Z., Gwaski, P. A., Gudusu, M., Kuburi, F. S., & Abadam, Y. I. (2015). Evaluation of phytochemical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State–Nigeria. *Journal of Pharmacognosy and Phytochemistry*, 3(5), 98-101.
- Cai, Y. Z., Sun, M., Xing, J., Luo, Q., & Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life sciences*, 78(25), 2872-2888.
- Dahiru, D., Sini, J. M., & John-Africa, L. (2006). Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodents. *African journal of biotechnology*, 5(10).
- Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *J. biol. Chem*, 73(2), 627-650.
- Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. (2013). Plant defense against insect herbivores. *International journal of molecular sciences*, 14(5), 10242-10297.
- Ghafoor, A. O., Qadir, H. K., & Fakhri, N. A. (2012). Analysis of phenolic compounds in extracts of *Ziziphus spina-christi* using RPHPLC method. *J Chem Pharm Res*, 4(6), 3158-3163.
- Graf, B. A., Milbury, P. E., & Blumberg, J. B. (2005). Flavonols, flavones, flavanones, and human health: epidemiological evidence. *Journal of medicinal food*, 8(3), 281-290.
- Groot, H. D., & Rauen, U. (1998). Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundamental & clinical pharmacology*, 12(3), 249-255.
- Guidi, I., Galimberti, D., Lonati, S., Novembrino, C., Bamonti, F., Tiriticco, M., ... & Scarpini, E. (2006). Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of aging*, 27(2), 262-269.
- Han, B. H., & Park, M. H. (1986). Folk medicine: The art and science. The American Chemical Society Washington, 206, 210.

Hyun, D. H., Hernandez, J. O., Mattson, M. P., & de Cabo, R. (2006). The plasma membrane redox system in aging. *Ageing research reviews*, 5(2), 209-220.

Kaaria, I. (1998). Seed production, dispersal and germination in *Cryptostegia grandifolia* and *Ziziphus mauritiana*, two invasive shrubs in tropical woodlands of Northern Australia. *Australia J. Ecology*, 21(3), 324-331.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry*, 47(10), 3954-3962.

Khaleel, S. M., Jaran, A. S., & Haddadin, M. S. (2016). Evaluation of Total Phenolic Content and Antioxidant Activity of Three Leaf Extracts of *Ziziphus spina-christi* (Sedr) Grown in Jordan. *British Journal of Medicine and Medical Research*, 14(6), 1.

Kinnula, V. L., & Crapo, J. D. (2004). Superoxide dismutases in malignant cells and human tumors. *Free Radical Biology and Medicine*, 36(6), 718-744.

Kirtikar K R and Basu B D (1984). Indian medicinal plants, Lalit Mohan Basu, Allahabad, P.593.

Koley, T. K., Kaur, C., Nagal, S., Walia, S., & Jaggi, S. (2011). Antioxidant activity and phenolic content in genotypes of Indian jujube (*Zizyphus mauritiana* Lamk.). *Arabian Journal of Chemistry*.

Lulekal, E., Asfaw, Z., Kelbessa, E., & Van Damme, P. (2013). Ethnomedicinal study of plants used for human ailments in Ankober District, North Shewa Zone, Amhara region, Ethiopia. *Journal of ethnobiology and ethnomedicine*, 9(1), 63.

Manzocco, L., Anese, M., & Nicoli, M. C. (1998). Antioxidant properties of tea extracts as affected by processing. *LWT-Food Science and Technology*, 31(7), 694-698.

Memon, A. A., Memon, N., Bhangar, M. I., & Luthria, D. L. (2012). Phenolic Acids Composition of Fruit Extracts of Ber (*Ziziphus mauritiana* L., var. Golo Lemai). *Pakistan Journal of Analytical & Environmental Chemistry*, 13(2), 6.

Michel A (2002). Tree, shrub and liana of West African zone. Margraf Publishers GMBH, Paris, P. 440.

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, 57(5), 1655-1666.

Motamedi, H., Safary, A., Maleki, S., & Seyyednejad, S. M. (2009). *Ziziphus spina-christi*, a native plant from Khuzestan, Iran, as a potential source for discovery new antimicrobial agents. *Asian Journal of Plant Sciences*, 8(2), 187.

Nuru, A., Awad, A. M., Al-Ghamdi, A. A., Alqarni, A. S., & Radloff, S. E. (2012). Nectar of *Ziziphus spina-christi* (L.) Willd (Rhamnaceae): dynamics of secretion and potential for honey production. *Journal of Apicultural Science*, 56(2), 49-59.

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), 270-278.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, 53(10), 4290-4302.

Ramakrishna, B. S., Varghese, R., Jayakumar, S., Mathan, M., & Balasubramanian, K. A. (1997). Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. *Journal of gastroenterology and hepatology*, 12(7), 490-494.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9), 1231-1237.

Ross, J. A., & Kasum, C. M. (2002). Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annual review of Nutrition*, 22(1), 19-34.

S.Al-Jassabi and M.S. Abdullah. Extraction, Purification and Characterization of Antioxidant Fractions from *Zizyphus spina-christi* Fruits. *American-Eurasian Journal of Toxicological Sciences* 5 (3): 66-71, 2013.

Sas, K., Robotka, H., Toldi, J., & Vécsei, L. (2007). Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *Journal of the neurological sciences*, 257(1), 221-239.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition*, 45(4), 287-306.

- Seyoum, A., Asres, K., & El-Fiky, F. K. (2006). Structure–radical scavenging activity relationships of flavonoids. *Phytochemistry*, *67*(18), 2058-2070.
- Singh, U., & Jialal, I. (2006). Oxidative stress and atherosclerosis. *Pathophysiology*, *13*(3), 129-142.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.
- Smith, M. A., Rottkamp, C. A., Nunomura, A., Raina, A. K., & Perry, G. (2000). Oxidative stress in Alzheimer's disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1502*(1), 139-144.
- Upston, J. M., Kritharides, L., & Stocker, R. (2003). The role of vitamin E in atherosclerosis. *Progress in lipid research*, *42*(5), 405-422.
- Williams, R. J., Spencer, J. P., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules?. *Free radical biology and medicine*, *36*(7), 838-849.
- Wu, C. S., Gao, Q. H., Kjelgren, R. K., Guo, X. D., & Wang, M. (2013). Yields, phenolic profiles and antioxidant activities of *Ziziphus jujube* Mill. in response to different fertilization treatments. *Molecules*, *18*(10), 12029-12040.
- Yossef, H. E., Khedr, A. A., & Mahran, M. Z. (2011). Hepatoprotective activity and antioxidant effects of El Nabka (*Zizyphus spina-christi*) fruits on rats hepatotoxicity induced by carbon tetrachloride. *Nature and Science*, *9*(2), 1-7.
- Zhao, H. X., Zhang, H. S., & Yang, S. F. (2014). Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Science and Human Wellness*, *3*(3), 183-190.
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), 555-559.

## **Appendices**

**Appendix A:** Total phenolic content results.

**Table 1:** Total phenolic content (TPC, mg Gallic acid/g DW) of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types.  $P < 0.05$ ,  $n=3$ .

2014 2015	TPC 2014			TPC 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leaves						
North	273.4±1.38 A,a *	217.5±8.4 B,a *	104.7±0.6 C,a *	352.4±25 B,a *	448±29 A,a *	338.5±25 B,a
Middle	266.9±12.2 A,a *	204.1±13 B,a *	101.2±4 C,a *	324±35.44 A,a *	315.8±29.5 A,b *	301.5±27.6 A,a
South	249.1±3.9 A,a *	196.4±3.5 B,a *	94.1±5 C,a *	381.2±37.2 BA,a *	436.2±13.7 A,a *	328.7±5.6 B,a *
Fruits						
North	227.2±9.5 A,b	99.8±5.4 B,b	58.5±2.1 C,b	90.6±10.35 B,a	173.4±11.2 B,b	339.8±5 A,a
Middle	148±3.1 C,c	276±33 B,a	421.7±24 A,a	97.3±13.7 B,a	164.9±26.3 B,b	289.9±18.85 A,a
South	286.8±1.27 A,a	125.7±3.9 B,b	63±1.22 C,b	113.5±13.8 B,a	271.8±10.11 A,a	248.4±23 A,a



**Appendix B:** Total flavonoid content results.

**Table 2:** Total flavonoid content (TFC, mg catechin /g DW) of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types. P< 0.05, n=3.

2014 2015	TFC 2014			TFC 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leave						
North	12±0.82 B,a*	89.2±22.8 A,a*	12.8±0.81 B,a*	13.9±1.55 B,b	39.6±3.77 A,a*	16.9±1.52 B,a*
Middle	11.1±0.2 B.a	118.7±1.72 A,a*	9.1±1.23 B,b	20.9±0.37 B,a*	27.7±2.48 A,a*	10.5±0.27 C,a*
South	11.65±0.11 B,a*	16.64±0.51 A,b*	10.2±0.76 B,a*	18.8±1.90 B,a*	38.8±0.23 A,a*	19.5±1.10 B,a*
Fruit						
North	6.6±01.26 A,b	3.7±0.14 B,b	3.5±0.36 B,b	13.9±0.30 B,a	16.7±0.30 A,a	4.6±0.17 C,a
Middle	11.2±0.63 A,a	9.7±0.40 BA,a	8.4±0.21 B,a	6.6±0.19 B,b	8.3±0.3 A,c	4.6±0.05 C,a
South	5.2±0.40 A,b	3.8±0.03 B,b	2.5±0.02 C,c	9.5±1.96 A,b	12.4±0.47 A,b	4.5±0.04 B,a

## Appendix C: FRAP results.

**Table 3:** FRAP antioxidant activity (mg Trolox/ g DW) of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types.  $P < 0.05$ ,  $n=3$ .

2014 2015	FRAP 2014			FRAP 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leaves						
North	10.2±0.74 A,a	11.2±1.37 A,a*	3.5±0.05 B,b*	9.6±0.6 B,a	13.05±0.5 A,a*	9.58±0.6 B,a*
Middle	11±0.5 A,a*	9.6±0.3 B,a	4.1±0.02 C,a*	7.4±0.34 C,b*	13.60±1.5 A,a*	9.5±0.75 B,a*
South	11.9±0.22 A,a*	10.1±0.83 B,a*	2.6±0.87 C,c	7.67±0.62 B,ba	13.60±0.6 A,a*	7.75±1.64 B,b*
Fruits						
North	11.2±0.62 A,a	3.8±0.09 B,b	1.3±0.02 C,c	9.7±0.77 B,b	16±0.83 A,b	3.1±0.10 C,a
Middle	6.4±0.66 B,c	8.8±0.45 A,a	3.6±0.13 C,a	15.8±0.14 A,a	9.8±0.25 B,c	3.1±0.24 C,a
South	8.4±0.2 A,b	5.9±1.35 A,b	1.6±0.04 B,b	8.1±0.87 B,b	21.1±1.0 A,a	3.2±0.2 C,a

## Appendix D: CUPRAC results

**Table 4:** Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types.  $P < 0.05$ ,  $n=3$ .

2014 2015	CUPRAC 2014			CUPRAC 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leaves						
North	124.5±4.35 A,a*	88±6.23 B,b*	56.7±0.80 C,a*	136±3.2 A,a*	104±3.0 C,a*	122±2.7 B,b*
Middle	43.9±8.0 B,b*	108.9±6.85 A,a*	57±1.78 B,a*	106.9±3.2 B,b*	98.5±0.97 C,a*	136±0.80 A,a*
South	35.6±2.50 C,b	124.4±4.74 A,a*	46.5±1.18 B,b*	91.1±1.41 B,c*	92.2±6.88 B,a*	139.8±0.42 A,a*
Fruits						
North	33.5±1.0 A,b	30.7±2.8 A,a	20.5±0.25 B,b	158.5±0.13 A,c	57±0.15 B,a	27.6±1.01 C,b
Middle	155.2±8.10 A,a	34.7±1.67 B,a	26.8±1.40 B,a	261.7±7.48 A,a	45.4±2.25 B,b	31.9±0.35 B,a
South	35.5±3.50 A,b	44.1±6.90 A,a	12.7±1.50 B,c	176.9±0.80 A,b	55.4±0.97 B,a	33.1±0.67 C,a

## Appendix E: DPPH results.

**Table 5:** Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south). Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types.  $P < 0.05$ ,  $n=3$

2014 2015	DPPH 2014			DPPH 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leaves						
North	54.3±5.38 B,ba	32.4±9.48 C,b*	84.5±0.20 A,a*	67.4±3.20 C,b*	134±1.22 A,a*	78.3±0.25 B,b*
Middle	62.6±1.28 B,a*	78.1±1.72 A,a	76.6±0.55 A,ba*	70.2±1.93 C,a*	131.7±0.09 A,a*	81±0.59 B,a*
South	50.2±0.70 B,b*	80.4±0.30 A,a	82±3.25 A,b*	74.9±0.85 B,a*	133.5±0.59 A,a*	76.7±0.59 B,b*
Fruits						
North	57.7±19.00 BA,a	87.3±1.96 A,a	29.2±1.0 B,a	81.5±0.80 B,a	141.7±1.0 A,a	30.5±0.83 C,a
Middle	45.3±0.81 B,a	79.1±2.62 A,a	28.1±1.77 C,a	62.3±1.73 B,a	111.4±7.45 A,b	29.3±2.50 C,b
South	74.5±6.43 A,a	82.6±3.28 A,a	29.8±1.84 B,a	93.9±21.53 A,a	128.9±0.62 A,a	35±1.60 B,a

**Appendix F:** results of ABTS.

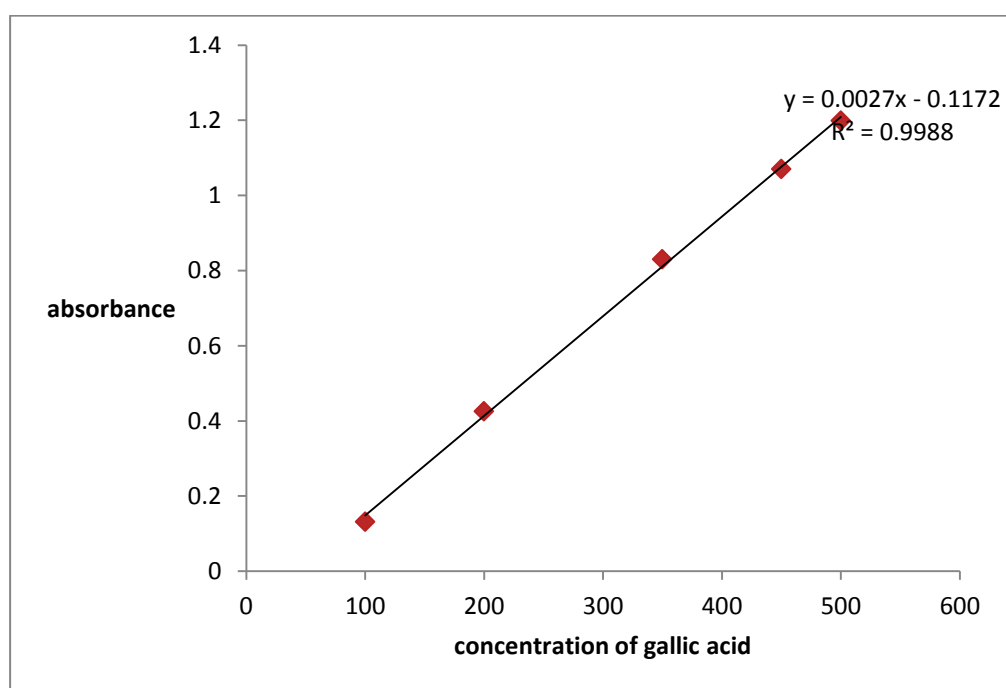
**Table 6:** Free radical scavenging activity (ABTS, mg Trolox/g DW of Ziziphus plant parts (leaves and fruits) in two years (2014 and 2015) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol) and geographical regions in the West Bank (north, middle and south), Comparing each year and each plant part separately, means within each row followed by the same capital letter are not significantly different. For each extraction method in each year and each plant part separately, means in the same column followed by the same small letter are not significantly different, \* indicates significant difference for a given extraction method in the same year within plant part types. P< 0.05.

2014 2015	ABTS 2014			ABTS 2015		
	D. water	80% Ethanol	99% Ethanol	D. water	80% Ethanol	99% Ethanol
Leave						
North	2.1±0.14 B,a	2.7±0.11 A,a	2.89±0.01 A,a	2.48±0.03 A,a	2.56±0.02 A,a	2.36±0.01 B,a
Middle	2.4±0.065 BA,a	2.1±0.30 B,b	2.83±0.08 A,a	2.7±0.01 A,a	2.27±0.02 B,b	2.1±0.06 C,b
South	1.3±0.06 B,b	2.8±0.02 A,a	2.67±0.04 A,b	2.07±0.14 A,b	2.10±0.01 A,c	2.14±0.02 A,b
Fruit						
North	2.6±0.08 BA,a	2.5±0.07 B,a	2.9±0.07 A,a	1.91±0.2 B,a	2.27±0.08 A,a	1.56±0.03 C,b
Middle	1.8±0.03 A,b	1.6±0.22 A,b	1.9±0.14 A,b	1.95±0.06 B,a	1.67±0.03 C,b	2.17±0.08 A,a
South	2.5±0.23 A,a	2.8±0.02 A,a	2.8±0.09 A,a	1.53±0.1 B,b	2.09±0.06 A,a	2.03±0.08 A,a

**Appendix G:** Total phenolic absorbance and calibration curve.

**Table 7:** absorbance of different concentration of Gallic Acid.

<b>Concentration of Gallic Acid (ppm)</b>	<b>Absorbance at <math>\lambda = (765) \text{ nm}</math></b>
<b>100</b>	0.132
<b>200</b>	0.426
<b>350</b>	0.830
<b>450</b>	1.070
<b>500</b>	1.199

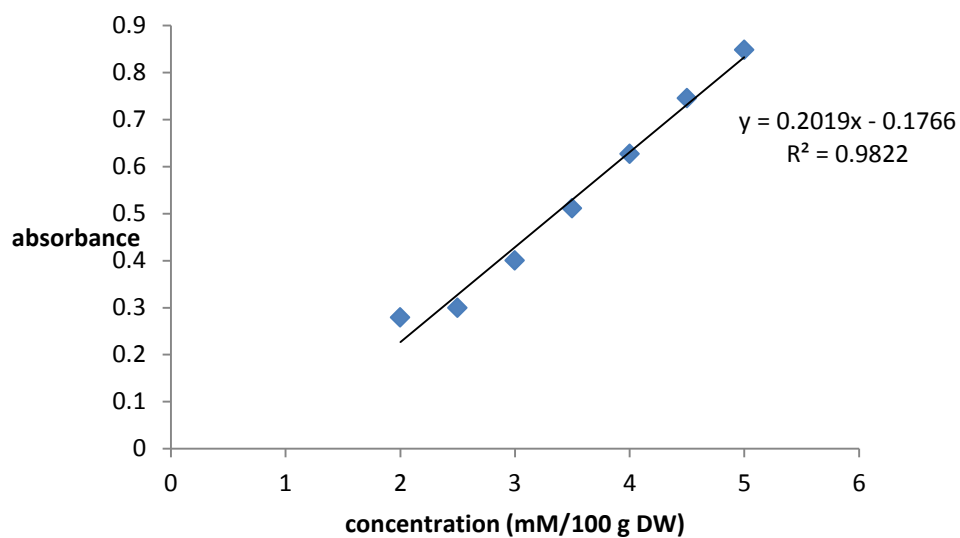


**Figure 1:** calibration curve for total phenols content.

**Appendix H:** FRAP absorbance (2 – 5 mM) FeSO<sub>4</sub>·7H<sub>2</sub>O), Fe (II) and calibration curve.

**Table 8:** absorbance of different concentration of Fe +2 (mM)

Concentration of Fe+2 (mM)	Absorbance (593 nm)
2	0.279
2.5	0.299
3	0.400
3.5	0.511
4	0.627
4.5	0.745
5	0.848

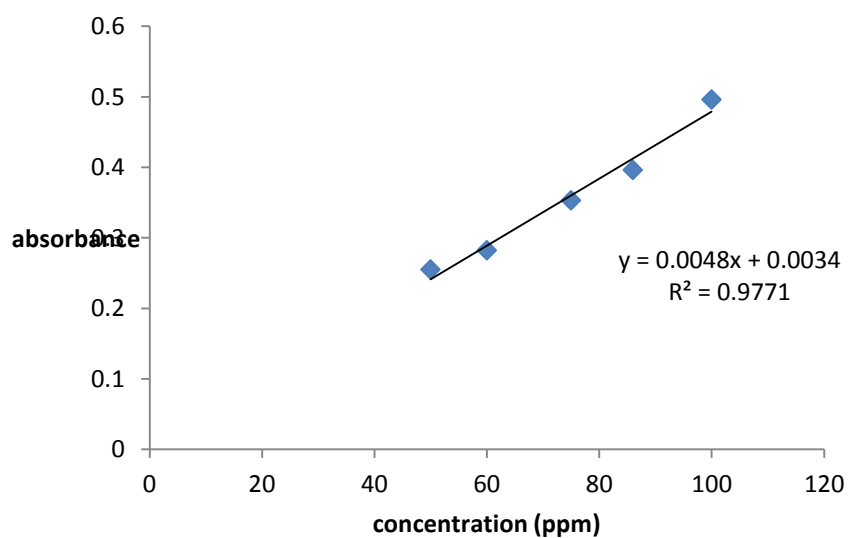


**Figure 2:** Calibration curve for FRAP.

**Appendix I:** Total flavonoid content absorbance and calibration curve.

**Table 9:** absorbance of different concentration of Catechin.

Concentration of catechin (ppm)	Absorbance (510 nm)
50	0.255
60	0.282
75	0.353
86	0.396
100	0.496



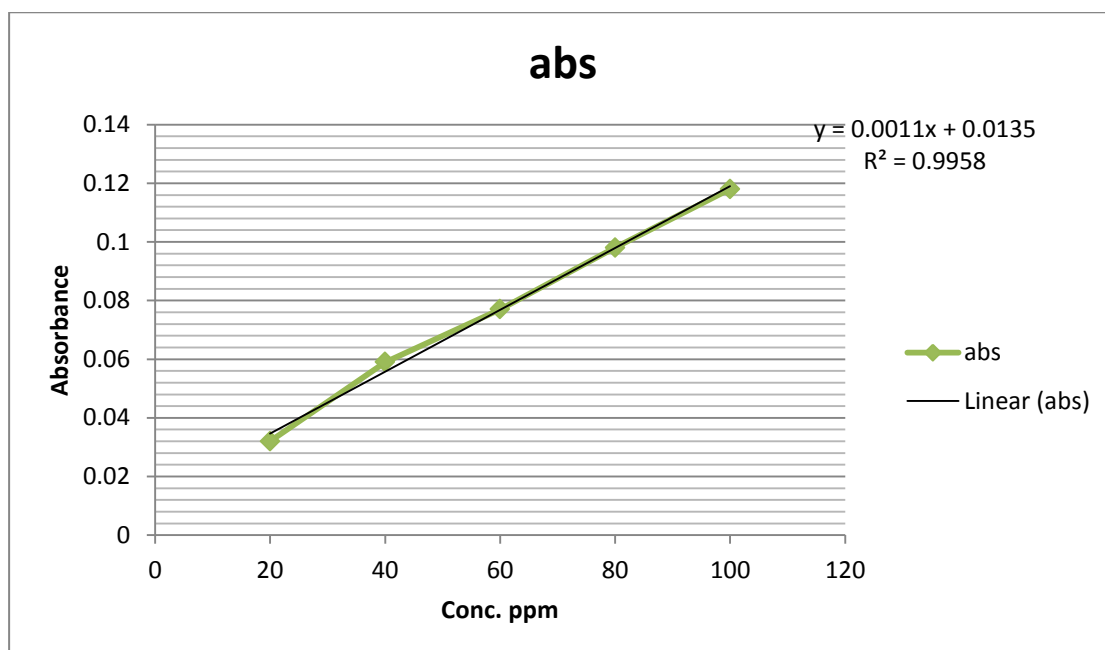
**Figure 3:** Calibration curve for total flavonoid content.



**Appendix J: CUBRAC absorbance and calibration curve.**

**Table 10:** absorbance of different concentration of Trolox.

Conc.(ppm) of Trolox	Abs. at 450 nm
20	0.032
40	0.059
60	0.077
80	0.098
100	0.118
120	0.142
140	0.168
160	0.204
180	0.246

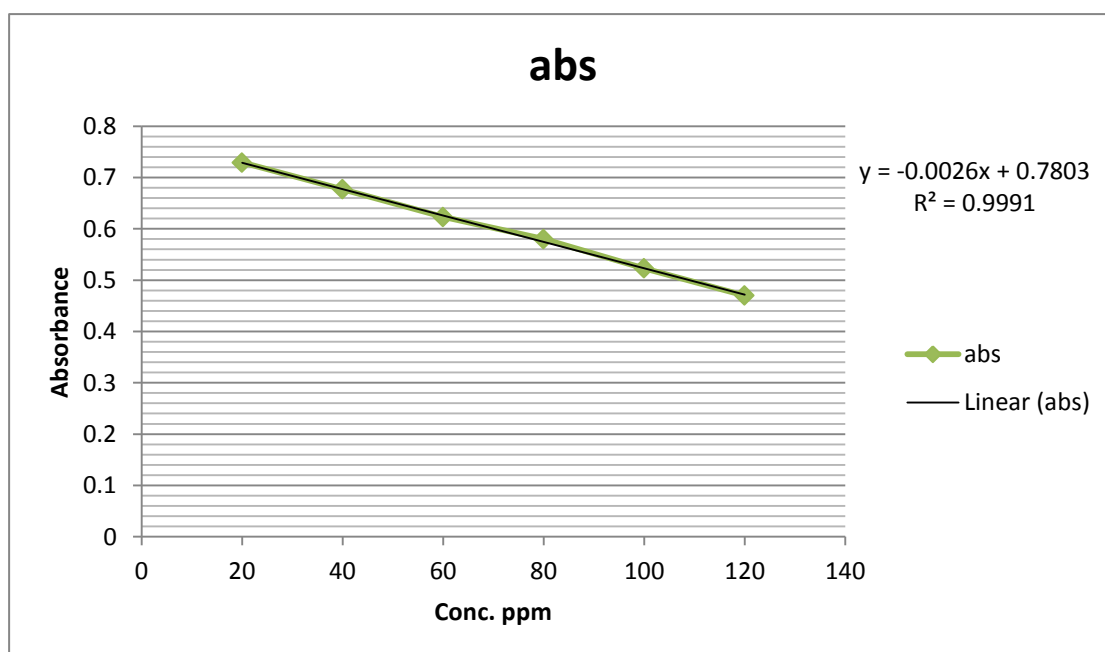


**Figure 4:** calibration curve for CUPRAC.

**Appendix K:** DPPH absorbance and calibration curve.

**Table 11:** absorbance of different concentration of Trolox.

Conc.(ppm) of Trolox	Abs. at 515 nm
20	0.729
40	0.677
60	0.623
80	0.580
100	0.523
120	0.470

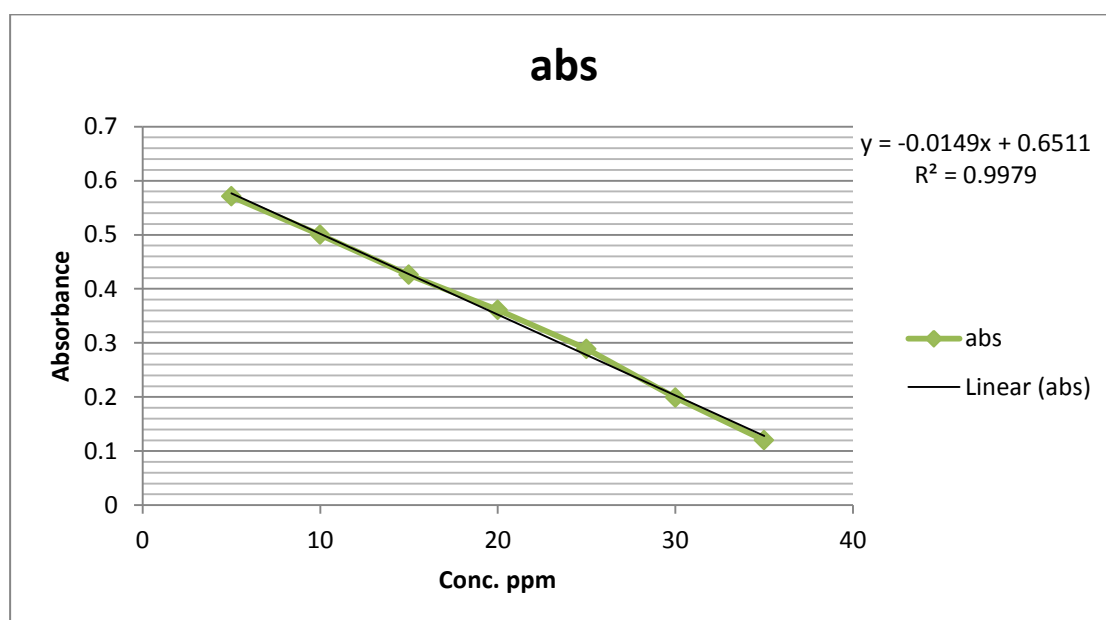


**Figure 5:** calibration curve for DPPH.

**Appendix L:** APTS absorbance and calibration curve.

**Table 12:** absorbance of different concentration of Trolox.

Conc. Ppm(Trolox)	Abs. (734 nm)
5	0.571
10	0.500
15	0.426
20	0.361
25	0.289
30	0.199
35	0.120
40	0.027



**Figure 6:** Calibration curve for ABTS.

**Appendix M: TPC assay results with different soaking time****Table 13:** Total phenolic content (TPC, mg Gallic acid/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	170.0	192.2	224.8
10 hrs.	368.6	320.4	282.4
72 hrs.	389.2	325.4	401.3
Fruits			
5 hrs.	75.2	60.1	98.5
10 hrs.	77.3	69.8	105.6
72 hrs.	77.7	74.2	100.6

**Appendix N: TFC assay results with different soaking time****Table 14:** Total flavonoid content (TFC, mg Catechin/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	13.7	9.3	11.9
10 hrs.	29.6	10.4	13.3
72 hrs.	28.8	11.2	12.6
Fruits			
5 hrs.	6.7	2.5	4.7
10 hrs.	6.7	2.9	7.0
72 hrs.	7.1	2.8	7.8

**Appendix O: FRAP assay with different soaking time****Table 15:** FRAP antioxidant activity (mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent \ Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	8.8	8.4	9.0
10 hrs.	10.3	10.0	9.4
72 hrs.	10.8	10.2	10.4
Fruits			
5 hrs.	6.7	2.8	7.5
10 hrs.	8.5	5.7	7.9
72 hrs.	8.9	5.9	10.0

**Appendix P: CUPRAC assay with different soaking time****Table 16:** Cupric reducing antioxidant power (CUPRAC, mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent \ Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	99.9	94.5	81.8
10 hrs.	112.3	101.5	104.6
72 hrs.	110.9	111.5	116.5
Fruits			
5 hrs.	61.5	55.96	31.5
10 hrs.	77.2	66.8	67.4
72 hrs.	109.7	63.8	76.5

#### Appendix Q: DPPH assay with different soaking time

**Table 17:** Free radical scavenging activity (DPPH, mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent \ Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	44.2	49.7	67.6
10 hrs.	71.0	69.6	69.2
72 hrs.	72.7	71.5	72.1
Fruits			
5 hrs.	77.9	36.0	20.8
10 hrs.	76.4	50.5	50.5
72 hrs.	77.6	54.0	58.0

#### Appendix R: APTS assay with different soaking time

**Table 18:** Free radical scavenging activity (ABTS, mg Trolox/g DW) of Ziziphus plant parts (leaves and fruits) with different soaking times (5, 10, and 72 hours) as affected by extraction methods (distilled water, 80% ethanol and 99% ethanol).

Solvent \ Time	80% Ethanol	99% Ethanol	D.Water
Leaves			
5 hrs.	2.45	2.08	2.13
10 hrs.	2.24	2.14	2.33
72 hrs.	2.48	2.31	2.37
Fruits			
5 hrs.	2.46	2.46	1.81
10 hrs.	2.64	2.08	1.62
72 hrs.	2.53	1.53	1.80

## مضادات الاكسدة والمحتوى الفينولي والمحتوى الفلييفونويدي في شجرة السدر في فلسطين

اعداد الطالب: شوقي محمود حراشنة

اشراف: د. فؤاد الريماوي

### الملخص

في هذا البحث في المختبر تم تقييم مضادات الاكسدة والمحتوى الفينولي والمحتوى الفلييفونويدي لمستخلص ورق وثمر شجرة السدر باستخدام ثلاث مذييات مختلفة (ماء مقطر وايتانول 80% وايتانول 99%) من ثلاث مناطق جغرافية مختلفة في الضفة الغربية ( الزبيدات في الشمال و اريحا في الوسط وبني نعيم في الجنوب) حيث تم قطف الورق في نيسان والثمر في ايار في سنتين(موسمين).

مستخلص الورق والثمر تم تحليله باستخدام طرق الفحص القياسية حيث تم فحص محتوى الفينول (TPC) بطريقة فولين(Folin-Ciocalteu) ومحتوى الفلييفونويد (TFC) بطريقة الفحص اللونية (Aluminium Chlorid method) , ومضادات الاكسدة تم فحصها باربع طرق مختلفة هي كالتالي: طريقة FRAP , DPPH , CUPRAC , APTS . وقد تم التحليل باستخدام جهاز (UV-Visible spectrophotometer).

وقد اظهرت الدراسة ان اوراق وثمار شجرة السدر تحتوي على قدر عالي من مضادات الاكسدة والمحتوى الفينولي والمحتوى الفلييفونويدي حيث : بلغ نسبة المحتوى الفينولي(TPC) في الورق 237.4 في الشمال مع الماء المقطر (مذيب) حتى 94.1 في الجنوب مع ايتانول 99% . وفي الموسم الثاني كانت نسبه المحتوى الفينولي 448 في الشمال مع الايتانول 80% حتى 301.5 في الوسط مع الايتانول 99% . في الثمر بلغت نسبه المحتوى الفينولي 421.7 في الوسط مع الايتانول 99% حتى 58.5 في الشمال مع الايتانول 99% . وفي الموسم الثاني بلغت النسبة 339.8 في الشمال مع الايتانول 99% حتى 90.6 في الشمال مع الماء المقطر . (نتائج (TPC) ب mg (of GAE/g DW).

نسبة المحتوى الفلييفونويد(TFC) في الورق 11.8.7 في الوسط مع الايتانول 80% حتى 9.1 في الوسط مع الايتانول 99% وفي الموسم الثاني بلغت النسبة 39.6 في الشمال مع الايتانول 80% حتى 10.5 في الوسط مع الايثامول 99% . في الثمر كانت نسبة الفلييفونويد 11.2 في الوسط مع الماء المقطر حتى 2.5 في الجنوب مع الايتانول 99% وفي الموسم الثاني كانت النسبة 16.7 في الشمال مع الايتانول 80% حتى 4.5 في الجنوب مع الايتانول 99% (نتائج (TFC) ب (mg of CE/g DW).

مضادات الاكسدة(AA) كانت النتائج على النحو التالي : نسبة مضادات الاكسدة في الورق باستخدام طريقة (FRAP) (2.6-11.9) وفي الموسم الثاني (7.4\_13.6) , بينما نسبة مضادات الاكسدة في الثمر (1.3-11.2)

وفي الموسم الثاني (3.1-21.1) . بطريقة CUPRAC كانت نسبة مضادات الاكسدة في الورق (35.6-124.5) وفي الموسم الثاني (91.1-139.8) , نسبة مضادات الاكسده في الثمر (12.7-155.2) وفي الموسم الثاني (27.6-261.7) . طريقة (DPPH) : نسبة مضادات الاكسده في الورق (32.4-84.5) وفي الموسم الثاني (67.4-134) , بينما في الثمر (28.1-87.3) وفي الموسم الثاني (29.3-141.7) . طريقة (APTS) نسبة مضادات الاكسده في الورق (1.3-2.89) وفي الموسم الثاني (2.07-2.7) , الثمر سجل نسبة (1.6-2.9) وفي الموسم الثاني (1.53-2.27)(كل نتائج مضادات الاكسدة كانت بوحدة (mg Trolox/g DW

هذه الدراسة اظهرت ان مستخلص ورق وثمر شجرة السدر يحتوي على كميات جيدة من المحتوى الفينولي والفليفونودي ومضادات الاكسدة. وان هذه النسبة اعلى في الورق بالمقارنة مع الثمر .