

**BIOCHEMICAL STUDIES OF POMEGRANATE
BIOACTIVE COMPOUNDS AND THEIR ROLE
IN OXIDATIVE STRESS REMEDY IN SOME
BIOLOGICAL SYSTEMS**

By

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ABSTRACT

Mariam Thabet Sawy Ibrahim: Biochemical studies of pomegranate bioactive compounds and their role in oxidative stress remedy in some biological systems. Unpublished, Doctor of Philosophy Dissertation, university of Ain Shams, Faculty of Agriculture, Depratment of Agric. Biochemistry, 2015.

The target of this study is to compare the antioxidant activity of three species of pomegranate (early wonderful, wonderful and white sweet) in different parts of their fruits (husk, peel and juice) at different maturity stages. The antioxidant activities of ethanolic extracts were measured by two different assays (DPPH and FRAP). The husk extract of early wonderful in pre-matured stage was the higher in their antioxidant activity. Using HPLC; the major bioactive compounds were Syringic (phenolic compound) and Hisperdin (flavonoid).

The second target of this study was to evaluate the role of pomegranate extract which has higher antioxidant activity on liver damage induced by carbon tetrachloride (CCl_4) in rats. The results showed that the administration of CCl_4 increased serum alanine transaminase (ALT), aspartate transaminase (AST) and bilirubin, but decreased superoxide dismutase (SOD), induced lipid peroxidation and caused depletion of liver reduced glutathione (GSH).

DNA damage was determined by random amplified polymorphic DNA (RAPD) method, the data revealed that the molecular genetic variability among the treated rats genomes and their controls were evaluated using 3 random primers (C1, H5 and P13). Primers gave positive and detectable bands and high level of polymorphism was generated using these primers.

Histopathological examination of liver tissues of treated group with CCl_4 shows steatosis of hepatocytes (ballooning of hepatocytes), apoptosis and inflammatory cells infiltration. On the other hand, liver

histology of rat treated with pomegranate extract at three concentrations (0.5, 1.0 and 2.0) treated group shows no histopathological changes.

Generally, it could be concluded that the pomegranate husk extract is an effective antioxidant as hepatoprotective agent.

Keywords: Pomegranate husk extract; Antioxidant activity; Liver damage; Carbon tetrachloride

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CONTENTS

	Page
LIST OF TABLES.....	III
LIST OF FIGURES.....	V
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	3
2.1. Chemical constituents of pomegranate antioxidants	3
2.1.1. Pomegranate flavonoids	5
2.1.2. Pomegranate Tannins and phenols	5
2.1.2.1. Punicalagins: characteristic, metabolism and their effects	6
2.1.2.2. Ellagic acid (EA): characteristic, metabolism and effects	7
2.2. Antioxidant activity of pomegranate	8
2.3. Hepatotoxicity and pomegranate protective effect	14
2.3.1. Carbon tetra chloride as hepatic oxidative stress inducer	15
2.3.2. Pomegranate extract prevent hepatotoxicity	17
3. MATERIALS AND METHODS.....	23
3.1. Plant material	23
3.2. Chemicals	23
3.3. Extraction of pomegranate fruit parts.....	23
3.4. Antioxidant activity of pomegranate fruit	23
3.4.1. DPPH assay	23
3.4.2. Ferric Reducing Antioxidant Power [FRAP]	24
3.5. Determination of total phenols	24
3.5.1. Determination of phenolic compounds by HPLC	25
3.6. Determination of Total flavonoids	25
3.6.1. Determination of flavonoid compounds by HPLC.....	25
3.7. Biological experiment	25
3.8. Biochemical analysis	27
3.8.1. Determination of alanine aminotransferase (ALT) activity.	27
3.8.2. Determination of aspartate aminotransferase (AST) activity	28
3.8.3. Determination of total bilirubin	28
3.8.4. Serum proteins	29
3.8.4.1. Determination of total serum protein	29
3.8.4.2. Determination of serum albumin	29
3.8.5. Superoxide Dismutase (SOD) activity	29

3.8.6. Glutathione content	30
3.8.7. Oxidative stress biomarkers	30
3.8.7.1. Lipid peroxidation (MDA assay).....	30
3.8.7.2. Protein damage	30
3.8.7.3. DNA damage	31
3.8.8. Histopathological examination	31
3.9. Statistical analysis	32
4. RESULTS AND DISCUSSION	33
4.1. Antioxidant activity of pomegranate	33
4.1.1. DPPH assay.....	33
4.1.2. FRAP assay	35
4.1.3. Total phenolic contents	38
4.1.4. Total flavonoid contents	40
4.1.5. Determination of phenolic compounds and flavonoids by HPLC	42
4.2. Hepatoprotective effect of pomegranate husk extract	45
4.2.1. Alanine aminotransferase (ALT).....	46
4.2.2. Aspartate aminotransferase (AST).....	48
4.2.3. Serum bilirubin concentration	49
4.2.4. Total protein and Albumin level	51
4.2.5. SOD enzyme	54
4.2.6. Glutathione content	56
4.2.7. Oxidative cell biomarkers	58
4.2.7.1. Lipid peroxidation	58
4.2.7.2. Protein damage.....	60
4.2.7.3. DNA damage	63
4.2.8. Histopathological examination.....	69
5. SUMMARY AND CONCLUSION.....	74
6. REFERENCES.....	80
ARABIC SUMMARY	

LIST OF TABLES

No.		Page
1	Chemical constituents and structure of the main antioxidants in pomegranate fruit	3
2	Absorbance values of known samples of AST activity.....	28
3	Free-radical scavenging activity of pomegranate fruits ethanol extract (measured by DPPH assay)	34
4	Antioxidant potential of pomegranate fruit extracts (FRAP assay).....	36
5	Total phenolic compounds in husk, peel and juice of pomegranate ethanol extracts.....	39
6	Total flavonoids profile of husk, peel and juice of pomegranate ethanol extract.....	40
7	HPLC analysis of phenolic compounds in ethanol husk extract of Early Wonderful type.....	43
8	HPLC analysis of flavonoid compounds in ethanol husk extract of Early Wonderful type.....	44
9	Serum liver enzymes and bilirubin level as affected by CCl ₄ and pomegranate extract.....	46
10	Serum albumin and total protein as affected by CCl ₄ and pomegranate extract.....	52
11	Effect of pomegranate extract on SOD, MDA, GSH and function of CCl ₄ intoxicated-rats.....	55
12	SDS-PAGE of experimental rats liver proteins (polypeptide) as affected by CCl ₄ and pomegranate extract	61
13	RAPD profile as affected by CCl ₄ and pomegranate extract treatments (C1).....	66

14	RAPD profile as affected by CCl ₄ and pomegranate extract treatments (H5)	67
15	RAPD profile as affected by CCl ₄ and pomegranate extract treatments (P13)	68

1- INTRODUCTION

The reactive oxygen species (ROS) are free radicals such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$). The production of ROS is a normal physiological event in various organs, including liver and kidney tissues. However, the over production of ROS causes structural damage of biological macromolecules including nucleic acids, proteins and lipids that results in the formation of cytotoxic secondary products such as malondialdehyde (MDA) (**Cestmir and Jitka, 2015**).

Endogenous protection against oxidative stress is achieved by enzymes that remove free radicals. This includes superoxide dismutase, catalase and glutathione peroxidase. Another possibility is the existence of low molecular weight agents that scavenge ROS like glutathione and α -tocopherol. There is an intimate relationship between nutrition and antioxidant defense system, as some exogenous low molecular weight antioxidant may be supplied by the diet. These two main systems of the antioxidant defense act in coordination, their levels being regulated by each other, to avoid oxidative stress events. Few years ago, a considerably large group of molecules widespread in plants has come into focus such as those present in pomegranate (**Ana et al., 2007**).

Pomegranate is an important source of bioactive compounds and has been used for folk medicine for many centuries. Pomegranate juice has been demonstrated to be high in its antioxidant activity, but it was found that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed (**Fatma, 2009**). Pomegranate peel exerted diverse pharmacological functions as antioxidant activity such as hepatoprotective activity (**Murthy et al., 2002**). Pomegranate peel extract contains phenolic punicalagine, gallic acid, catechin, quercetin, rutin and other flavonols, flavones, flavonones and anthocyanidins (**Julie-Jurenka, 2008**). These compounds are known for their properties in scavenging free radical and inhibiting lipid peroxidation *in vitro* (**Noda et al., 2002**).

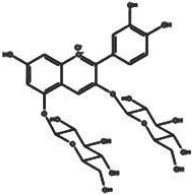
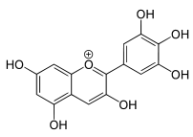
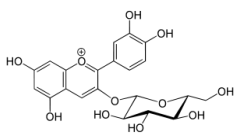
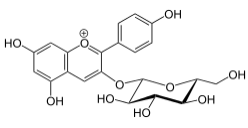
The aim of the present investigation is: studying the bioactive compounds of different pomegranate extracts; their free radical scavenging activity and their expected role to avoid oxidative stress, especially liver oxidative damage induced by CCl₄ in rats.

2- REVIEW OF LITERATURE

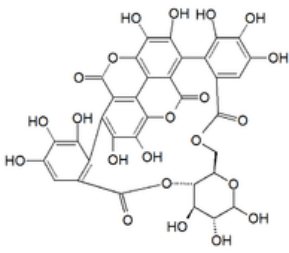
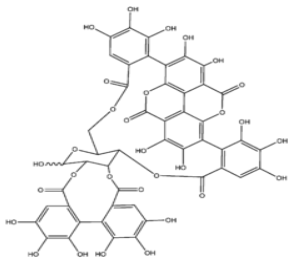
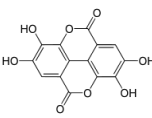
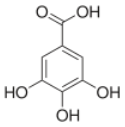
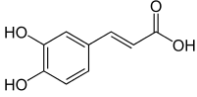
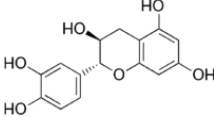
2.1. Chemical constituents of pomegranate antioxidants:

Monica *et al.*, (2013) declared that the high antioxidant capacity of pomegranate has been mostly attributed to its high levels of polyphenolic compounds, such as anthocyanin, tannins and phenols:

Table (1): chemical constituents and structures of the main antioxidants in pomegranate fruit

Constituent	Chemical structure	Part of fruit
Cyanidin 3,5-diglucoside		Seed, peel, juice
Delphinidin 3-glucoside		Seed, peel, juice
Cyanidin 3-glucoside		Seed, peel, juice
Pelargonidin 3-glucoside		Seed, peel, juice

Anthocyanine

		
Punicalin		Seed, peel, juice
		
Punicalagin		Seed, peel, juice
tannins		Seed, peel, juice
		juice
Gallic acid		
		juice
Caffeic acid		
phenols		Peel, juice

2.1.1. Pomegranate flavonoids:

Flavonoids isolated from pomegranate include flavones, flavonols, anthocyanidins and flavan-3-ols. The brilliant colors of pericarp and juice are attributed to anthocyanidins and flavan-3-ols, of which their content decrease or increase with the time of ripening.

Anthocyanidine reported in pomegranate usually present in the form of glycoside with aglycons of delphinidin, cyanidin, epicatechin, epigallocatechin and their derivatives. Flavones and flavonols constitute the major flavonoids of pericarp and leaves, which frequently exist as glycosides with aglycons of luteolin, kaempferol, quercetin, epigenin and naringin (Wang *et al.*, 2010).

Ali *et al.*, (2014) determined the total phenolic and flavonoid contents of different parts of pomegranate fruit (peel, flesh, seed, and whole fruit) using different solvent (methanol and ethyl acetate). The highest total phenolic and flavonoid contents was detected in peel methanol extract (103.2 mg/g dw and 132.4 mg/g dw) respectively. Using HPLC analysis the major phenolic compounds in pomegranate peel methanolic extract were investigated. Among the phenolic compounds, chlorogenic acid, rutin and coumaric acid are present predominantly in the peel extract.

2.1.2. Pomegranate Tannins and phenols:

Pomegranate contains different bioactive compounds. Hydrolysable tannins of diverse structures including ellagitannins and gallotannins constitute the most prevalent compounds presenting in various parts of pomegranate. Ellagitannins are mainly found in the pericarp, bark, seeds and flowers. For example, punicalin, punicalagin and ellagic acid are the major constituents of pericarp (Seeram *et al.*, 2005 and Wang *et al.*, 2010).