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Study The Role of Polyphenolic Extracts of Prunus Domestica L. Wall Nuts As Hypolipidemic, Antioxidant and Antibacterial agents

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By

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وزارة التعليم العالي والبحث العلمي
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LIST OF CONTENTS

Tittles NO.	Contents	Page NO.
Contents Index		**7**
Tables Index		**11**
Figures Index		**12**
Abbrevitions		**14**
Summary		**18**
	Chapter one	
1	**Introduction**	**21**
1.1	***Prunus domestica* L.**	**23**
1.1.1	**Scientific Classification: *Prunus domestica* L.**	**25**
1.2	**Polyphenols**	**25**
1.2.1	**Phenolic Acids**	**26**
1.2.2	**Flavonoids**	**28**
1.2.3	**Polyphenolic Amides**	**29**
1.2.4	**Other Polyphenols**	**30**
1.3	**Biosynthesis of Polyphenols**	**32**
1.4	**Phenolic Compounds and Human Health**	**34**
1.4.1	**Polyphenols as Antioxidant**	**34**
1.4.2	**Polyphenols and Cardiovascular Disease**	**35**
1.4.3	**Other Uses**	**36**
1.5	**Plasma Lipids and Plasma Lipoproteins**	**36**
1.5.1	**Lipids Profile and Chronic Diseases**	**37**
1.6	**Lipid Peroxidation (LPO)**	**39**
1.7	**Antioxidants (an overview)**	**41**
1.7.1	**Ceruloplasmin(Cp)**	**44**

1.7.2	Albumin(Alb)	45
1.8	Antibacterail Activity	45
1.8.1	Antibacterial Activity of Polyphenols	47
1.9	Aim of the Study	48

	Chapter Two	
2	Materials and Methods	49
2.1	Materials	49
2.1.1	Chemicals	49
2.1.2	Equipments and Apparatus	51
2.1.3	Plants Collection	52
2.1.4	Experimental Animal	52
2.2	Methods	52
2.2.1	Extraction of Polyphenols	52
2.2.2	Qualitative Tests of Some Plants Extracts	53
2.2.2.1	Detection of Phenolic Compounds in Plants Extracts	53
2.2.2.2	Detection of Flavonoids in Plants Extracts	53
2.2.2.3	Detection of Carbohydrates in Plants Extracts	53
2.2.2.4	Detection of Alkaloids in Plants Extracts	53
2.2.2.5	Detection of Tannins in Plants Extracts	54
2.2.2.6	Detection of Saponins in Plants Extracts	54
2.2.2.7	Detection of Glycosides in Plants Extracts	54
2.2.2.8	Detection of Triterpinoids in Plants Extracts	55
2.2.2.9	Detection of Triterpins and Setrols in Plants Extracts	55
2.2.3	Investigation of Polyphenolic Extract by UV-VIS. (Spectrophotometer).	55

2.2.4	Investigation of Polyphenolic Extract by HPLC Technique - Polyphenolic extract	56
2.2.5	Acute Toxicity Study for PDR and PDY Polyphenolic Extracts	57
2.2.6	Effects of PDR and PDY Polyphenolic Extracts on Boody Weight	58
2.2.7	Biochemical Parameters	59
2.2.7.1	Determination of Serum Lipid Profile	59
2.2.7.1.1	Determination of Serum Total Cholesterol	59
2.2.7.1.2	Determination of Serum Triglyceride Concentration	61
2.2.7.1.3	Determination of Serum HDL Concentration	63
2.2.7.1.4	Determination of LDL and VLDL	65
2.2.7.1.5	Determination of Atherogenic Index Plasma	45
2.2.7.2	Determination of Oxidative Stress Parameters	65
2.2.7.2.1	Determination of Serum Malondialdehide Level (MDA)	65
2.2.7.2.3	Determination of Antioxidant Parameters	67
2.2.7.2.3.1	Determination of Serum Ceruloplasmin Concentrations	67
2.2.7.2.3.2	Determination of Serum Albumin Concentrations	68
2.2.8	Investigation of Antibacterial Activity of Polyphenolic Extract	70
2.2.8.1	Culture Media	70
2.2.8.2	Pathogenic Bacterial Strains	70
2.2.8.3	Assessment of Antibacterial Activity of Extracts	70
2.2.9	Statistical Analysis	70
	Chapter Three	
3	Results &Discussion	71

3.1	**Plants Extracts**	**71**
3.2	**Qualitative Tests of Some Active Compounds in Plant Extracts**	**72**
3.3	**UV-Visible Spectrum**	**74**
3.4	**Identification of Plant Polyphenol Compounds by HPLC Technique**	**77**
3.5	**Acute Toxicity Study**	**82**
3.6	**Effects of Polyphenolic Extracts on Boody Weight**	**83**
3.7	**Effects of Polyphenolic Extracts on Serum Lipid Profile**	**85**
3.7.1	**Serum Cholesterol Concentration**	**85**
3.7.2	**Serum Triglycerides Concentration**	**87**
3.7.3	**High Density Lipoprotein (HDL)**	**89**
3.7.4	**Low Density Lipoprotein (LDL)**	**91**
3.7.5	**Very Low Density Lipoprotein(VLDL)**	**93**
3.7.6	**Atherogenic Index Levels**	**95**
3.8	**Effects of Polyphenolic Extracts on Serum Oxidant – antioxidant Status**	**96**
3.8.1	**Malondialdehyde (MDA) Concentration**	**96**
3.8.2	**Serum Ceruloplasmin (CP) Concentration**	**99**
3.8.3	**Serum Albumin *(*Alb*)* Concentration**	**101**
3.9	**Antibacterial Properties of Polyphenolic Extracts**	**103**

	Conclusion and Future Research	
	Conclusion	**110**
	Future Research	**111**
	References	**112**

	Summary in Arabic Language	

Tables. NO.	TABLE	Page. NO.
1-1	Both endogenous and exogenous antioxidants in organ	42
2 – 1	Chemicals and their suppliers	49
2 – 2	The Equipments and their manufacturers	51
3-1	Percentage of extracts in wall nuts	71
3-2	Qualitative tests of some active compounds in plants both polyphenolic extract	73
3-3	Retention time of standard polyphenolic compounds	78
3-4	Concentration of polyphenolic compounds in PDR polyphenolic extract	79
3-5	Concentration of polyphenolic compounds in PDY polyphenolic extract	80
3-6	Names and Structure of polyphenolic compounds in PDR and PDY polyphenolic extracts	81
3-7	Acute toxicity effect of PDR polyphenolic extract	82
3-8	Acute toxicity effect of PDY polyphenolic extract	83
3-9	The body weight in the all studied groups	84
3-10	Serum TC concentration in the all studied groups	86
3-11	Serum TG concentration in the all studied groups	88
3-12	Serum HDL concentration in the all studied groups	90
3-13	Serum LDL concentration in the all studied groups	92
3-14	Serum VLDL concentration in the all studied groups	94
3 -15	The Atherogenic index levels in the all studied groups	96
3 -16	Serum MDA concentration in the all studied groups	98
3-17	Serum CP concentration in the all studied groups	100
3-18	Serum Alb concentration in the all studied groups	102
3-19	Diameters of inhibition zone (mm) for all extracts	106

Figures Index

Fig. NO.	Legend	Page. NO.
1-1	Classification of phytochemicals	21
1-2	*Prunus domestica* L.	24
1-3	Typical phenolic acids in food	27
1-4	The main flavonoid classes	29
1-5	Some Polyphenol amides structures	30
1-6	Some Other Polyphenols structures	31
1-7	Phenolic compounds biosynthesis	33
1-8	Poly unsaturated fatty acid (PUFA) oxidation	39
1-9	Radical chain reaction mechanism of lipid peroxidation	40
1-10	Diseases and damages caused by reactive oxygen species	43
3-1	Absorption spectrum of PDR polyphenolic extract by (UV- SCAN) in water	65
3-2	Absorption spectrum of PDY polyphenolic extract by (UV- SCAN) in water	66
3-3	HPLC chromatogram of standard polyphenolic compounds	68
3-4	HPLC chromatogram of PDR polyphenolic extract	69
3-5	HPLC chromatogram of PDYpolyphenolic extract	70
3-6	The body weight in the all studied groups	74
3-7	Serum TC concentration in the all studied groups	77
3-8	Serum TG concentration in the all studied groups	79
3-9	Serum HDL concentration in the all studied groups	81
3-10	Serum LDL concentration in the all studied groups	83
3-11	Serum VLDL concentration in the all studied groups	84

3-12	**The Atherogenic index levels in the all studied groups**	**86**
3-13	**Serum MDA concentration in the all studied groups**	**88**
3-14	**Serum CP concentration in the all studied groups**	**100**
3-15	**Serum Alb concentration in the all studied groups**	**102**
3-16	**Chemical structure of ciprofloxacin**	**104**
3-17	**The activity of PDR and PDR polyphenolic extracts against(*E.Coli*) bacteria**	**106**
3-18	**The activity of Ciprodar against (*E.Coli*) bacteria**	**106**
3-19	**The activity of PDR and PDR polyphenolic extracts against (*Pseudo*.) bacteria**	**107**
3-20	**The activity of Ciprodar against (*Pseudo*.) bacteria**	**107**
3-21	**The activity of PDR and PDR polyphenolic extracts against(*Staph.*)bacteria**	**108**
3-22	**The activity of Ciprodar against (*Staph.*) bacteria**	**108**
3-23	**The activity of PDR and PDR polyphenolic extracts against (*Bacillus*.) bacteria**	**109**
3-24	**The activity of Ciprodar against (*Bacillus*.) bacteria**	**109**

ABBREVITION

µg	**Microgram**
µL	**Micro letter**
µm	**Micrometer**
ACAT	**Acyl-CoA cholesterol transferase**
Alb	**Albumin**
AMP-activated protein kinase	**5' adenosine monophosphate-activated protein kinase**
B.W	**Body weight**
Bacillus	***Bacillus subtilis***
C-18	**Column-18**
CFU	**Colony forming unit**
CHD	**Coronary heart disease**
CM	**Chylomicrons**
Conc	**Concentration**
Cp	**Ceruloplasmin**
Ctrl	**Control**
CVD	**Cardiovascular Disease**
D.W	**Distilled water**
DMSO	**Dimethyl sulphoxide**
DNA	**Deoxyribonucleic acid**
E.coli	***Escherichia coli***
Fe^{+2}	**Ferrous iron**
Fe^{+3}	**Ferric iron**

FLC	**Fast liquid chromatography**
gm	**Gram**
H	**Hour**
HDL	**High density lipoproteins**
HMG-CoA reductase	**3-hydroxy-3-methyl-glutaryl-CoA reductase**
HPLC	**High performance liquid chromatography**
IDL	**Intermediate density lipoproteins**
IUPC	**International Union of Pure and Applied Chemistry**
KD	**Kilodalton**
L	**Liter**
LCAT	**Lecithin Acyl Transferase**
LDL	**Low Density Lipoprotein**
LPO	**Lipid peroxidation**
M	**Molarity**
MDA	**Malondialdehyde**
min	**minute**
mL	**Milliliter**
mm	**millimeter**
NADH	**Nicotinamide adenine dinucleotide**
NADPH	**Nicotinamide adenine dinucleotide phosphate**
nm	**Nanometer**
nmol	**Nanomole**
ORAC	**Oxygen radical absorbance capacity**
oxLDL	**LDL oxidation**

PAL	**Phenylalanine ammonia-lyase**
PDE	**phosphodiesterase**
PDR	***Prunus domestica* red**
PDY	***Prunus domestica* yellow**
PL	**phospholipids**
PPD	**Para-phenylene diamine**
Pseudo	***Pseudomonas aeruginosa***
PUFA	**Poly unsaturated fatty acid**
Q10	**ubiquinone**
RNS	**Reactive nitrogen species**
ROS	**Reactive oxygen species**
rpm	**Rotor per minute**
S	**Standard**
SD	**Standard Deviation**
SOD	**Superoxide dismutase**
Staph	***Staphylococcus aureus***
TBA	**Thio barbituric acid**
TC	**Total cholesterol**
TCA	**Trichloro acetic acid**
TG	**Triglycerides**
THF	**Tetrahydrofuran**
US	**United States**

UV	**Ultra-Violate**
Vitamin C	**ascorbic acid**
Vitamin E	**γ-tocopherol**
Vitamine A	**Aquasola**
VLDL	**Very low density lipoproteins**
ε Cp	**The extinction coefficient of Cp**
ε MDA	**molar absorption coefficient of MDA**

SUMMARY

The present study investigate the effect of polyphenolic extracts of *Prunus domestica* red (PDR) and *Prunus domestica* yellow (PDY) wall nuts on some biochemical parameters in female rats and anti-bacterial *in vitro*.

The work involved the following studies:

Analytical study: involved extraction, isolation and identification of polyphenolic content of PDR and PDY extracts. The UV-Vis Spectra and high performance liquid chromatography of extracted polyphenols are proved the presence of (tannic acid, gallic acid, caffeic acid, vanillic acid, ferulic acid, chlorogenic acid and amygdalin) in both extracts.

Acute toxicity: *in vivo* study included of both extracts was performed on four groups of rats (6 rats in each group). After treatment of different concentration of PDR and PDY polyphenolic extracts (25,50 and 100 mg/kg B.W) and after 72 hr of treatment, no mortality in all rats of experiments can be observed. This indicated that both extracts are orally non toxic.

Boody weight : all rats are divided into normal control group (A) treated with (0.2 mL) DMSO for 30 days, group (B) treated with daily high cholesterol diet for 30 days, and the rest of the two groups (C & D) treated with (25 mg/kg B.W) for PDR and PDY polyphenolic extracts respectively besides high cholesterol diet for 30 days. Group (B) rats showed a significant increase ($P < 0.01$) in the body weight compared with normal control group(A). PDR and PDY polyphenolic extracts are significantly decreased the body weight in groups (C & D). PDR polyphenolic extract reduces the body weight more effective than PDY polyphenolic extract.

Biochemical studies: blood samples collects after 30 days. Lipid profile measured: Group (B) rats showed a significant increase ($P < 0.01$) in serum TC, TG, LDL and VLDL levels compared with normal control group(A). Serum HDL levels are a significant decreases in group (B) ($P < 0.01$) compared with normal control group(A). PDR and PDY polyphenolic extracts are significantly decreased the levels of these parameters in groups (C & D) compared with group(B). Consequently the PDR polyphenolic extract reduces the lipid levels more effective than PDY polyphenolic extract.

Also the *in vivo* included evaluation of serum oxidant-antioxidant status: Group (B) rats showed a significant increase ($P < 0.01$) in serum MDA and Cp levels compared with normal control group(A). The serum Alb level in group(B) are a significant decreases ($P < 0.01$) compared with normal control group(A). PDR and PDY polyphenolic extracts are significantly decreases ($P < 0.01$) the levels of MDA and Cp in groups (C & D) compared with group(B). PDR polyphenolic extract reduces serum MDA and Cp levels more effective than PDY polyphenolic extract. Whereas PDR and PDY polyphenolic extracts are a significant increase ($P < 0.01$) the levels of Alb in groups (C & D) compared with group(B) . PDR polyphenolic extract increase serum Alb levels more effective than PDY.

Antibacterial study: four types of bacteria, two Gram positive (*Staphylococcus aureus***,** *Bacillus subtilus*) and two Gram negative (*Escherichia coli***,** *Pseudomonas aeruginosa*) have been used to investigate the antibacterial activity of both extracts. Both extracts are actively worked against both types of bacteria.

Furthermore, the highest activity of extracted polyphenolic for PDR against *Pseudomonas aeruginosa* (30 mm) is used, whilst the same extraction had equally inhabited zone against *Escherichia coli***,** *Staphylococcus aureus and Bacillus subtilus* (20 mm).

Finally, for extracted polyphenolic of PDY founded that highest activity against *Staphylococcus aureus* (13 mm) and lowest activity against *Bacillus subtilus*.

The final conclusion is that the PDR and PDY extracts are rich in polyphenols. Besides, the polyphenolic compounds non toxic, have affect the hypolipidemic, antioxidant activities and antibacterial.

1. Introduction

For thousands of years natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (Phillipson, 2001).

Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world. Higher plants contribute no less than 25% of the total (Ameenah, 2006).The role of the natural products is particularly relevant in the infectious diseases area, where as over 60% of the antimicrobial agents that used in therapy are from natural origin (Newman *et al.*, 2003; Newman and Cragg, 2007). Natural products have been a major source of new drugs (Vuorela *et al.*, 2004). Researches on natural products have significantly progressed over the last decades, mainly on plants corroborating their importance to the discovery of new biological and medicinal agents (Calixto, 2000; Rates, 2001; Newman *et al.*, 2003). Medicinal plants are a source for a wide variety of natural products, such as flavonoids and phenolic acids which are very interesting for their antioxidant properties (Farhan *et al.*,2012). The use of plants as medicines has a long history in the treatment of various diseases.The nearest known records for the use of plants as drugs are from Mesopotamia in 2600 B.C., and these still are a significant part of traditional medicine and herbal remedies(Koehn and Carter, 2005). Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hashim *et al.*, 2010).

Application of Medicinal plants are known to be used as food preservative due to its antimicrobial activity and antioxidant (Sunilson *et al.*, 2009).

Several plant species have been investigated and bioactive ingredients extracted to treat various human diseases. Plant parts used included leaves, flowers, stems, roots and seeds (Said and Azaizeh, 2008; Said and Saad, 2008). Fruits and vegetables contain ascorbic acid, carotenoids, tocopherols, some metal compounds and phenolic compounds show antioxidant properties (Prior , 2003; Rohrmann *et al.*, 2007).

The "phyto-" of the word phytochemicals is derived from the Greek word phyto, which means plant. Therefore, phytochemicals are plant chemicals (Figure1-1). Phytochemicals are produced by the plants in self-defence to protect them from pest, microbes and environmental stress factors. In past, plants and herbs have been reported to contain different phytochemical compounds with a wide range of activities, which may help in the development of new drugs (Shoeb , 2006; Menichini *et al.*, 2009; Lei *et al.*, 2011).

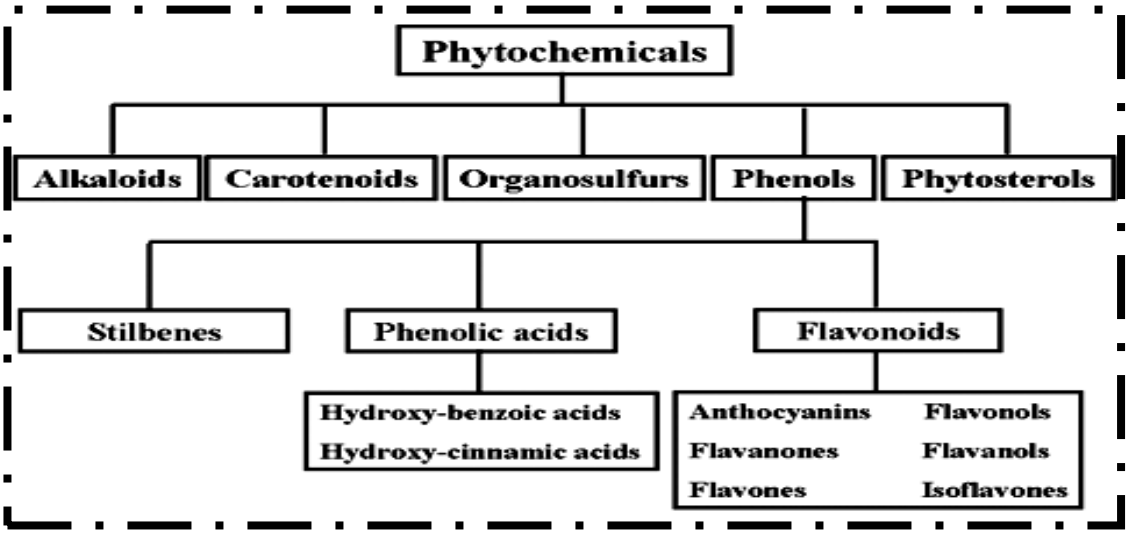


Figure (1-1): Classification of phytochemicals.

In fact, many phytochemicals, such as polyphenols, are rapidly degraded and metabolized in the human body. Furthermore, genetic variation in pathways affect absorption, metabolism, and distribution of these natural substances (Ross, 2007; Manach *et al.*, 2009). More than 10,000 phytochemicals have been described (Maria *et al.*, 2010). Phytochemicals are plants-derived chemical compounds, which are non-essential nutrients, some of which show health promoting properties (Dharmesh, 2010), namely primary constituents which includes amino acids, sugar , protein and chlorophyll etc and secondary constituents consists of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds (Alinmoladun *et al.*, 2007). Majority of phytochemicals have been known to have valuable treatment activities like insecticidal ,antifungal, antibacterial, spasmolytic, (Edeogo *et al.*, 2005) and antioxidant activities (Krishnaiah *et al.*, 2007).

1.1 *Prunus domestica* L.

Prunus domestica commonly known as Plum Alu-Bukhara, Alucha found commonly in Pakistan, India, Afghanistan and Persia (Gupta 2003; Narayan and Kumar ,2003). *P.domestica* belongs to rosaceae family which is one of the largest family includes 100 genera and 200 species, researchers have paid attention towards rosaceae family because many plant of the family possess immense therapeutic potential (Mishra *et al.*, 2012). *P.domestica* are considered as healthy food because of lower fat contents and contain considerable amount of important nutrients like carbohydrates, vitamins and minerals.

Consumption of fruits, like prunes, is useful in blood circulation problems, measles,anticancer, antidiabetes, antiobesity, cardiovascular problems, dyspepsia, nausea, vomiting, thirst, in bilious fevers, headache, jaundice and hepatitis, leucorrhea, miscarriage, antioxidant, antihyperlipidemic, anxiolytic, asthma (Li, 2008; Qaiser and Naveed 2011; Soni *et al.*, 2011).

P. domestica are high in potassium contents and have beneficial effects in cardiovascular problems (Stacewicz *et al.*, 2001). Prunes are a significant source of major nutrients, including carbohydrates, several amino acids, vitamin A, vitamin B , vitamin K, calcium, magnesium, zinc, copper, manganese, selenium, boron and dietary fibers. Prunes fiber consist mainly of soluble fraction (80%) including pectin, hemicellulose, cellulose and lignins. Drying process increases the total dietary fibers (Siddiq, 2006). Malic acid is the predominant acid, although citric, tartaric, benzoic and boric acid were also identified in prunes (Qaiser and Naveed, 2011). *P.domestica* are fruits rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than oranges, apples or strawberries (Kayano *et al.*, 2002; Leong and Shui, 2002). Figure (1-2) explained the *Prunus domestica* red (PDR) and *Prunus domestica* yellow (PDY).

Figure (1-2): *Prunus domestica* L.

1.1.1. Scientific Classification: *Prunus domestica* L.

Kingdom	***Plantae* – Plants**
Subkingdom	***Tracheobionta* – Vascular plants**
Superdivision	***Spermatophyta* – Seed plants**
Division	***Magnoliophyta* – Flowering plants**
Class	***Magnoliopsida* – Dicotyledons**
Subclass	***Rosidae***
Order	***Rosales***
Family	***Rosaceae* – Rose family**
Genus	***Prunus* L. – plum**
Species	***Prunus domestica* L.**

1.2. Polyphenols

Polyphenols are a large group of phytochemicals wide spread in the plant kingdom (María *et al.*, 2011). In plants, polyphenols minimize the effects of UV radiation and protection against herbivory (Daglia, 2012),as wall as, are important for pigmentation, reproduction, growth, and protection against pathogens. In industry, polyphenols are used in the production of paints, paper, and cosmetics as well as in food additives (Bravo , 1998). Phenolic compound seem to play an important role in the natural defense mechanisms in fruit (e.g., antifungal effects)(Tomas-Barberan *et al.*, 2001). Polyphenols are usually solid, crystalline and with low solubility in water. Many of these substances are classified as natural antioxidants and have therapeutic properties, being present in food and medicine plants (Boudet, 2007). Phenolic compound is the large group of phytochemicals present in plant, so far more than 8 000 structurally known phenolic compounds have been reported (Gaurav *et al.*, 2013). Phenolic compounds are secondary metabolites which synthesize in plants. They posses biological properities such as:antioxidant, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function (Han *et al.*, 2007).

Polyphenols are classified into two major classes: Flavonoids and Non-Flavonoids (Cheynier, 2005).

1.2.1. Phenolic Acids

Phenolic acids are non-flavonoid polyphenolic compounds which can be further divided into two main types, benzoic acid(7 carbon atoms) and cinnamic acid (9 carbon atoms) derivatives based on C1–C6 and C3–C6 backbones (Figure 1-3). Phenolic acids are secondary metabolites widely spread throughout the plant kingdom. Phenolic compounds confer unique taste, flavour, and health-promoting properties found in vegetables and fruit (Tomas-Barberan *et al.,* 2001). While fruits and vegetables contain many free phenolic acids, in grains and seeds (Kim *et al.,*2006; Chandrasekara and Shahidi, 2010). Epidemiologic studies have suggested an association between the consumption of phenolic acid rich foods or beverages and the prevention of many diseases (Morton *et al.*, 2000)**.**

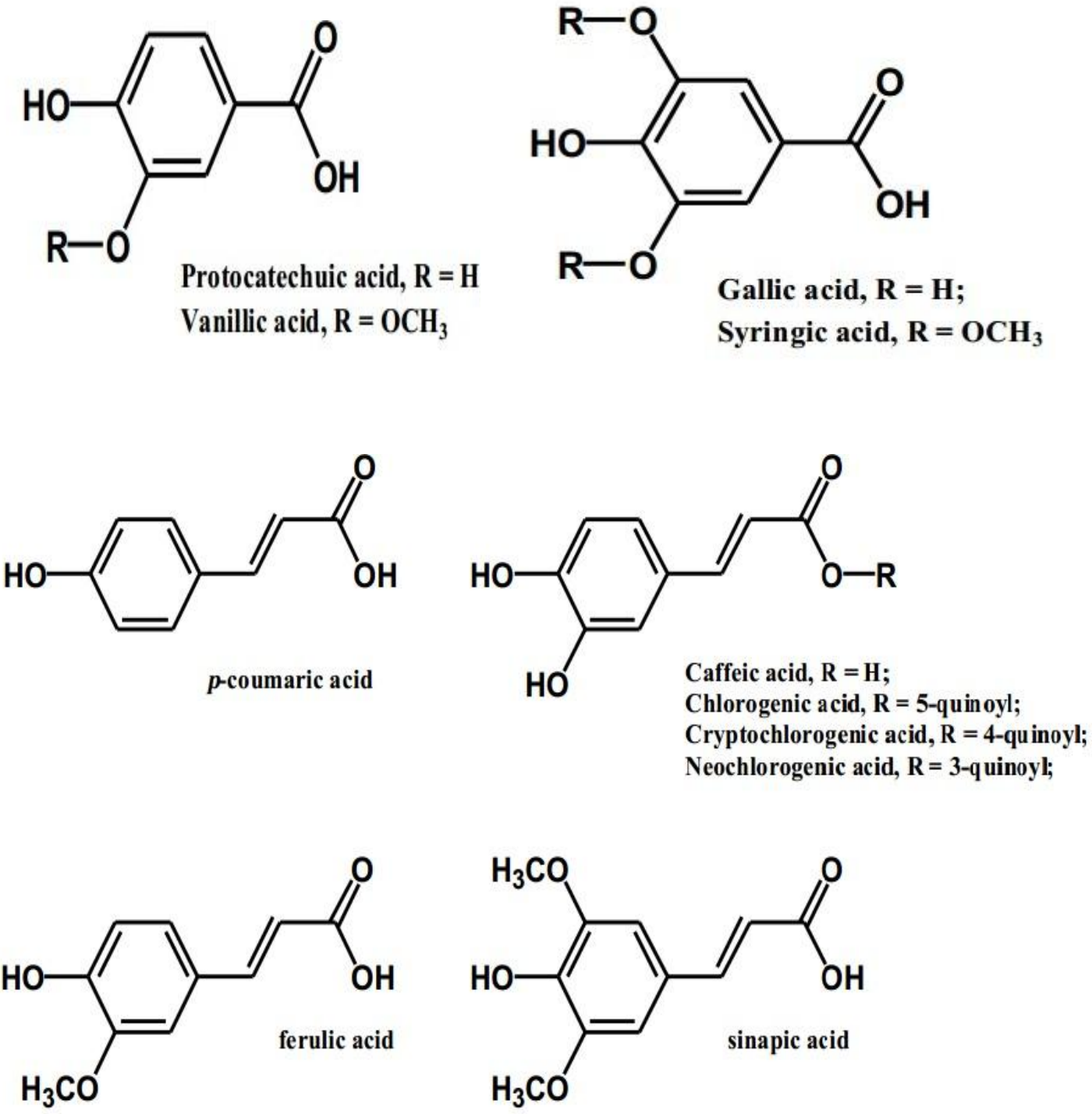
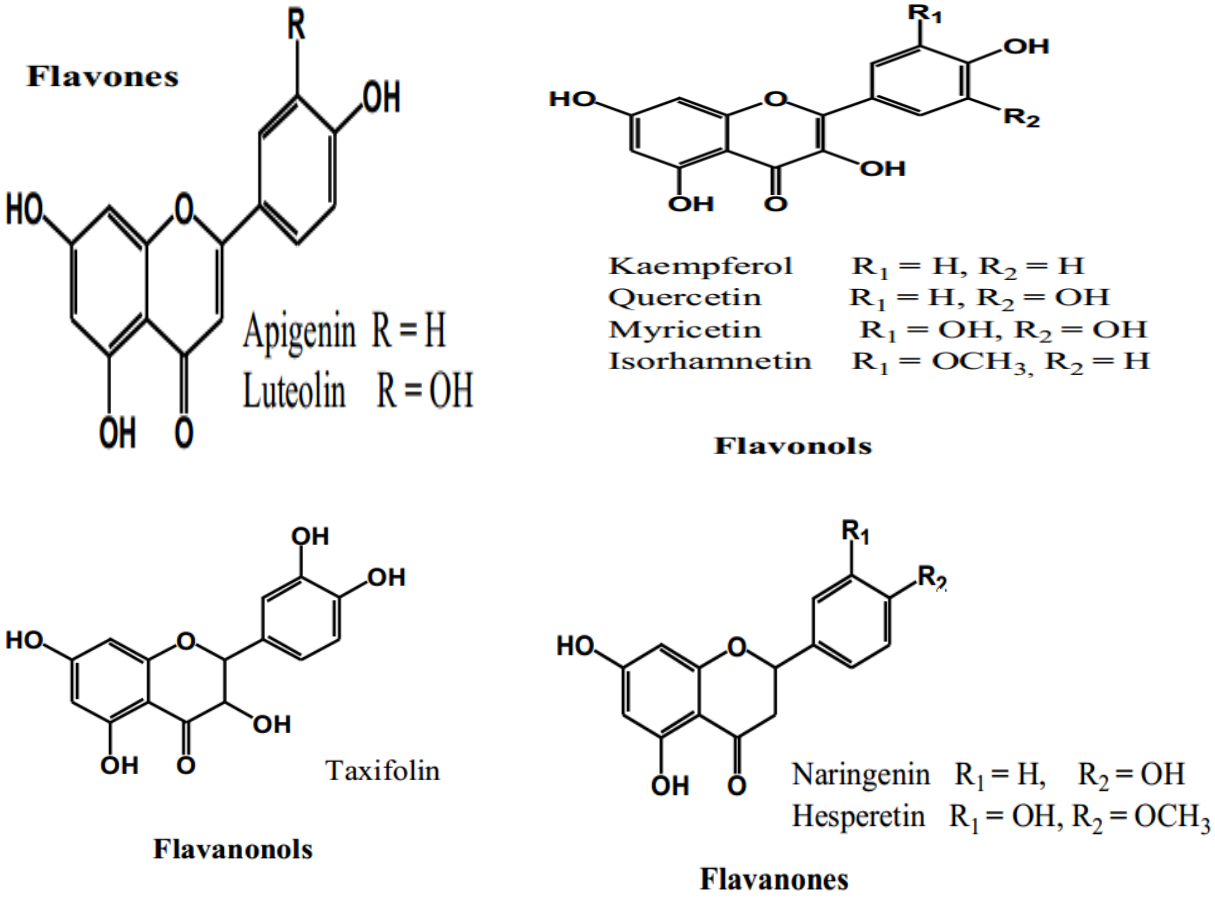
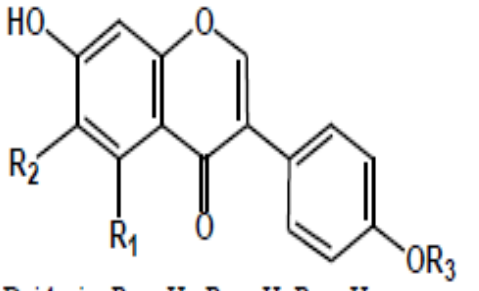


Figure (1-3): Typical phenolic acids in food (Tsao ,2010).

1.2.2. Flavonoids

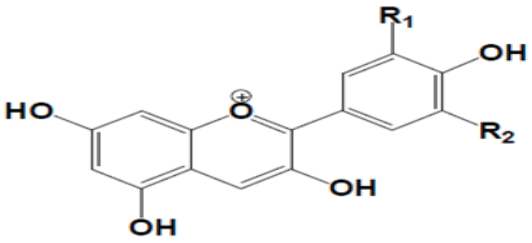
Flavonoids comprise a large group of low-molecular-weight polyphenolic plant metabolites that are found in fruits, vegetables (Toshio and Ryo, 2013). Flavonoids, which share a common structure consisting of two aromatic rings (A and B) that are bound together by three carbon atoms that form an oxygenated heterocycle (ring C), are classified into six subclasses: flavones (including: luteolin and apigenin), flavonols (Kaempferol, Quercetin and Myricetin), flavanonols (Taxifolin), flavanones (Hesperetin, Naringenin), isoflavones (Daidzein and Genistein)and anthocyanidins(Figure 1-4)(Cyanidin and Pelargonidin) (Toshio and Ryo, 2013).





Daidzein: $R_1 = H$; $R_2 = H$; $R_3 = H$
Formononetin: $R_1 = H$; $R_2 = H$; $R_3 = OCH_3$
Glycitein: $R_1 = H$; $R_2 = OCH_3$
Genistein: $R_1 = OH$; $R_2 = H$, $R_3 = H$
Biochanin A: $R_1 = OH$; $R_2 = H$; $R_3 = OCH_3$

Isoflavones



Cyanidin $R_1 = OH$; $R_2 = H$
Delphinidin $R_1 = OH$; $R_2 = OH$
Pelargonidin $R_1 = H$; $R_2 = H$
Malvidin $R_1 = OCH_3$; $R_2 = OCH_3$
Peonidin $R_1 = OCH_3$; $R_2 = H$
Petunidin $R_1 = OH$; $R_2 = OCH_3$

Anthocyanidins

Figure (1-4): The main flavonoid classes(Tsao ,2010).

1.2.3. Polyphenolic Amides

Some polyphenols may have N-containing functional substituents. Two such groups of polyphenolic amides are of significance for being the major components of common foods: capsaicinoids in chili peppers (Davis *et al.*, 2007) and avenanthramides in oats (Bratt *et al.*, 2003) (Figure 1-5). Capsaicinoids such as capsaicin are strong antioxidant and anti-inflammatory properties, and they modulate the oxidative defense system in cells. Antioxidant activities including inhibition of LDL oxidation by avenanthramides have also been reported.

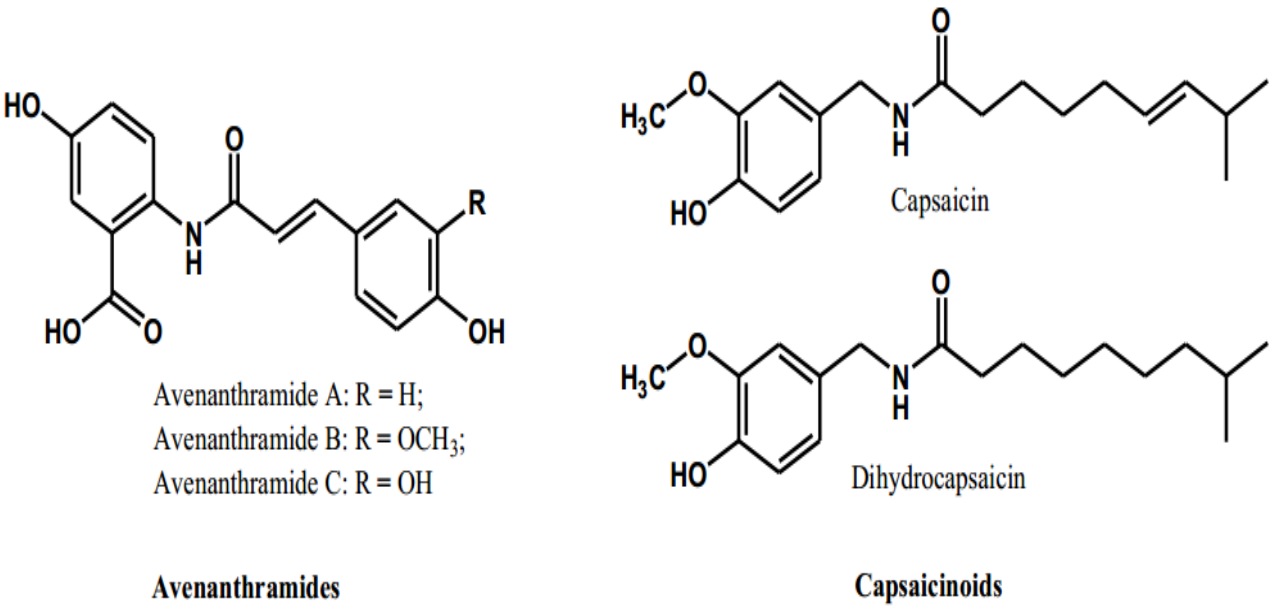
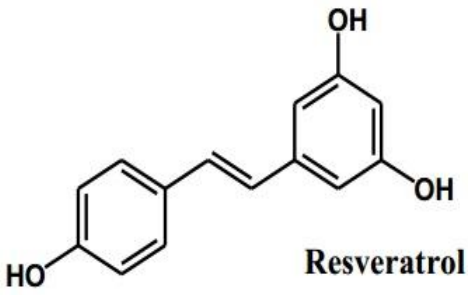


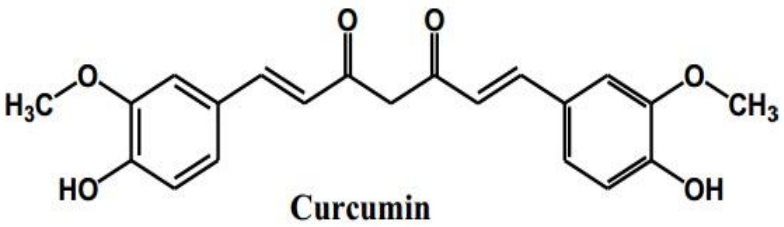
Figure (1-5): Some Polyphenol amides structures (Tsao ,2010).

1.2.4. Other Polyphenols:

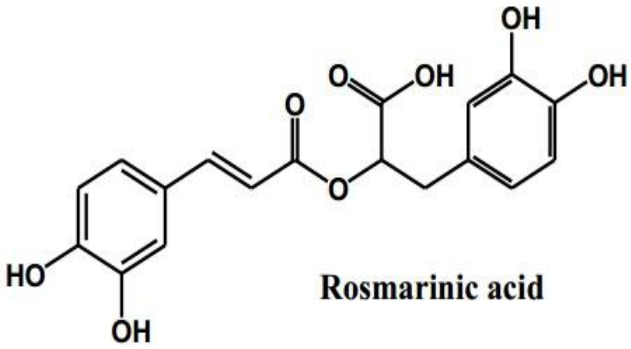
There are several non-flavonoid polyphenols found in foods that are considered important to human health. Among these, resveratrol is unique to the grapes and red wine; ellagic acid and its derivatives are found in strawberries and raspberries, Lignans exist in the bound forms of flax, sesame and many grains; structures shown in (Figure 1-6). Curcumin is a strong antioxidant from turmeric (Figure 1-6). Rosmarinic acid is a dimer of caffeic acid, and ellagic acid is a dimer of gallic acid. While both gallic acid and ellagic acid are found in the free forms, their glucose esters, a group known as hydrolysable tannins, also exist in different plants(Tsao , 2010).



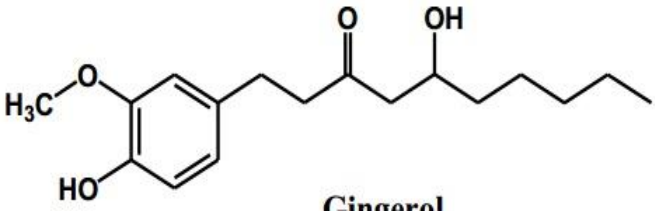
Resveratrol



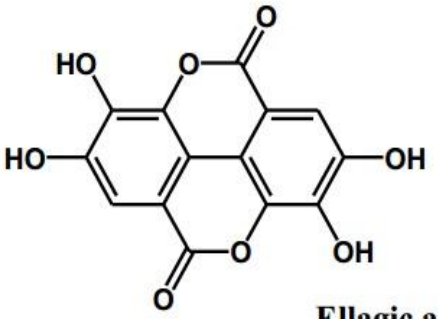
Curcumin



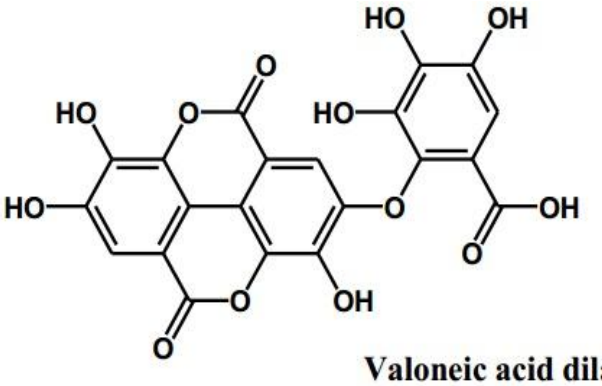
Rosmarinic acid



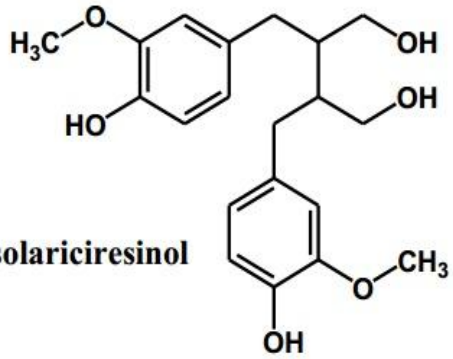
Gingerol



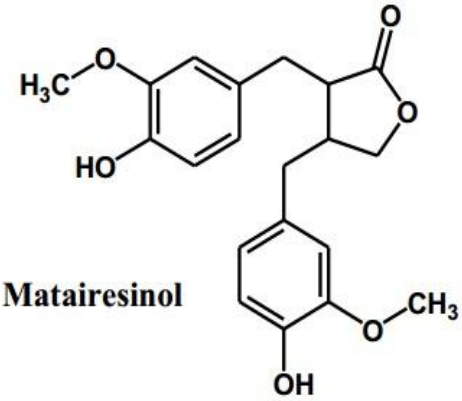
Ellagic acid



Valoneic acid dilactone



Secoisolariciresinol



Matairesinol

Figure (1-6): Some Other Polyphenols structures(Tsao,2010).

1.3. Biosynthesis of Polyphenols

Phenolic compounds are generally synthesised via the shikimate pathway, which starts with the formation of phenylalanine and tyrosine. In Figure (1-7), phenylalanine is deaminated to form cinnamic acid in a reaction catalysed by phenylalanine ammonia-lyase (PAL), the key enzyme in phenolic biosynthesis (Tomas-Barbern and Espin, 2001 ; Chen *et al.*, 2009). Hydroxycinnamoyl and *p*-coumaroyl coenzyme A esters are the common structural precursors of various phenolic classes. The cleavage of acetate leads to hydroxybenzoic acids, hydroxylation and methoxylation to hydroxycinnamic acids (Macheix *et al.*, 1990). The final products of the flavonoid branch are anthocyanins, starting with the formation of chalcones and with the rest of flavonoids being formed from intermediates of anthocyanin biosynthesis (Schijlen *et al.*, 2004).

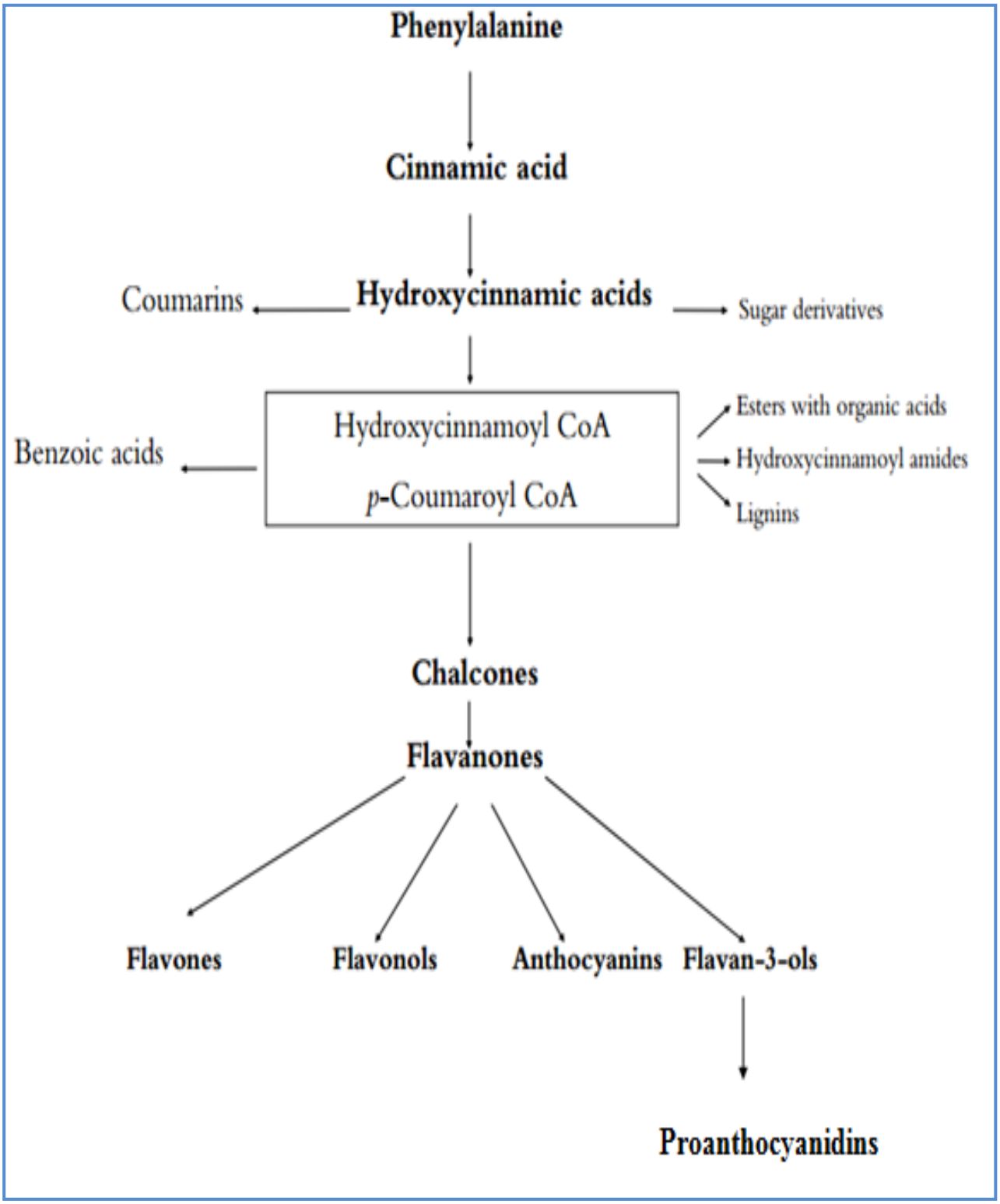


Figure (1-7): Phenolic compounds biosynthesis (Macheix *et al.*, 1990).

1.4. Phenolic Compounds and Human Health

Plant extracts and their components have been known to exhibit biological activities, especially antimicrobial (Iscan *et al.*,2002), antifungal (Sokovic´ *et al.*, 2009), antibacterial (Kanatt *et al.*, 2008) and antioxidant activities (Seun-Ah *et al.*,2010). In general, a diet rich in fruit and vegetables has been linked to various beneficial effects on human health, such as reducing the risk of developing coronary heart disease (Dauchet *et al.*, 2006), cancer (Zhang *et al.*,2009), hypertension(Mignone *et al.*, 2009), diabetes, and inflammatory processes (Zafra-Stone *et al.*, 2007). The constituents responsible for these protective effects include some vitamins (*e.g*. A, C, E), minerals (*e.g*. potassium, zinc, selenium), carotenes and phenolic compounds (Muhammad *et al.*, 2006 ; Anderson *et al.*, 2009).

1.4.1. Polyphenols as Antioxidant

The antioxidant activity of polyphenols is very important for human health and food stability. Recent research is mainly focused on the study of the antioxidants in fruits extracts, particularly their characteristics and protecting effects against free radicals. Free radicals are responsible for human ageing and food deterioration. The only way to slow down or prevent such effects is to use the antioxidants, such as polyphenols (Prior , 2003). Basically, polyphenols are compounds used to delay free radical accumulation and strengthen oxidative stability of food and human body (Jeszka *et al.*, 2010). Phenolic compounds have been shown to possess an antioxidant activity based on their (hydroxyl group) donation to free radicals (Marinova *et al.*, 2005; Ibrahim and Jaafar, 2011).

The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms:

(1) scavenging radical species such as ROS/ reactive nitrogen species (RNS).

(2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production.

(3) up regulating or protecting antioxidant defense (Cotelle, 2001).

Antioxidant activities of fruits correlate with total contents of phenolic compounds (Wang *et al.,* 2000), and the activity of fruits such as strawberry, plum, orange, and grapefruit is high (Wang *et al.,* 1996) . Among the molecules exerting antioxidant activity in plums, the contribution of phenolic compounds was found much greater than that of vitamin C and carotenoids (Gil *et al.,* 2002). Polyphenols are the most abundant antioxidants in the diet; their intake is 10 times greater than that of vitamin C and 20 times that the carotenoids (Mar *et al.,* 2013).

1.4.2. Polyphenols and Cardiovascular Disease

Numerous epidemiological and human intervention studies have suggested that regular consumption of polyphenol-rich foods, such as fruits and vegetables may exert cardio-protective effects in humans (Nakachi *et al.,*2000 ; Arts *et al.,* 2001; Mink *et al.,* 2007). Plant-derived foods and beverages are rich in phenolic compounds which show protection properties against cancer, cardiovascular disease and aging (Piotr *et al.,* 2012). The treatment of hypercholesterolemia and related cardiovascular diseases with medicinal plants has increased in recent years (Asaolu *et al.,* 2010). Phenolic compounds have been proven to be successful in attenuating hypercholesterolemia (Bok *et al.,* 1999; Kumar *et al.,* 2005; Rehrah *et al.,* 2007).

LDL oxidation (oxLDL) is considered to be a major risk factor for the development of atherosclerosis and CVD (Witztum, 1994). Both human and animal *in vivo* studies have shown that the level at which LDL oxidizes, decrease with increasing phenolic concentration (Weinbrenner *et al.*, 2004; Marrugat *et al.*, 2004 ; Covas *et al.*, 2006).

1.4.3. Other Uses

Epidemiological studies suggest that consumption of polyphenolic compounds is associated with decreased risk of chronic diseases, such as heart disease (Chong *et al.*, 2010), cancer (Thomasset *et al.*, 2006), as well as neuro-degenerative diseases, such as Parkinson's and Alzheimer's diseases (Aquilano *et al.*, 2008), by lowering blood pressure (Hooper *et al.*,2008*;* Desch *et al.*,2010*;* Erlund *et al.*,2008) , by improving endothelial function (Schroeter *et al.*,2006; Heiss *et al.*, 2007; Widlansky *et al.*, 2007), by inhibiting platelet aggregation (Erlund *et al.*,2008) and by reducing inflammatory responses (Mao *et al.*, 2002; Schramm *et al.*, 2003).

1.5. Plasma Lipids and Plasma Lipoproteins

The major lipids are fatty acids, triglycerides (TG), cholesterol (free and esterified cholesterol) and phospholipids (PL). They are important in maintaining the structure of cell membrane (cholesterol and phospholipids), steroid hormone synthesis (cholesterol), and energy metabolism (TG and fatty acid)(Liu , 2002). The term lipid has been loosely defined as any of a group of organic compounds that are insoluble in water but soluble in organic solvents (Smith, 2000). Because lipids are water-insoluble molecules, they cannot be transported in aqueous solutions, such as plasma. For that reason, lipids are transported in plasma as macromolecular complexes known as lipoproteins (Bauer, 2004; Johnson, 2005).

Plasma lipoproteins are typically classified into five major subclasses on the basis of their densities **(**Betteridge,2000**)**.

1. High density lipoproteins (HDL) collect cholesterol from the body's tissues, and bring it back to the liver.
2. Low density lipoproteins (LDL) carry cholesterol from the liver to cells of the body.
3. Intermediate density lipoproteins (IDL) are intermediate between VLDL and LDL. They are not usually detectable in the blood.
4. Very low density lipoproteins (VLDL) carry (newly synthesized) triacylglycerol from the liver to adipose tissue.
5. Chylomicrons (CM) transport exogenous lipids to liver, adipose, cardiac, and skeletal muscle tissue.

1.5.1. Lipids Profile and Chronic Diseases

The term hyperlipidemia refers to hypercholesterolemia ,hypertriglyceridemia and hyperlipoproteinemia, is a major risk factor for the development of cardiovascular diseases. Hyperlipidemia, mainly an increased level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) along with a decrease in high-density lipoprotein cholesterol (HDL-C), is the predictor of coronary artery disease, fatty liver disease, and carcinogenesis, which is associated with the formation of reactive oxygen species (Roberts *et al.*, 2006).

Hypercholesterolemia encourages atherosclerosis and therefore represents a major risk factor for cardiovascular disease (Liu *et al.,* 2006).

Lipemia is a result of hypertriglyceridemia, but not hypercholesterolemia (Ford,1996; Johnson, 2005). lipemia is apparent when serum triglyceride concentrations exceed 2.26 mmol/L (200 mg/dL) (Bauer, 1995).

Atherosclerosis is a chronic inflammatory condition that commonly affects coronary arteries, leading to coronary heart disease (CHD). It is characterised by foam cell formation in the subendothelial space of large and medium arteries (Ross, 1993).The coronary arteries are especially susceptible to atherogenesis; atherosclerosis of the coronary arteries may lead to angina pectoris and myocardial infarction (Falk , 2005). Atherosclerosis is a multifactorial disease, exacerbated by external factors such as dietary foods high in cholesterol, sedentary lifestyle, smoking and stress (Cooper *et al.,* 2000).

Atherosclerosis develops When LDL become oxidized (ox-LDL) by free radicals, particularly ROS (Ross *et al.,* 1999). The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the ox-LDL forming specialized foam cells(VanderWal *et al.,* 1989).

Cardiovascular disease is a growing health problem throughout the world and represents a leading cause of death and disease in humans (Crews, 2007). Several factors, such as a high caloric diet, age, lack of exercise, smoking, alcohol consumption, and genetic predisposition have been linked with cardiovascular disease (Asaolu *et al.,* 2010). Elevated cholesterol levels predispose patients to a condition known as hypercholesterolemia (Durrington, 2003),which increases the risk of deadly and non-deadly coronary heart disease in people over the age of 50 years (Marks *et al.,* 2003).

1.6. Lipid Peroxidation (LPO)

LPO, a chain process which affects unsaturated fatty acids in cell membranes, in which end product as malondialdehyde (MDA) is generated (Janero, 1990). LPO is one of the major interrelated derangements of cell metabolism which is produced by oxidative stress. Therefore, it is a well established mechanism of cellular injury in human and is used as an indicator of oxidative stress in cell and tissues (Halliwell, 1991). LPO increase the tendency of blood to clot by stimulating thrombin generation (Desrumaux *et al.*, 2010) and they may be involved in atherogenesis (Esterbauer *et al.*, 1993). The role of lipid peroxidation of plasma lipoproteins in the development of human chronic-degenerative diseases has been widely investigated (Ferretti *et al.*, 2006; Itabe, 2009; Levitan *et al.*, 2010). LPO process, that results in production of mainly malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4- HNE), **(**Montillet *et al*., 2005 ; Garg and Manchanda ,2009) Figure(1-8). MDA the products of lipid peroxidation are easily detected in the blood plasma and have been used as a measure of oxidative stress. Approximately 20% of end- products derived from oxidative damage of lipids *in vitro* are MDA (Wu *et al*., 2004).

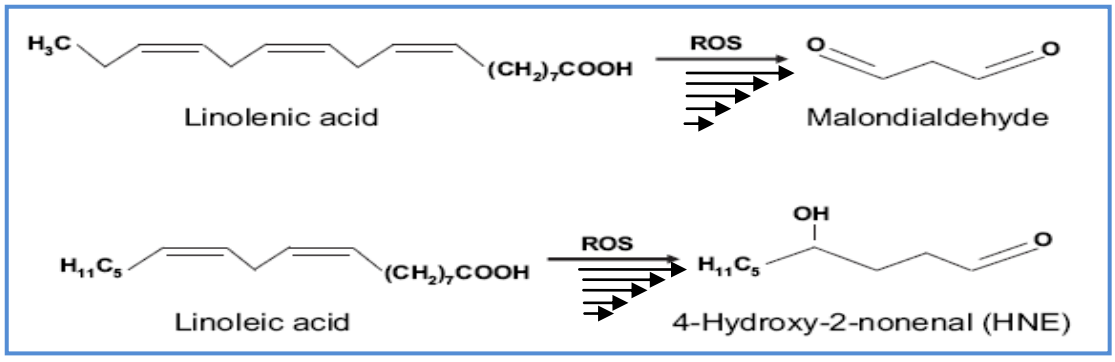


Figure (1-8): Poly unsaturated fatty acid (PUFA) oxidation.(Gill and Tuteja ,2011).

The initial reaction of •OH with polyunsaturated fatty acids produces a lipid radical (L•), which in turn reacts with molecular oxygen to form a lipid peroxyl radical (LOO•). The LOO• can abstract hydrogen from an adjacent fatty acid to produce a lipid hydroperoxide (LOOH) and a second lipid radical (Buettner ,1993). Further, reducing agents, such as Fe^{2+}, causes reductive cleavage of LOOH producing lipid alkoxyl radical (LO•). The chain reaction of lipid peroxidation stimulated by both alkoxyl and peroxyl radicals by abstracting additional hydrogen atoms (Figure 1-9) (Green and Reed, 1998).

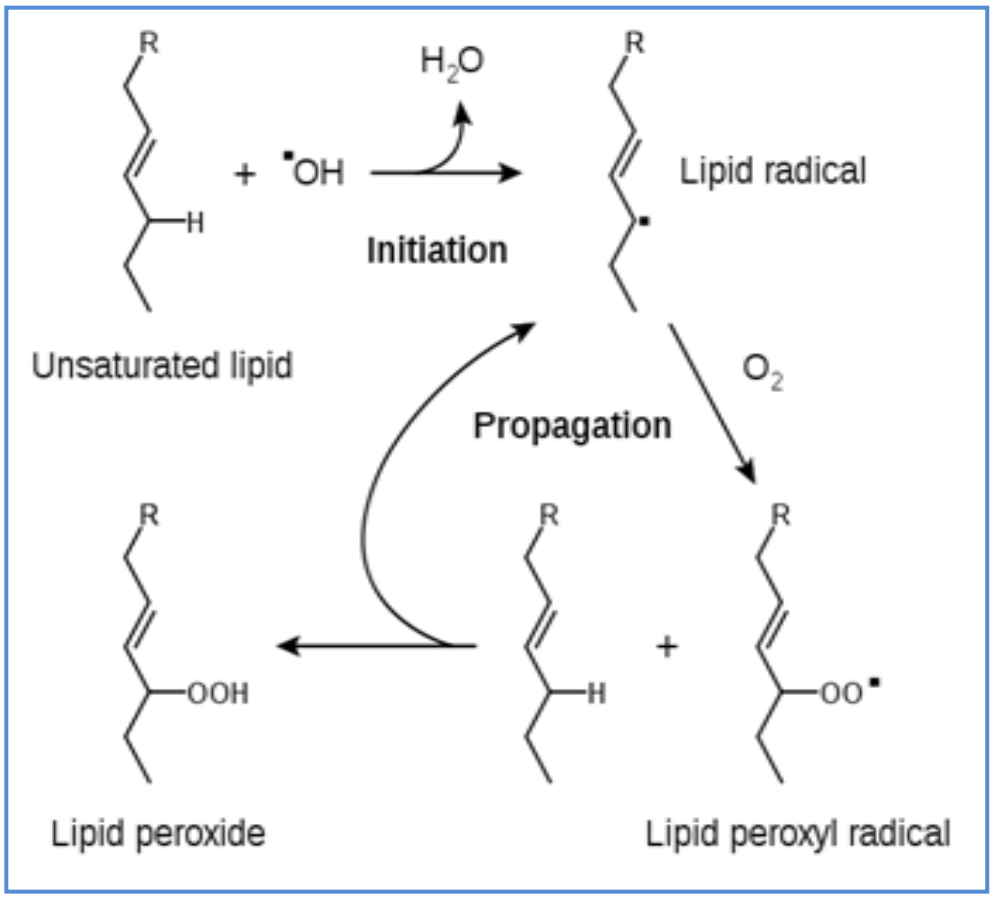


Figure (1-9): Radical chain reaction mechanism of lipid peroxidation(Tim Vickers *et al.*,2001).

Oxidative stress is defined as an "imbalance between oxidants and antioxidants in valid of the oxidants, potentially leading to damage" (Sies, 1991). Oxidative damage to cellular components weakens physiological functions. Reactive species can be ROS or reactive nitrogen species (RNS) (Sies,1991; Halliwell and Gutteridge,2007). Reactive oxygen species include superoxide (O_2•−), hydroxyl (•OH) and hydrogen peroxide (H_2O_2), while RNS are nitric oxide (NO), nitrogen dioxide (NO_2•−) and peroxy nitrite (OONO−) (Sies, 1991; Halliwell and Gutteridge, 2007). Oxidative DNA damage has been implicated as a cause of cancer (Huang, 2003), aging and neurodegenerative diseases such as Alzheimer's and Parkinson's (Drew and Leeuwenburgh, 2002 ; Markesbery and Lovell, 2006), cardiovascular diseases such as artherosclerosis (Steinberg,1997; Vokurkova and Touyz, 2007).

1.7. Antioxidants (an overview)

Antioxidants are organic molecules which can prevent or delay the progress of lipid oxidation. Recently, the interest in using antioxidants of natural origin in food has increased, because they also appear to be suitable antioxidants for the prevention of diseases associated with the process of LPO (Stahl, 2000; Valenzuela *et al.*,2003). There are several classifications of antioxidants, including endogenous antioxidants and exogenous antioxidant Table(1-1) (Jeppsson *et al.*, 2007).

Table(1-1): Both endogenous and exogenous antioxidants in organ(Jeppsson *et al.*,2007).

1-Endogenous Antioxidants:-
Bilirubin, Thiols, e.g., glutathione, NADPH and NADH, Ubiquinone (coenzyme Q10), Uric acid and Enzymes: – copper/zinc and manganese-dependent superoxide dismutase (SOD) – iron-dependent catalase – selenium-dependent glutathione peroxidase
Metal Binding Proteins
Albumin (copper), Ceruloplasmin (copper), Metallothionin (copper), Ferritin (iron), Myoglobin (iron) and Transferrin (iron)
2-Exogenous Antioxidant:-
Dietary Antioxidants
Vitamin C, Vitamin E, β-Carotene and other carotenoids , lipoic acid and Polyphenols, e.g., flavonoids

One of the main causes of food deterioration is oxidation, due to free radical production from its components, especially lipids. To prevent oxidation of foods, several synthetic antioxidants have been used widely by the food industry (Cubero *et al.*, 2002). However, scientific evidence that synthetic antioxidants produce toxic effects on consumers has led to the search of natural antioxidants sources. Generally, fruits and vegetables are the natural origins of antioxidant components such as flavonoids, phenolic acids, tannins, coumarins, lignans and their derivatives, and they show strong antioxidant activity (Potterat, 1997; Cao *et al.*, 1998).

Especially, the antioxidant activity of prunes is very high in comparison to the antioxidant activities of other fruits and vegetables on the basis of the oxygen radical absorbance capacity (ORAC) (Shin-Ichi *et al.*, 2003). The antioxidants comprise of a range of substances that play a role in protecting biological systems against the deleterious effects of oxidative processes on macromolecules, such as proteins, lipids, carbohydrates and DNA (Figure 1-10) (Argolo *et al.*, 2004; Tepe *et al.*,2005).

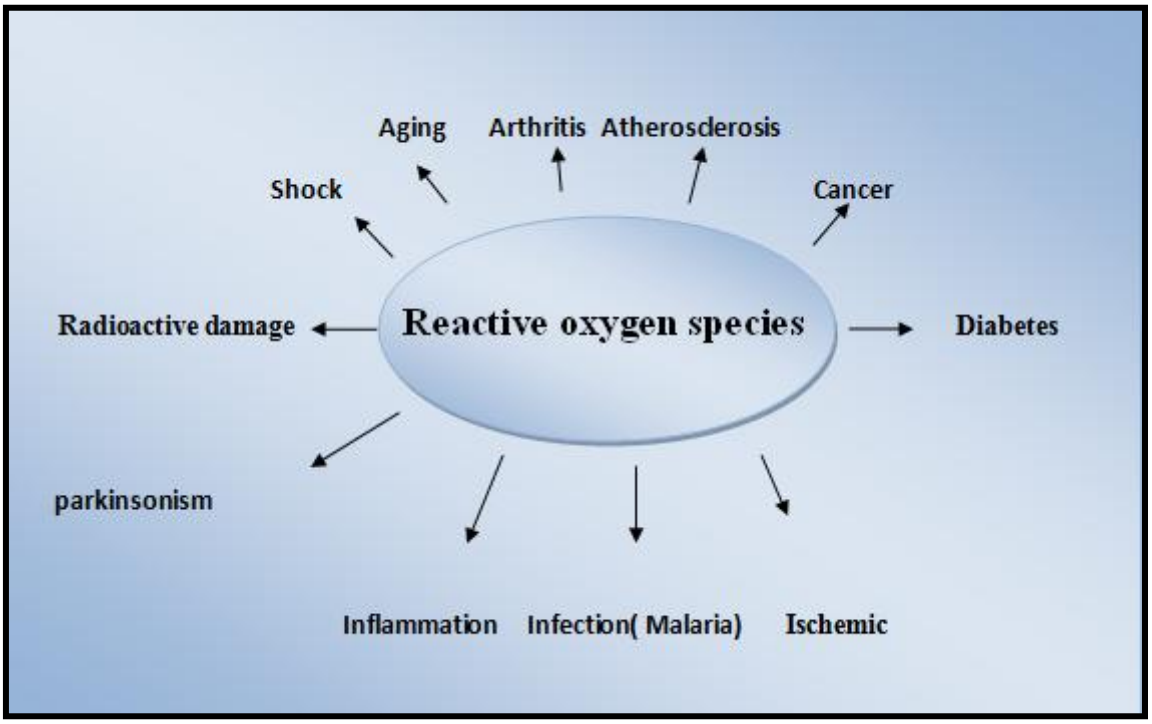


Figure (1-10): Diseases and damages caused by reactive oxygen species.

1.7.1. Ceruloplasmin(Cp)

Ceruloplasmin (Cp) is an α2-globulin that contains approximately 95% of total serum copper. Each molecule of Cp contains six to eight copper atoms, most of which are tightly bound while others are relatively free (Fleming and Gitlin, 1990; Fox *et al.*, 1995). The molecular weight of human ceruloplasmin is 34 KD (Gutteridge,2005). Several researchers suggested that Cp scavenge $O^{\cdot-}_2$ radicals via a dismutation reaction similar to SOD (Goldstein *et al.*, 1988; Plonka and Metodiewa, 1989). Cp increased in pregnancy and reduced in Wilson's disease (Bhagaven, 1978).

The following have all been reasonably suggestedas physiological functions of Cp (Pamela and Lilia,2002).

- Plasma Ferroxidase Activity: Iron Homeostasis
- Copper Transport and Storage
- Antioxidant Activity: Defence Against Oxidant Stress
- Ascorbate Oxidase Activity
- Degradation of Organic Substrates
- Pro-oxidant Activity

Cp exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of Fe^{2+}(ferrous iron) into Fe^{3+}(ferric iron), therefore assisting in its transport in the plasma in association with transferrin, which carry iron in the ferric state (Vinayak *et al.*,2010). Cp carries about 70% of the total copper in human plasma while albumin carries about 15% (Royle *et al.*, 1987).

The antioxidant effects of Cp could have important implications for various neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease in which iron deposition is known to occur (Vinayak *et al.*, 2010). Cp is also important in the control of membrane lipid oxidation, probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation (Keles *et al.*, 2001).

1.7.2. Albumin(Alb)

Albumin(Alb) is the most abundant plasma protein in human. It accounts for about 60% of the total plasma proteins, the liver is the primary site of albumin synthesis, and small amounts of albumin may be synthesized in the mammary glands and skeletal muscle (Doweiko and Nompleggi,1991; Rothschild *al.*,1972),its half-life in plasma is 20 days (Whitby *et al.*, 1984). Alb is a single polypeptide consisting of 585 amino acids with M.W. of approximately 66,248 Dalton (Thomas and McCarty, 1999; Wikipedia, 2007). One of physiological function to Alb is binding of free metal ions such as (copper and iron), where the plasma free copper ions bind tightly to serum Alb (Pickart and Thaler,1980).

1.8. Antibacterail Activity

Infectious disease caused by bacteria, fungi ,viruses and parasites are still a major risk to public health, despite of the great progress in human medicine bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the rise (Buvaneswari *et al.*, 2011). Medicinal plants is very important one in human health, it will act as an antibactericide activity against the bacterial pathogens (Soniya *et al.*, 2013).

Plants-derived products contain a great variety of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds (Cowan,

1999). These compounds possess numerous health related effects such as antibacterial (Bidlack *et al*., 2000). In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Nimri *et al*., 1999; Saxena and Sharma, 1999). Most bacteria can be divided into two groups according to a stain developed by the Danish physician Hans Christian Gram in 1884 (Volk *et al.,* 1991). This procedure is based on the fact that Gram-positive bacteria retain a crystal violet-iodine complex through decolorization with alcohol or acetone and Gram-negative bacteria do not (Benson, 2004). Gram-positive bacteria are generally more sensitive in the extracts than Gram negative bacteria (Lopez *et al*., 2005). A possible explanation for these observations may be in the significant differences in the outer layers of Gram-positive bacteria. The permeability of the cell wall of the Gram-negative is generally less efficient than Gram-positive may be because of the presence of the high level of phospholipids in the cell wall compared with Gram-positive bacteria (Nikaido, 1996). Smallest activities of the extracts against Gram-negative bacteria were observed (Holetz *et al.,* 2002; Aguiar *et al*., 2008). This can be justified because the Gram-negative bacteria present an external surrounding membrane that restricts the diffusion of hidrofobics compounds through the lipopolysaccharide of cellular membrane. Besides, the periplasmatic space contains enzymes that can prevent strange molecules from introducing from outside (Duffy and Power, 2001; Laciar *et al*., 2009).

1.8.1. Antibacterial Activity of Polyphenols

The several studies have proved a correlation between antibacterial activity and chemical composition: (alkaloid, saponin, phenol)(Akgul and Gulshen, 2005; Doughari and Manzara, 2008).The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Bhengraj *et al*., 2008 ; Vuuren and Naidoo, 2010). Flavonoids and phenolic compounds act as antibacterial agent against many pathogenic bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi* and *Escherichia coli* (Kamal *et al*., 2010). Polyphenols antimicrobial activity found in plant foods and medicine plants has been widely investigated against a wide range of microorganisms (Daglia, 2012).

Phenolic acids with a variety of bio-activities, including antimicrobial, are widely distributed in the plant kingdom (Jiang *et al*., 2005; Balasundram *et al*., 2006). The phenolic acids, which can inhibit pathogens growth and have little toxicity to host cells are also promising candidates for developing new antimicrobial drugs. Consequently, there is growing interest in developing many plant-derived drugs with multiple biological functions to use for the treatment of various infectious diseases (Parekh *et al*., 2005). Members of the Rosaceae families are also rich in polyphenolic compounds with antimicrobial activity (Jiang, 2009).

1.9. Aim of the Study:

The present study is undertaken to assess the effect of polyphenolic extracts (both PDR and PDY extract) in the treatment of hyperlipidemia as bellow:

1. Extraction of polyphenols from *Prunus domestica* red (PDR) and *Prunus domestica* yellow (PDY) wall nuts.

2. Identification of polyphenols containing in each extract.

3. Determination of polyphenols containing in each extract.

4. Investigation of the acute toxicity of both extracts *in vivo*.

5. Investigation of the boody weight of both extracts *in vivo.*

5. Investigation of anti-hyperlipidemia of both extracts *in vivo*.

6. Investigation of the antioxidant activity of both extracts *in vivo*.

7. Investigation of antibacterial activity of both extracts *in vitro.*

2. Materials & Methods

2.1. Materials

2.1.1. Chemicals

The study used specific chemicals which are shown with their suppliers in Table (2 – 1).

Table (2 – 1): chemicals and their suppliers.

No.	Material	Supplied company
1.	Acetic acid	BDH(England)
2.	Acetic acid anhydrate	BDH(England)
3.	Ammonia	G.C.C(U.K)
4.	Albumin Kit	Biolabo (France)
5.	Ciprodar	ABI Chemicals, GmbH(Germany)
6.	Chloroform	BDH(England)
7.	Cholesterol	BDH(England)
8.	Cholesterol Kit	Spinreact, Spain
9.	Copper sulfate	BDH(England)
10.	Dichloromethane	BDH(England)
11.	Dimethyl sulfoxide	G.C.C(U.K)

12.	Ether	BDH(England)
13.	Glacial acetic acid	BDH(England)
14.	Ferric chloride	BDH(England)
15.	Filter papers Whattman No. 1	TEAR(India)
16.	Hydrochloric acid	BDH(England)
17.	HDL Kit	Biolabo (France)
18.	Iodide	Fluka(Switzerland)
19.	Lead acetate hydrate	BDH(England)
20.	HPLC n-methanol	BDH(England)
21.	Mercury chloride unhydrate	BDH(England)
22.	Mueller Hintom Agar	Himedia(India)
23.	n-hexane	BDH(England)
24.	n-propanol	BDH(England)
25.	Methanol	BDH(England)
26.	Para pheneline di amine(PPD)	BDH(England)
27.	Potassium citrate	BDH(England)
28.	*Prunus domestica L.* nuts	
29.	Potassium hydroxide	BDH(England)

30.	Potassium iodide	BDH(England)
31.	Sodium acetate	BDH(England)
32.	Sodium azide	Reidledehain(Germany)
33.	Sodium carbonate unhydrate	BDH(England)
34.	Sodium chloride	BDH(England)
35.	Sodium hydroxide	BDH(England)
36.	Sulfuric acid	G.C.C(U.K)
37.	Thio barbituric acid (TBA)	BDH(England)
38.	Trichloro acetic acid (TCA)	BDH(England)
39.	Triglyceride Kit	Biolabo(France)
40.	α-naphthol	BDH(England)

2.1.2. Equipments and Apparatus

Table (2 –2) explained the equipments and apparatus which are used in this study.

Table (2 –2): The equipments and their manufactures.

No.	Equipments	Supplied company
1.	Animals balance_Stanton 461	(Germany)
2.	Autoclave	YX-2803, (Germany)
3.	Centrifuge	Universal 16 A, (Germany)
4.	Digital vernier	(China)
5.	HPLC	Shimadzu 10 AVP. (Japan)
6.	Incubator	Memmert , (Germany)
7.	Mixer	(China)
8.	Oven	Gallen Kamp, (England)
9.	Rotary evaporator	Buchi (switzland)
10.	Sensitive balance	Denver(Germany)
11.	UV/VIS spectrophotometer	T 60,PG Instrumente Ltd, (Germany)
12.	Vacuum pump	PG Instruments Ltd, (England)
13.	Water bath	Thomas Sc.Ap. (England)

2.1.3. Plants Collection

Prunus domestica L. wall nuts were collected in July 2012 from local markets in Nasiriyah city at Iraq, then it was authenticated and specimen of plant was classified in biological department-college of science at university of Thi -Qar in Iraq by Asst. prof. Hayder Radhi . The nuts were cleaned , washed by distilled water, dried at room temperature for four weeks, ground as powder and kept in dark glass containers for further use.

2.1.4. Experimental Animal

Twenty four healthy female rats *(Rattus norvegicus*) weighing (85-110 g) of 8-9 weeks old were used in the present study. Animals were housed in the animal house of Biology Dept. Science College, Thi-Qar University. Experiments were achieved between May-2013 & June-2013. Animals were housed in iron boxes bedded with wooden chips. During the experimental period six animals were kept in each box and they were housed under standard laboratory conditions (12h light: 12h dark photoperiod (LD) at 22 ± 2 Cº and relative humidity 45-55%. Animals were fed on standard rat pellet and tap water *ad libitum*.

2.2. Methods

2.2.1. Extraction of Polyphenols

(500 g) of the dry powder wall nuts were defatted by washing five times with n-hexane(1L) at (60°C) , then it was macerated with (800mL) of acetic acid (2% v/v), the mixture were placed in conical flask volume (2000mL) and put in water bath (60°C) for 8 hrs, then the extraction process done by reflex condenser. The mixture was heated at 50°C (water bath) for 15 min and left to cool.

The suspension was filtered by Buchner funnel by Whatman No.1 filter paper and by the use of vacuum pump. The precipitate was canceled and the filtrate volume was measured. n-propanol was added in to filtrate with the same volume of filtrate. Then (NaCl) was added until to become solution super saturated. Then, it was evaporator by using rotary evaporator until drying (Gayon, 1972).

2.2.2. Qualitative Tests of Some Plants Extracts:

2.2.2.1. Detection of Phenolic Compounds in Plants Extracts:

Filter paper (Witman No.1) was wetted in (2mL) of extract and some drops of ferric chloride (1%) was added to it after that it was exposed to ammonia vapor, appearance blue or green color indicates presence of phenolic compounds (Gayon, 1972).

2.2.2.2. Detection of Flavonoids in Plants Extracts:

(1mL) of alcohol potassium hydroxide reagent (Ethanolic KOH (5N)) was added to (1mL) of extract , appearance of yellow precipitate indicates presence of flavonoids (Harbone, 1984) .

2.2.2.3.: Detection of Carbohydrates in Plants Extracts:

Molisch Reagent: (0.5mL) of α-naphthol (dissolve 1 gm of α-naphthol in 25 mL of methanol) was added to (1mL) of extract in test tube with shaking, there after concentrated sulfuric acid was added slowly with slope , appearance violet ring indicates presence of carbohydrates (Hawk *et al.*,1954).

2.2.2.4. Detection of Alkaloids in Plants Extracts:

Wagner reagent: it was prepared by dissolving of (2g) of iodine and (6g) of potassium iodide in (100mL) of distilled water.

Some drops of this reagent was added to (1mL) of extract , appearance reddish brown precipitate indicates presence of alkaloids (Harbone, 1984).

2.2.2.5. Detection of Tannins in Plants Extracts:

1. Lead acetate hydrate (1%) reagent:

(1mL) of Lead acetate hydrate was added to (1mL) of extracts, appearance of white or light brown precipitate indicates presence of tannins.

2. Ferric chloride (1%) reagent:

Some drops of this reagent was added to (1mL) of extract, appearance bluish green color indicates presence of tannins (Harbone, 1984).

2.2.2.6. Detection of Saponins in Plants Extracts:

(1mL) of extract was added to (1mL) of HgCl2 (5%),appearance white precipitate indicated presence of Saponins (Harbone, 1984).

2.2.2.7. Detection of Glycosides in Plants Extracts:

Benedict reagent : it was prepared by dissolving (173 g) of potassium citrate and (100 g) of sodium carbonate anhydrate in (800mL) of distilled water with heating (100C°), after that this solution was cooled and filtered. Copper sulfate[it was prepared by dissolving (17.30 g) of copper sulfate in (100mL) distilled water and completed the volume to (1L)] . Then the two prepared solutions were mixed with (1:1) volume ratio.

1.Before glycoside dissociation:

(1mL) of Benedict reagent was added in test tube contains (1mL) of extract and heated in boiling water bath to (10min),appearance of red or brown precipitate indicates presence of reduced saccharides.

2. After glycoside dissociation:

Several drops of HCl was added to (5mL) of extracts and heated in water bath in (40°C) at (25 min) to cleavage the glycoside bonds, the solution of adjusted by added sodium hydroxide (2M) , there after equal volume from Benedict reagent was added to extract and heated at (10 min), appearance of red or brown precipitate more than the precipitate that appearance in first test indicates presence of glycosides(Al-khazrajy,1991).

2.2.2.8. Detection of Triterpinoids in Plants Extracts:

(1mL) of concentration sulfuric acid was added to (1mL) of extract that was dissolved in chloroform, appearance of purple red color indicates presence of triterpinoids (Harbone, 1984).

2.2.2.9. Detection of Triterpins and Setrols in Plants Extracts:

(10) drops of acetic acid anhydrate was added to(2mL) of solution of extracts in test tube thereafter two drops of Con.H_2SO_4 was added, this solution was mixed carefully. If color of solution becomes dark blue and gradually converts to green , this indicates presence of triterpines and setrols (Harbone, 1984).

2.2.3. Investigation of Polyphenolic Extract by UV-VIS. (Spectrophotometer):

The absorption spectra of plant constituents was measured in dilute solution against a blank solvent by using an automatic recording spectrophotometer. The solvent was used for UV spectroscopy is water : the method was performed by using polyphenolic extract. The sample solutions absorbance (A), was recorded by measuring the range scan from 190nm to 800nm on a double beam UV-VIS spectrophotometer. (Ikbal,2004).

2.2.4. Investigation of Polyphenolic Extract by HPLC Technique

The extract were analyzed on FLC (Fast Liquid Chromatography) column, C-18, 3 µm particle size (50×4.6 mm ID), mobile phase 40:60, 0.01% (methanol : water : acetic acid, V/V) using linear gradients from 0-100%B in 10 minutes, detection UV set at 264 nm ,flow rate 1.4 mL/min the sequences of the eluted material of the standard were as follow, each standard was 25 µg/mL (Ikbal,2004). 10 g of each sample was weighed, then dissolved in 10 mL HPLC methanol, the sample shaking and agitated in ultrasonic bath for 10 minutes, then concentrated by evaporating the solvent with stream of liquid N_2 until reach nearly 0.5 ml , then 20 µL were injected on HPLC column . The concentration of each compound were quantitatively determined by comparison the area under peak of the standard with that of the samples(Ikbal,2004).

Calculation:

$$\text{Concentration of sample } \mu g/mL = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. Standard} \times \text{dilution Factor}$$

The separation occurred on liquid chromatography Shimadzu 10 AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer.

2.2.5. Acute Toxicity Study for PDR and PDY Polyphenolic Extracts

Administration of Laboratory Animals:

Experimental animals were divided into four groups (6 rats in each group) upon the following designed:

- **Group A**: control (normal) that were treated with (0.2mL) DMSO
- **Group B**: Rats were treated with (25 mg/kg) of PDR polyphenolic extracts.
- **Group C**: Rats were treated with (50 mg/kg) of PDR polyphenolic extracts.
- **Group D**: Rats were treated with (100 mg/kg) of PDR polyphenolic extracts.

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD/OCDE) guidelines. The animals were fasted for 4hrs with free access to water only . Polyphenolic extract was administered orally as above doses and mortality if any was observed for 3 days(Ecobichon,1997). The animals were let for two weeks without treatment after that the same groups of investigation of PDR extract toxicity were treated with PDY

polyphenolic extract and in the same steps and concentrations that were used previously(Ecobichon,1997).

2.2.6. Effects of PDR and PDY Polyphenolic Extracts on Boody Weight

- Method of Food Preparing (High Cholesterol Diet)

5% of high cholesterol diet prepared from 50 g of cholesterol dissolved in 200 g of olive oil and heated in a water bath, and after soluble cholesterol in the oil were added to 1 kg of feed, then was cut into small pieces fit with the size of the holes in the lid iron to boxes, to facilitate the process taken up by rats (Cook *et al.*, 1950).

-Laboratory Animals:

Experimental animals were divided into four groups (6 rats in each group) upon the following designed:

- **Group A**: control (normal) that were treated daily with (0.2 mL DMSO)

- **Group B**: Rats were treated with daily high cholesterol diet for 30 days (Cook *et al.*, 1950) .

- **Group C**: Rats were daily treated with (25 mg/kg). of PDR polyphenolic extract besides high cholesterol diet for 30 days. (2.37 mg of extract dissolved in 0.2 mLof DMSO, and the rats were given daily oral)

- **Group D**: Rats were daily treated with (25 mg/kg). of PYR polyphenolic extract besides high cholesterol diet for 30 days. (2.37 mg of extract dissolved in 0.2 mL of DMSO, and the rats were given daily oral)

Measured the weights of animals in the first and last day of the experiment using animals balance_stanton461, then calculate the difference was the weight of the animals.

2.2.7. Biochemical Parameters

-Laboratory Animals:

Experimental animals were divided into four groups (6 rats in each group) as in (2.2.6).

Blood Samples-

5mL of blood were drawn from each animal of experimental groups, by heart puncture method after 12 hours fast. Using 60 gauge syringes, the sample was transferred into clean tube, left at room temperature for 15 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in a clean tube in the refrigerator at 2-8ºC until the time of assay. Several considerable methods were used to measure the studied parameters. It is notable that all measurements were duplicated for each sample.

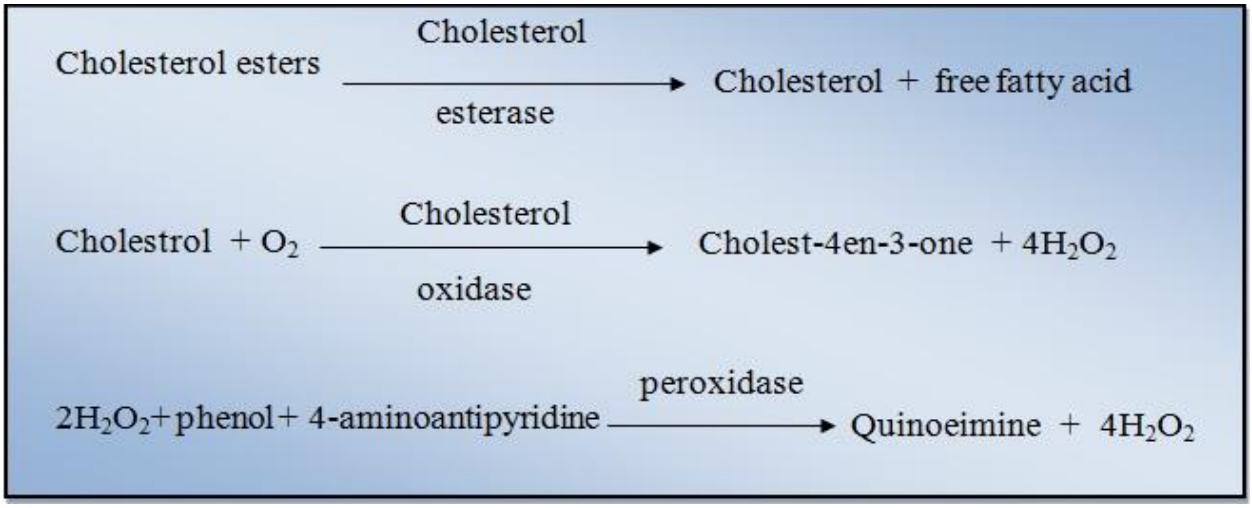
2.2.7.1. Determination of Serum Lipid Profile

2.2.7.1.1. Determination of Serum Total Cholesterol

The used reagents were supplied by Biolabo (France) , and Serum total cholesterol was measured according to (Allan and Dawson ,1979**)**.

Principle:

Enzymatic method described by (Allian *et al.*,1974) which the reaction scheme is as follows :-



Reagents composition:

Reagent	**Composition**	
Reagent 1 Buffer	Phosphate buffer Chloro-4-phenol Sodium cholate Triton x 100 Preservative	100 mmol/L 5 mmol/L 2.3 mmol/L 1.5 mmol/L
Reagent 2 enzymes	Cholesterol oxidase Cholesterol estrase Peroxydase	≥ 100 IU/L ≥ 170 IU/L ≥ 1200 IU/L

	4-aminoantipyrine	0.25 IU/L
	PEG 6000	167 µmol/L

Manual procedure:

1. Reagent $_1$ and reagent $_2$ were mixed to form working solution .

2. Reagent and sample were left stand at room temperature.

	Blank	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
D.W	10µL	……..	……..
Standard	……..	10µL	……..
Sample	……..	……..	10µL

Wait for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculation :

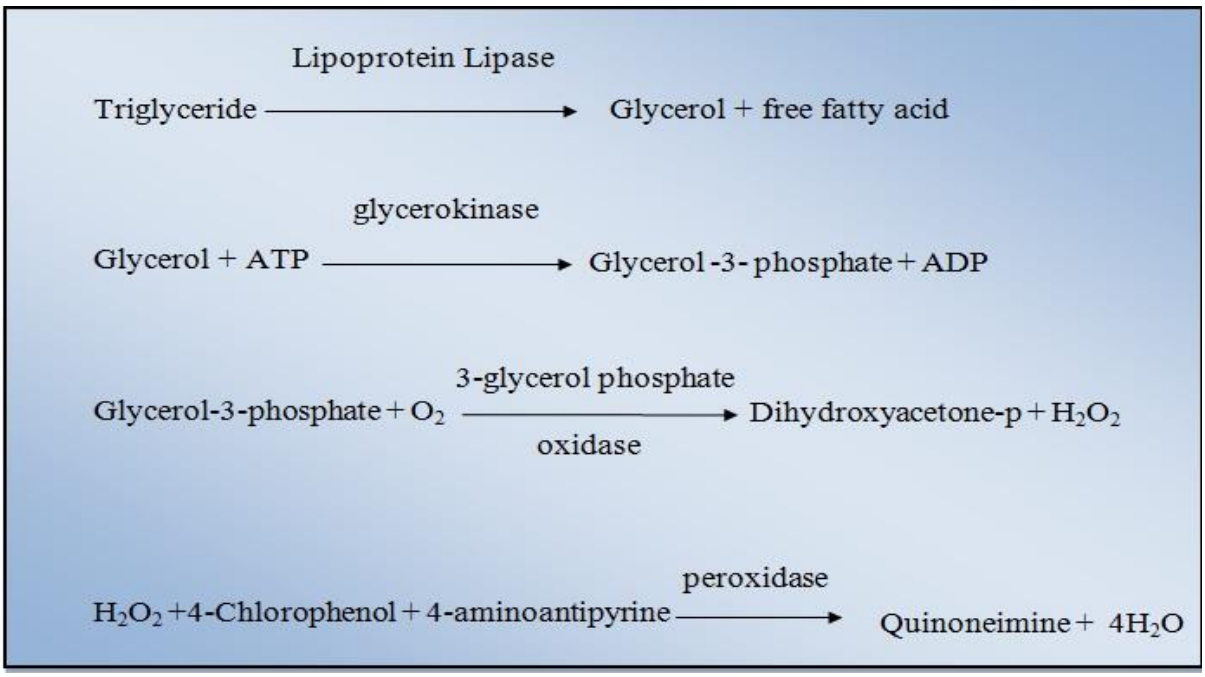
$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \text{ X } 200 \text{ (standard concentration)}$$

2.2.7.1.2. Determination of Serum Triglyceride Concentration

The used reagents were supplied by Biolabo (France) ,and Serum TG was measured according to (Tietz *et al.*, 1999).

Principle:

Fossati and Prencipe method associated with Trinder reaction scheme is as follows (Trinder,1969 ; Fossati and Prencipe ,1982) :



Reagents composition:

Reagent	Composition		
Reagent1 Buffer	Pipes	100	mmol/L
	Magnesium chloride	9.8	mmol/L
	Chloro-4-phrnol	3.5	mmol/L

Reagent2 enzymes	Lipase	≥ 1000 IU/L
	Peroxidase	≥ 1700 IU/L
	Glycerol-3-phosphate oxidase	≥ 3000 IU/L
	Glycerol kinase	≥ 660 IU/L
	4-aminoantipyrine	0.5 mmol/L
	ATP	1.3 mmol/L
Reagent3 standard	Glycerol	2.28 mmol/L (200 mg/dl)

Manual procedure:

1. Reagent $_1$ and reagent $_2$ to were mixed form working solution .

2. Reagent and sample were left stand at room temperature.

	Blank	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
D.W	10 µL	……..	……..
Standard	……..	10 µL	……..
Sample	……..	……..	10 µL

Wait for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculation :

The result was calculated as follows:

$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \text{ X } 200 \text{ (standard concentration)}$$

2.2.7.1.3. Determination of Serum HDL Concentration

The used reagents were supplied by Biolabo (France) , and serum HDL was measured according to (Lopes-Virella,1977) .

Principle:

According to (Tietz ,1999) , low density lipoproteins (LDL) , very low density lipoproteins (VLDL) and chylomicrons from the sample are precipitated by phosphotungstic acid and magnesium chloride . HDL obtained in supernatant after centrifugation is then measured with total cholesterol reagent.

Reagents composition:

Reagent	Composition	
Reagent 1 precipitant	Phosphotungstic acid	13.9 mmol/L
	Magnesium chloride	570 mmol/L
Reagent 2 standard	Cholesterol	2.58 mmol/L

Manual procedure:

Sample preparation (without treating standard) :

Pipetted in centrifuge	Micromethod
Sample	500 µL
Precipitant	50 µL

Mixed vigorously , Wait for 10 minutes at room temperature , centrifuged for 15 minutes at 3500 – 4000 RPM , aspirate the supernatant. Reagent and supernatant was left stand at room temperature .

	Blank	Standard	Assay

Reagent2(Cholesterol assay)	1 mL	1 mL	1 mL
D.W	25 µL	……..	……..
Standard	……..	25 µL	……..
Supernatant (HDL)	……..	……..	25 µL

Wait for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculate the result as follows:

$$\text{Result(mg/dl)} = \frac{\text{Abs (assay)}}{\text{Abs (standard)}} \text{ X standard concentration X 1.1}$$

2.2.7.1.4. Calculation of LDL and VLDL

According to (Friedwald *et al*.,1972) , LDL and VLDL concentrations were measured as follows :

LDL(mg/dl) = Total cholesterol – (HDL + VLDL)

VLDL(mg/dl) = serum TG /5

2.2.7.1.5. Calculation of Atherogenic Index Plasma

Atherogenic Index was calculated by the following equation:-

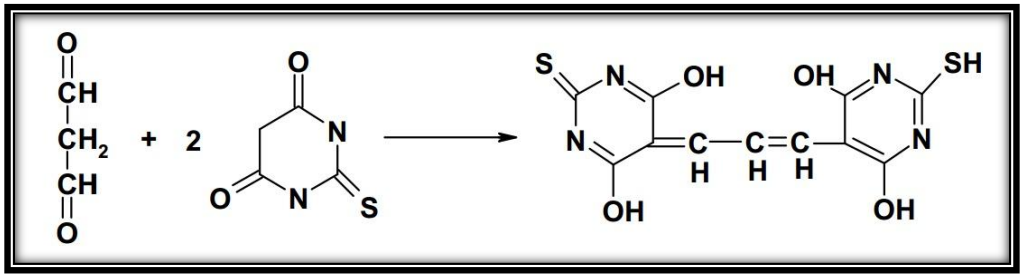
Atherogenic Index = LDL / HDL

2.2.7.2. Determination of Oxidative Stress Parameters

(2-2-7-2-1): Determination of Serum Malondialdehyde Level (MDA)

Principle:-

LPO was determined by using the thiobarbituric acid method. In this method, MDA formed from the breakdown of polyunsaturated fatty acids was identified as the product of LPO that react with thiobarbituric acid (TBA), in coexisting trichloro acetic acid (TCA), to give a red chromophore absorbing at 535 nm (Fong *et al.*, 1973). MDA concentrations were calculated, using the extinction coefficient of MDA ($\mathcal{E}_{MDA}$) equal to 1.56 x10^5 L. mol^{-1}. cm^{-1} (Wills,1969).



Procedure:-

	Sample	Blank
Plasma	0.5 mL	……..
TCA	2.5 mL	2.5 mL
TBA	1 mL	1 mL
D.W	1 mL	1 mL
Good mixing was important each addition. The mixture boiled by water bath for 30 min		
Butanol	4ml	4ml
Centrifuged at 2000 rpm for at least 5 min and the absorbance of clear supernatant is read at 535 nm.		

The concentration of plasma MDA computed according to the following equation:-

$$Plasma\ MDA\ (nmol/ml) = \frac{A\ sample - A\ blank}{\varepsilon_{MDA}} = \frac{\Delta A}{0.156} \times \text{dilution Factor}$$

2.2.7.3. Determination of Antioxidant Parameters

2.2.7.3.1. Determination of Serum Ceruloplasmin Concentration

Principle:-

Ceruloplasmin concentration in serum was measured by Menden *et al.*,(1977). It is based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidize form. The reaction is followed photometrically and the blank value is determined after inhibition of the enzyme with sodium azide at ($0^{o}C$).

$$\textit{Reduced PPD} + 2H^{+} + \tfrac{1}{2} O_2 \leftrightarrow \textit{Oxidized PPD} + H_2O$$

(colorless) ***(blue)***

A mixture of serum, substrate and acetate buffer at pH = 6.0 is incubate at 37°C for 15 min. The reaction is stopped by the addition of sodium azide, and the absorbance of the purple color formed (*Oxidized PPD*) in the diluted test mixture is read at 525 nm against blank solution. The corrected absorbance is directly related to the concentration of Cp.

Reagents :-

● ***Substrate solution :-***

50 mg of PPD was dissolved in 5 mL(4 mL of D.W and 1 mL of glacial acetic acid) . In another container 8.15gm sodium acetate trihydrate was dissolved in 30 mL of D.W then added to the first solution, mixed and completed the volume to 50 mL with D.W. This solution is stable for three hours and is kept refrigerated in a dark bottle(Menden *et al.*,1977)

● ***Inactivating solution :-***

100 mg of sodium azide was dissolved in (500 mL) of D.W then was kept cold in refrigerator prior to use, this solution must be prepared every week.

Procedure :-

- 1 mL of substrate was pipetted into test tubes, incubated at 37°C for 1 min.
- 0.1 mL of plasma was added, incubated at 37°C for 15 min, then tubes were removed and placed in iced-bath for 30 min.
- 5 mL of cold inactivating solution was added, mixed and the temperature was brought to 25°C in water bath.
- Blank was prepared by combining the substrate, inactivating solution, and 0.1 mL of D.W, then the mixture was incubated as above.

The absorbance of test and blank was read in spectrophotometer at 525nm.

Calculations:-

$$\text{Serum Cp (g/L)} = \frac{A_{test} - A_{blank}}{\varepsilon_{Cp}} \times 10 = \frac{\Delta A \times 10}{0.68}$$

ε_{Cp} = The extinction coefficient of Cp equal to 0.68

2.2.7.3.2. Determination of Serum Albumin Concentration

Principle:-

The concentration of albumin in serum sample was measured by the bromcresol green a colorimetric method.

Albumin in the presence of bromcresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The absorption of the colour formed is proportional to the albumin concentration in the sample(Webster,1974).

Reagents:-

Reagent	Contents	Concentrations of Solutions
R1 (Bromocresol Green)	Succinic acid	83mmol/L
	Bromocresol green	167µmol/L
	Sodium hydroxide	50mmol/L
	Polyoxyethylene monolaurylether	1gm/L
R2(standard)	Bovine albumin	750µmol/L

Procedure:-

The following reagents were pipetted into a curette.

Mixed and incubated for 10 min at room temperature (15-25ºC) read the absorbance (A) of the samples and standard against the blank at (630)nm.

The colour is stable 1 hour at room temperature.

Calculations:-

$$\text{Serum Alb (g / L)} = \frac{A_{sample}}{A_{standard}} \times 50$$

	Blank	Standard	Assay
Reagent	2.5mL	2.5mL	2.5mL
D.W	5µL	……..	……
Sample	……	……..	5µL
standard	……	5µL	……

2.2.8. Investigation of Antibacterial Activity of Polyphenolic Extract

2.2.8.1. Culture Media

Muller Hinton Agar was supplied from Himedia company (india), it was used as a culture medium and was prepared depending on information determining by manufacturing company .

2.2.8.2. Pathogenic Bacterial Strains

Staphylococcus aureus and *Bacillus subtilus* (positive to Gram stain), *Escherichia coli* and *Pseudomonas aeruginosa* (negative to Gram stain) were used as pathogenic bacteria strains and they were identified in Biology Dept. College of Science, Thi- Qar University.

2.2.8.3. Assessment of Antibacterial Activity of Extracts

Bacteria suspension of each tested bacteria (107 CFU/mL) was spread onto the surface of Muller-Hinton agar plates. Eight mm cork borer was used to punch wells into the plates and 100 µL of each extract dissolved in DMSO (10 mg/mL) as well as were applied to each well. The plates were incubated for 18 h at 37°C. The inhibition zones diameter for each extract was measured and the phenol coefficient was calculated (NCCLS, 1999).

2.2.9. Statistical Analysis

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA was used to compare parameters in different studied groups. P-values ($P < 0.01$) were considered statistically significant.

Results & Discussion

3.1. Plant Extracts

As shown in table (3-1), the weight of dry powder material of *Prunus domestica L.* nuts was (500g). The product dry powder PDR polyphenolic extract were (3.1g) and PDY polyphenolic extract were (2.9g) .

Table (3-1): Percentage of extracts in wall nuts

Material	PDR extract	PDY extract
Wall nuts Weight	500 g	500 g
Extract weight	3.1 g	2.9 g
Percentage	0.62%	0.58%

3.2. Qualitative Tests of Some Active Compounds in Plant Extracts

The results of qualitative tests were shown in table(3-2) . Polyphenolic extract, it was found to contain each polyphenols, glycosides and carbohydrate. Whereas it does not contain , flavonoids, terpenoids , terpenes and setrols. This is due to the defatting step that was performed firstly with hexane solvent (Andersen and Markham, 2006). Many phenolic compounds are normally present as glycosides in plants(Ani *et al.*, 2006).

Table (3-2): Qualitative tests of some active compounds in plant both polyphenolic extract

Active principle	Test	PDR Polyphenolic Extract	PDY Polyphenolic Extract
Polyphenols	$FeCl_3$(1%)+ ammonia vapor	+	+
Flavonoids	Alcohol KOH	-	-
Carbohydrates	Molisch	+	+
Alkaloids	Wagner	-	-
Tannins	$PbCl_3$ (1%)	-	-
	$FeCL_3$ (1%)	-	-
Saponins	$HgCL_2$ (1%)	-	-
Glycosides	Before analysis	+	+
	After analysis	+	+
Triterpenoids	$CH_3Cl+H_2SO_4$	-	-
Triterpenes and setrols	Liebermann – Burchard	-	-

3.3. UV-Visible Spectra

The UV-Visible analysis of the polyphenolic extracts were recorded at the range of 190-800 nm, as shown in figures (3-1) and (3-2).

Figures (3-1) and (3-2) illustrates the UV-Vis spectra for PDR and PDY polyphenolic extracts with λ_{max} at (202nm) and another peak at (284 nm) (280nm) respectively, the spectrum of UV-Vis of polyphenols shows intensity and very clear absorption peak at (202nm) for π-π^* electronic transitions due to presence of multi double bounds in aromatic rings for these compounds. The other peak at (284 and 280 nm) which was low intensity appears in wave length longer from the peaks due to n- π^* electronic transitions due to presence of non bonding electrons for oxygen atoms in these compounds.

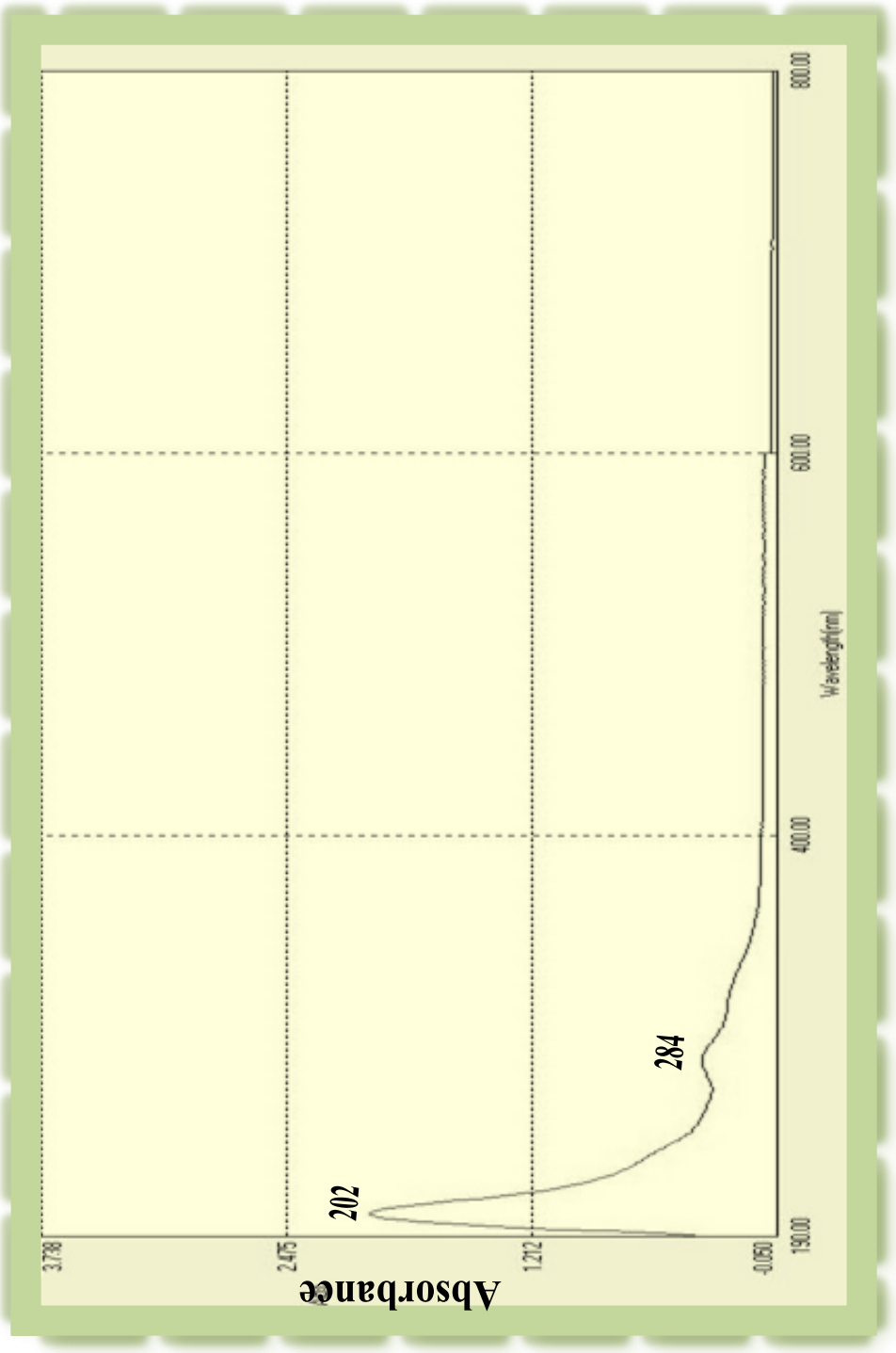


Figure (3-1):Absorption spectrum of PDR polyphenolic extract by (UV-SCAN) in water.

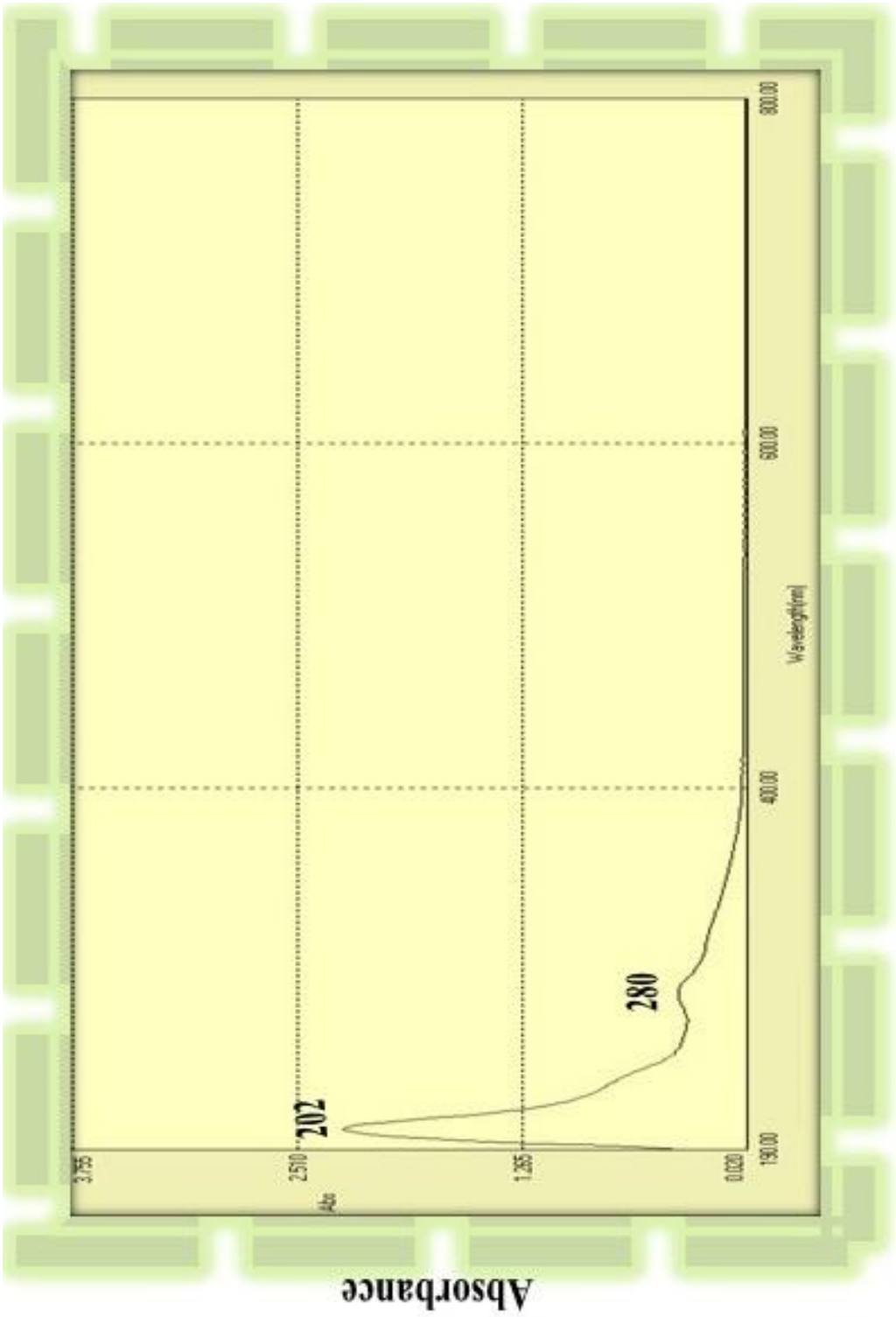


Figure (3-2):Absorption spectrum of PDY polyphenolic extract by (UV-SCAN) in water.

3.4. Identification of Plant Polyphenol Compounds by HPLC Technique

Figures (3-3),(3-4) and (3-5) showed that the retention time of sample agrees with the retention time of the standard for most contents in each extract, and the tables (3-3),(3-4) and (3-5) showed the retention time of standards and PDR , PDY polyphenolic extracts respectively.

In this study and from HPLC results it was found that extracts for PDR and PDY polyphenolic extracts contains some important compounds that include ***(*tannic acid, gallic acid, caffeic acid, vanillic acid, ferulic acid, amygdalin, chlorogenic acid*)*** as shown in figures (3-4) and (3-5) and the structures of these compounds were shown in table (3-6). The peaks of the mentioned chromatogram also pointed to presence of some unknown compounds that are thought represent derivatives of polyphenolic compounds. Donovan *et al.*, (1998) and Kimura *et al.*, (2008) shows that the phenolic compounds of prunes consist mainly of chlorogenic acid, neochlorogenic acid, caffeic acid, coumaric acid, rutin and proanthocyanidin.

High performance liquid chromatography (HPLC) is the most widely employed chromatographic technique in polyphenolic compounds analysis (Shylaja *et al.*, 2008). It has added a new dimension to the investigation of polyphenolic compounds in each extract, the ability to obtain both qualitative and accurate quantitative data in one operation and the great speed of analysis (Poucheret *et al.*, 2006). The extracts were analyzed to estimate their contents of polyphenol compounds. The identification of each compound was based on retention time in comparison with pure commercial standards (Shindalkar *et al.*, 2005). Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors (Stalikas, 2007).

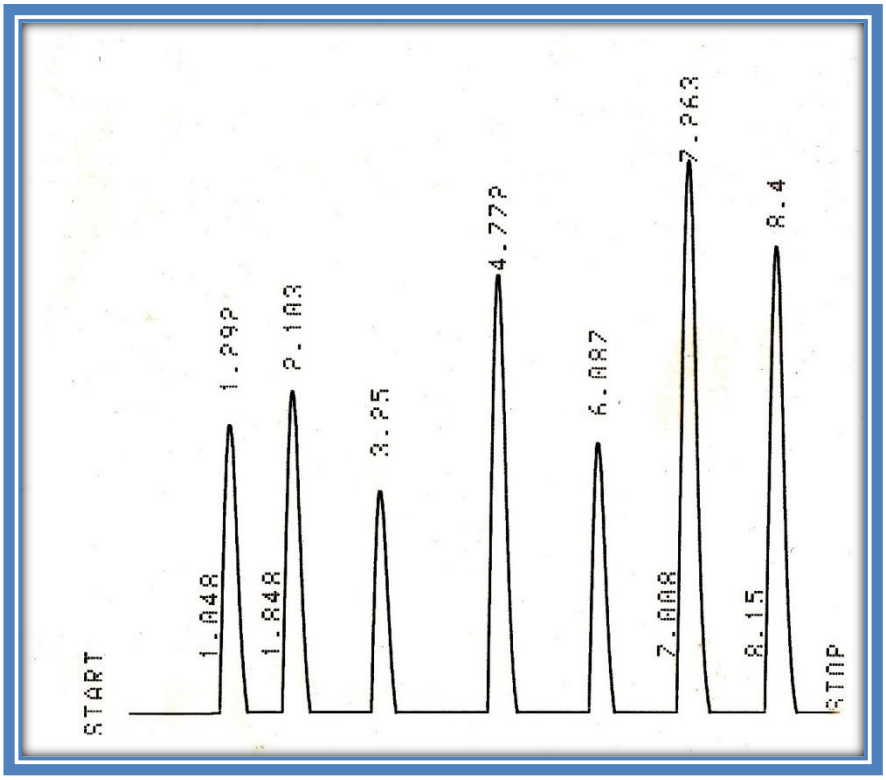
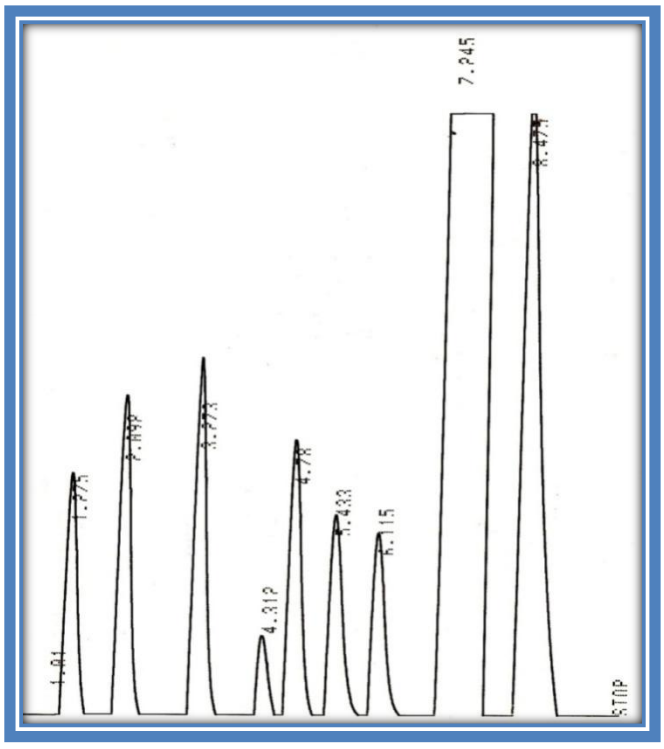


Figure (3-3) : HPLC Chromatogram of standard polyphenolic compounds

Table (3-3): Retention time of standard polyphenolic compounds

seq	Polyphenolic contents in the standard	Retention time (min)	Area
1	**Tannic acid**	**1.29**	**28145**
2	**Gallic acid**	**2.10**	**33825**
3	**Caffeic acid**	**3.25**	**18984**
4	**Vanillic acid**	**4.77**	**35799**
5	**Ferulic acid**	**6.08**	**31299**
6	**Amygdalin**	**7.26**	**48537**
7	**Chlorogenic acid**	**8.40**	**35543**



Figure(3-4): HPLC Chromatogram of PDR

Table (3-4): Concentration of polyphenolic compounds in PDR

seq	Polyphenolic contents in the extract	Retention time (min)	Area	Conc. of polyphenolic compounds µg/mL	polyphenolic compounds µg/gm
1	**Tannic acid**	**1.29**	**49466**	**131.81**	**0.0131**
2	**Gallic acid**	**2.10**	**75466**	**167.33**	**0.0168**
3	**Caffeic acid**	**3.25**	**83245**	**328.88**	**0.0329**
4	**Vanillic acid**	**4.77**	**63266**	**132.56**	**0.0133**
5	**Ferulic acid**	**6.08**	**81783**	**195.97**	**0.0196**
6	**Amygdalin**	**7.26**	**746118**	**1152.91**	**0.1152**
7	**Chlorogenic acid**	**8.40**	**171890**	**362.71**	**0.0363**

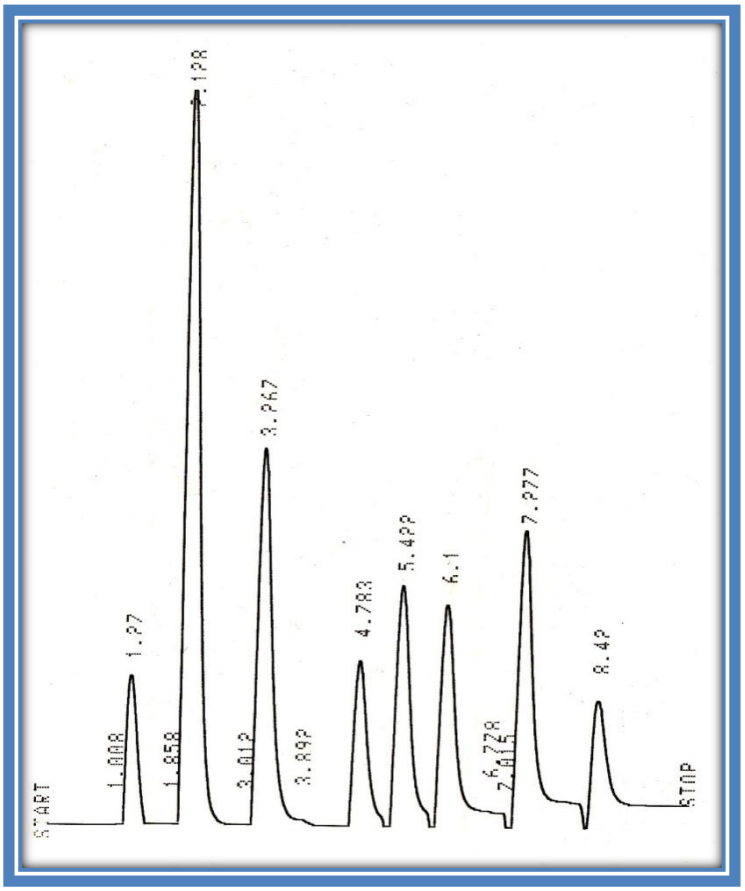
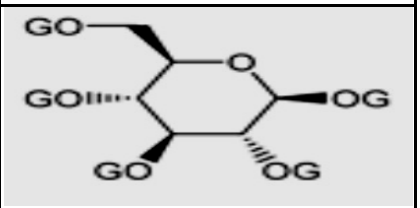
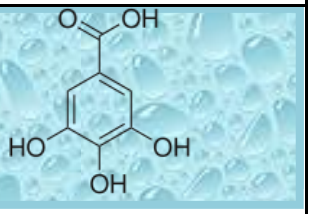
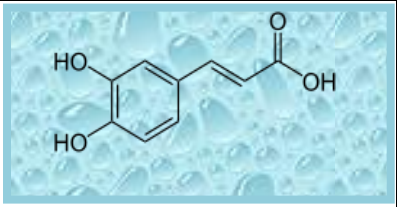
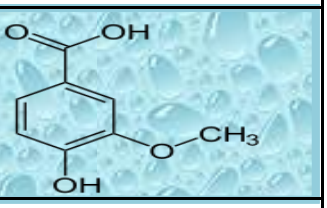
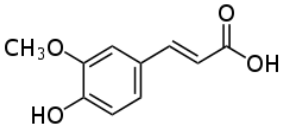


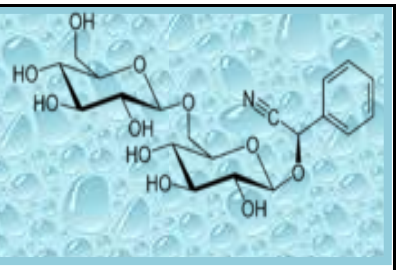
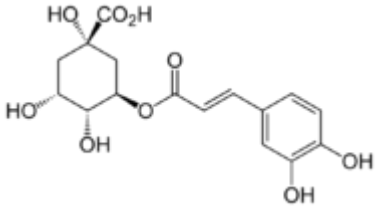
Figure (3-5): HPLC Chromatogram of PDY

Table (3-5): Concentration of polyphenolic compounds in PDY

seq	Polyphenolic contents in the extract	Retention time (min)	Area	Conc. of polyphenolic compounds µg/mL	polyphenolic compounds µg/gm
1	**Tannic acid**	**1.29**	**32518**	**86.65**	**0.0087**
2	**Gallic acid**	**2.10**	**106438**	**236.00**	**0.0236**
3	**Caffeic acid**	**3.25**	**49111**	**194.02**	**0.0194**
4	**Vanillic acid**	**4.77**	**22201**	**46.51**	**0.0047**
5	**Ferulic acid**	**6.08**	**32814**	**78.63**	**0.0079**
6	**Amygdalin**	**7.26**	**41608**	**64.29**	**0.0064**
7	**Chlorogenic acid**	**8.40**	**11311**	**23.86**	**0.0023**

Table (3-6): Names and structure of polyphenolic compounds in PDR and PDY

Communally name	Organizational name	Structure
Tannic acid	**3,5-dihydroxy-2-(3,4,5 trihydroxybenzoyl)oxy-6-[(3,4,5-trihydroxybenzoyl)oxymethyl]oxan3,4,5-trihydroxybenzoate**	
Gallic acid	**3,4,5-trihydroxybenzoic acid**	
Caffeic acid	**3-(3,4-Dihydroxyphenyl)-2-propenoic acid**	
Vanillic acid	**4-Hydroxy-3-methoxy benzoic acid**	
Ferulic acid	**3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid**	

Amygdalin	**[(6-O-β-glucopyranosyl-β-D-glucopyranosyl)oxy](phenyl)acetonitrile**	
Chlorogenic acid	**(1*S*,3*R*,4*R*,5*R*)-3-{[(2*Z*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid**	

3.5. Acute Toxicity Study

These results indicated that, the doses (25,50 and 100 mg/kg) of PDR and PDY polyphenolic extracts employed for acute oral toxicity studies were found to be non-toxic, no abnormal symptoms and no death of the rats was observed as shown in tables (3-7) and (3-8). This result is similar to the result of OECD (2001) which reported that polyphenolic and flavonoidic extracts can be classified as non toxic since the limited dose of an acute toxicity is generally considered to be 5.0 mg/kg B.W (Assam *et al.*, 2010). Polyphenolic extract did not show any untoward effects on behavioral response, normal reflexes and so on, after the daily treatment (150 mg/kg and 300 mg/kg) for 14 days. According to OECD guidelines for acute oral toxicity, an LD_{50} dose of 2000 mg/kg and above is characterized as unclassified and hence the drug is found to be safe (Chidambaram *et al.*, 2007).

Table (3-7): Acute toxicity effect of PDR polyphenolic extract

Groups	Number of rats	Number of death after 72 h
Group A	6	0
Group B	6	0
Group C	6	0
Group D	6	0

Table (3-8): Acute toxicity effect of PDY polyphenolic extract

Groups	Number of rats	Number of death after 72 h
Group A	6	0
Group B	6	0
Group C	6	0
Group D	6	0

3.6. Effects of Polyphenolic Extracts on Boody Weight

The body weight changes were shown in table (3-9) and figure (3-6). During 30 days, there was a significant increase in the body weight in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in body weight in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in the body weight in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

These results are similar to the result of Shu-yuan (2009) who reported that the chlorogenic acid treated group was found 31% lower the body weight than that of the control group. Similar effects were also reported in previous work of other investigators. In the mice on standard normal diet, oral administration of chlorogenic acid (60 mg/kg/day for 14 days) showed a tendency to reduce body weight(Shimoda *et al*.,2006). After absorbed into blood, dietary fat is packaged inside Chylomicrons and transported to extra-hepatic tissues such as muscles and adipose tissue for disposal. Chlorogenic acid and Gallic acid suppressed the weight gain during the high fat diet and inhibited fatty acid biosynthesis (David *et al*.,2013).

Table (3-9): Changes in the body weight in the all studied groups

Groups	N	ΔWeight (gm) Mean ± S.D	% Increase in body weight
A	6	35.50 ± 6.72 [b]	42.85%
B	6	65.33 ± 8.61 [a]	78.08%
C	6	39.33 ± 9.70 [b]	47.29%
D	6	44.16 ± 7.38 [b]	53.20%
L.S.D		13.45	

Note: Each value represents (mean ± SD) values with non identical superscript (a, b or c …etc.) were considered significantly different (P ≤ 0.01). n= no. of animals

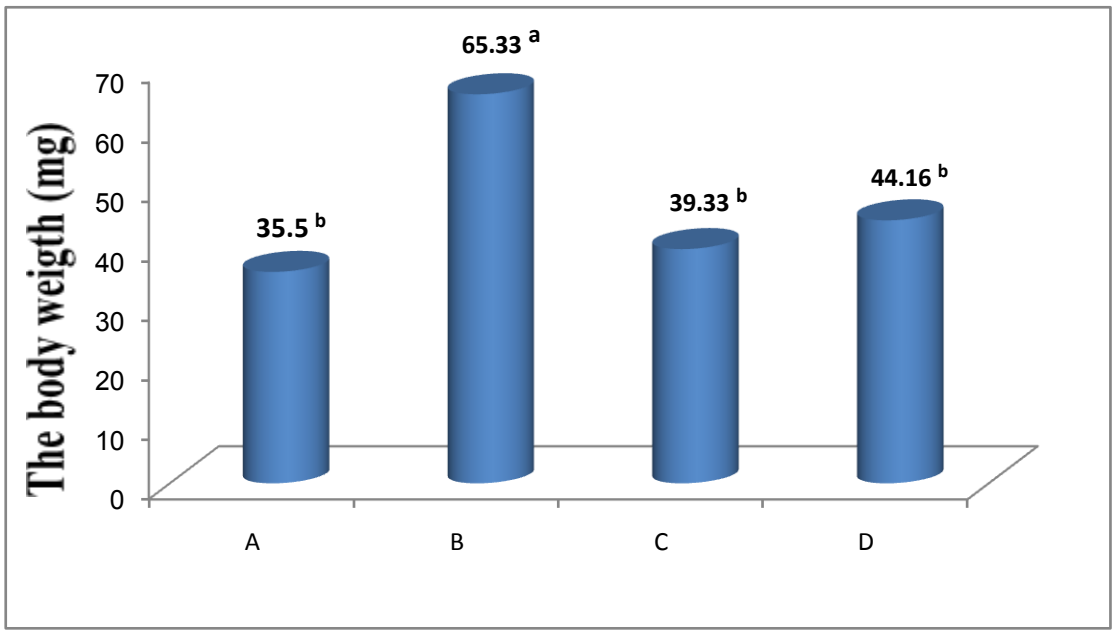


Figure (3-6): Changes in the body weight in the all studied groups

3.7. Effects of Polyphenolic Extracts on Serum Lipid Profile

3.7.1. Serum Total Cholesterol (TC) Concentration

In the current study, the investigation of the protection role for extracted polyphenols against induced hyperlipidemia in rats, during 30 days of cholesterol intake, was done.

Serum TC concentration changes were shown in table (3-10) and figure(3-7).

During 30 days, there was a significant increase in the serum concentration of TC in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the serum concentration of TC in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in TC concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

The increase in the serum concentration of TC in group (B) was due to rats were treated with daily high cholesterol diet for 30 days. These results are similar to the result of Nishi, *et al.* (2013) that reported that more pronounced effect was found with low-dose chlorogenic acid than with high dose in elevated plasma cholesterol. The caffeic acid and chlorogenic acid lowered HMG-CoA reductase and acyl-CoA cholesterol transferase (ACAT) activities compared to the high-fat control group, while they elevated fatty acid β-oxidation activity (Ae-Sim *et al.*2010).Various studies have reported that the Chlorogenic acid from walnut leaf indirectly interferes in cholesterol synthesis pathway and inhibit HMG-CoA reductase enzyme, reduce cholesterol production, also increase its bile secretions (Namasivayam,2002). HMG-CoA reductase is the enzyme which catalyses the

conversion of HMG-CoA to mevalonate using NADPH as reducing equivalent and is the major rate limiting step in cholesterol biosynthesis (Kedar and Chakrabarti, 1982) .

Rodrigues de Sotillo and Hadley (2002) found a significant reduction in plasma total cholesterol in rats treated with chlorogenic acid. It was also reported that prune intake prevented hypercholesterolemia (Edralin *et al*.,2000).

In a previous study, a more pronounced effect was found with low-dose ferulic acid than with high dose in elevated plasma lipid (Sri Balasubashini *et al*., 2003). Reports have shown that ferulic acid exerts hypolipidemic activity by decreasing the HMG-CoA reductase and ACAT (Kim *et al*., 2003).

The mechanisms with which polyphenols exert hypolipidemic effect were attributed to the stimulation of AMP-activated protein kinase, and inhibition of LDL-receptor in liver (Zang, 2006).

Table (3-10): Serum TC concentration in the all studied groups

Groups	n	TC (mg/dL) Mean ± S.D
A	6	101.21 ± 2.49 [b]
B	6	138.89 ± 3.86 [a]
C	6	103.46 ± 2.24 [b]
D	6	105.52 ± 3.19 [b]
L.S.D		4.95

table (3-9) Legend as in

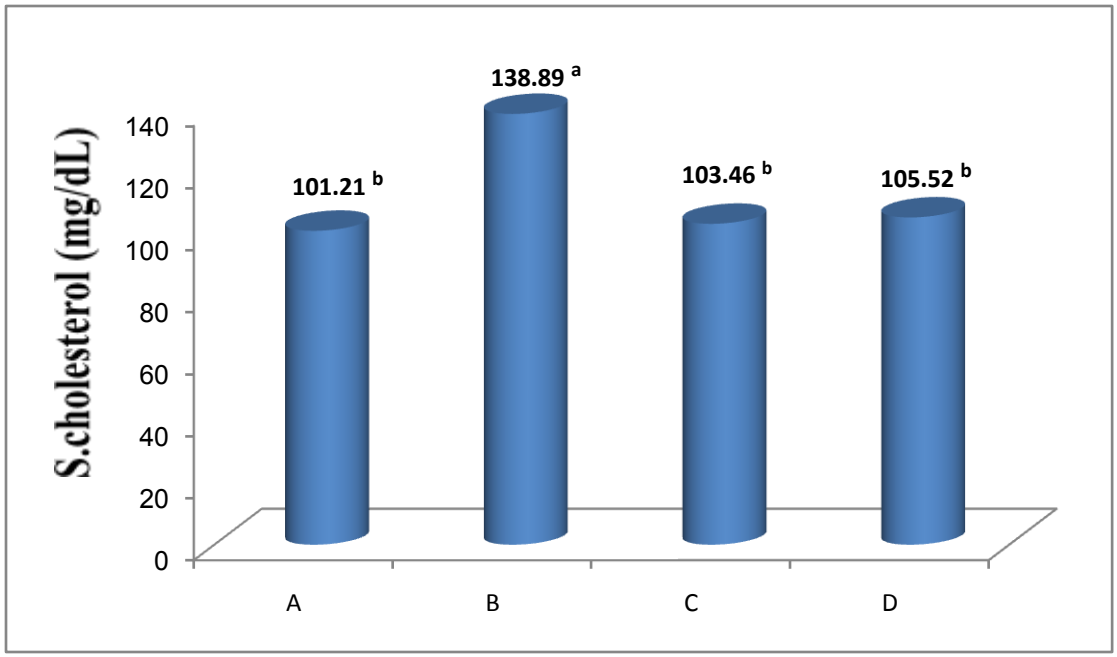


Figure (3-7): Serum TC concentration in the all studied groups

3.7.2. Serum Triglycerides (TG) Concentration

The statistical data were reported in table (3-11) and figure(3-8). During 30 days, there was a significant increase in the serum concentration of TG in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the serum concentration of TG in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in TG concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group (D) ($p < 0.01$). On the other hand, non significant differences can be observed between group(C) compared to control group (A). While, there was a significant increase in TG of group (D) compared with group (A) ($P < 0.01$).

This result was matched with the study of Nishi, *et al*. (2013). The mechanism of the anti-hyperlipidemic activity of phenolic compounds could be the inhibition of lipid absorption (Johnston *et al.*,2003). In a previous study, more pronounced effect was found with low-dose of ferulic acid than with high dose in serum concentration of TG (Sri Balasubashini *et al*., 2003). Other study also showed that TG are independently related to coronary heart diseases (Bainton *et al.*, 1992), and most of the anti-hyperlipidemic drugs don't decrease TG levels, but polyphenolic extract lowered it significantly and this effect might be related to increase the lipoprotein lipase that hydrolysis the TG into free fatty acids. Lipoprotein lipase playing a major role in the transport and metabolism of TG of exogenous origin (Chidambaram *et al.*, 2007).

Table (3-11): Serum TG concentration in the all studied groups

Groups	n	TG (mg/dL) Mean ± S.D
A	6	42.41 ± 2.02 [c]
B	6	61.06 ± 1.29 [a]
C	6	43.08 ± 1.38 [bc]
D	6	45.33 ± 1.13 [b]
L.S.D		2.45

table (3-9) Legend as in

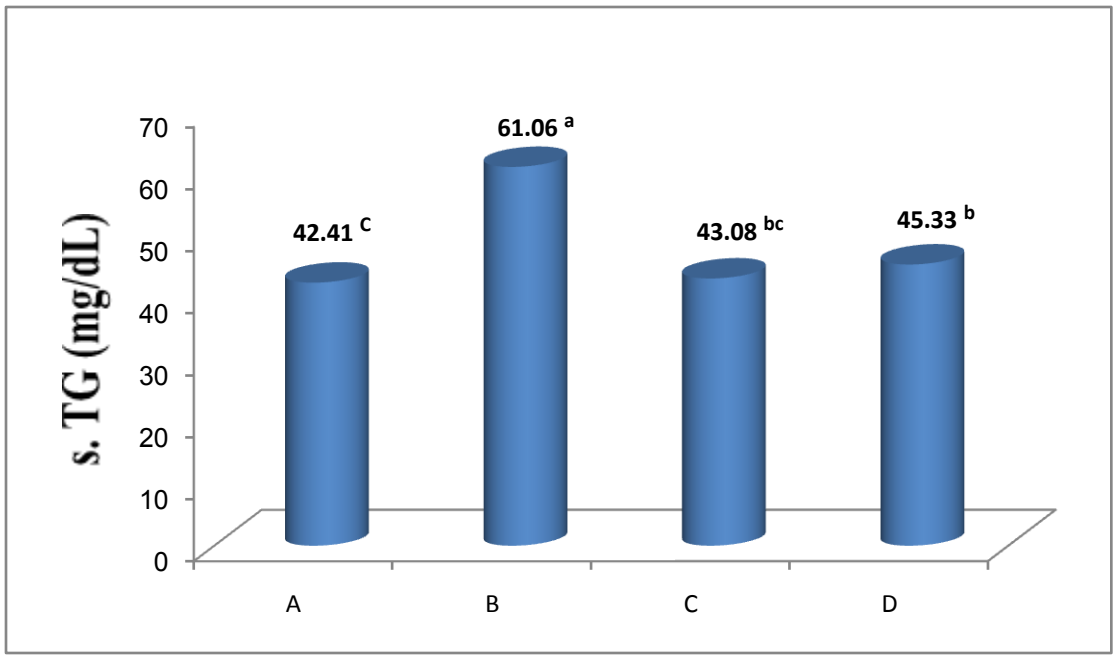


Figure (3-8): Serum TG concentration in the all studied groups

3.7.3. High Density Lipoprotein (HDL)

Table (3-12) and figure (3-9), illustrate serum TG concentration changes. During 30 days, there was a significant decreas in the serum concentration of HDL in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant increase in the serum concentration of HDL in groups (C and D) as compared with group (B) ($P < 0.01$), with greater increment in HDL concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group (D) ($p < 0.01$). On the other hand, non significant can be observed between(C and D) groups compared to control group (A).

These results are similar to the result of Nishi, *et al*. (2013). Studies in both rats and humans also reported that intake of some polyphenols suppressed LDL cholesterol concentrations and susceptibility of LDL to oxidation and increased HDL cholesterol concentrations (Fuhrman and Aviram,2001: Zhan and Ho,2005). Various studies have reported that polyphenols can also increase plasma HDL cholesterol concentration (Murphy *et al.*,2003 ; Abe *et al.*,2011). The reason of this rise in HDL may be due to activation of Lecithin acyl transferase (LCAT), by polyphenols that leads to integration of free cholesterol with HDL leading to raise the level of HDL in blood (Ghule, 2006). Also polyphenols can activate lipoprotein lipase enzyme that involved in the metabolism of proteins of HDL (Hemilä, 1992).

Table (3-12): Serum HDL concentration in the all studied groups

Groups	n	HDL (mg/dL) Mean ± S.D
A	6	35.86 ± 1.58 [a]
B	6	30.58 ± 1.34 [b]
C	6	36.03 ± 1.79 [a]
D	6	35.93 ± 1.54 [a]
L.S.D		2.57

table (3-9) Legend as in

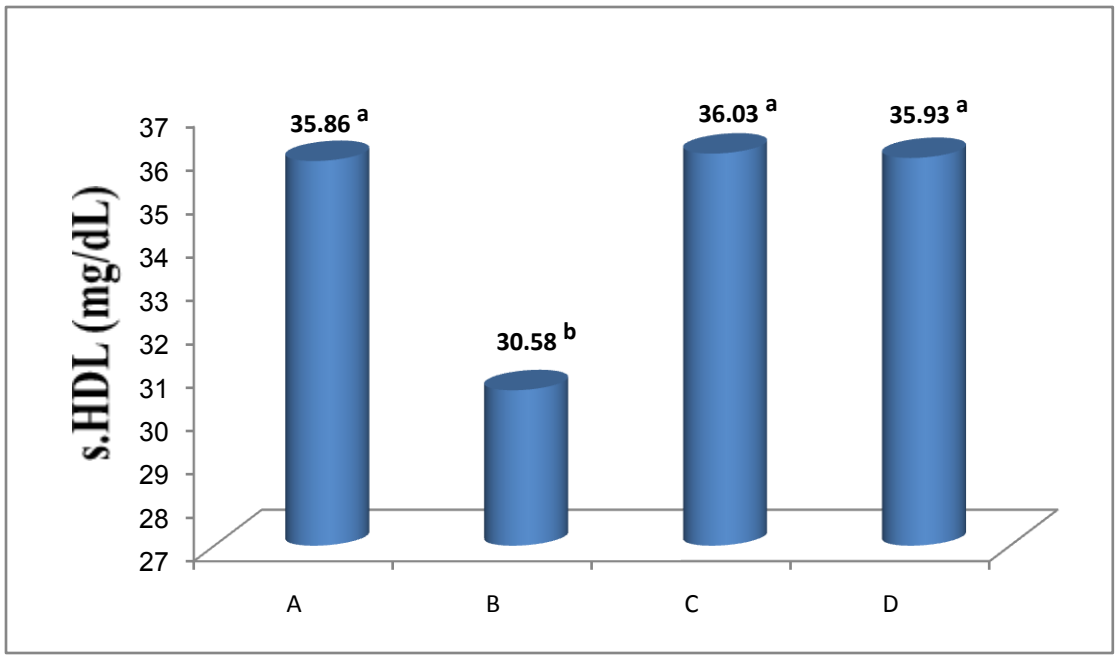


Figure (3-9): Serum HDL concentration in the all studied groups

3.7.4. Low Density Lipoprotein (LDL)

Serum LDL concentration changes were shown in table (3-13) and figure (3-10). During 30 days, there was a significant increase in the serum concentration of LDL in group (B) as compared with normal control group(A) ($P < 0.01$). At these times, there was a significant reduction in the serum concentration of LDL in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in LDL concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

The decrease of LDL levels that reported in this study was compatible with finding of Nishi, *et al*. (2013). On the other hand, several studies have confirmed the predominant phenolic compounds in prunes also inhibited LDL oxidation the strong antioxidant activity of chlorogenic acid toward LDL (Nardini *et al.,* 1995; Rice-Evans *et al.,* 1996). Other phenolic components in prunes such as caffeic acid have also been reported to be active inhibitors of human LDL oxidation (Nardini *et al.,* 1995; Rice-Evans *et al.,* 1996; Teissedre *et al.,* 1996). In general, chlorogenic acid and caffeic acid inhibit oxidation of LDL *in vitro* (Laranjinha *et al.,*1994; Nardini *et al.,*1995) and might therefore protect against cardiovascular disease. Polyphenols were shown to exhibit strong antioxidant properties that could protect LDL from oxidation (Frankel *et al.,*1993; Vinson *et al.,*2001). In a rat model, caffeic acid spared vitamin E and enhanced the resistance of LDL towards oxidative stress (Nardini *et al.,* 1997). Also polyphenols can activate paraoxonase enzyme that runs inhibition of oxidized LDL uptake by the cells.

Table (3-13): Serum LDL concentration in the all studied groups

Groups	n	LDL (mg/dL) Mean ± S.D
A	6	56.86 ± 2.80 [b]
B	6	96.09 ± 3.99 [a]
C	6	58.81± 2.99 [b]
D	6	60.53 ± 3.02 [b]
L.S.D		5.31

table (3-9) Legend as in

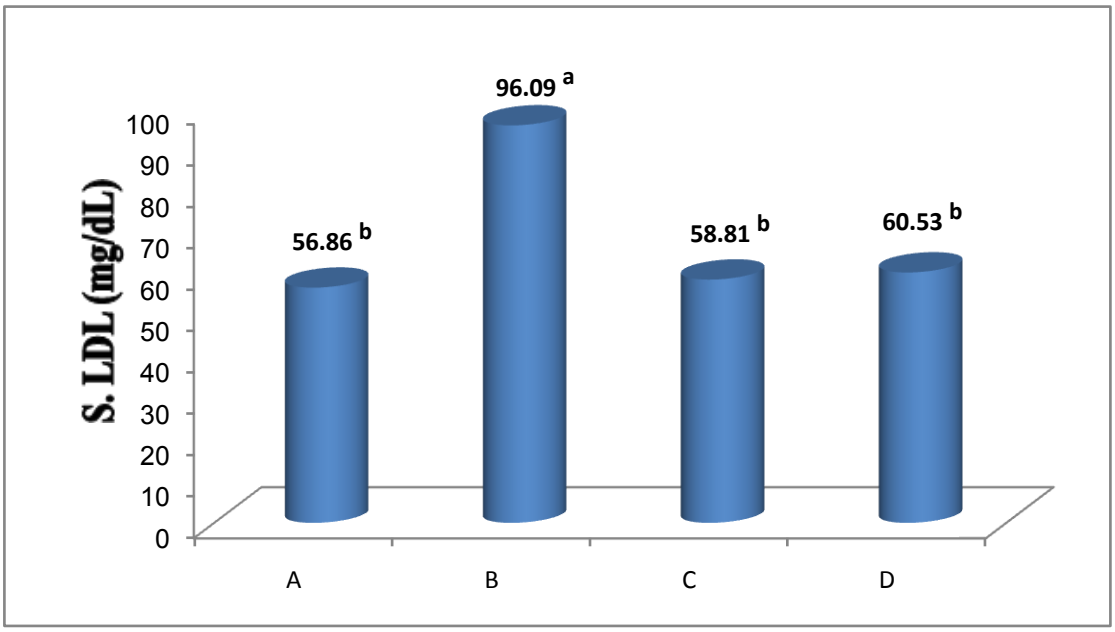


Figure (3-10): Serum LDL concentration in the all studied groups

3.7.5. Very Low Density Lipoprotein(VLDL)

Serum VLDL concentration changes were shown in table (3-14) and figure (3-11). During 30 days, there was a significant increase in the serum concentration of VLDL in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the serum concentration of VLDL in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in VLDL concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group (D) ($p < 0.01$). On the other hand, non significant differences can be observed between group(C) compared to control group (A). While, there was a significant increase in VLDL of group (D) compared with group (A) ($P < 0.01$).The decrease of VLDL levels that reported in this study was compatible with finding of Nishi, *et al*. (2013). Polyphenolic extract exhibited a significant hypolipidemic effect through the reduction of VLDL levels (Chidambaram *et al.*,2007).

Table (3-14): Serum VLDL concentration in the all studied groups

Groups	n	VLDL (mg/dL) Mean ± S.D
A	6	8.48 ± 0.40 [c]
B	6	12.21 ± 0.26 [a]
C	6	8.62 ± 0.27 [cb]
D	6	9.06 ± 0.23 [b]
L.S.D		0.49

table (3-9) Legend as in

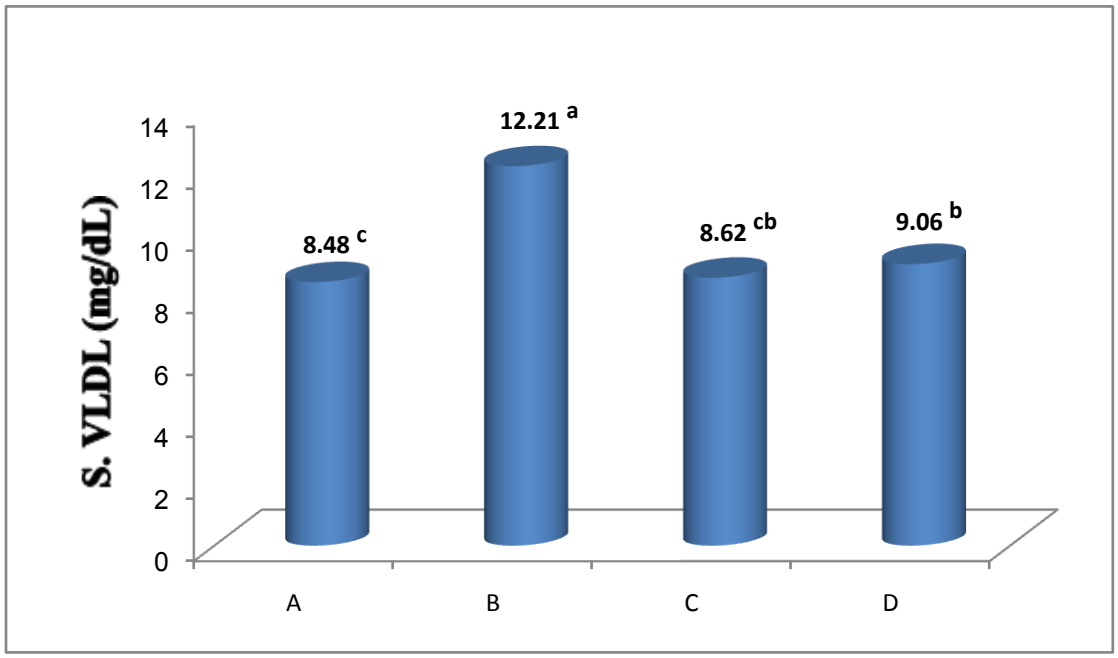


Figure (3-11): Serum VLDL concentration in the all studied groups

3.7.6. Atherogenic Index Levels

Changes in Atherogenic Index levels are shown in table (3-15) and figure (3-12). During 30 days, there was a significant increase in the Atherogenic Index levels in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the Atherogenic Index levels in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in Atherogenic Index levels concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

The decrease of Atherogenic Index levels that reported in this study was compatible with finding of Nishi, *et al*. (2013). Several studies have been shown that polyphenolic extracts of red wine have antiatherogenic properties (Fremont, 2000 ; Vinson *et al.*,2001).

Table (3-15): The Atherogenic Index levels in the all studied groups

Groups	n	Atherogenic Index Mean ± S.D
A	6	1.59 ± 0.12 [b]
B	6	3.14 ± 0.22 [a]
C	6	1.64 ± 0.14 [b]
D	6	1.69 ± 0.12 [b]
L.S.D		0.25

table (3-9) Legend as in

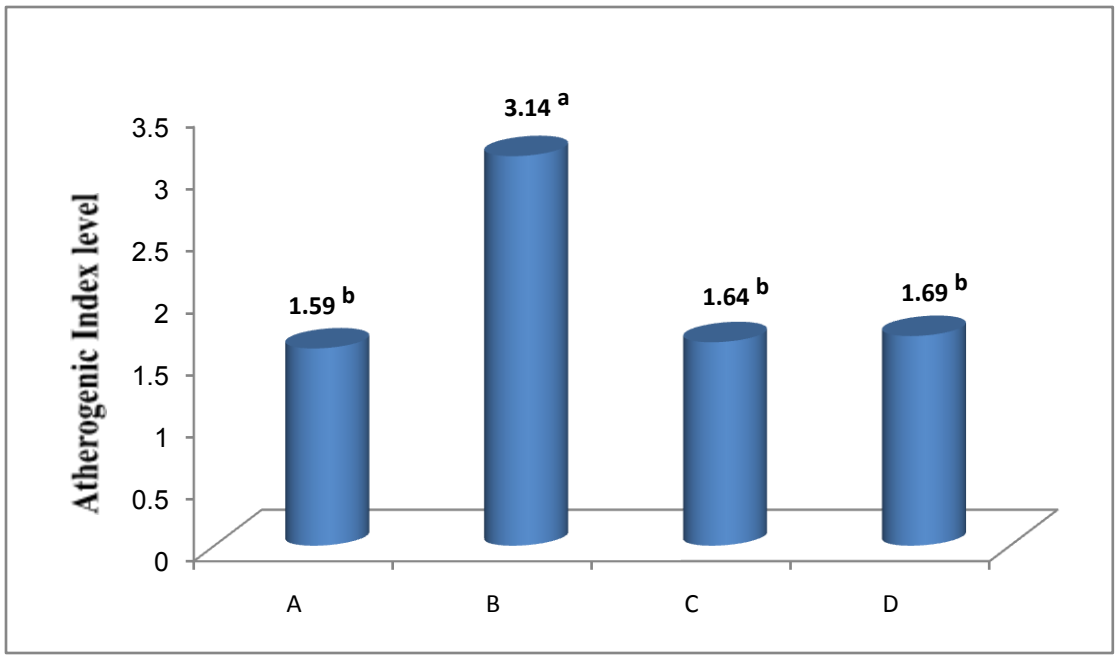


Figure (3-12): The Atherogenic Index levels in the all studied groups

3.8. Effects of Polyphenolic Extracts on Serum Oxidant – Antioxidant Status

3.8.1. Serum Malondialdehyde (MDA) Concentration

Serum MDA concentration changes were shown in table (3-16) and figure (3-13). During 30 days, there was a significant increase in the serum concentration of MDA in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the serum concentration of MDA in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in MDA concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

MDA is general biomarker of biological oxidative stress (Kadiiska *et al.*, 2005). The increase of MDA levels that reported in the present study in group (B) agrees with the results of Hung *et al.*,(2006).

The decrease of MDA levels that reported in the present study in groups (C and D) agrees with the results of Raid (2010) , who reported that both gallic and tannic acids are considered as a free radical scavengers and consequently as a lipid peroxidation inhibitors. This activity of polyphenols is due to presence of multiple hydroxyl groups in each compound which are able to donate their protons to finally break the chain reaction of free radicals (Van Acker *et al.*,1996). Ferulic acid has a high antioxidant potential due to its resonance-stabilized phenoxy radical structure. It is an effective scavenger of free radicals and it has been approved in certain countries as a food additive to prevent lipid peroxidation (Graf,1992). Protective roles of chlorogenic acid against lipid peroxidation status (Ohnishi *et al.*,1994) and elimination of hydroxyl free radical were shown (Ishii *et al.*,1990). Caffeic acid inhibits lipoxygenase activity and suppresses lipid peroxidation (Gutteridge,1995).

In other studies, polyphenols activate the enzymes which inhibit (LPO) like kinases and in that time polyphenols inhibit the enzymes that activate (LPO) such as phosphodiesterase (PDE). Phenolic structures often have the potential to strongly interact with proteins, due to their hydrophobic benzenoid rings and hydrogen-bonding potential of the phenolic hydroxyl groups. This gives phenolics the ability to act as antioxidants also by virtue of their capacity to inhibit some enzymes involved in radical generation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase (Parr and Bolwell,2002).

Table (3-16): Serum MDA concentration in the all studied groups

Groups	n	MDA (nmol/mL) Mean ± S.D
A	6	18.47 ± 1.12 [b]
B	6	27.12 ± 1.20 [a]
C	6	19.13 ± 1.07 [b]
D	6	20.30 ± 1.13 [b]
L.S.D		0.47

table (3-9) Legend as in

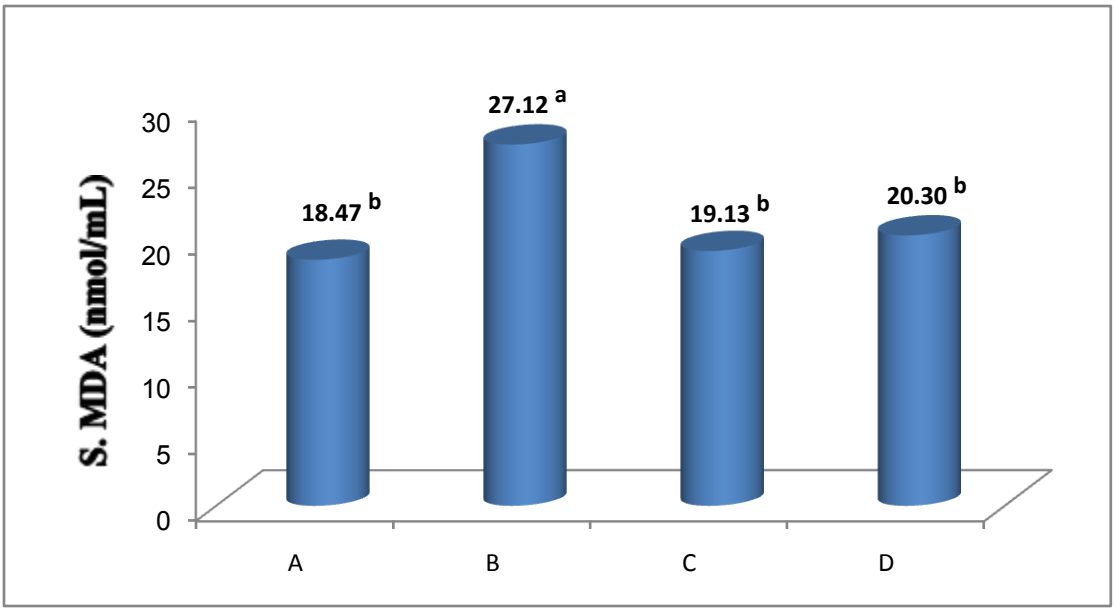


Figure (3-13): Serum MDA concentration in the all studied groups

3.8.2. Serum Ceruloplasmin (Cp) Concentration

Table (3-17) and figure (3-14), illustrate Serum Cp concentration changes. During 30 days, there was a significant increase in the serum concentration of Cp in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant reduction in the serum concentration of Cp in groups (C and D) as compared with group (B) ($P < 0.01$), with greater decrement in Cp concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group(D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

The increase in concentration of serum ceruloplasmin in group (B) was probably due tocatalysis of the liver cells synthesis of Cp against iron overload status (Tobe *et al.*, 2002) and elevation in serum copper level (Butris and Ashood, 1996) as a defiance function. The decrease of CP levels that reported in this study was compatible with finding of Sirajwala *et al.*, (2007) , who reported polyphenols decrease an activity of numerous proteins associated with oxidative stress. Also the reduction in Cp concentration could be to counter balance of the ROS radicals generated in the lipid peroxidation processes and presence of iron or copper ions.

Table (3-17): Serum Cp concentration in the all studied groups

Groups	n	CP (g/L) Mean ± S.D
A	6	1.71 ± 0.31 [b]
B	6	3.20 ± 0.51 [a]
C	6	2.31 ± 0.43 [b]
D	6	2.43 ± 0.52 [b]
L.S.D		0.74

table (3-9) Legend as in

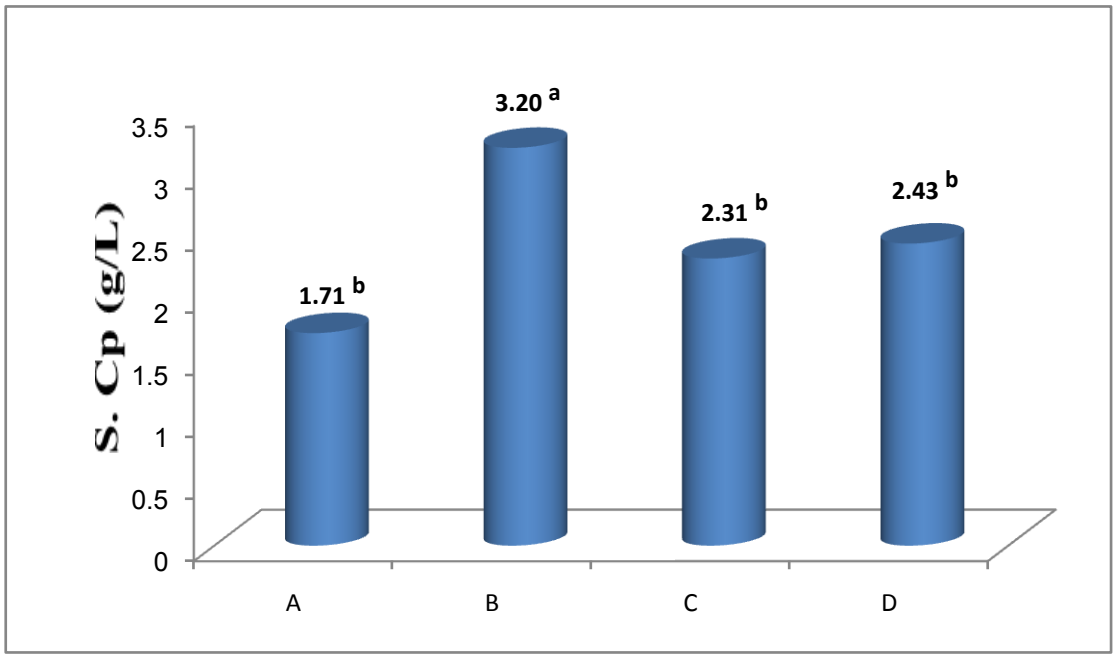


Figure (3-14): Serum Cp concentration in the all studied groups

3.8.3. Serum Albumin *(*Alb*)* Concentration

Serum Alb concentration changes were shown in table (3-18) and figure (3-15). During 30 days, there was a significant decrease in the serum concentration of Alb in group (B) as compared with normal control group(A) ($P < 0.01$) . At these times, there was a significant increase in the serum concentration of Alb in groups (C and D) as compared with group (B) ($P < 0.01$), with greater increment in Alb concentration in PDR polyphenolic extract treated group (C) than this with PDY polyphenolic extract treated group (D) ($p < 0.01$). On the other hand, non significant differences can be observed between(C and D) groups compared to control group (A).

These results are similar to the result of Al-Hashem, (2009) that reported that polyphenols could decrease of LPO processes as well as increase in the activities of plasma protein thiols as albumin and other serum proteins in both animal and human. Vlassara *et al*. (2001) has been reported that this decrease is due to the increase in synthesis of lipid peroxide and elevation in formation of free radicals which result in increasing of membranes permeability and leaking the proteins outside the vascular system. Ferulic acid is a good antioxidant against protein oxidation. It also possesses some activity against LPO damage (Castellucio *et al.*1995: Bourne and Rice-Evans,1997). Baziramakenga *et al.,* (1997) and Hegab (2005) proved the stimulation of protein synthesis and activation of antioxidant enzymes with the application of phenolic acids at low doses.

Table (3-18): Serum Alb concentration in the all studied groups

Groups	n	Alb(g/L) Mea±n S.D
A	6	46.49 ± 2.16 [a]
B	6	36.97 ± 2.88 [b]
C	6	44.95 ± 1.76 [a]
D	6	42.49 ± 3.17 [a]
L.S.D		4.19

table (3-9) Legend as in

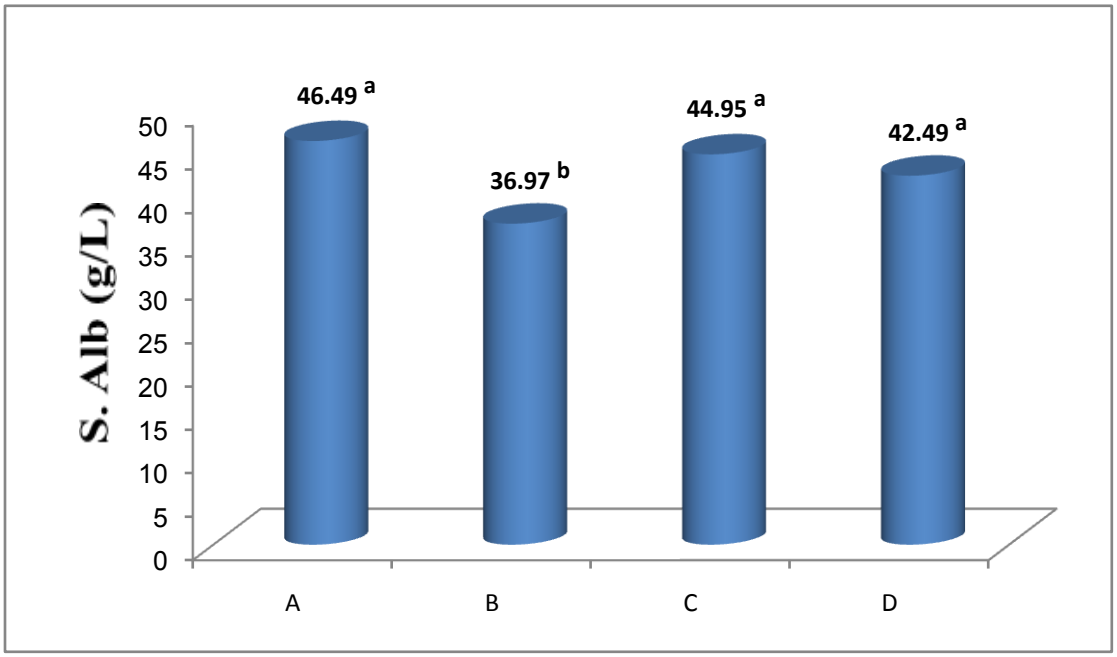


Figure (3-15): Serum Alb concentration in the all studied groups

3.9. Antibacterial Properties of Polyphenolic Extracts.

As shown in table (3-19), the extracted polyphenols have antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram - negative), *Staphylococcus aureus* and *Bacillus subtilus* (Gram- positive). PDR polyphenolic extract showed maximum activity against pathogens ***Pseudo*** (30mm), and equal inhibition zone against ***E.coli , Staph*** and ***Bacillus*** (20mm).

On the other hand, PDY polyphenolic extract showed maximum activity against pathogens like ***Staph*** (13mm) **,** ***Pseudo*** (10mm), ***E.coli*** (10mm) and the minimum activity was against ***Bacillus*** (1mm). This results are similar to the result of Zahra, *et al*. (2013). These findings confirmed by other workers who reported strong antibacterial activity against food borne pathogens (Seyhun *et al.,* 2009).

The control (Ciprodar) showed maximum activity against ***Staph*** (28mm)and against ***Pseudo*** (25mm). *In vitro* studies have shown that plant phenolic acids can rapidly kill many human-pathogenic bacteria (Friedman *et al.,* 2003; Kim *et al.,* 2003), Such as *E.coli*, which is inhibited by phenolic acids (Zaldivar and Ingram,1999). Polyphenols and flavonoids are reported to be important antimicrobial components (Chung *et al*., 1998; Karou *et al*., 2005). There are also enough documented results, which suggest that there is a positive correlation between total phenolic content and antimicrobial activity (Shan *et al*., 2007). The potentially antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes. The -OH groups in phenolic compounds are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of bacterial growth. Phenolic compounds are also involved in protein and cell wall binding, inactivation of bacterial enzymes, and intercalation into the bacterial DNA during replication (Sung-Jin *et al.*,2013).

The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes (Damintoti *et al.*,2005).

In general, the Gram-positive strains of bacteria tested appeared to be more sensitive to the extracts. However, this study also records a significant sensitivity of some of the examined Gram-negative bacteria. According to literature, the antimicrobial activity could be influenced by the phenolic compounds (Alberto *et al.,* 2001). The differences in the antimicrobial effects between Gram-positive and Gram-negative bacteria are mainly due to their different cell wall structures. The cell wall of Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multilayered structure bounded by an outer cell membrane (Yao and Moellering, 1995).

The phenolic acids, which can inhibit pathogens growth and have little toxicity tohost cells are also promising candidates for developing new antimicrobial drugs. Consequently, there is growing interest in developing many plant-derived drugs with multiple biological functions to use for the treatment of various infectious diseases (Parekh *et al.*,2005).

Ciprodar(Ciprofloxacin hydrochloride), the IUPC name is 1-cyclopropyl-6 fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid. The structure of ciprodar shown in figure(3-16).

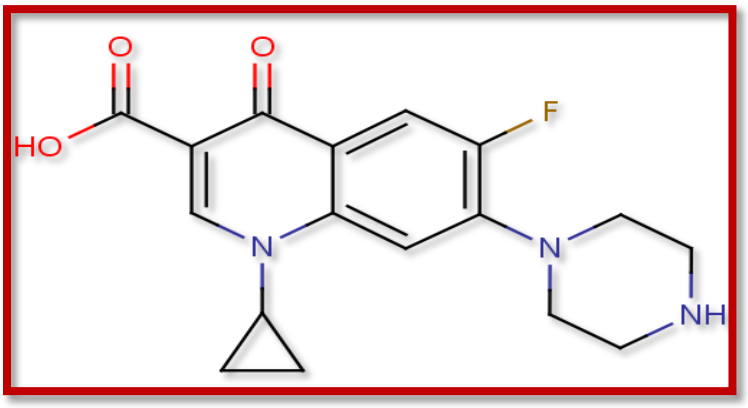


Figure (3-16): Chemical structure of ciprofloxacin

Ciprofloxacin hydrochloride a broad-spectrum fluoroquinolone,is effective against both gram-negative and gram-positive bacteria,and is used widely to eradicate Mycoplasma. Anaerobes (Sherris,1994). It is available in more than 100 countries, where it is approved for the treatment of 14 types of infections, especially urinary tract infections such as acute uncomplicated cystitis and chronic bacterial prostatitis, and lower respiratory infections. Ciprofloxacin destroys microorganisms by interfering with their DNA gyrase ,an enzyme that is critical to bacterial chromosome replication, and will also kill bacteria in both the active and inactive growth phases (Wolfson and Hooper,1985).

The values of inhibition zones of ciprodar against both Gram positive and Gram negative of this study was shown in table (3-19).

Table (3-19): Diameters of inhibition zone (mm) for all extracts

Name of organisms	Zone of inhibition in mm			
	Gram stain	PDR Polyphenolic Extracts	PDY Polyphenolic Extracts	Control (Ciprofloxacin)
E.coli	-	20	10	20
Pseudomonas	-	30	10	25
Staph.aureus	+	20	13	28
Bacillus subtilus	+	20	1	20
DMSO		0	0	

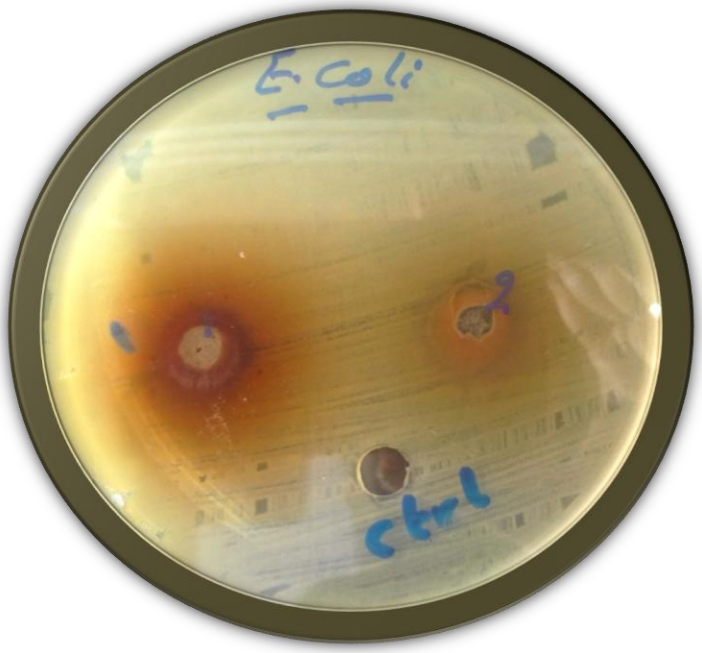


Figure (3-17): The activity of PDR and PDR Polyphenolic extracts against(*E.Coli*) bacteria

1: PDR polyphenolic extract 2: PDR polyphenolic extract Ctrl: (DMSO)

Figure (3-18): The activity of Ciprodar against (*E.Coli*) bacteria

E.Coli: Escherichia coli

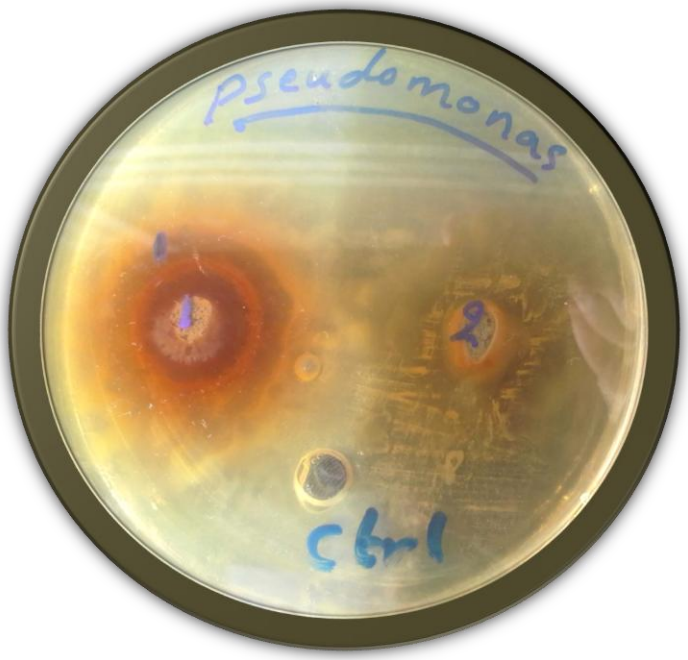


Figure (3-19):The activity of PDR and PDR Polyphenolic extracts against (*Pseudo*.) bacteria

Figure (3-20): The activity of Ciprodar against (*Pseudo*.) bacteria

Pseudo*: *Pseudomonas aeruginosa

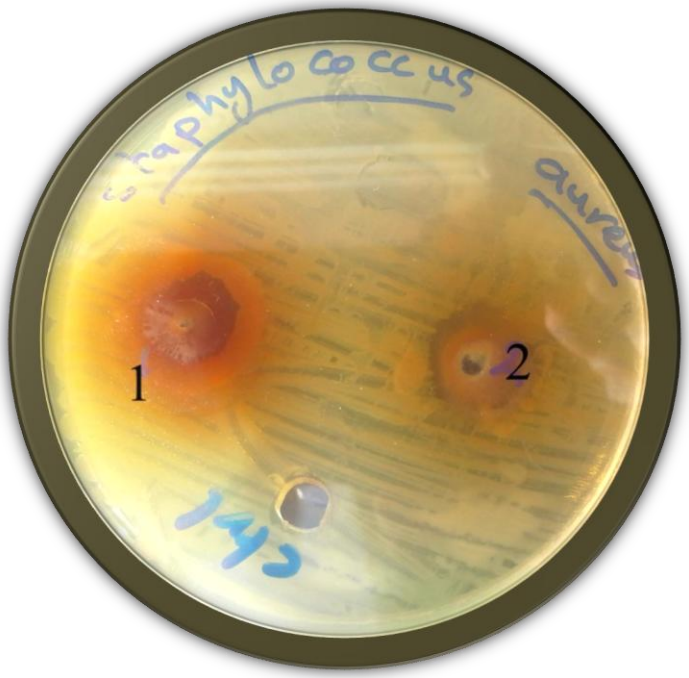


Figure (3-21): The activity of PDR and PDR Polyphenolic extracts against(*Staph.*)bacteria

Figure (3-22): The activity of Ciprodar against (*Staph.*) bacteria

Staph*: *Staphylococcus aureus

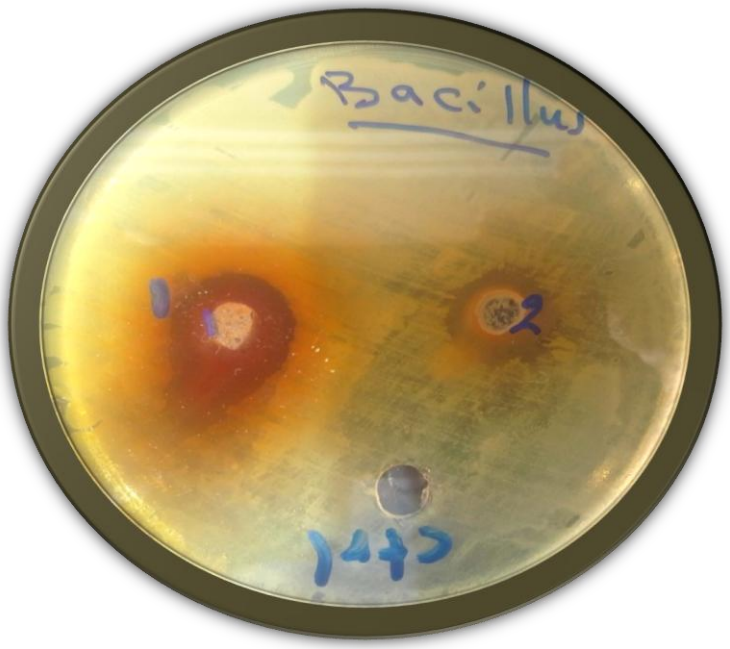


Figure (3-23):The activity of PDR and PDR Polyphenolic extracts against (*Bacillus*.) bacteria

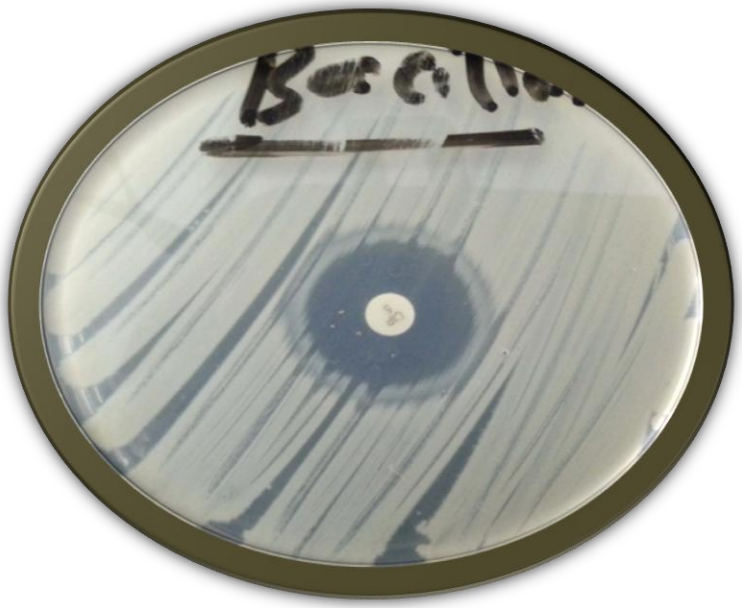


Figure (3-24): The activity of Ciprodar against (*Bacillus*.) bacteria

Bacillus. : Bacillus subtilus

Conclusion

At the end of this thesis points below can be concluded:

- Polyphenolic extract isolated from *Prunus domestica* red (PDR) and *Prunus domestica* yellow (PDY) wall nuts in this study giving the immediately yields.
- Compounds that are exist in each extract identified by using UV-Visible spectrum, also appearance of several peaks indicating to the existing of phenolic acids.
- Compounds are determined through using HPLC technique, where in polyphenolic extract six phenolic acids and one glycosides (tannic acid, gallic acid, caffeic acid, vanillic acid, ferulic acid, chlorogenic acid and amygdalin) have been extracted from PDR and PDY wall nuts.
- The extracted compounds were tested for their acute toxicity properties and it was not appearance any toxic effect on the rats so it is safely.
- PDR extract has greater effect on reduce serum lipid profile and atherogenic index levels approximately to normal values than PDY polyphenolic extract.
- PDR extract has greater effect on reduce serum MAD and Cp approximately normal values than PDY polyphenolic extract.
- PDR extract has greater effect on increase serum Alb approximately to normal values than PDY polyphenolic extract.
- The extracted compounds have been tested for their inhibitory activities in compare with various bacteria, these extracts have irregular effects on various bacteria compared with control.

Futuer Research

The following can be recommended for the next studies :

1. Study of another parts of this plant such as seeds, juice, steam and root .
2. Study of more biological activities for these extracts such as anti-inflammatory, antifungal and antivirus.
3. Further works are needed to study effect of these extracts as a hypoglycemic and hypotension.
4. Fractionation of each compounds extract and determination of the potency and efficacy of active constituents that have the effect larger on serum lipid profile.
5. Determination of Lethal dose(LD_{50}) for these studied compounds.

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