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1. Saoudi S, Khennouf S, Amira S, Dahamna S.2017. Polyphenols contents and antioxidant activity of *Fragariaananassa, Maluscommunis, Musa paradisiaca*fruit extracts. *Revues des regions arides*. 43(3): 601-606.

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1.Saoudi S, Khennouf S, Amira S,Dahamna S. Polyphenols contents and antioxidant activity of *Fragariaananassa, Maluscommunis, Musaparadisiaca* fruit extracts. Poster.5th International Meeting on Plant Biotechnology in Arid and Oasis Zones. 19-21 december, 2016, Zarzis, Tunis.

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List doctorialday

1. Saoudi S, Khennouf S,Amira S,Dahamna S. polyphénol et activité antioxydante de l'extrait des fruits de *Musa paradisiaca*. Poster.Doctorial day. 31 may, 2016, Setif, Algeria.

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الملخص

تملكالانواعالأكسجينيةالنشطةقدرةعاليةعلىالحاقأضرارتمستقريباكافةالمكوناتالخلوية,ممايفسردورهافياحداثالكثيرمنالامراض ،ولهذافإنتزويدالجسمبموادخارجيةمضادةللأكسدةيبدومهماجدًاللدفاعضدهذهالانواعالأكسجينيةالضارة.تعتبر الفواكهمصدرامهمالمضاداتالأكسدةالخارجية والتييمكنأنتقللمنالضررالتأكسدي لهذه الجذور الحرة. تهدفهذهالدراسة إلىتقديرمحتوى عديدات الفنول والفلافونويدات و الدباغ والسكرياتو البروتيناتوكذا تعريفبعضالمركباتالفينوليةوالنشاطية المضادةللأكسدةوسميةالكبدلمستخلصالميثانوليللعديدمنالفواكهالمستهلكة في الجزائر. أظهرالتحليل الكيمائيان هناك اختلاف كبير بين الفواكه. ويعتبر حمضالغليكالمركبالرئيسي الموجود في كل المستخلصات. أظهرت النتائج ان مستخلص الفراولة يحتويكميةمعتبرة من عديدات الفنول و الفلافونويدات. بينما كمية الدباغ الأكبركانت متواجدة في مستخلص الرمان. كماوجد المحتوى العالي للسكريات في ثمار التين. تم تقدير النشاطية المضادة للأكسدة باستعمال اختبار DPPHو ABTS و استخلاب الحديد و القدرة الارجاعية ونظام β-كاروتان وتيوسيانيت الحديد و حمض التيوباربيتيك.وجد أن فاكهة الفراولة هي الافضل في القدرة على التقاط الجذور الحرة. بينما يعتبر التين الأفضل في القدرة في استخلاب الحديد. كما تملك فاكهة الفراولة قدرة عالية على تثبيط أكسدة الليبيدات. تم تقدير النشاطية المضادة للأكسدة حيويا بجرعات 200 مع/كغ و 600 مع/كغ عند الجرذان بعد المعاملة بالمستخلصات لمدة 14 يوما على القدرة المضادة للأكسدة في البلازما و مؤشرات الاجهاد التأكسدي في الكبد و الكلى و على بعض المؤشرات البيوكميائيةبالبلازما. أظهرت النتائج ان جميع المستخلصات عند جرعة 600 مع/كغ لها تأثيرا قويا على مؤشرات الاجهاد التأكسدي. في هذه الدراسة تم تقدير السمية الكبدية باستعمال رباعي كلوريد الكربون. اظهرت النتائج ان هذا المركب يؤثر بشكل كبير على الكبد. كما أدت المعاملة بمستخلصات كل الفواكه إلىوقاية نسيج الكبد والكلية من التأثير السام لهذا المركب. أدى اعطاء الجرعتين 200 و 600 مع/كغ من مستخلص التين إلى اظهار تأثير وقائي على الكبد وإلى التأثير على مؤشرات الإجهاد التأكسدي وعلى بعض المؤشرات البيوكميائية في البلازما. ونستخلص من نتائج هذه الدراسة أن مستخلصات الفواكه المستعملة تمتلك نشاط مضاد للأكسدة وواقي للأنشطة مرتبطا بالمحتوى الفينولي و الفلافونويدات.

الكلمات المفتاحية : مستخلصات الفواكه،عديدات الفنول، الفلافونويدات،الاجهادالتأكسدي، السمية الكبدية.

Résumé

Les espèces réactives oxygénées (ERO) ont une grande capacité d'endommager presque tous les types de constituants cellulaires. Ce qui explique leur implication dans l'induction de plusieurs pathologies. La supplémentation de l'organisme par des antioxydants exogènes s'avère très utile pour lutter contre ces espèces nocives. Les fruits sont une source importante d'antioxydant exogène qui peut minimiser les dommages oxydatifs. Le but de cette étude est d'évaluer les teneurs en polyphénols, flavonoïdes, sucres, tannin et protéines, l'identification de certains composés phénoliques, l'activité antioxydante et l'hépatotoxicité d'extraits méthanoliques de plusieurs fruits consommes en Algérie. L'identification par UPLC et HPLC / MS a montré une forte différence entre les extraits de fruits. L'acide gallique est le principal composé dans tous les extraits de fruits études. Les résultats ont révélé que l'extrait de *Fargariaananassa*contient une grande quantité de polyphénol, de flavonoïde et de protéine. Le contenu élevé de tannin se trouve dans l'extrait de *Punicagranatum*. Tandis que, le contenu élevé de sucres est trouvé dans le *Ficus*. L'activité antioxydante a été évaluée *in vitro*par les tests de DPPH, d l'ABTS, radical hydroxyle, pouvoir réducteur, chélation des ions ferreux, système β-carotène / acide linoléique, la méthode de thiocyanate ferrique et la méthode à l'acide thiobarburique. Les fraises présentent l'extrait le plus active dans l'activité scavenger des radicaux libres. Cependant, les figues sont le meilleur chélateur comparé aux autres fruits. *Fargaria* peut inhiber la peroxydation lipidique de différentes manières. L'activité antioxydante*in vivo*a été évaluée par la voie orale chez le rat pendant 14 jours(200 et 600 mg / kg) sur la capacité antioxydante plasmatique (PAC), les marqueurs du stress oxydatif dans le foie et les reins et certains paramètres biochimiques. Les résultats ont montré que tous les extraits à 600 mg / kg ont un bon effet contre le stress oxydatif. Dans la présente étude, l'effet hépatoprotecteur des extraits de fruits a été évalué chez des rats intoxiqués par CCl_4. Les résultats ont montré que cet agentinduisait une hépatotoxicité remarquable. Tous les extraits de fruits ont un effet protecteur important et réduisent les dommages aux tissus hépatiques et rénaux. L'administration de 200 et 600 mg / kg de *Ficus carica* a montré un effet hépatoprotecteur important, il améliore les marqueurs du stress oxydant (GSH et MDA) et les paramètres biochimiques hépatiques, les résultats sont confirmés par un examen histologique. En conclusion, les extraits de fruits examinés dans cette étude ont montré une très bonne activité antioxydante, liée à leur teneur en polyphénols et flavonoïdes.

Mot clés: Extraits de fruits, polyphenols, flavonoides, stress oxydant, hepatotoxicité.

Abstract

Reactive oxygen species (ROS) have high potential to damage almost all types of cellular constituents, which explains their involvement in the induction a number of pathologies. The supplementation of the body by exogenous antioxidants seems to be very helpful to fight these harmful species.Fruits are an important source of exogenous antioxidant which can minimize oxidative damage. The aim of this studyis the evaluationpolyphenols, flavonoids, tannin, sugars and protein contents, the identification of some phenolic compounds, the antioxidant and hepatotoxicity activity of methanolic extracts of the several fruits consumed in Algeria.The identification of phenolic compounds by UPLC and HPLC/MS showed deference between fruits extracts. Gallic acid is the major compounds present in all fruits extracts. The results revealed that *Fargariaananassa*contains high amount of polyphenols, flavonoids and protein. The high level of tannin was found in *Punicagranatum*.While, the high level of sugars was found in *Ficus*.The antioxidant activity was evaluated *in vitro* using the DPPH, ABTS, hydroxyl radicals,reducing power, ferrous ion chelating tests, β-carotene/ linoleic acid model system, ferric thiocyanate method and thiobarburic acid method. Strawberries extracts were the best one can scavengers of free radicals.However *Ficus*extracts was the better chelator compared to other fruits. *Fargaria*can inhibit lipid peroxidation by deferent ways. The antioxidant activity of extracts at doses of 200 and 600 mg/kg*in vivo*was assessed in rats for 14 days, on plasma antioxidant capacity (PAC), oxidative stress markers in liver and kidney and on some biochemical parameters. Results showed that all extracts at 600 mg/kg were effectives against stress oxidative. The hepatoprotective effect of fruits extracted was evaluated in rats intoxicated by CCl_4. Results showed that CCl_4induced remarkable hepatotoxicity. All fruits extracts have an important protective effect and reduce hepatic and renal tissues damage induced by this toxic agent. The administration of 200 and 600 mg/kg of *Ficuscarica*showed a significant hepatoprotective effect, it improve the oxidative stress markers (GSH and MDA) and hepatic biochemical parameters and this results confirmed by histological examination. In conclusion, Fruit extracts examined in this study showed a very high antioxidant activity and hepatoprotective properties which wererelated to their contents in polyphenols and flavonoids.

Key words: Fruits extracts, polyphenols, flavonoïds, oxidative-stress, hepatotoxicity.

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List of abbreviations

ABTS: 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

AD: Alzheimer's disease

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

BHT: Butylatedhydroxytoluene

DAD: Diode array detector

DNA: Deoxyribonucleic acid

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl

EDTA: Ethylene diamine tetra acetic acid

ELISA: Enzyme-linked immunosorbent assay

FAD: Flavin adenine dinucleotide

FRAP: Ferric reducing ability of plasma

GAE: Gallic acid equivalents

GC/MS: Gas Chromatography coupled to Mass Spectrometer

GGT:γ-Glutamyltransferase

GPX: Glutathione peroxidase

GR: Glutathione reductase

GSH: Glutathione

GSSG: Glutathione disulfure oxidized

GST: Glutathione-S- transferase

H_2O_2: Hydrogen peroxide.

I%: Inhibition percentage.

IC_{50}%: Inhibitory concentration for 50% of activity

LDL: Low density lipoprotein

MDA: Malondialdehyde

NAD: Nicotinamide adenine dinucleotide.

NOS: Nitric oxide synthase

NOX: NADPH oxidase.

PDA: Photodiode array detector

QE: Quercetin equivalents

RNS: Nitrogen reactive species

ROS: Oxygen reactive species

SEM: Standard error of the mean

SOD: Superoxide dismutase

TAE: Tannic acid equivalents

TAC: Total antioxidant capacity

AU: Uric acid

XDH: Xanthine dehydrogenase

XO: Xanthine oxidoreductase

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Materials and methods

INTRODUCTION

Introduction

Oxygen is a very importantmolecule in the body. It is used in many biological systems such as mitochondria, which reduce the O_2in the synthesis of the adenosine triphosphate (ATP), during this reaction free radicals were product.

Free radicals are instable molecules contain one or more unpaired electron in their externe orbital. Free radicals are produced by endogenous or exogenous sources.Reactive species are compounds related to two types ofmolecules, the reactive oxygen species (ROS) and the reactive nitrogen species (RNS). Free radical produced by the body in the low level but overproduction of these molecules induced oxidativestress.

Oxidative stress is a status of an excessive production of reactive oxygen species in the organism and the low concentration of antioxidant. It has been demonstrated that reactive oxygen species (ROS) are key players in the hepatic and kidney pathophysiologic changes caused by chemicals.

The oxidative stress is associated with various diseases such as hypertension, cardiovascular disease, atherosclerosis, diabetes, cancer and arthritis. To deal with these species, the body is equipped with an effective defense system, which includes: enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), and glutathione reductase (GR); high-molecular-weight antioxidants such as albumin, ceruloplasmin, and ferritin; and low-molecular-weight antioxidants such as ascorbic acid, α-tocopherol, β-carotene, glutathione (GSH) and uric acid.

Carbon tetrachloride (CCl_4) has been widely used for the induction of liver and kidney damage in experimental animals. CCl_4 is converted by the cytochrome P450 in the CCl_3OO^* radical. This induced an increase of the level of peroxyde radical. This radical is responsible for lipid peroxidation.

Polyphenols effects on health are based on the results obtained from bioactivity studies, which in turn, has increased the interest in the consumption of foods rich in polyphenols. Such bioactive effects include antioxidant properties, prevention of oxidative stress associated diseases like cardiovascular, neurodegenerative diseases and cancer and their role in long term health protection by reducing the risk of chronic and degenerative diseases. Fruits and vegetables are rich sources of some micronutrients (vitamins, minerals), fibers and a wide array of phytochemicals that individually or in combination benefit human health.Several studies have linked vegetable consumption, especially fruits with a reduced risk for cancer

and cardiovascular disease, thus characterization of polyphenols is essential to increase the knowledge on fruits contents and their related beneficial effects.

The present study was conducted in order to identify polyphenols compounds and evaluate the *in vivo* and *in vitro* antioxidant activity and the protective effect of some fruits growing in Algeria and consumed largely in Setif on some oxidative stress indicators and some biochemical parmeters.

The aim of this study is:

Phytochemical analysis of fruit extracts by UPLC/DAD and HPLC/MS (LC/MS) to identify bioactive compounds in these fruits.

Determination of polyphenols, flavonoids, tannin, sugars, and protein contents in deferent methanolic fruits extracts.

Evaluation of the *in vitro* antioxidant activity of extracts using different assay (DPPH, ABTS, reducing power, chelation of iron, β-carotene and peroxidation lipidic).

Evaluation of the *in vivo* antioxidant activity of fruits extracts.

Evaluation of MDA and GSH levels in the liver and kidney of rats, treated with fruits extracts.

Evaluation of some biochemical parameters levels in plasma of rats treated with fruits extracts

Evaluation of protective effect of fruits extracts against hepatotoxicity and nephrotoxicity induced by CCl_4.

Review of literature

1 Oxidative stress

1.1 Definition of oxidative stress

Oxidative stress defined as a disturbance in the balance between the production of reactive oxygen and nitrogen species and antioxidant defenses (Cabello-Verrugio *et al.,* 2017). This disturbance is resulted from the overproduction of free radicals and the low level of antioxidant (Gammaz *et al*., 2018).

1.2 Free radicals

Free radicals are defined as molecules formed in the human body under physiologic and pathologic conditions, and having an unpaired electron in the outer orbit, generally is unstable and very reactive (Fang *et al*., 2002; Labo *et al*., 2010) such as oxygen reactive species (superoxide, hydroxyl, peroxyl (RO2•), alkoxyl (RO•), and hydroperoxyl (H_2O_2•) radicals. Examples for species nitrogen reactive (Nitric oxide and nitrogen dioxide (•NO2)). ROS and RNS include radical and non-radical species (Evans and Halliwll, 2001). Oxygen and nitrogen radicals can be transformed to non-radical, such as hydrogen peroxide, hypochlorous acid (HOCl), hypobromous acid (HOBr) and peroxynitrite (ONOO) (Kumar, 2011).

1.3 Formation of free radical

Free radicals are molecules occur by several mechanisms including endogenous and exogenous sources.A potential endogenous source of reactive oxygen species is the mitochondrial electron transport chain, inflammation, excessive exercise, ischemia, infection, cancer and aging (Hrycay and Bandiera, 2015). Exogenous free radical are the result of air and water pollution, cigarette smoke, alcohol, transition metals, certain drugs, industrial solvents, and radiation (Hrycay and Bandiera, 2015).

1.3.1 Internal sources of free radicals

ROS and RNS mainly produced by NADPH oxidases (Nox), Xanthine oxidoreductase, mitochondrial respiratory chain enzymes and NO synthases (NOS) (Liguori *et al*., 2018).

A) Mitochondrial respiratory chain enzymes

Mitochondria have important role in the number of fundamental function such as: respiration and oxidative energy production, regulation of the intracellular calcium concentration and

control of the fatty acid β-oxidation. The major role of mitochondria is the production of energy. They used about 95% of oxygen to produce ATP by the oxidizing substances contained in food by transfer of electrons to electron carriers such as NAD^+ and FAD (Navarro and Boveris, 2007).

The electron is transformed by the Complexes I and II carriers in the inner mitochondrial membrane associated with the formation of ROS. The first ROS produced by the mitochondria is the superoxide radical anion by 2% of oxygen. This converted to H_2O_2 in the presence of superoxide dismutase (SOD). The hydrogen peroxide is turned in the hydroxyl radical via the Fenton reaction (Mohamed *et al*., 2015; Di Meo *et al*., 2016). As described in the following reactions:

$$\mathbf{O_2 + e^- \longrightarrow O_2^{\bullet -}}$$

$$\mathbf{2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2}$$

$$\mathbf{Fe^{2+} + H_2O_2 \longrightarrow OH^{\bullet} + OH^- + Fe^{+3}}$$

B) NADPH oxidases (Nox)

NADPH oxidase (NOX) plays a pivotal role in the production of ROS, it catalyze the conversion of oxygen to superoxide (Muzza and Fugazzola, 2017)

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet -} + NADP^+ + H^+$$

NADPH oxidase (NOX) is a transmembrane enzyme and there are a number of isoforms: Fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac monocytes and thyroid tissue nonphagocytic (NOX1-5 and Duox1-2).Muscle cells and fibroblasts account for the majority of $O_2^{\bullet -}$ produced in the normal vessel wall . The different NOX isoforms in humans are involved in a wide range of cellular processes, including apoptosis, host defence, cellular signal transduction, oxygen sensing and angiogenesis. Although NOX is ubiquitously expressed the distribution of different isoforms is cell or tissue specific, allowing each NOX a distinct physiological and pathological function (Giardino *et al*., 2017).

C) Xanthine oxidoreductase

Xanthine oxidoreductase is a flavoprotein that contains molybdenum, nonheme iron and labile sulfur. The enzyme is present in two forms, one with dehydrogenase activity (xanthine dehydrogenase XDH) and the other with oxidase activity (Xanthine oxidase XO). XDH can

be converted to XO either by reversible thiol oxidation or by irreversible proteolytic cleavage (Hare *et al*., 2008). During the reactions of the oxidation of hypoxanthine to xanthine, and the oxidation of xanthine to uric acid, XOR normally produces two molecules of hydrogen peroxide and two molecules of superoxide. This enzyme plays an important role in the catabolism of purines in some species including human (Laurindo, 2018).

$$\textbf{Hypoxanthine} + H_2O + O_2 \rightarrow \textbf{xanthine} + H_2O_2$$

$$\textbf{Xanthine} + H_2O + O_2 \rightarrow \textbf{uric acid} + H_2O_2.$$

D) Nitric oxide synthases (NOS)

Nitric oxide ($NO^{\bullet}$) and L-citrulline are produced from a guanidine nitrogen of L-arginine via electron transfer from NADPH in two successive steps. The enzyme responsible for this reaction exists in three isoforms: neuronal (nNOS, type I, NOS-I or NOS-1), endothelial (eNOS, type III, NOS-III or NOS-3) and inducible (iNOS, type II, NOS-II or NOS-2). Although all three NOS isoforms (two constitutively-expressed isoforms; neuronal (nNOS) and endothelial (eNOS) and one inducible isoform; (iNOS) produce identical products, the function of the NO varies widely in terms of physiological functions due to the varied localization of the isoforms within different cell populations of the body (Miller, 2004). nNOS and eNOS are constitutively expressed, but their activity is regulated by the intracellular Ca^{2+} concentration. nNOS exhibits NADPH-diaphorase (NADPH-d) activity (Mohammed *et al*., 2015).

1.3.2 External sources

Reactive oxygen species can be produced by exogenous processes. The environmental sources include ionizing radiation and pollutants such as chemicals that promote the formation of superoxides such as quinones, nitroaromatic and herbicides (Elkhateeb and Alshammary, 2018)

A) Cigarette smoke

Cigarette smoke contained 4800 compounds, approximately 400-500 compounds present in the gaseous phase of Cigarette smoke. Its composition is complex, it contains carbon monoxide, nitrogen oxide, hydrogen cyanide as well as many volatile organic compounds and particulate matter. Various compounds may be associated with particulate matter, such as nicotine, tobacco-specific nitrosamines and polyaromatic hydrocarbonsmany (Baker, 2006)

.The vapor phase and particules matter of cigarette smoke contains free radicals including reactive oxygen species, reactive nitrogen species, superoxide, hydroxyl radicals, nitric oxide and hydrogen peroxide. These compounds can enter the bloodstream and generating ROS especially through interact with enzyme such as NADPH oxidase, then the result is stress oxidative is increased (Tostes *et al*., 2008). Semiquinone radicals associated with tar can reduce oxygen to produce superoxide as well as hydrogen peroxide and hydroxyl radicals. Moreover cigarette contains metal ion which can generate hydroxyl radicals from hydrogen peroxide (Pryor, 1997). It damages the liver, causes cirrhosis, damages the heart, causes myocardiopathy and damages the brain. All these free radicals that damage the lung so that almost every smoker gets bronchitis and emphysema by time (Pham-Huy *et al*., 2008).

B) Metal ions

Heavy metal ions, such as iron, copper, cadmium, Mercury, can induce the generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA (Birben *et al.*, 2012).

C) Air pollution

Air pollution contains gaseous phase, particulate matter, Ozone, nitrogen oxide, carbon monoxide and sulfurdioxide. Particulate matter can penetrate deep into the respiratory tract and can cause cytotoxicity by inducing oxidative stress, which may lead to oxidative damage of DNA, mutagenesis, and stimulation of proinflammatory factors. In addition to oxidative stress (due to oxygen free radicals), air pollution may also produce nitrosative stress (stress caused by reactive nitrogen species such as nitrous oxide) (Oh *et al*., 2011**).**

D) Radiation

Ionizing radiation such as UV, X or gamma ray produced hydroxyl radical, superoxide and organic radicals (Gaston, 2016). Free radicals, especially reactive oxygen species, can be generated by UV radiation that can damage DNA. Therefore, air pollution may act in synergy with UV radiation of sunlight in causing skin damage through the production of oxidative stress (Burke and Wei, 2009; Pfeifer and Besarantinia, 2012).

E) Dietary factors

Additives, alcohol, foods that have been barbecued, fried, grilled, foods that have been browned or burned, hydrogenated vegetable oils, processed foods containing high levels of lipid peroxides can also produce free radicals (Birben *et al*., 2012).

Alcohol consumption and alcoholism is one important cause of an acute illness and chronic disease worldwide. It is also observed that excess levels of ROS resulting in oxidative stress and have been implicated in a variety of human diseases.Many studies have demonstrated that alcohol increases lipid peroxidation as well as the modification of proteins. There are many processes and factors involved in causing alcohol induced free radical generation and oxidative stress (Elkhateeb and Alshammary, 2018).

Alcohol induced increases in the activity of the enzyme cytochrome P_{450} 2E1 (CYP2E1), which metabolizes alcohol and other molecules and generates ROS in the process. Alcohol induced increases in the levels of free iron in the cell (iron that is not bound to various proteins), which can promote ROS generation (Elkhateeb and Alshammary, 2018).

Biochemical reactions generating an alcohol derived radical (the hydroxyethyl radical and also in the conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS. It has also been observed that alcohol deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the reactive oxygen species generated during the various activities of the respiratory chain (Mukherjee, 2014).

Fast foods are characterized as quick, easily accessible and cheap alternatives to home-cooked meals such as foods that have been barbecued, fried, grilled, foods that have been browned or burned. Fast foods are a part of the new life. Fast foods contain high level of sugars, fat and salt (sodium). If the human consume high level of carbohydrates the blood sugar is increased. Frequent spikes in blood sugar may be a contributing factor in insulin resistance and type 2 diabetes. Excess sodium in fast foods also increases risk of developing osteoporosis (thin, fragile bones) (Elkhateeb and Alshammary, 2018).

High calories and fat induced High cholesterol and high blood pressure. It is considered a factor of heart disease (Young and Woodside, 2001).

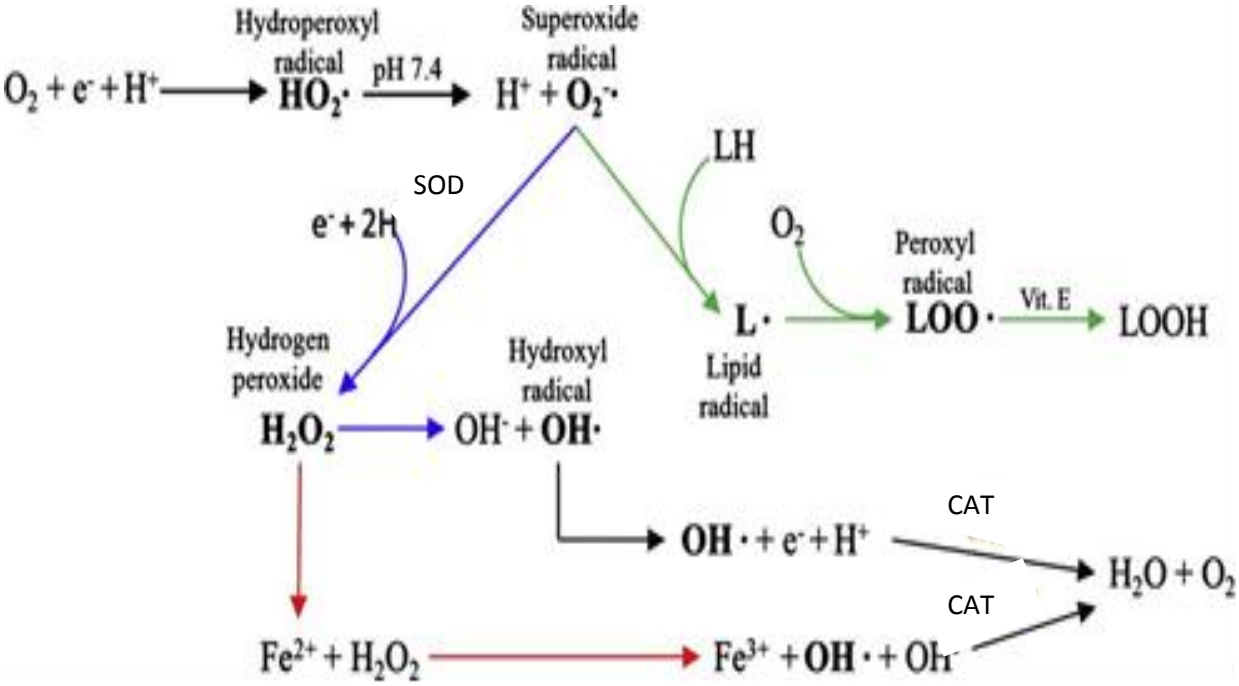


Figure 1: Overview of the reactions leading to the formation of ROS. Green arrows represent lipid peroxidation. Blue arrows represent the Haber–Weiss reactions and the red arrows represent the Fenton reactions. The bold letters represent radicals or molecules with the same behavior (H_2O_2). SOD refers to the enzyme superoxide dismutase and CAT refers to the enzyme catalase. Adapted from Ferreira *et al.*, 2009 and Flora ,2009.

1.4 Oxidative stress and pathologies associated to free radicals

Free radical have role important in human body, it is product normally with low amount by various physiological mechanisms. Excess formation of free radical can initiate series of chemical reaction and cause damage to cellular components such as: damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors (Das Sarma *et al*., 2010; Shastri *et al*., 2018) and production of proinflammatory and anti-inflammatory cytokines (Birben *et al*., 2012). However, when the cell can't produce the antioxidant for repair the damage the oxidative stress occur. The important relationship between oxidative stress and a wide variety of human diseases has placed this stress factor at the forefront of diseases research. Indeed, diseases such as rheumatoid arthritis, Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and cancer are all related to oxidative stress (Liguori *et al*., 2018).

1) Cancer:

It is one of the leading causes of death in humans. Free radicals cause different types of chemical changes in DNA, thus they could be mutagenic and involved in the etiology of cancer. Cancer cells in particular, in comparison to normal cells, have higher levels of ROS and are more susceptible to mitochondrial dysfunction due to their higher metabolic rate. Cancer cells display elevated levels of oxidative stress indicators due to the activation of oncogenes and loss of tumor suppressors. ROS by altering the growth signals and gene expression, cause continuous proliferation of cancer cells. ROS can damage DNA by inducing base modifications, deletions, strand breakage and chromosomal rearrangements (Cairns *et al*., 2011; Acuna *et al*., 2012).

2) Alzheimer's disease

Free radical damage induced strong damage in Nervous tissue due to high content of lipids especially polyunsaturated fatty acids. In Alzheimer's disease (AD) biochemical and histological studies have provided evidence for increased levels of oxidative stress and membrane lipid peroxidation. Alterations in levels of antioxidant enzymes such as catalase and CuZn- and Mn-SOD in neurons in AD patients increased stress. Peroxidation of 4-hydroxynonenal (4-HNE) in the cerebrospinal fluid of AD patients increase lipid peroxidation. Iron (Fe^{2+}) likely contributes to increase lipid peroxidation in AD. Lipid peroxidation may promote neuronal death in AD by multiple mechanisms that include impairment of the function of membrane ion-motive ATPases (Na^{+}/K^{+}-ATPase and Ca^{2+}-ATPase), glucose transporters and glutamate transporters. Lipid peroxidation leads to production of hydroxynonenal that appears to play a central role in the neurotoxic actions of amyloid β-peptide (Sivanandham, 2011).

3) Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world. Extensive postmortem studies have provided evidence to support the involvement of oxidative stress in the pathogenesis of PD; in particular, these include alterations in brain iron content, impaired mitochondrial function, alterations in the antioxidant protective systems (most notably superoxide dismutase (SOD) and reduced glutathione (GSH)) and evidence of oxidative damage to lipids, proteins, and DNA. Iron can induce oxidative stress and intranigral injections have been shown to induce a model of progressive parkinsonism. The nature of the free radical species responsible for cell death in PD remains unknown, but there

is evidence of involvement of hydroxyl radical, peroxynitrite and nitric oxide (Jenner and Olanow, 1996).

4) Rheumatoid Arthritis (RA)

Several lines of evidence suggest a role for oxidative stress in the pathogenesis of RA. Both ROS and RNS damage cartilage. Tissue injury in inflammation results in NO· production by articular chondrocytes and synovial fibroblasts and elevated levels of NO· are observed in the serum and synovial fluid of RA patients. The free radicals particularly NO· and $O2^{\bullet-}$, inhibit the synthesis of matrix components like proteoglycans by chondrocytes and also damage the extracellular matrix through activation and up regulation of matrix metalloproteinases (Vasanthi *et al*., 2009; Phaniendra *et al*., 2015).

5) Diabetes

Diabetes mellitus (type 1 and 2) is a metabolic disease associated with increased formation of free radicals and decreased antioxidant potential. Increased intracellular glucose leads to an increased RONS production, which exceeds the antioxidant capability of the cell to neutralize them. RONS induce, in this way, the activation of three important molecular pathways involved in hyperglycemia induced oxidative tissue: activation of protein kinase C (PKC), increased hexosamine pathway flux and increased polyol pathway flux (Liguori *et al*., 2018).

6) Cardiovascular disease

NADPH oxidases (Nox) are the important source of ROS. It is catalyze the transfer of electrons from NADPH to molecular oxygen to generate $O_2^{\cdot-}$. Important roles have been shown for NADPH oxidases in redox signaling events involved in hypertension, atherosclerosis, endothelial activation, and angiogenesis, well as in endothelial dysfunction. The close functional association between NADPH oxidase and the renin-angiotensin system may be of particular relevance in linking oxidative stress to hypertension. Nox2 and Nox4 are the most abundant NADPH oxidases in the heart and are expressed in cardiomyocytes, endothelial cells and fibroblasts. The over production of ROS induced the development of CVDs, particularly atherosclerosis (Puddu *et al*., 2008; Violi *et al*., 2009).

Oxygen free radicals are highly reactive compounds causing peroxidation of lipids and proteins and are thought to play an important role in the pathogenesis of reperfusion abnormalities including myocardial stunning, irreversible injury and reperfusion arrhythmias (Das Sarma *et al*., 2010).

7) Aging

Aging is the progressive loss of tissue and organ function over time. The free radical theory of aging, later termed as oxidative stress theory of aging, is based on the structural damage-based hypothesis that age-associated functionallosses are due to the accumulation of oxidative damage to macromolecules (lipids, DNA and proteins) by RONS. The exact mechanism of oxidative stress-induced aging is still not clear, but probably increased RONS levels lead to cellular senescence, a physiological mechanism that stops cellular proliferation in response to damages that occur during replication (Carocho *et al*., 2018).

8) Kidney disorders

Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. Iron catalyzed free radical formation and lipid peroxidation are accepted mechanisms of heme protein-induced acute renal failure. However, the source of those free radicals which trigger lipid peroxidation in proximal tubular cells remains unknown (Das sarma, 2010).

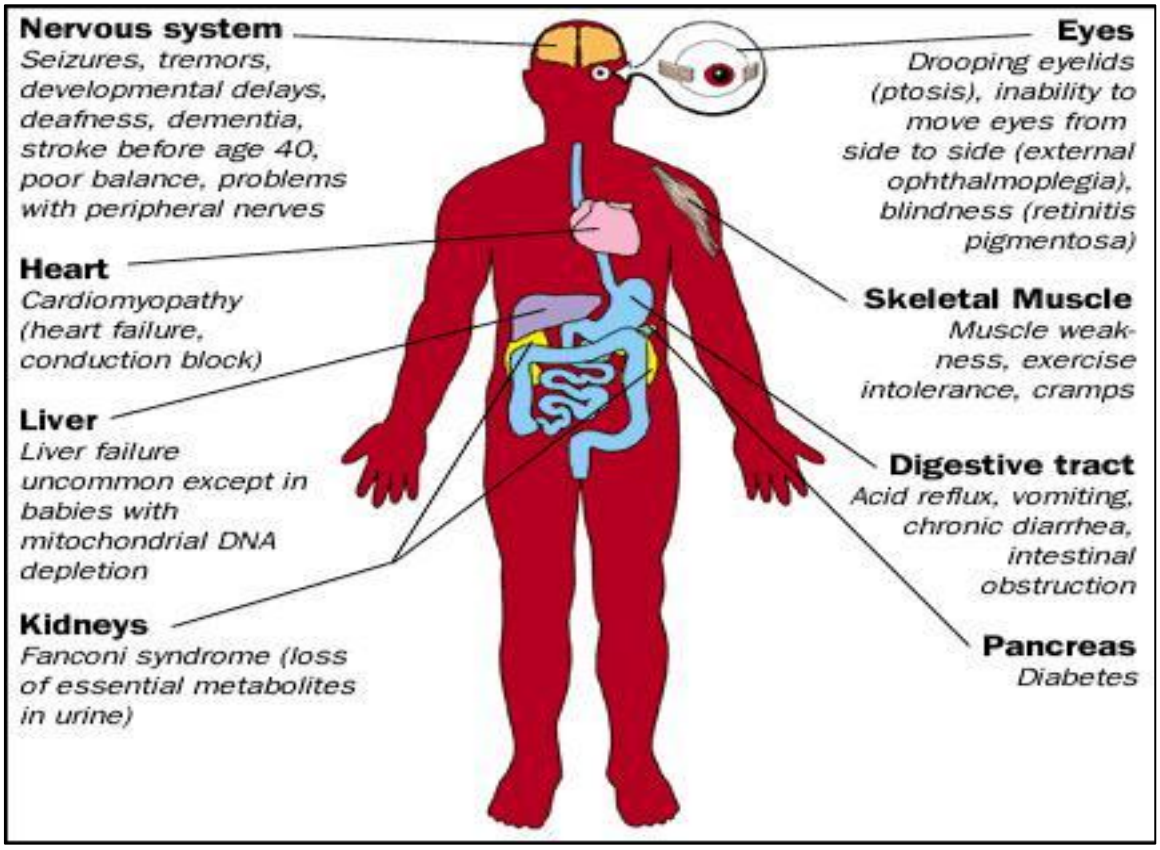


Figure 2: Overview of free radical damage (Das Sarma *et al.*, 2010)

1.5 Antioxidants

The term antioxidant was defined as the molecule which can give an electron to free radicals to neutralize them and reduces their damage capacity. This molecule is a stable substance and protects cell against any damage effect of free radicals (Carocho andFerreira, 2013).

The damaging effect of free radicals is limited by numerous cellular antioxidant defense mechanisms in the body. Common antioxidants include: enzymes such as SOD, CAT and glutathione system, non-enzymes such as vitamins (C, E), carotenoids, flavonoids, glutathione and other minerals antioxidants (copper, ferritin, zinc, manganese, selenium etc.) (Lozovoy *et al*., 2013).

1.5.1 Enzymatic antioxidants

A) Superoxide dismutase (SOD)

Superoxide dismutase is a group of metalloenzymes which are essential to protect cells under aerobic conditions. This enzyme catalyzes the transfer of an electron from one molecule of the superoxide anion to dioxygen, which rapidly combines with two hydrogen ions to produce hydrogen peroxide. There are three forms of SOD in the human body:

• SOD1 in the cytoplasm: copper and zinc (Cu-Zn-SOD).

• SOD2 in the mitochondria: Manganese superoxide dismutase (Mn-SOD) plays a crucial role in antioxidant responses to environmental stress.

• SOD3 extracellular: copper and zinc (Cu-Zn-SOD) (Docampo and Moreno, 2017).

B) Catalase (CAT)

Catalase (CAT) is a 240 kilodalton (kDa) tetrameric protein. CAT is a common antioxidant enzyme present almost in all living tissues that utilize oxygen. This enzyme is found mainly in mitochondria and peroxisomes. The enzyme was originally regarded as a monofunctional peroxisomal enzyme that efficiently catalyzes the dismutation of hydrogen peroxide to water and oxygen and uses either iron or manganese as a cofactor. In the last year catalase is considered a multifunctional enzyme, which exhibits not only classic catalase activity, but also peroxidase and oxidase functions such as: the formation of compound I, degradation of NO. Catalase has protective effects in the cardiovascular system and delayed the development of atherosclerosis (Castaldo *et al*., 2016).

C) Glutathione system

This system includes three enzymes: GSH reductase, GSH peroxidase (GPx) and glutathione-S-transferase (GST)(Lumb, 2017).

1. GSH reductase (GR)

GSH reductaseis a group of flavoproteins and it is homodimer containing FAD coenzyme in its active site that catalyzes the transfer of electrons from NADPH to FAD, ultimately to the reactive disulfide bridge center that reduces GSSG to GSH. This enzyme maintains GSH levels in the cell (Gregg and Prchal, 2018).

2. GSH peroxidase(GP_x)

The enzyme activity depends on a micronutrient cofactor known as selenium. The biochemical function of GPx is to reduce free hydrogen peroxide to water and to reduce lipid hydroperoxides to their corresponding alcohols and in turn oxidizes glutathione to glutathione disulfide (Ighodaro and Akinloye, 2017). There are four isoforms of GPx

1. Cellular GPx (GPx1): reduces H_2O_2 and fatty acid peroxides
2. Gastrointestinal GPx (GPx2): is localized in the gastrointestinal epithelial cells.
3. Extracellular GPx (GPx3): is the only member of the GPx family that exists in the extracellular compartment, and is believed to be the most important extracellular antioxidant enzyme in mammals
4. Phospholipids hydroperoxide GPx (GPx4): esterified lipids are reduced by membrane-bound GPx-4 (Tabet and Tyouz, 2007).

3. GSH-S-transferase (GST_s)

This is another antioxidant enzyme family, which inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST, and membrane-associated microsomal GST (Birben *et al*., 2012). GST used the GSH reduced by GSH reductase to neutralize diverse electrophilic and reactive compounds by conjugating them with GSH. Glutathione-S-transferases also metabolize H_2O_2 and many other endogenous hydroperoxides (Hrycay and Bandiera, 2015).

1.5.2 Non enzymatic antioxidants

A) Glutathion (GSH)

GSH is a tripeptide including Glutamate, cysteine and glycine. This tripeptide is formed by two steps. The first, γ-glytamylcysteine by γglytamylcysteine ligase (GSH1) then the GSH sentethase add glycine to dipeptide. GSH can regenerated from the oxidized GSSH in the

presence of GR. GSH is present in plants in the cytosol, chloroplast, mitochondria and nucleus. It is the major antioxidant in these cell compartments. Protective roles of glutathione against oxidative/nitrosative stress are that it can act as a co-factor for several detoxifying enzymes, participate in amino acid transport across plasma membrane and scavenge hydroxyl radical and singlet oxygen directly (Csiszár *et al*., 2016).

B) Vitamin C (Vit C)

Vit C is a major water soluble antioxidant in the body. It quenchs the activity of some free radicals by donating electron such as hydroxyl and superoxide, and scavenge other ROS such as singlet oxygen, superoxide, hydroxyl, water soluble peroxyl radical, and hypochlorous acid. Vit C has an important role in the regeneration of vitamin E and protects biomembranes from peroxide damage. It increases the level of GSH in the cell. Fresh fruits and vegetables contain high amounts of vitamin C (Marin *et al*., 2018).

C) Vitamin E

Vitamin E is a lipid soluble vitamin. It is an important antioxidant having various physiological functions, including the maintenance of plasma membrane integrity, cell signaling and cell cycle regulation, cell adhesion, platelet aggregation, smooth muscle cell proliferation and immune function. The major role of vit E is re-evaluated by the European Food Safety Authority expert panel(Raederstorff *et al*., 2015). It can scavenge free radicals by the hydroxyl group specifically with the acyl chains of polyunsaturated fatty acids in the membrane. There are four isoform of vit E: α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol (Temiz *et al*., 2018).

D) Carotenoids

Carotenoids are groups of pigments found in most fruits and vegetables, plants, algae and photosynthetic bacteria and more than 600 different carotenoids have been identified and characterized. Carotenoids include β-carotene, lutein, zeaxanthin, lycopene, astaxanthin and canthaxanthin. Lycopene showed potent antioxidant activity *in vitro* and reduces serum cholesterol in animal studies (Müller *et al*., 2016). Carotenoids have the ability to neutralize singlet oxygen and reactive oxygen species, protect against UV-induced peroxidation and reduce the formation of lipofuscin and associated oxidative-stress induced damage (Eggersdorfer and Wyss, 2018).

E) Coenzyme Q10

CoQ10 is an ubiquinone, it is a lipid-soluble benzoquinone. CoQ10 is considered as intracellular antioxidant that protects membrane phospholipids, mitochondrial membrane proteins, and low-density lipoproteins from free radical-induced oxidative damage. Coenzyme Q10 is added to support mitochondrial functions such as shuttling electrons, serving as a potent antioxidant and working as an electron transport chain to generate ATP (Mousavinejed *et al*., 2018).

F) Uric acid (UA)

Uric acid is a heterocyclic organic compound. It is the end product of purine metabolism or purine nucleotides (guanosine and adenosine) catabolism in humans. In rats and mice, UA is converted by the enzyme uricase to produce allantoin. This is 100 times more water-soluble than UA and consequently has more efficient urinary excretion route than UA. Various studies have shown that uric acid is a potent antioxidant against oxidative stress. It is capable particularly of neutralizing superoxide, peroxyl, hydroxyl radicals (OH), hypochlorous acid (HOCl) and protects vascular endothelium from external oxidative stress (Banihani, 2018). Uricases also prevents peroxynitrite induced protein nitrasation, lipid and protein peroxidation and used a cofactor for endothelial nitric oxide synthase (Ndrepepa, 2018).

G) Selenium

It is a trace mineral that supports the body in many ways.Selenium has unique multi-functional function: catalytic, structural, and regulatory. It activates the action of many enzymes, vitamins, hormones and thus ensures the normal functioning of various biological systems. Selenium is one of the most important antioxidants in the human body. The biochemical functions of selenium are determined by the selenium proteins that contain selenocysteine, which have antioxidant functions and include enzymes such as glutathione peroxidase, a potent free radical scavenger. Selenium acts to destroy peroxides and thus protects lipid membranes like vitamin E (Sobolev *et al*., 2018).

H) Zinc

It is an essential mineral for human health, it is considered as co-factor for over 300 enzymes and 2000 transcription factors. This mineral is a mediator of cellular signaling. In addition, the zinc has an important role in the antioxidant defense system. This molecule protects the cell against the oxidative damage. It also inhibits the NADPH-oxidase enzyme, regulation of

GSH peroxidase and the expression of metallothioneim, used as co-factor for SOD and reduces chronic inflammation and hyperglycemia (Marreiro *et al*., 2017).

I) Polyphenols

Polyphenols are important group of natural compounds they contained heterogeneous group of molecules with feature structure. Polyphenols are constituted of aromatic ring and several hydroxyl groups. They are synthetized by two pathways, the first is the Shikimic acid pathway and the second is the acetic acid pathway. Based on the chemical structure, polyphenols are divided into five groups as flavonoids, phenolic acid, stilbenes, lignans and tannins (Belščak-Cvitanović *et al*., 2018)

1. Flavonoids

Flavonoids came from the Latin word « flavus» wich means yellow. They are the biggest group of polyphenol present in the plant with low molecular weight. Generally they have a common C6-C3-C6flavone skeleton, which contained two aromatic ring (A and C) and heterocyclic (B). Depending on the oxidation and the saturation in the C ring flavonoids mainly are divided into flavanols, flavones, flavonones, isoflavone, flavonol and flavanonol. In fact, the majority of flavonoids exist in fruits and vegetables as glycosides (Vicente and Boscaiu, 2018).

2. Tannin

Tannins are water-soluble phenolic compounds having a molecular weight between 500 and 3000 Dalton. Tannins are classified into two major groups on the basis of their structure: hydrolysable and condensed tannins.

a) Hydrolysable tannins: Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexahydroxydiphenic acid, also called ellagitannins (Sarni-Manchado and cheynier, 2006).
b) Condensed tannins: Condensed tannins are oligomers or polymers composed of flavan-3-ol nuclei. They are also called proanthocyanidins, because they are decomposed to anthocyanidins in heated ethanol solutions (Dangletter, 2007).

They characteristic properties of tannin as include forming of insoluble complexes with proteins, polysaccharides, nucleic acids and alkaloids. Within this general character, tannins exhibit a number of various bioactivities, which are often related to their antioxidant activity (Koleckar *et al*., 2008)

Antioxidant activity study of tannins *in vitro* and *in vivo* showed that they are effective scavengers of free radicals and they inhibit the tissues oxidation better than vitamin C, vitamin E and β-carotene. The basic concept of free radical scavenging activity of polyphenols, including tannins, is the ability of the antioxidant to donate electron to a free radical and produce a more stable and therefore less harmful radical structure. DPPH (1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazyl) or ABTS ((2, 2-azinobis)3-ethyl-2,3-dihydrobenzothiazol-6-sulphonic acid) radicals are often used for in the *vitro* evaluation of free radical activity of plants extracts (Hatano *et al*., 2002). Procyanidins are the most active on the basis of scavenging ABTS radical, hypochlorous acid, or in FRAP test for the evaluation of reducing power (Koleckar *et al*., 2008).

In vitro conditions have been shown that condensed tannins have a preference for neutralizing the hydroxyl free radical ($OH^{\cdot}$). It has been demonstrated that they have the capacity to act as noncompetitive inhibitors of the enzyme xanthine oxidase, one of the biggest generators of free radicals in cellular metabolism. Additionally, they act as scavengers of free radicals and inhibitors of superoxides formation and nitric oxide synthases (NOS) (Fogliani *et al*., 2005; Koleckar *et al*., 2008). Chandranayagam *et al* (2013) demonstrated that the gavage of tannin with dose of 30-300 mg/kg protect the liver.

3. Phenolic acids

Phenolic acids belong to a highly diversified group of phytochemicals, phenolics which are found in all foods of plant origin in the human diet. Phenolic acids are non-flavonoid polyphenolic compounds which can be further divided into two main types: benzoic acid and cinnamic acid derivatives based on C1–C6 and C3–C6 backbones. Hydroxybenzoic acids include gallic, p-hydroxybenzoic, vanillic, syringic, and protocatechuic acids. The hydroxycinnamic acids commonly found in foods and beverages are p-coumaric, caffeic, ferulic, sinapic and cinnamic acids. While fruits and vegetables contain many free phenolic acids, in grains and seed particularly in the bran or hull—phenolic acids are often in the bound form . These phenolic acids can only be freed or hydrolyzed upon acid or alkaline hydrolysis, or by enzymes (Nazck and Shahidi, 2006; Chandrasekara and Shahidi, 2010).

In the process of free radical scavenging, phenolic acids produce phenolic radicals and they can be stabilized by establishing intra-molecular hydrogen bonds and extending delocalization and conjugation of the electrons enhanced by resonance stabilization (Chen *et al*., 2015).

Ferulic acid has demonstrated *in vitro* free radical scavenging properties against hydroxyl radical, peroxynitrite and LDL oxidation (Ogiwara *et al*., 2002). Caffeic acid has the ability of scavenging several reactive species, such as DPPH, peroxyl, hydroxyl, superoxide radical anion, peroxynitrite and singlet oxygen (Gulcin, 2006). Chlorogenic acid has been shown to scavenge organic free radicals, hypochlorous, superoxide, hydroxyl radicals and peroxynitrite (Chandrasekara, 2018). Ferulic acid treatment has shown beneficial effects by reducing oxidative stress in diabetic. It decreases the levels of thiobarbituric acid reactive substances (TBARS), hydroperoxides and free fatty acids. It increases the level of glutathione and antioxidant enzymes activity such as glutathione peroxidase, superoxide dismutase and catalase (Balasubashini *et al*., 2004).

4. lignans

Lignans are members of phenylpropanoids, and their basal structures contain dimers of coniferyl alcohol that is defined as a "C6–C3" unit. The phenylpropane units are covalently bound by the central carbons of the side chains. Depending on structural patterns such their carbon backbones, the position of oxygen incorporated into the skeletons and the cyclization styles lignins are divided into eight groups: furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene and dibenzylbutyrolactol (Satake *et al*., 2013, 2015, 2016, 2017). Lignan have preventive or reductive effects on extensive life-related diseases, including cancers, diabetes and hypertension. There are simple lignans and cyclic lignans that fight against the damaging effects of free radicals and whose compounds like enterodiol and enterolactone have anticancer potential that mimics the functions of human hormones, in addition to inhibiting the growth of breast and prostate tumors (Goyal *et al*., 2014; Kajla *et al*., 2015; Schmidt *et al*., 2012). These compounds can use a supplements food. Indeed, sesame lignan is the most functional supplements available commercially due to its beneficial effects on human health, such as antihypertension and protection of the liver based on reduction of lipid oxidation (Satake *et al*., 2013, 2015, 2016).

5. Stilbenes

Stilbene is a family has structure C6–C2–C6, like flavonoids, are polyphenolic compounds. Stilbenes are phytoalexins, compounds produced by plants in response to attack by fungal, bacterial and viral pathogens. Resveratrol is the most common stilbene. It occurs as both the cis and the trans isomers and is present in plant tissues primarily as trans-resveratrol-3-O-glucoside. The major dietary sources of stilbenes include grapes, wine, soya and peanut

products. Trans-resveratrol and its glucoside are found in especially high amounts in the Itadori plant (Polygonum cuspidatum). Trans-resveratrol is considerate a contributors to the cardioprotective effects and can inhibit LDL oxidation, the initial stage of parthenogenesis of atherosclerosis (Crozier *et al*., 2006).

1.6 Some fruits rich in polyphenols and flavonoids

Several studies suggest that inverse relationship between the consumption of fruits and vegetables and chronic disease such as cancer, cardiovascular and neurodegenerative diseases. There is lot of evidence showed that when people consume sufficient quantities of fruits and vegetables the risk of these diseases is lower. In addition, the interest in understanding the type, number and action mechanism of the different components of fruits and vegetables that confer nutritional and health benefits is also increasing (Yahia *et al*., 2019).

The nutritional values of fruits come mostly from their micronutrient content(vitamins and minerals), nonnutritional bioactive substances and bioactive compounds. Phytochemicals present in fruits and vegetables are very diverse, such as ascorbic acid, carotenoids and phenolic compounds (Laura, 2010). Polyphenols is the important group of phytochemicals. They have various benefits human health. Which can considered as antioxidant, which may be protective against a wide variety of conditions, ranging from heart disease to cancer and diabetes. The consummation of polyphenols in foods may help preventing chronic disease and minimizes the risk of oxidative stress (Laura, 2010).

Table 1: Sources and potential effects of some phytochemicals on human health and some pathologies.

Phytochemical	**Sources**	**Potential Effects on Human Health**
Phenolic compounds		
1. Flavonoids		
Anthocyanidins (cyanidin, malvidin, delphinidin, pelargonidin, peonidin, petunidin)	Red, blue, and purple fruits (apple, blueberry, blackberry, cranberry, grape, nectarine, peach, plum, prune,raspberry, pomegranate, strawberry)	Heart disease, cancer initiation diabetes, cataracts, blood pressure, allergies (Yahia *et al*., 2019)
Flavan-3-ols (epicatechin, epigallocatechin, catechin, gallocatechin)	Apples, apricots, blackberries, plums, raspberries, strawberries, cherries.	Reduce systemic inflammation markers and decrease oxidative stress (Si *et al*., 2011). Decrease plasma oxidized lipoprotein cholesterol (Inami *et al*., 2007)
Flavanones (hesperetin, naringenin, eriodictyol)	Citrus such as oranges,lemons, Grape fruits, Tangerines.	Heart disease (Agrawal *et al*., 2014). Decrease glucose levels (Mahmoud *et al*., 2012). Restore lipid profile changes, increase antioxidant state in liver (Priscilla *et al*., 2015).
Flavones (luteolin, apigenin, chrysin)	Guava, Juniper berries	Increase of antioxidant enzymes activities enzymes

		and reduce serum triacylglycerol and total cholesterol (Wang *et al*., 2012) Protect tissues from glucose damage and modulate lipid peroxidation (Hossain *et al*., 2014)
Flavonols (kaempferol, myricetin, quercetin, rutin)	cranberry, apples, cherry, Grape	Inhibit growth in a dependent manner. Induce chromatin condensation and fragmentation. Induce DNA double-strand breaks and endoplasmic reticulum stress (Xu *et al*., 2016). Decrease blood lipids. Restore insulin resistance. Reduce hepatic inflammatory lesions (Luo *et al*., 2015)
2. Phenolic acids		
Hydroxybenzoic acids (gallic acid, protocatechuic acid, syringe acid, vanillic acid)	Black olive, black raspberry, dates (dried, fresh), kiwi, strawberry.	Endothelial dysfunction, Hypertension Reduction of oxidative stress Decrease of cholesterol levels, and hepatic triacylglycerol accumulation (Hsu and yen, 2007).
Hydroxycinnamic acids (caffeic acid, ferulic acid, sinapic acid, chlorogenic,	Apple, blueberry, cherry, cranberry, grapes, lemon, pear, orange, grape fruit,	Atherosclerosis, antimicrobial effect, anti-inflammatory and cancer.

acid, coumaric acid)	peach.	Decrease oxidative stress markers in kidneys and albumin excretion and albumin creatinine ratio (Choi *et al*., 2011).
3. Tannins		
Proanthocyanidins	Apple, Pomegranate and grape.	Cancer (Yahia *et al*., 2019)

1.6.1. Antioxidant activity of polyphenols and flavonoids in fruits

Mechanisms of the antioxidant action of polyphenols can include direct scavenging and can neutralize of reactive-free radicals by the donating of electron or hydrogen atom (Makris and Boskou, 2014).

Polyphenols are also known as metal chelators. A number of polyphenols having a 1,2-dihydroxy, a α-hydroxyketo, or β-hydroxyketo substitution efficiently chelate trace metal ions, such as Al^{3+}, Fe^{3+} and Cu^{+}, that play an important role in oxygen metabolism and free radical formation (Dangles, 2006). Chelation of transition metals can directly reduce the rate of Fenton reaction.

In vitro antioxidant activity of polyphenols can be increased by polymerization of flavonoid monomers, resulting in the formation of proanthocyanidins or condensed tannins. Due to the increased number of hydroxyl groups those polymers are very potent antioxidants *in vitro*. Glycosylation significantly decreases the antioxidative capacity of polyphenols (Belščak-Cvitanović *et al*., 2018).

Flavonoids are able to activate of antioxidant enzymes such as NADPH-quinone oxidoreductase, glutathione S-transferase, and UDP-glucuronosyl transferase, which are the major defense enzymes against electrophilic toxicants and oxidative stress (Ago *et al*., 2003)

Ability to inhibit oxidases such as xanthine oxidase, NADH oxidase, and protein kinase C. Flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase and microsomal succinoxidase (Procházková *et al*., 2011).

Polyphenols may well exert most of their antioxidant activity in the gastrointestinal tract, for instance, by inhibiting the peroxidation of dietary polyunsaturated fatty acids initiated by heme iron (Vulcain *et al*., 2005).

Epicatechin and quercetin accumulated mostly as glucuronide and sulfate conjugates in blood plasma after oral administration in rats. No intact quercetin was found in the circulation. However, on the oral administration of these flavonoids, the antioxidative ability of rat plasma was enhanced indicating that conjugated metabolites participate in the antioxidant defense (Procházková *et al*., 2011).

1.6.2 Effects of polyphenols and flavonoids on some biochemical parameters

A) Uric acid levels

Cao *et al*. 1998 described the significant increase in plasma or serum urate after consumption of strawberries, spinach or red wine. Lotito and Frei (2006) suppose that the large increase in plasma total antioxidant capacity observed after the consumption of flavonoid rich foods is not caused by the flavonoids themselves, but is likely the consequence of increased uric acid levels, which is a major contributor to plasma total antioxidant capacity .

B) Blood glucose level

Previous studies of ferulic acid nargenin and hesperdin, keampfrol treatment describe reduced blood glucose levels (Jung *et al*., 2007) and weak -ened lipid peroxide by increasing the antioxidant capacity (Sri Balasubashini *et al*., 2003). In type 2 diabetic people, total polyphenol and total flavonoid but not phenolic acid intake was associated with lower Hemoglobin levels. Lignan was associated with elevated fasting blood glucose (Sakaki *et al*., 2019).

C) Levels of cholesterol, triacylglycerol and LDL

Keamphrol decreased significantly the circulating levels of serum triacylglycerol, cholesterol and LDL. Some studies show that oral administration of flavonoids decreases plasma cholesterol and/or TG levels in rats (Wang, 2012). Catechin and epicatechin reduce the level Of LDL (Koleckar *et al*., 2008).

D) Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and G-glutamyltransferase (GGT)

Animal studies have shown that administering apricot to rats chronically feeding on ethanol decreased the levels of ALT and AST in the serum. Studies have shown that coadministration of grapes to rats decrease in the levels of AST, ALT, ALP and GGT in the liver. *In vivo* studies with rats have also shown that ferulic acid and quercetin is shown to decrease the

elevated serum levels of AST, ALT, ALP and GGT in rats subjected to ethanol induced hepatotoxicity (Shivashankara *et al*., 2011).

C) Creatinine and urea

Orhan *et al*., 2007 demonstrated that the feding with Vitis inhibit cytochrome P450, which convert the CCl_4 on CCl_3 radical. This last induce the decrease in the level of creatinine and urea.

1.7 Assay of antioxidant activity *in vitro*

Various assays for measuring total antioxidant capacity of antioxidants in food constituents or biological samples are based on electron transfer reaction such as ABTS radical scavenging assay, the ferric ion reducing antioxidant power assay (FRAP), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. There is other method for measuring the total antioxidant capacity for example Superoxide anion radical, Hydroxyl radicals, the nitric oxide radical scavenging capacity. The most common metal chelating assay is based on ferrous ion chelating capacity. In fact, antioxidant activities in complex systems cannot be evaluated satisfactorily using a single test, and several test procedures may be required (Laura 2010; Dasgupta and Klein, 2014).

In the $ABTS^{\cdot+}$ radical scavenging assay (an electron transfer-based assay), the 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$), have dark blue color which is reduced by an antioxidant into colorless ABTS, which can be measured spectrophotometrically. In the original assay, metmyoglobin reacted with hydrogen peroxide to generate ferrylmyoglobin, which reacted with ABTS to form the $ABTS^{\cdot+}$ radical cation. However, other antioxidants can also be used for oxidation of ABTS, including manganese dioxide potassium persulfate and horseradish peroxidase (Erel, 2004).

The ferric ion reducing antioxidant power assay (FRAP) is based on reducing power of an antioxidant to reduce a ferric salt (Fe^{3+}) to ferrous salt (Fe^{2+}) by electron transfer reaction and blue color is appeared. FRAP assay can also be carried out using ferricyanide [Fe^{3+} $(CN^{-})_6$], in which an antioxidant donates an electron and converts fer-ricyanide to ferrocyanide [$Fe^{2+}(CN^{-})_6$], which in the presence of excess ferric ion (ferric chloride) produces an intense blue color (Perl's Prussian blue) (Gulcin, 2012).

In DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay the presence of antioxidant the DPPH radical is reducedand the color is changed. The concentration of antioxidant needed to reduce DPPH concentration by 50% is termed IC_{50} (moles of antioxidant per mole of DPPH) (Jimenez-Escrig *et al*., 2000).

Xanthine oxidase is an endogenous source of ROS *in vivo*. In normal tissue, xanthine oxidase transfers electrons to nicotinamide adenine dinucleotide (NAD), it oxidizes xanthine or hypoxanthine to uric acid. In stress conditions, the dehydrogenase is converted to an oxidase enzyme and produces superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide. At pH 7.4 xanthine oxidase plus xanthine or hypoxanthine used to generate the $O_2^{\bullet-}$ anion (MacDonald-Wicks *et al*., 2006), which reduces nitroblue tetrazolium (NBT) into formazan. The reduction of absorbance is estimated as $O_2^{\bullet-}$ scavenging activity compared to the value obtained with no test added sample (Sanchez-Moreno, 2002).

In the Fenton reaction the hydroxyl radical ($OH^{\bullet}$) is formed by the combination of Fe (II) and hydrogen peroxide. The original method of the $OH^{\bullet}$ scavenging used 2-deoxy-d-ribose. The OH• radical is formed by combining ascorbic acid, hydrogen peroxide, ferric chloride ($FeCl_3$), and ethylenediaminetetraacetic acid (EDTA) in aqueous solution. $OH^{\bullet}$ radical react with deoxyribose and donated series of fragments. Which combine with thiobarbituric acid (TBA) and formed a pink chromogen when heated (absorbance=532 nm). Scavengers of the hydroxyl radical prevent the formation of this chromogen by competing for the radical. This method has been used by some authors to study the antioxidant activity in several vegetables (Chu *et al*., 2002) and berries (Pantelidis *et al*., 2007).

1.8 Assay of antioxidant activity *in vivo*

Various methods are used for measuring total antioxidant capacity of a compound or a mixture of compounds such as food, but many of these methods can also be used for determining antioxidant capacity of human serum or plasma (Huang *et al*., 2005). In other hand, there is other study considered to measure the oxidative damage in the biomolecules such as lipid, DNA, protein and other enzymes (table). Moreover Vit C, GSH and β-carotene in the blood can directly measure their antioxidant capacity (Dasgupta and Klein, 2014).

Thiobarbituric Acid–Reactive Substance (TBARS) Assay

This test is used for both *in vitro* and *in vivo* determinations. It is the best assay for measure the lipid peroxidation. The antioxidant can effect in the deferent steps in the production of

TBARS.It involves reacting thiobarbituric acid (TBA) with malondialdehyde (MDA), produced by lipid hydroperoxide decomposition, to form a red chromophore with peak absorbance at 532 nm (Moselhy *et al*., 2013).

Table 2: Methods for assessing antioxidant activity *in vivo* and *in vitro*

Principle	Marker	Method	References
Oxidative stress	Lipid oxidation	TBARS: Spectrophotometry (532–535 nm) Malondialdehyde (thiobarbituric acid reactive substances)	Dasgupta and Klein, 2014
	Protein peroxidation	GC/MS, HPLC. Spectrophotometry (540nm)	Laura, 2010
	DNA damage 8-	Hydroxydeoxyguanosine: HPLC, ELISA	Laura, 2010
Antioxidant enzymes	Superoxide dismutase Catalase	Spectrophotometry (240 nm) Spectrophotometry (420 nm)	Dasgupta and Klein, 2014
Other markers	Glutathione	Spectrophotometry (412nm)	
Scavenging radical Species	Hydrogen peroxide	Spectrophotometry (230 nm)	Laura, 2010
	Hydroxyl radical	Spectrophotometry (532 nm)	
	Peroxyl radical	Carotene bleaching: Spectrophotometry (470 nm)	
	ABTS radical cation DPPH radical Electron	Spectrophotometry (660–820 nm) Spectrophotometry (515–528 nm)	
Other procedure	Folin–Ciocalteu FRAP	Spectrophotometry (750 nm) Spectrophotometry (593 nm)	

2. Study the toxicity of extracts

2.1 Hepatoprotective and Hepatotoxicity of plant extracts and natural products

2.1.1 Structure and Function of the Liver

The liver is the largest organ in the human body, accounting for approximately 2% to 3% of average body weight. It is weighs approximately 1500 g and is located in the upper right corner of the abdomen. The organ is closely associated with the small intestine. The falciform is divided the liver in two lobes: the right lobe occupied 2/3 volume total of liver, for the left lobe is occupied 1/3 volume total of liver. Each lobe is divided into lobules. The liver is protected by the Glisson capsule (McCuskey *et al*., 2012). The liver is contained several cell types, which not interact only with each other but also are adapted to perform specific functions. The principal cell type is the hepatic parenchymal cell or hepatocyte, which accounts for 60% of the total cell population and 80% of the volume of the organ. Approximately 80% of the blood entering the liver is poorly oxygenated and is supplied by the portal vein. This is the venous blood flowing from the intestines, pancreas, spleen and gallbladder. The remaining 20% of the blood supply is well oxygenated and delivered by the hepatic artery (Saxena *et al*., 2003).

The liver is a vital organ only found in vertebrates. It has a wide range of functions, including detoxification of various endogenous metabolites and exogenous toxic substances, protein synthesis, and the production of biochemicals necessary for digestion. It also plays a critical role in metabolism, including the regulation of glycogen storage and lipid homeostasis (Su *et al*., 2018).

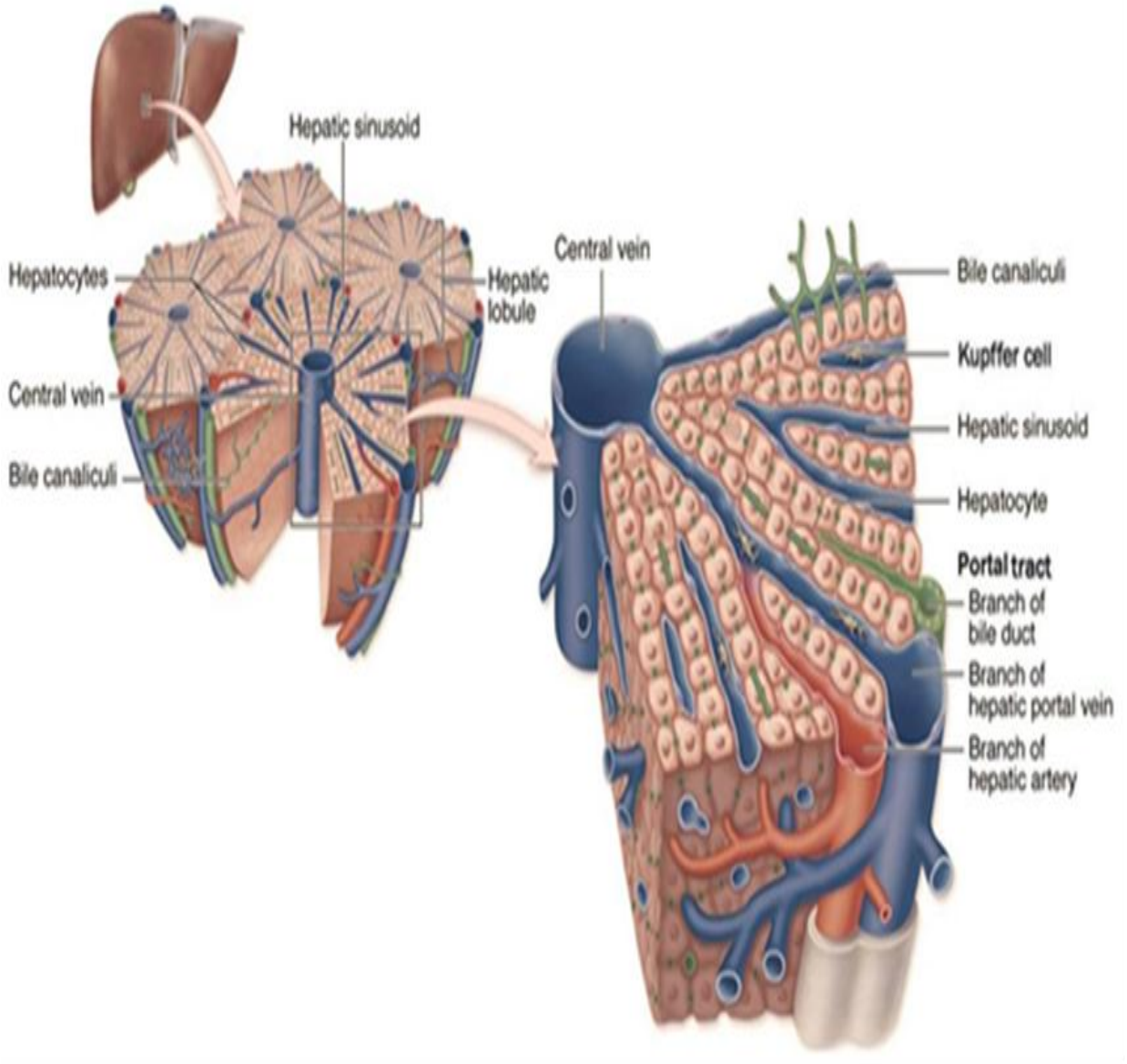


Figure 3: Anatomy of the liver and the hepatic microstructures (Mescher, 2010).

2.1.2 Tetrachloride Carbone solvent

Carbon tetrachloride (CCl_4) is an aliphatic halogenated hydrocarbon, derived from methane. It is few soluble in the water but miscible with many organic solvents. Carbon tetrachloride (CCl_4) is a toxic chemical agent, which is used to induce kidney and liver injury in animals, after body exposure. Kidney and liver tissue has great affinity for CCl_4 due to the predominant presence of the cytochrome P_{450} in the renal cortex and in the liver. It is converted to CCl^{*}_{3} radical by the cytochrome (P450 2E1) (El-haskoury *et al*., 2018). CCl^{*}_{3} react with molecular oxygen and formed CCl_3OO^{*} radical, which react with macromolecule (DNA, lipid, protein) and induced auto-oxidation (Safhi, 2018).

2.1.3 Hepatotoxicity

The liver is important organ for the elimination the toxic substances. There are various studies which used many toxicants to induce liver injuris. The potent hepatotoxic agent is CCl_4. This drug induce the disintegration the membrane of hepatocytes with subsequent release of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and G-glutamyltransferase (GGT) marker enzymes of hepatotoxicity (Saad *et al*., 2014).

2.1.4 Hepatoprotectives agents

The herbal medicine was used for the treatment of various diseases for centries. A many 250,000 higher plant species, about 70,000 species have been used in different traditional medicinals. Plants are a great source of pharmaceutical products. For long time medicinals plants and their derivative, were used for the treatment of liver disease. Great interest of the scientific and pharmaceutical community over the therapeutic use of plantbased materials used in various ethnobotanical practices have led to purification and characterisation of various bioactive compounds, which have proven to be hepatoprotective (Dey *et al*., 2013) .

2.2 Nepheroprotective and Nepherotoxicity of plant extracts and natural products

2.2.1 Structure and Function of the kidney

The kidneys are a paired and are considered to be retroperitoneal organs because they lie behind the peritoneum that lines the abdominal cavity. It is bean-shaped organ found along the posterior muscular wall of the abdominal cavity with the left kidney located below the spleen and slightly more superior than the right because of the larger size of the liver on the right side of the body. The weight of each kidney is 125-175 g in males and 115-155g in females. They are about 11-14 cm in the length, 6 cm wide and 4cm thick and directly covered by a fibrous capsule. This capsule is covered by a shock- absorbing layer of adipose tissue called the renal fat pad. On the superior aspect of each kidney is the adrenal gland. This is influenced in renal function by the production of hormone aldosterone which stimulates sodium reabsorption (Sands and Verlander, 2017)**.**

A frontal section through the kidney reveals an outer region called the renal cortex and an inner region called the medulla. The renal columns are connective tissue extensions that radiate downward from the cortex through the medulla to separate the most characteristic

features of the medulla, the renal pyramids and renal papillae. The papillae are bundles of collecting ducts that transport urine made by nephrons to the calyces of the kidney for excretion. The renal columns also serve to divide the kidney into 6–8 lobes and provide a supportive framework for vessels that enter and exit the cortex. The pyramids and renal columns taken together constitute the kidney lobes (Silverthorn, 2007).

The nephron is the functional unit of kidney. The number of nephron is varied between species. The number of nephron is 1 million in the human and approximately 30,000 are found in the rat kidney. It is contained glomerulus which have two structures the Bowman's capsule and the actual glomerulus. The nephron contained proximal tubule, loop of Henle, distal tubule and collecting tubules (Ekor, 2014).

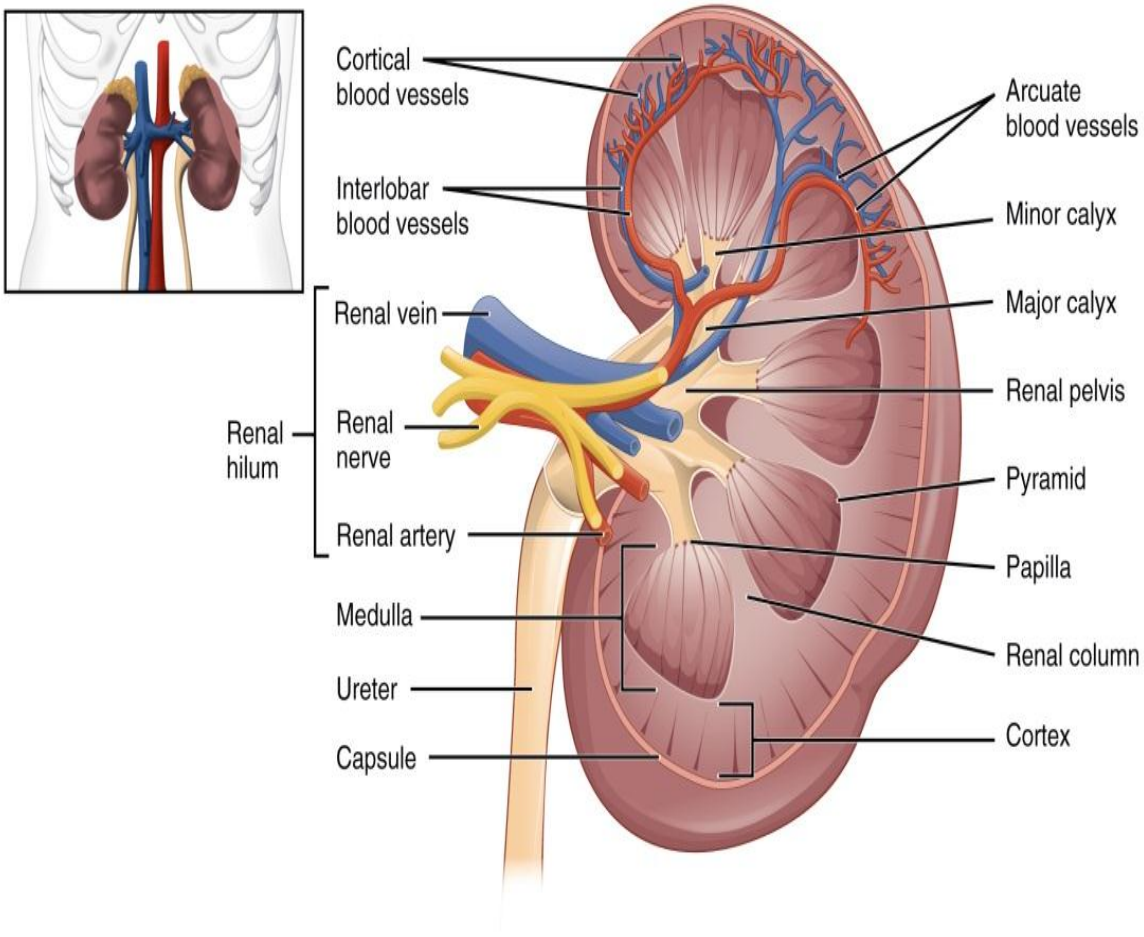


Figure 4: Anatomy of kidney (Feher, 2017)

The kidney is an essential organ which performs several important physiological functions including clearance of endogenous waste products, control of volume status, maintenance of electrolyte and acid base balance, and endocrine function. Other major functions include metabolism and excretion of exogenously administered therapeutic and diagnostic agents as well as environmental exposures. Furthermore, the kidney is responsible for the synthesis of erythropoietin and this underscores its direct involvement in the formation of erythrocytes (Sands and Verlander, 2005; Ferguson *et al*., 2008).

2.2.2 Nepherotoxicity

The kidney contains many xenobiotic metabolizing enzymes and plays a central role in metabolizing drugs and foreign compounds in the body. It is eliminates the metabolic waste, xenobiotic and regulates the water and ion content in the blood. The kidneys receive approximately 25% of the cardiac output, and renal tubules have a high tendency for drugs uptake via transporter proteins or endocytosis (Perazella, 2009). This can result in high intracellular levels of various drugs and substances that then metabolized, leading to formation of reactive oxygen species (ROS) and toxic metabolites. Excessive ROS production and oxidative stress have been demonstrated to play a role in drug induced renal damage and tubular necrosis. The kidney is a highly vulnerable organ to injury caused by ROS, possibly due to the richness of long chain polyunsaturated fatty acids in the composition of kidney lipids (Srivalli kumari *et al*., 2011). Nephrotoxicity is one of the most common renal problems that especially occur when the body is exposed to drugs or chemical reagents such as CCl_4. CCl_4 induced increase the serum uric acid, urea and creatinine level. This biochemical parameter is marker of kidney diseases (Aly *et al*., 2017). Herbal medicines and related natural products are an important and unregulated source of potentially nephrotoxic substances and a large number of them have long been recognized as capable of causing renal failure (Ekor, 2014).

2.2.3 Nepheroprotectiveproperties of natural products

Many plants are believed to prevent kidney damage and often are used in the traditional system of medicine for the treatment of kidney failure in different parts of the world. Vegetables and fruits are also stated to reduction the risk of degenerative diseases and could have a protective efficacy against various oxidative stress insults. The defending role of glutathione, as an antioxidant and detoxifying agent, has been established in some clinical investigations (Sen *et al*., 2011). Several studies demonstrated that polyphenols have good effect in kidney injury. Ferulic acid, an antioxidant of plant cell wall, was detected to prevent

functional and pathological abnormalities in the kidney of diabetic rats reducing inflammation and oxidative stress (Srivalli Kumari *et al*., 2011). There are various herbal plants have antioxidant ability and which can diminished the formation of free radical and promotion of endogenous antioxidant enzyme activity in the kidney (Aleebrahim-Dehkordy *et al*., 2016). Hence it is possible that, *Ocimum basilicum*, possesses significant renoprotective capability with minimal toxicity and thus has a hopeful role in the treatment of acute kidney injury induced by various nephrotoxins, particularly cisplatin and gentamicin (Gamaniel, 2000). *Curcumin* protected against renal damage by suppressing free radicals and increasing renal glutathione content and glutathione peroxidase activity (Youn *et al*., 2010). Quercetin is the most potent scavenger of ROS in the family of flavonoids, including O_2^- and RNS like NO and $ONOO^{-\cdot}$, thanks to two antioxidant pharmacophores present in quercetin the catechol group in the B ring and the OH group at position 3 of the AC ring, quercetin has high antioxidant ability (Bugrim *et al*., 2004).

Materiels and methods

MATERIALS AND METHODS

1. Materials

1.1. Plant material

In this study, fruits of *Fargaria ananassa and Prunus persica*, *Malus communis* (two varieties), *Musa paradisiaca* and *Pyrus communis* were purchased from commercial market in Amoucha from Setif (Algeria) on April and July 2016. *Purnus armeniaca, Ficus carica*, *Punica granatum* and *Vitis vinifera* fruits were harvested from Tizi N'bachar in Sétif region on 2016. Fruits were used freshly.

1.2 Fruit used in this study

In recent years the scientists gave an important interest to fruit and their benefits in human health. Fruits are an important source of phytochemicals. In this study, is some fruits are used based on epidemiologic study which showed most consumed fruits in Setif region.

Table 3: Fruit used in this study

English name	Arabic name	Scientific Name	Family	Location	Part used
Strawberry	الفراولة	*Fargaria ananassa*	*Rosaceae*	Market of Amoucha	Fruit
Apricot	المشمش	*Prunus armeniaca*	*Rosaceae*	Tizi N'bachar	Fruit without nucleus
Peach	الخوخ	*Prunus persica*	*Rosaceae*	Market of Amoucha	Fruit without nucleus
Fig	التين	*Ficus carica*	*Moraceae*	Tizi N'bachar	Fruit
Pomegranate	الرمان	*Punica granatum*	*Lythraceae*	Tizi N'bachar	Fruit without peel
Red Grape	العنب الأحمر	*Vitis vinifera*	*Vitaceae*	Tizi N'bachar	Fruit
Banana	الموز	*Musa paradisiaca*	*Musaceae*	Market of Amoucha	Fruit without peel
Red Apple	التفاح الأحمر	*Malus communis*	*Rosaceae*	Market of Amoucha	Fruit without nucleus
Yellow Apple	التفاح الأصفر	*Malus communis*	*Rosaceae*	Market of Amoucha	Fruit without nucleus
Pear	الاجاص	*Pyrus communis*	*Rosaceae*	Market of Amoucha	Fruit without nucleus

1.3 Animals

Male Wister rats (150-200g) were purchased from Pasteur institute, Algiers. They were kept in cages at room temperature for one week. Animals were allowed to free commercial diet and tap water. Ethics committee of the faculty of nature and life sciences, university Ferhat Abbas, Sétif 1 approved the experimental protocol.

1.4 Chemicals

Linoleic acid, ammonium thiocyanate, β-carotene, butylated hydroxytoluene (BHT), were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), gallic acid, 2.2´-Azino-bis(3-ethylbenzenothiazoline 6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, Potassium persulphate, potassium ferricyanide (K3FeCN6), trichloroacetic acid (TCA), thiobarbituric acid (TBA), Ferrozine, ferrous and ferric chloride, Furosemide, DTNB(5-5'-dithiobis2-nitrobenzoïc acid), H_2O_2 , reduced glutathion (GSH). These chemicals were obtained from Sigma Chemical Co. (St.Louis, MO) or Merck. All other reagents were of analytical grade.

2. Methods

2.1. Preparation of fruits extracts

The extraction of phenolic compounds was carried out according to Markham (1982) with slight modification. 100g of the consumed parts of the fruits were washed with water, then homogenized and mixed with 1 liter of methanol (85:15 v/v, 50:50 v/v) and kept at room temperature for 5 days. The resulting solution was then filtered and the supernatant was evaporated using a vacuum rotary evaporator at 40 C° to obtain crude methanol extract. The crude extract was dried and stored at 4° until use.

2.2. Determination of total polyphenols in fruits extracts

The amount of total phenolic content in fruit samples was estimated using the Folin–Ciocalteu reagent as described by Li (2007) with slight modification. In brief 200µl of samples were mixed with 1000µl of Folin–Ciocalteu reagent (1:10 diluted with distilled water). The mixture was allowed to react for 5 min, and then 800µL of sodium carbonate (7.5%) was added to the mixture. The reaction was incubated at room temperature in the dark for 90 min. the absorbance was measured against a blank at 760 nm using a UV-Visible spectrophotometer.

The standard curve was prepared using 0-160 µg/ml solution of gallic acid. The amount of total phenolic was expressed as mg equivalent gallic acid /g dry extract.

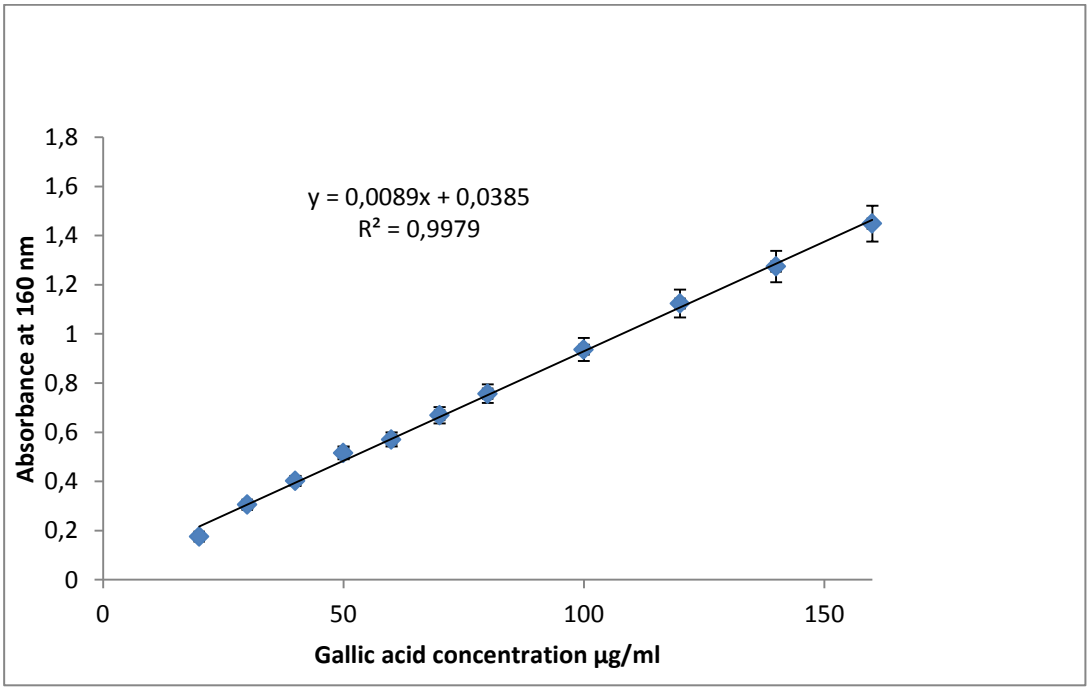


Figure 5: Standard curve of gallic acid for the determination of total polyphenols in fruits extracts. Each value represents mean ± SD (n=3).

2.3. Determination of flavonoids content in fruits extracts

Aluminum chloride colorimetric method adapted from Bahorun (1996) was used for the determination of total flavonoids. 1 ml of 2% $AlCl_3$ solution was added to an equal volume of extract. After mixing, the mixture was incubated for 10 min at ambient temperature in the dark. The absorbance was determined against the same mixture without the extract as a blank at 430 nm. The results are expressed in milligram of quercetin equivalent per gram dried extract.

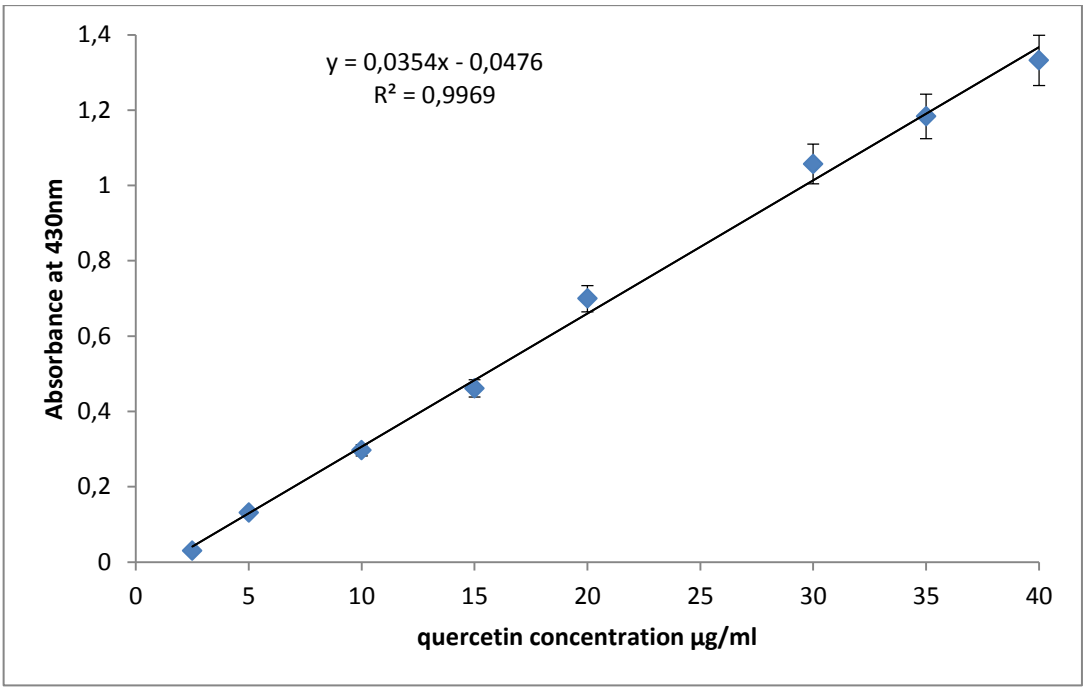


Figure 6: Standard curve of quercetin for the dtermination of flavonoids in fruits extracts. Each value represents mean ± SD (n=3)

2.4. Determination of tannin contents in fruits extracts

The amount of tannins was determined using the method described by Gharzouli *et al.* (1999).This method is based on the capacity of the tannin to precipitate hemoglobin. Briefly, a volume of samples mixed with an equal volume of hemolysed bovine blood (absorbance = 1.6). After 20 min of incubation at room temperature, the mixture was centrifuged at 4000 rpm for 10 min, and the absorbance of the supernatant was determinedat 576 nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

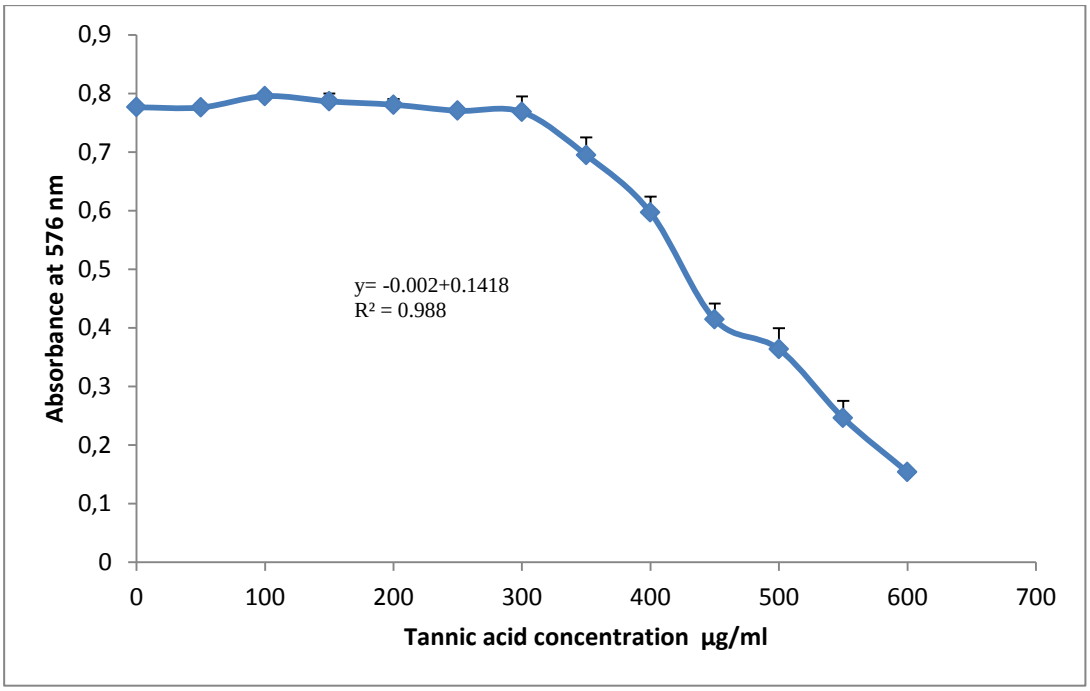


Figure 7: Standard curve of tannic acid for the determination of tannins in fruits extracts. Each value represents mean ± SD (n=3).

2.5. Determination of protein in fruits extracts

The amount of proteins in the extracts was estimated by Commasie bleu reagent described by Bardfordf (1976). The blue of Commassie 0.004 % was dissolved in 4% ethanol (96 %) and 10% phosphoric acid (85%). SDS 0.1% was added to the mixture. 100 µl of extract was added to 2 ml of reagent. Tubes were vortexed, and the absorbance was measured against a blank at 595 nm. The standard was prepared using 0.1-2 mg/ml solution of BSA in water. The results were expressed as mg per g extract.

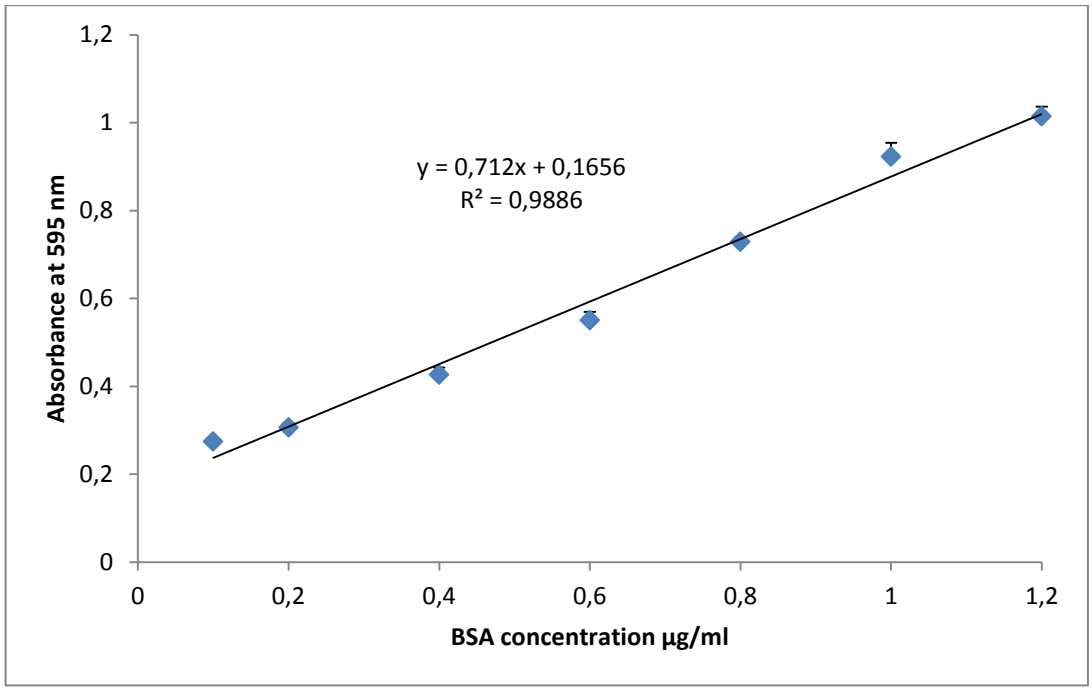


Figure 8: Standard curve of BSA for the determination of protein in fruits extracts. Each value represents mean ± SD (n=3).

2.6. Total soluble sugars content in fruits extracts

Total soluble sugars were determined by the method described by Dubois *et al*. (1956). In brief, 1 ml of samples was treated with 1 ml of 5% phenol and 5ml of concentrated sulphuric acid. The absorbance was recorded at 490 nm in UV/VIS spectrophotometer, against a blank (without sample). D-Glucose was used as standard and the amount of sugar was expressed in mg/g dried weight.

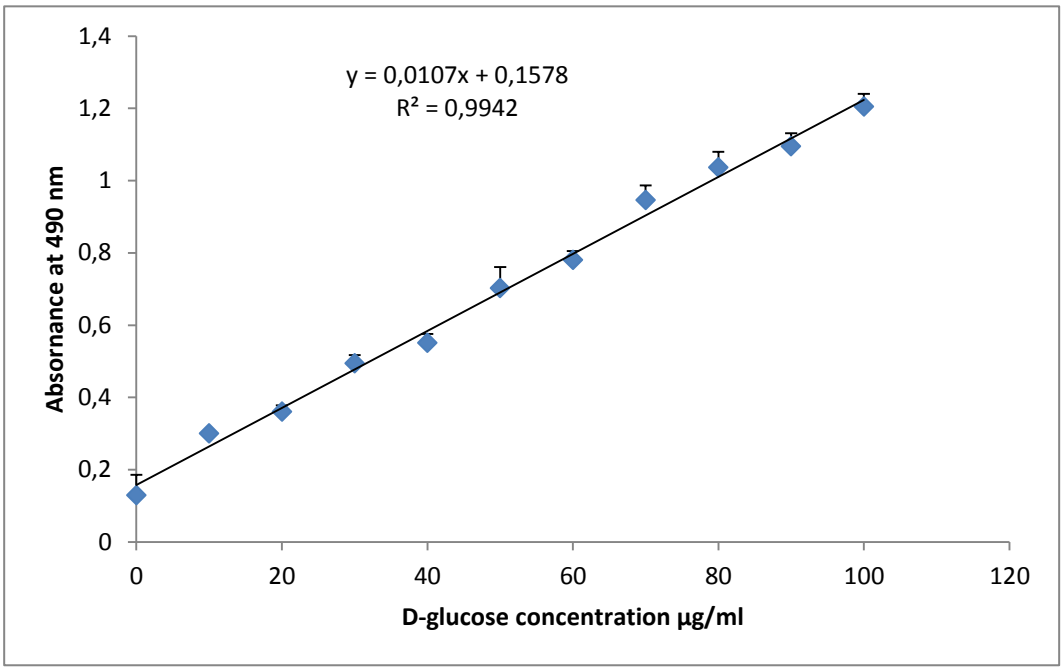


Figure 9: Standard curve of D-glucose for the determination of sugars in fruits extracts. Each value represents mean ± SD (n=3).

2.7 Identification of phenolic compounds by UPLC-DAD

The phenolic compounds in samples were analyzed by UPLC-DAD system (Perkin Elmer series 275), a model LC-200 micro pump High pressure Binary with series 200 autosmpler, a model Hypersil Gold reversed phase column(1.9µm* 3nm* 50mm) and a model diode array detector. The flow rate was kept constant throughout the analysis at 0.6 ml/min and the injection volume was 20 µl. The operating condition were a follows: mobile phase water (A) and acetonitrile (B): gradiant 5% B from 0 to 1 min, 5%-21% B from 1 to 5 min, 21%-50% B from 5 to 7 min, 50%-100% B from 7 to 10min, 5% B from 10 to 13 min. The column was maintained at 30° and UV detection was recorded in the range 165 nm- 365 nm. Phenolic compounds were identified by comparing retention time and spectrograms of samples with standards.

2.8 Identification of phenolic compounds by HPLC /MS

Identification of phenolic compounds were performed out using an agilent 1100 series HPLC system coupled with a photodiode array detector (PDA), a triple quadrupole mass spectrometer type Micromass Autospec Ultima Pt (Kelso, UK) and an ESI ion source working in negative and positive mode was used for the identification of phenolic compounds. Separation of samples are achieved on a 12.5 cm x 2 mm i.d, 4 µm, (Agilent Technologies,

Rising Sun, MD) column at 45° C, with 0.1% acetic acid (A) and acetonitrile (B) as the mobile phases. The gradient program started with: 5min 0-2% B; 75 min 2-88 % B; 90 min 88- to 2% B. The chromatograms were recorded at 200 nm- 800nm, and ions in the m/z range of 100-1000 were detected using a scan time of 1s.ESI was performed using an capillary voltage of 3.2 kV and a cone voltage of 115 V, probe temperature, 350°C and ion source temperature, 110°C. Data acquisition wasacquired and processed using MassLynx 4.0 software. The phenolic compounds were characterized by their UV and mass spectra, and the comparison between the retention time and fragmentation pattern with those of authentic standard. The characteristics were compared with the literature (Mejri *et al*., 2018).

2.9 Determination of the *in vitro* Antioxidant activity of fruits extracts

2.9.1 Phosphomolybdate assay (Total Antioxidant Capacity)

Total antioxidant capacity assay is a method used for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex, this method was described by Prieto *et al*. (1999). An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. The total antioxidant capacities were expressed as µg ascorbic acid equivalent per mg dry extract.

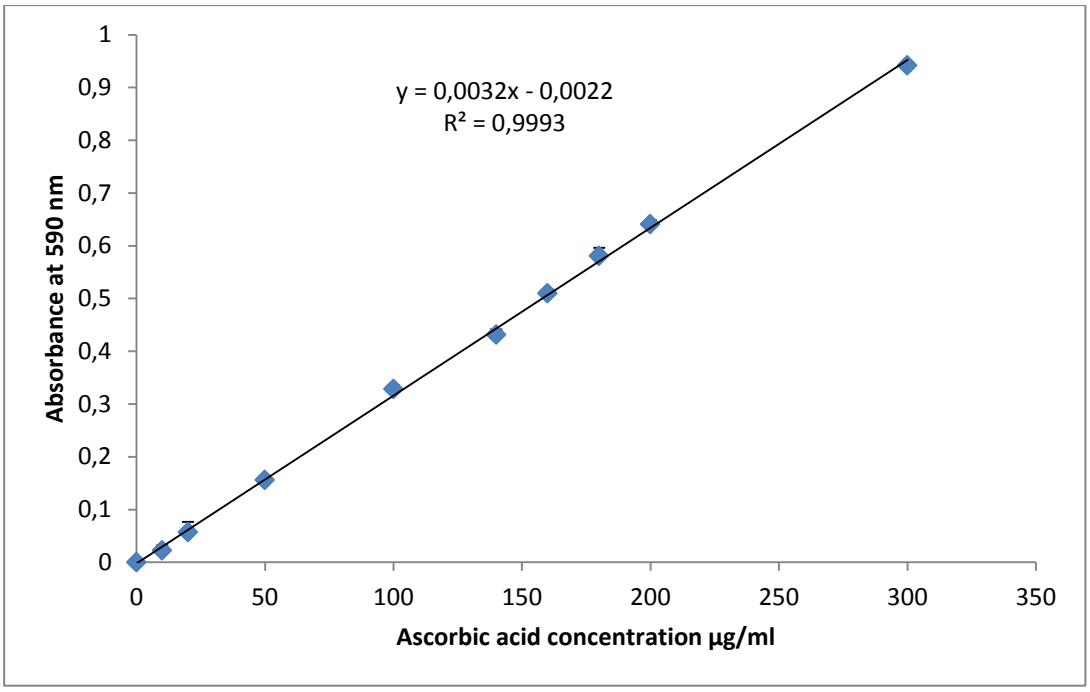


Figure 10: Standard curve of ascorbic acid for the determination of Total Antioxidant Capacity in fruits extracts. Each value represents mean ± SD (n=3).

2.9.2 DPPH radical scavenging assay

Free radical scavenging activity of extracts against stable DPPH (2-diphenyl-2-picrylhydrazyl hydrate) was determined using the method described by Yardpiroon *et al.* (2014). 1 mL of the extract was added to 2.0 mL of 0.1 mM DPPH solution. The mixture was strongly shaken and left to stand at room temperature for 30 min. The changes in color (from deep-violet to light-yellow) and the Absorbance of samples were measured at 517 nm.The percentage of radical scavenging activity was calculated using the following equation:

Radical scavenging activity (%) = $[A_{control} - A_{sample}] / A_{control} \times 100$.

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except theSample). $A_{sample}$is the absorbance of the extract.A curve of percent inhibition or percent scavenging effect against samples concentrations was plotted and the concentration sample required for 50% inhibition was determined. The value for each test sample was presented as the inhibition curve at 50% or IC_{50}.

2.9.3 Free radical scavenging ability of ABTS

The free-radical-scavenging activity was determined by ABTS radical cation decolorization assay Re *et al*., (1999). Briefly, $ABTS^{+}$ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the

dark for 16 h at room temperature. The solution was then diluted by mixing ABTS solution with methanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm. Then, 50 µl of sample was mixed with 1ml of $ABTS^{+}$ solution and kept for 30 min at room temperature. The absorbance of reaction mixture was measured at 734 nm. The ABTS scavenging capacity of the extract was compared with that of Vit C and the percentage inhibition was calculated as ABTS radical scavenging activity (%) = [($Abs_{control} - Abs_{sample}$) /(Abscontrol)] x 100 where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

2.9.4 Ferrous ion chelating activity of the extracts

The method described by Decker and Welch (1990) was used to investigate the ferrous ion chelating ability of different extracts. This activity ferrous ion chelating ability was monitored by the absorbance of the ferrous iron ferrozine complexe at 562 nm. The mixture contained 500 µl sample or EDTA, 100 µl FeCl2 (0.6 mM in water) and 900µl methanol. Same mixture without the extract or EDTA was considered as a control. The mixture was shaken well and allowed to react at room temperature for 5 min; 100 µL of ferrozine (5 mM in methanol) was then added. The chelating effect was calculated as a percentage, using the same equation as that described for the DPPH assay.

2.9.5 Hydroxyl radical scavenging assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. Hydroxyl radical scavenging activity was measured by the ability of the different fruit extracts to scavenge the hydroxyl radicals according the method described by Smirnoff and Cumbes (1989) with slight modifications. The reaction mixture consists of 100 µL of varying concentration of samples or standard antioxidants, 1 ml of FeSO4 (1.5 mM), 0.7 ml of H2O2 (6 mM), 0.3 ml of sodium salicylate (20 mM). This mixture was incubated at 37 C° for 1 h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as flows:

Scavenging rate = [Acontrol – Asample] / Acontrol× 100.

Where Acontrol was the absorbance of the control (without sample) and Asample was the absorbance in the presence of the sample

2.9.6 Reducing power of extracts

The reducing power of the fruit extract was estimated according to the method described by Ebrahimzadeh *et al.*, (2010) .In brief, 100 µl of the extract with various concentration were mixed with an equal volume of 0.2 M phosphate buffer (PH= 6.6) and 1% of potassium ferricyanide[K_3Fe (CN_6)]. The reaction was incubated at 50°C in a water bath for 20 min and the reaction was terminated by the addition of 250 µl of 10% trichloroacetic acid followed by centrifugation for 10 min at 3000 rpm. 250 µl of the upper layer of solution was mixed with 250µl of distilled water and 500µl of $FeCl_3$ and the absorbance was measured at 700 nm against a blank. Higher absorbance indicates higher reducing power. BHT was used as positive control.

2.9.7 β-Carotene bleaching assay

In this test, the antioxidant capacity of the extracts is determined by measuring the inhibition of the oxidative decomposition of β-carotene (discoloration) by the products of oxidation of the linoleic acid according to the method described by Gursoy *et al* (2012). The emulsion of β-carotene/ linoleic acid is prepared by solubilization of 0,5mg β-carotene in 1ml of chloroform, 25µl of the linoleic acid and 200mg of Tween 40 are added, after that 100ml of distilled water saturated with oxygen was then added to the reaction. 350µl of extracts or BHT solubilized in methanol (2mg/ml) was mixed with 2,5ml emulsion. The same procedure was repeated with MeOH and H_2O as negative control. The absorbance was measured at 490 nm after: 1heure, 2h, 3h, 4h, 6h and 24h of incubation at room temperature in the dark .The percentage of inhibition of β-carotene decomposition by the extracts antioxidant was measured as follows:

$AA\% = ABS_{test} / ABS_{BHT} \times 100$

AA%: Percentage of the antioxidant activity.

ABS_{test}: Absorbance in the presence of the extract (test).

ABS_{BHT}: Absorbance in the presence of positive control BHT.

2.9.8 Ferric thiocyanate (FTC) method

The antioxidant capacity of fruits extracts towards the peroxidation of linoleic acid was tested by the thiocyanate method described by Yen *et al.*, (2003). In this test, the concentration of peroxide decreases as the antioxidant activity increases. The mixture contained 0.5 ml of samples, 2.5 ml of 0.02M linoleic acid emulsion at pH 7.0 and 2 ml of 0.2 M phosphate buffer at pH 7.0. The emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20, and 50 ml of phosphate buffer. The reaction mixture was incubated for 5 days at 37 °C.

0.1 ml of the reaction mixture is transferred to a test tube and 75% EtOH (4.7 ml), 30% ammonium thiocyanate (0.1 ml), 0.02 M ferrous chloride in 3.5% HCl (0.1 ml) were added to tubes each 24 h intervals. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of the resulting mixture (red color) is measured at 500 nm every 24 h until the absorbance of the control reached its maximum. BHT and vitamin C were used as positive controls and the mixture without the sample is used as the negative control. Inhibition (%) of lipid peroxidation is calculated by the 45 following equation:

Inhibition (%) = [Ac – As / Ac × 100].

Where, As is the absorbance of the sample on the day when the absorbance of the control is maximum and Ac is the absorbance of the control on the day when the absorbance of the control is maximum.

2.9.9 Thiobarbituric Acid (TBA) assay

According the method of Kikuzaki and Nakatani 1993, the TBA was measured on the final day of FTC assay. This method based on the determination of the in levels of malonaldehyde (MDA) formed during lipid peroxidation. The sample contained the same elements used in the lipid peroxidation. 1 ml of sample solution was mixed with 2 ml of trichloroacetic acid (20%) and 2 ml of thiobarbituric acid solution. The mixture was then placed in a boiling water bath for 10 minutes, after cooling tubes were centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm and recorded after it has reached its maximum.

2.10 Determination of the *in vivo* antioxidant activity of fruits extracts

2.10.1 Experimental design

Rats were divided into 14 groups of 6 rats in each group: Group 1: received 0.9% of NaCl. Group 2: received vitamin C (200 mg/kg); Group 3: received the dose 200mg /kg of *Fargaria ananassa* extract. Group 4: received the dose 600mg /kg of *Fargaria ananassa* extract. Group 5: received the dose 200mg /kg of *Purnus armeniaca* extract. Group 6: received the dose 600mg /kg of *Purnus armeniaca* extract. Group 7: received the dose 200mg /kg of *Purnus persica* extract. Group 8: received the dose 600mg /kg of *Purnus persica* extract. Group 9: received the dose 600mg /kg of *Ficus carica* extract. Group 10: received the dose 600mg /kg of *Ficus carica* extract. Group 11: received the dose 600mg /kg of *Punica granatum* extract. Group 12: received the dose 600mg /kg of *Punica granatum* extract. Group 13: received the

dose 600mg /kg of *Vitis vinifera* extract. Group 14: received the dose 600mg /kg of *Vitis vinifera* extract.

At the end of the experimental period (15 days), rats were sacrificed. Blood was collected in heparinized tubes and centrifuged at 3000 rpm for15 min. Plasma was kept in the freezer until use. Tissues are also kept in freezer until use.

2.11 Effect of fruits extracts on the hepatotoxicity induced by CCl_4

The hepatotoxicity activity was induced according to the method of Wang *et al*., (2004) with slight modification. Mal Wister rats weighting 200-250g were divided into 16 groups of seven rats.

Group 1: (normal group) received distilled water

Group 2: received distilled water

Group 3: received distilled water

Group 4: treated with vit C at a dose 200 mg/kg.

Group 5: treated with *Fargaria ananassa* extract at a dose 200 mg/kg.

Group 6: treated with *Fargaria ananassa* extract at a dose 600 mg/kg.

Group 7: treated with *Prunus armeniaca* extract at a dose 200 mg/kg.

Group 8: treated with *Prunus armeniaca* extract at a dose 600 mg/kg.

Group 9: treated with *Prunus persica* extract at a dose 200 mg/kg.

Group 10: treated with *Prunus persica* extract at a dose 600 mg/kg.

Group 11: treated with *Ficus carica* extract at a dose 200 mg/kg.

Group 12: treated with *Ficus carica* extract at a dose 600 mg/kg.

Group 13: treated with *Punica granatum* extract at a dose 200 mg/kg.

Group 14: treated with *Punica granatum* extract at a dose 600 mg/kg.

Group15: treated with *Vitis vinifera* extract at a dose 200 mg/kg.

Group 16: treated with *Vitis vinifera* extract at a dose 600 mg/kg.

Animals were treated by oral gavage for 7 days. On the seventh day the groups 3-16 injected by the fresh mixture (CCl_4 olive oil, 3ml/kg V: V) after 30 min of gavage. The second group was gaved with olive oil (3ml/kg). After 24 hours, all animals were scarified by cervical dislocation ways. Collected blood was centrifuged at 3000 rpm for 10 min 4°C. Rat kidney and livers were remeoved and washed by physiological water (0.9%) and weighted. Portion of organs were kept in formalin (10%) for histopathological analysis, other portion were kept in – 4°C for biomarker enzymes (GSH) and lipid peroxidation (further biochemical and enzymatic analysis).

2.11. 1 Effect of fruits extracts on biochemical parameters

Serum levels of the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ glutamyl transferase (GGT), alkaline phosphatase (ALP) as markers of hepatic damage and function were determined. The kidney damage was assessed by the dtermination of the levels of creatinine (CREA), uric acid (AU) and urea. Plasma levels of Cholesterol and triglyceride were measured according to standard procedure using pictus 200 Random Access Clinical Analyzer (Diatron). The determination of all biochemical parameters was done using commercial kits (spinreact) and only GGT we used commercial kit (biomeghreb).

1. Determination of aspartate aminotransferase

The principle of the determination of aspartate aminotransferase or glutamate oxaloacetate was based on the transfer of amino group from aspartate to α-ketoglutarate forming gulutamate and oxaloacetate. Malate dehydrogenase in the presence of NADH reduces oxaloacetate to malate. This method uses two reagents (R1 buffer and R2 the substrate). R1 is the L-Aspartate and R2 is mixture between the NADH, lactate dehydrogenase, malate dehydrogenase and α-ketoglutarate. The working reagent was prepared by the mixed one tablet of R2 with 15 ml of R1. It is incubated in 2-8°C for 21 days. 100 µl of serum mixed with 1 ml of reagent. The mixture incubated for 3 minute at 37 °C.

2. Determination of alanine aminotransferase

Alanine aminotransferase ALT or Glutamate pyruvate transaminase catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase and NADH according the following reaction:

$$\text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

The preparation of working reagent needs two substances (R1 is the buffer and R2 is the substrate). R1 contains L- alanine and tris pH 7.8. R2 contains NADH, Lactate dehydrogenase and α-ketoglutarate. In brief, one tablet of R2 is dissolved in 15 ml of R1. This reagent was kept at 2-8 °C and can be used for 21 days. The determination of ALT in samples is conducted by the mixy of 100 µl of samples and 1ml of working reagent for 3 min. The value is automatically determined.

3. Determination of γ glutamyl transferase (GGT)

Gamma-glutamyl transferase (GGT) is a cellular enzyme located in the kidney, pancreas, liver and prostate. In this method, this enzyme catalyzes the transfer of G-glutamyl group from G-glutamyl-p-nitroanilide to acceptor glycylglycine, according to the following reaction:

G-L-Glutamyl-3-carboxy-4-nitroanilide + Glycylglycine $\xrightarrow{\text{GGT}}$ G-L-Glutamyl-glycylglycine + 2-Nitro-5-aminobenzoic acid.

In the middle reaction, 100 µl of samples was mixed with 1 ml of working reagent at 37°C. The concentration of GGT was indicated after 1 min automatically.

4. Determination of alkaline phosphatase (ALP)

Alkaline phosphatase is an enzyme present in almost organisms. It catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4 liberating p-nitrophenol and phosphate. The reagent was prepared by mixing of two reactive one vial of R1 (buffer which contained Diethanolamine and magnesiumchloride) and one tablet of R2 (substrate is p-nitrophenylphosphatase). 20 µl of samples was mixed with 1.2 ml of reagent.

5. Determination of Creatinine (CREA) levels

Creatinine is the product of the degradation of creatine. The method of determination of this product is based on the reaction between the Creatinine and the sodium picrate. Creatinine reacts with alkaline picrate and produce red color. This is proportional to the concentration of the creatinine in the samples. In this assay the working reagent contains always two references (R1: pinic acid and R2: sodium hydroxide). This working reagent is prepared by the mixing equal volumes of R1 and R2. In brief 100 µl of serum was mixed with 1 ml of working reagent after 1 min values were determined automatically by the system.

6. Determination of uric acid (AU)

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$) which under the influence of POD, 4-aminophenazone (4-AP) and 2-4 Dichlorophenol sulfonates (DCPS) forms a red quinoneimine compound:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2.$$

$$2H_2O_2 + 4\text{-AP} + \text{DCPS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O.$$

The intensity of the red color is proportional to the uric acid concentration in the samples. In the middle reaction, 25µl of sample was mixed with 1 ml of working reagent, which contains two reagents (R1 is the Buffer and R2 contains enzymes). This working reagent is prepared by the mixing the two reagents, one vial of R2 with one bottle R1. This is kept at 2-8 °C for 1 month.

7. Determination of urea levels

Urea is formed in the liver and it is the final product of the metabolism of proteins. Urease hydrolyzes urea to ammonia and CO_2. In the presence of Nitroprusside ammonia, were reacts with salicylate, hypochlorite and forms indophenol. In the reaction, 10µl was mixed with 1 ml of working reagent, after 5 min at 37 °C the concentration is determined automatically.

8. Determination of cholesterol levels

The cholesterol is determined after the transformation of Cholesterol esters to free cholesterol, then the oxidation of the cholesterol and the generation of H_2O_2, Which reacts with phenol and 4- aminophenazone to produce Quinonimie in the presence of peroxidase as shown in the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}.$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{4-Cholestenona} + H_2O_2.$$

$$H_2O_2 + \text{phenol} + \text{4- aminophenazone} \xrightarrow{\text{Peroxidase}} \text{Quinonimie} + 4\,H_2O.$$

9. Determination of triglyceride levels

In this assay, triglyceride is incubated with lipoproteinlipase (LPL) and liberates glycerol and free fatty acid. Glycerol is phosphorylated by the glycerol kinase to glycerol-3-phosphate (G3P). Glycerol phosphate dehydrogenase is converted to dihydroxyacetone and generates of

H_2O_2. This reacts with 4- aminophenazone and p-chlorophenol in the presence of peroxidase to produce a red color Quinone. The concentration of triglyceride in the samples is proportional to the intensity of color.

2.11.2 Histopathological examination of liver and kidney tissues

A portion of liver and kidney tissues from each rats in the experimental group were fixed in 10% neutral formalin solution. They were dehydrated in graduated ethanol, cleared in xylene, and embedded in paraffin. Tissues section (5 µm thick), were prepared with a Microtome (Leica, RM 2145). These sections were spread on slides and dried for one hour at 37°C, then stained with heamatoxylin and eosin. Finally, the sample was analyzed by assessing the morphological changes under a microscope, and their photomicrographs were obtained. Histological preparations were carried out in the laboratory of histopathology of the University Hospital Sétif (Bensalem-Bendjelloul, 1998).

2.11.3 Effect of extracts on plasma antioxidant capacity using DPPH radical

In this assay, the ability of plasma to scaveng DPPH radical was measured by the method described by Burists and Bucars (2000) with slight modifications. Briefly, 50 µl of plasma was mixed with DPPH solution (0.004%). The mixture was incubated for 30 min and then tubes were centrifuged at 3000 rpm for 15 min. The absorbance was measured at 517 nm, and the plasmatic antioxidant capacity was calculated as follows:

% scavenging activity = $[(Abs_{control}-Abs_{sample})/Abs_{control}] \times 100$

A control: is the absorbance of the blank solution.

A sample: is the absorbance in the presence of plasma.

2.11.4 Effect of extracts on plasma reducing power

According the method of Chung *et al.* (2005), the reducing power was evaluated. 0.1 ml of plasma was mixed with 0.1 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.1 ml of potassium ferricyanide (1 %). The mixture was incubated for 20 min at 50°C. After that, 0.250 ml of trichloroacetic (1%) was added. The mixture was then centrifuged for 10 min at 3000 rpm. An aliquot (0.250 ml) of the upper layer was mixed with 0.250 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance at 700 nm was measured. Higher absorbance indicates a higher reducing power.

2.11.5 Preparation of liver and kidney homogenate

After scarifying the animals, the homogenate of liver were prepared by homogenizing 0.5g of liver tissues in 4.5 ml cold KCl solution (1.15%) using homogenizer on ice. The homogenate was centrifuged at 4000 rpm for 15 min at 4°C and the supernatant was used for the determination of (GSH) activity and lipid peroxidation (MDA).

2.11.6 Assessment of reduced glutathione concentration

GSH was measured using a previously described procedure by Ellman (1959). GSH can react with 5, 50-dithio-bis (2-nitrobenzoic acid) (DTNB) and forms yellow color. In brief, 50 µl of tissue homogenate was diluted in 10 ml phosphate buffer (0.1 M, PH 8). 3 ml of this mixture was mixed with 20 µl of DTNB. The developed yellow color was then measured immediately after 5 min at 412 nm against a blank (without tissue homogenate). GSH concentrations were calculated using the standard curve of GSH (figure 11). It was expressed as µmol/ g tissue.

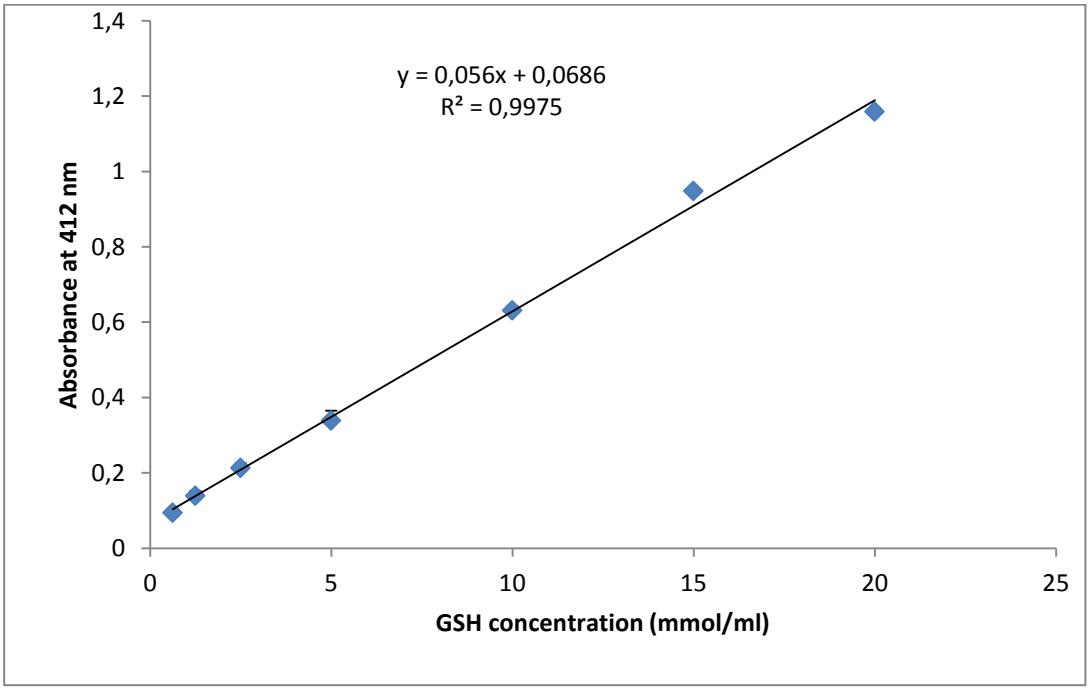


Figure 11: Standard curve of reduced glutathione (GSH). Each value represents mean±SD (n=3).

2.11.7 Assessment of lipid peroxidation in tissues homogenate

According the method of Okhawa *et al.* (1979) lipid peroxidation rate was determined by measuring malondialdehyde level (MDA). This assay was based on the reaction between TBA and MDA in acidic conditions and at a higher temperature (90-100°C) to form a pink MDA-

$(TBA)_2$ complex (Figure 12) . In brief, to 0.5 ml of tissue homogenate, 1 ml of TBA (0.67 %) was added. The mixture was incubated for 15 min in boiling water bath. 4 ml of n-butanol was added to the mixture, after cooling, tubes were then centrifuged at 3000 rpm for 15 min. The amount of TBARS formed in each sample was assessed by measuring the optical density of the supernatant at 532 nm against a blank. The concentration of MDA was determined from a standard curve of 1, 1, 3, 3 tetraethoxypropane in the same conditions and it was expressed as nmol/ g tissue.

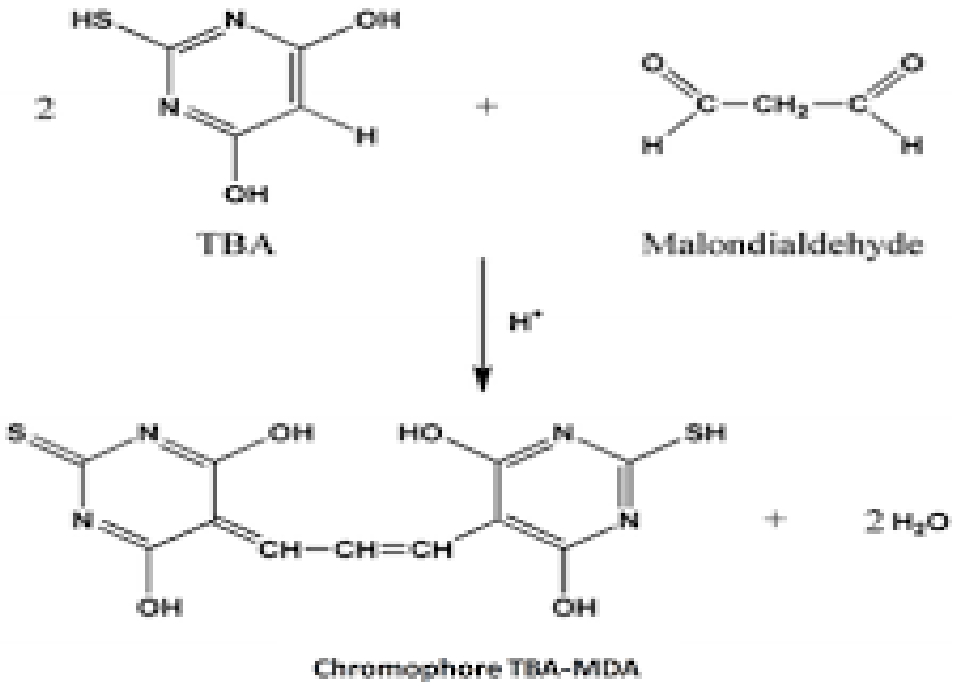


Figure 12 : Malondialdehyde formation (Santos-Fandila *et al*., 2014)

2.12 Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). *In vitro* results were expressed as mean ± standard deviation (SD) and were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's test. The pharmacological results were presented as mean ± standard error of mean (S.E.M.) of six experiments. In all cases, The P-values less than 0.05 were considered statistically significant.

Result and discussion

Result and discussion

1. Total polyphenols, flavonoids and tannins contents in the extracts

Fruits and vegetable are considered as a major source of some essential micronutrients and fiber. They are recognized as important sources of phytochemicals, which are individually or in combination have health benefits. Most of these compounds have antioxidants effects such as polyphenols, flavonoids, protein and vitamins (Laura, 2010). Polyphenols are a group of phytochemicals and secondary metabolites present in fruits. Phenolic compounds contribute also to the sensory quality of fruit such as color, astringency, bitterness and flavor, scent and oxidative stability (Brahem *et al*., 2017). The health benefits of polyphenols depend on the type of polyphenol, the concentration and the time it is administered or consumed in the diet. People are advied to take a balanced diet with high content of fruits, vegetables and cereals because this food has high nutritional contributions and containing polyphenols and other phytochemicals whos provide benefits for health. In other hand, scientifics showed that polyphenols offer a synergic effect with health benefits effects (Calderon-oliver and Ponce-Alquicira, 2018). These substances have an important role in the prevention of various diseases and are strong natural antioxidants (Kaur and Kapoor, 2001). In the present study, total phenolic, flavonoids and tannins contents in different extracts are shown in table 4.

Total phenolic compounds are determined by the Folin–Ciocalteu reagent. This method can determine the content of phenolic including tannins (Wang *et al*., 2007). Results are expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW). In this study, we noticed that fruits extracts have important quantity of polyphenols. Total phenolic content ranged between 47.5 ± 0.006 and 310 ± 0.003 µg GAE /mg DW, and the results showed that *Fargaria* is rich on polyphenols and the low amount is flowed by the *Ficus carica.*

Flavonoids are the most and important group of phenolic compounds, which are characterized by a benzo-pyrone structure (Bakar *et al*., 2009). Total flavonoids content was determined using the $AlCl_3$ method. Total flavonoids contents were expressed as mg quercetin equivalents per gram of dry weight (mg QE/g DW). *Fargaria* extract exhibited the highest flavonoids content with a value of 14.78 ± 0.002 mg QE/g DW. Also the *Ficus* extracts containsthe lowest content of flavonoids (4.78 ± 0.002).

Tannins are naturally occurring phenolic compounds which precipitate protein. They are found in legumes and fruits and are considered as an important source of dietary antioxidant

(Koleckar *et al*., 2008). The quantification of tannins contents showed that *Punica granatum* extract contained the highest tannins concentration with the value of 168.75 ± 0.005 µg TAE /mg extract. The lowest tannins content was noticed for *Pyrus communis* extract with a value of 62.83 ± 0.03 µg TAE /mg extract (Table 4).

Table 4: Total phenolics, flavonoids and tannins contents in fruits extract

Extract	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg QE/ g DW)	Total tannins (mg TAE/g DW)
Fargaria ananassa	310 ± 0.003	14.78 ± 0.002	81.5 ± 0.10
Prunus armeniaca	233.33 ± 0.02	5.68 ± 0.002	127.90 ± 0.003
Prunus persica	208.33 ± 0.02	6.02 ± 0.003	62.82 ± 0.005
Ficus carica	47.5 ± 0.006	4.87 ± 0.002	30.51 ± 0.005
Punica granatum	151.87 ± 0.006	4.71 ± 0.001	168.75± 0.04
Vitis vinifera	207.5 ± 0.003	5.07 ± 0.005	61.63 ± 0.06
Musa paradisiaca	54.83 ± 0.02	7.86 ± 0.003	20.35 ± 0.003
Malus communis (gala)	143.5 ± 0.009	8.35 ± 0.12	163 ± 0.002
Malus communis (aris)	103.1 ± 0.003	5.08 ± 0.001	31.38 ± 0.006
Pyrus communis	172.5 ± 0.005	5.34 ± 0.003	22.62 ± 0.011

Results are expressed as means ± SD (n=3).

Various factors can change the fruit phenolic contents such as the variety, the stage of maturation, the area, the harvesting time and the part of the fruit as well as the types and quantity of phytochemicals (Aydemir *et al*., 2000). During the second stage of maturation, the fruit have a high phenolic content, which might be associated with an amplified polyphenol oxidase activity (Lako *et al*., 2008). In addition, both genetic and agronomic or environmental factors play a role in the phytochemical composition and nutritional quality of the crops (Hind

et al., 2003). Climate has an important role on the quality, including the nutritional value of fruit and vegetables. Light intensity, temperature and water availability affect the antioxidant activity in different fruit and vegetables, and the deficit irrigation influences their polyphenol content (Liu *et al*., 2015) for these reasons we were interested in determing polyphenols contents in fruits growing in Sétif. Flavonoids have high antioxidant effect, which depend to the environment condition. Various factors may change the action of flavonoids and product alteration in their efficacy as antioxidant (Chuang and Mcintosh, 2011).

Tannins bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. This tannin protein complex can provide persistent antioxidant activity. The amount of tannin can be depending on their chemical nature, the solvent used and the experimental condition (Mahmoudi *et al*., 2012).

2. Protein and Sugar contents in the extracts

The most important fruit biochemical compounds, such as vitamin C, phenolic compounds, and sugar content, play also an important role to minimize the effect of oxidative stress, which cause cancer and cardio-vascular diseases, helping in the gastrointestinal health (Forcada *et al*., 2019; Zhou *et al*., 2018). The nature and the concentration of these constituents in fruits are of interest due to their important influence on organoleptic properties (Zhao *et al*., 2015). Sugar composition is one of the most important parameters for food energy level. Table 5 presents the level of total sugars and protein. In this study the result obtained suggest that all fruits extracts have high level of total sugars and low amount of proteins. *Ficus* contains the highest amount of sugars with a value of 963 ± 0.05 mg D-glucose/ g, flowed by *Fargaria* 963 ± 0.05 mg D-glucose/ g. The lowest amount was observed in *Vitis* (268.8 ± 0.008 mg D-glucose/g). Total protein content was high in *Fargaria* 2.73 ±0.04 mg /g) and low in *Pyrus communis* 1.35 ± 0.005mg/g.

Table 5: Total sugars, protein contents in fruits extracts.

Fruits	Total sugars (mg D-Glucose E/g)	Total Protein (mg/g)
Fargaria ananassa	958 ± 0.04	2.73 ± 0.004
Prunus armeniaca	285 ± 0.07	2.12 ± 0.02
Prunus persica.	610 ± 0.08	1.55 ± 0.001
Ficus carica	963 ± 0.05	1.75 ± 0.006
Punica granatum	653 ± 0.01	1.94 ± 0.006
Vitis vinifera	268.8 ± 0.008	2.22 ± 0.004
Musa paradisiaca	678 ± 0.02	1.49 ± 0.004
Malus communis (gala)	310 ± 0.02	1.91 ± 0.002
Malus communis (aris)	795 ± 0.05	2.37 ± 0.002
Pyrus communis	883.33 ± 0.01	1.35 ± 0.005

Result are expressed as mean ± SD (n=3).

Sugars are important food constituents and instant source of energy for the body. A high sugar level of a fruit also serves as an index of maturity so fully ripe fruit had higher total sugar (Zhaou *et al*., 2015). The amount of sugars is increased during the later stages of fruit development. An overall view of the obtained data showed variations in sugar levels that might be due to genetic factors, responsible for differences in composition among different varieties (Ali *et al*., 2011). The sugars and protein have antioxidant effect (Wong *et al*., 2006). The temperature is an important source for the best growing of fruit. The production of sugars by photosynthesis depends on the degree of temperature (Kumar *et al*., 2004). Decrease in sugar content may occur because of the formation of maillard reaction products, which are formed by the reaction between sugars, amino acids, and proteins (Martins *et al*., 2001). Furthermore, the effects of cultural, chemical and physical treatments on plant species resulted in a reduction in the level of sugars (Sujak *et al*., 2009). Some studies showed that

the advanced stages of fruit maturity in apple, strawberry, and grape have high level of sugars (Basson et al., 2010; Zhang *et al*., 2010; Wu *et al*., 2011).

Diba *et al*., 2014 demonstrated that the level of protein changes with stages of maturity.

3. Identification of different phenolic and flavonoids s in fruits extracts

3.1 Identification of different phenolic and flavonoids in fruits extracts by UPLC- DAD

UPLC is the most used technique for achieving the separation, identification, and quantification of polyphenols from natural complex samples. The chromatographic separation depends on several factors such as stereochemistry, molecular weight, polarity, degree of polymerization of polyphenols (Alonso-Carrillo *et al*., 2017). There is not one single HPLC method that can separate all different types of polyphenols, in fact, the stationary phase, mobile phase, and gradient elution should be optimized for each group of compounds separately. The acidification of the mobile phase is necessary to suppress the ionization of phenolic hydroxyl groups to get shaper peaks and minimized peak tailing (Struck *et al*., 2016). Polyphenols are commonly quantified and identified by coupling the UPLC column to diode array detection (DAD) because it is cheap and robust. Depending on the structure, polyphenols can absorb at different wavelengths. For instance, phenolic compounds in general are detected at 240–285 nm, flavones and flavonols at 350–365 nm, and anthocyanins at 460–560 nm (Lorrain *et al*., 2013). To carry out the identification of each polyphenol, its spectral data and retention time are compared with the data obtained from commercial standards. Phenolic compounds are very important fruits constituents because of their scavenging ability due to their hydroxyl groups (Uddin *et al*., 2014).

In this study, the identification of polyphenols demonstrated large deference between fruit extracts. For the fruit belonging in the same family the UPLC chromatogram suggest that the fruit contains a proximally the same compounds with small deferent.

The UPLC chromatogram of *Fargaria ananassa*, *Prunus persica*, *Prunus armeniaca*, *Malus* communis (red and yellow apple) and *Pyrus communis* fruits extracts belong to *Rosaseae* family revealed the presence of various phenolic acid and flavonoids such as gallic acid, protocachuic acid, caffeic acid and flavon-3-ols. Phenolic compounds were identified according to their retention times, molecular masses, fragmentation patterns, characteristic spectra, and bibliographical sources. The major compound in this family is gallic acid and protocachuic acid. Catechin was detectable in *Fargaria ananassa, Prunus persica. Prunus persica* and *armeniaca* contained ferulic acid. Chlorogenic acid was found in *Prunus armeniaca*, *Malus communis and Pyrus communis*.

Fargaria extract showed the presence of some compounds which are absent in the other fruit such as p-coumaric, cinamic acid. The chromatogram of yellow apple revealed the presence of syringic acid and naringenin. For the vanilic acid is present particularly in apricot. In the pear the presence of hydrobenzoic acid and kaempferol were noticed.

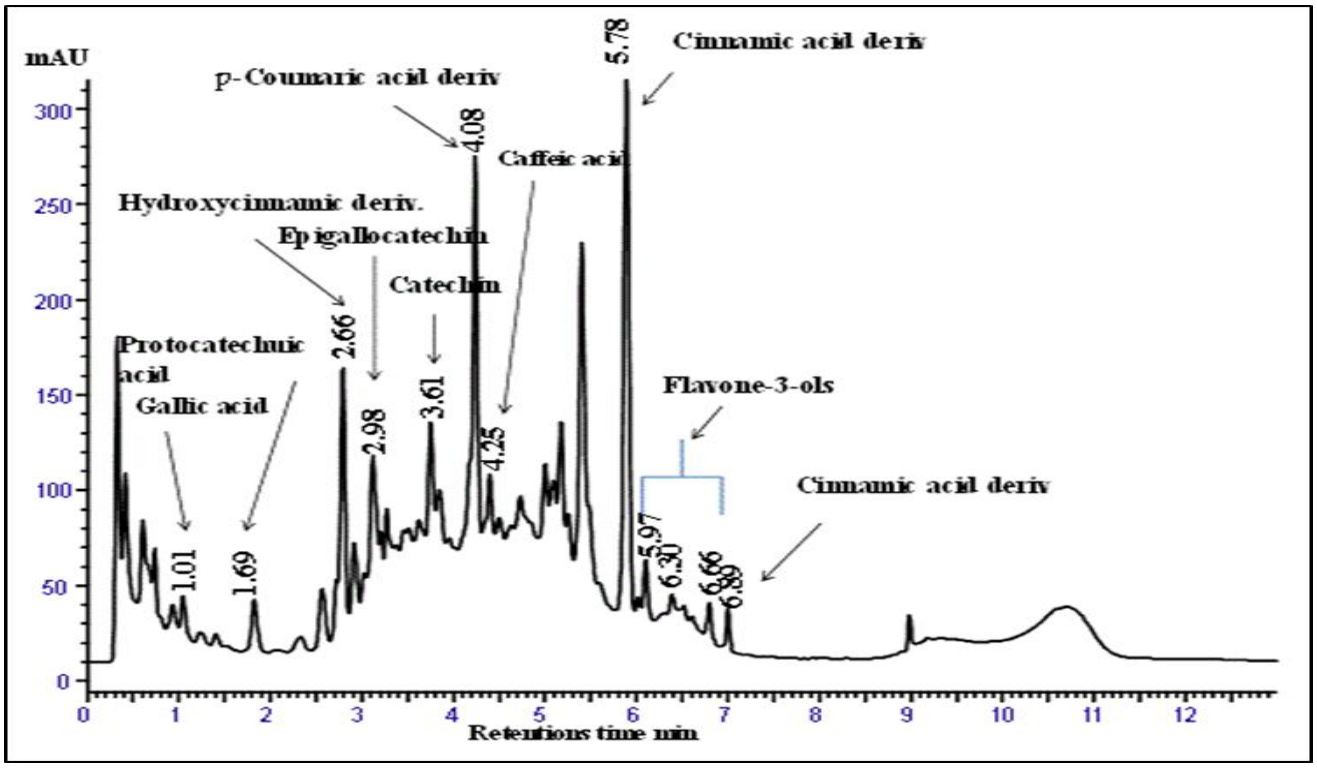


Figure 13: UPLC chromatogram of *Fargaria ananassa* fruit methanol extract.

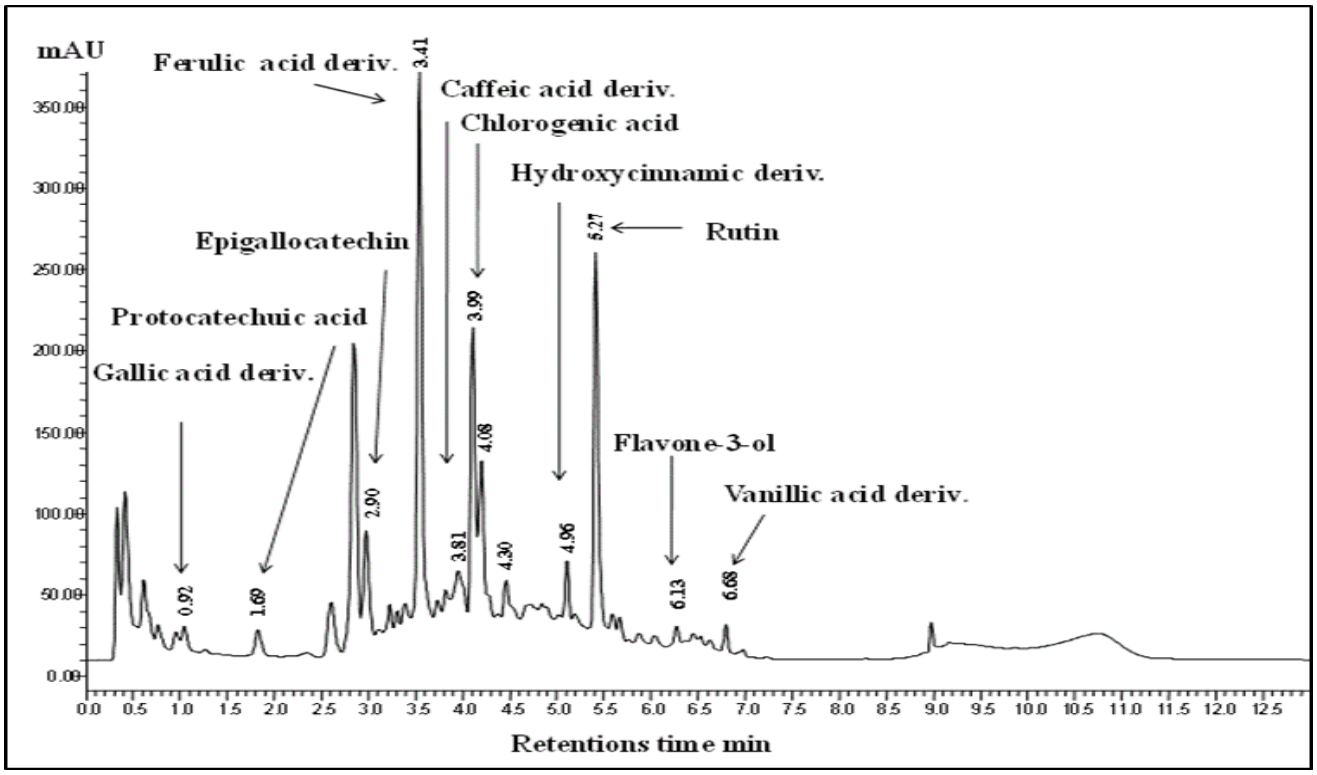


Figure 14: UPLC chromatogram of *Prunus armeniaca* fruit methanol extract.

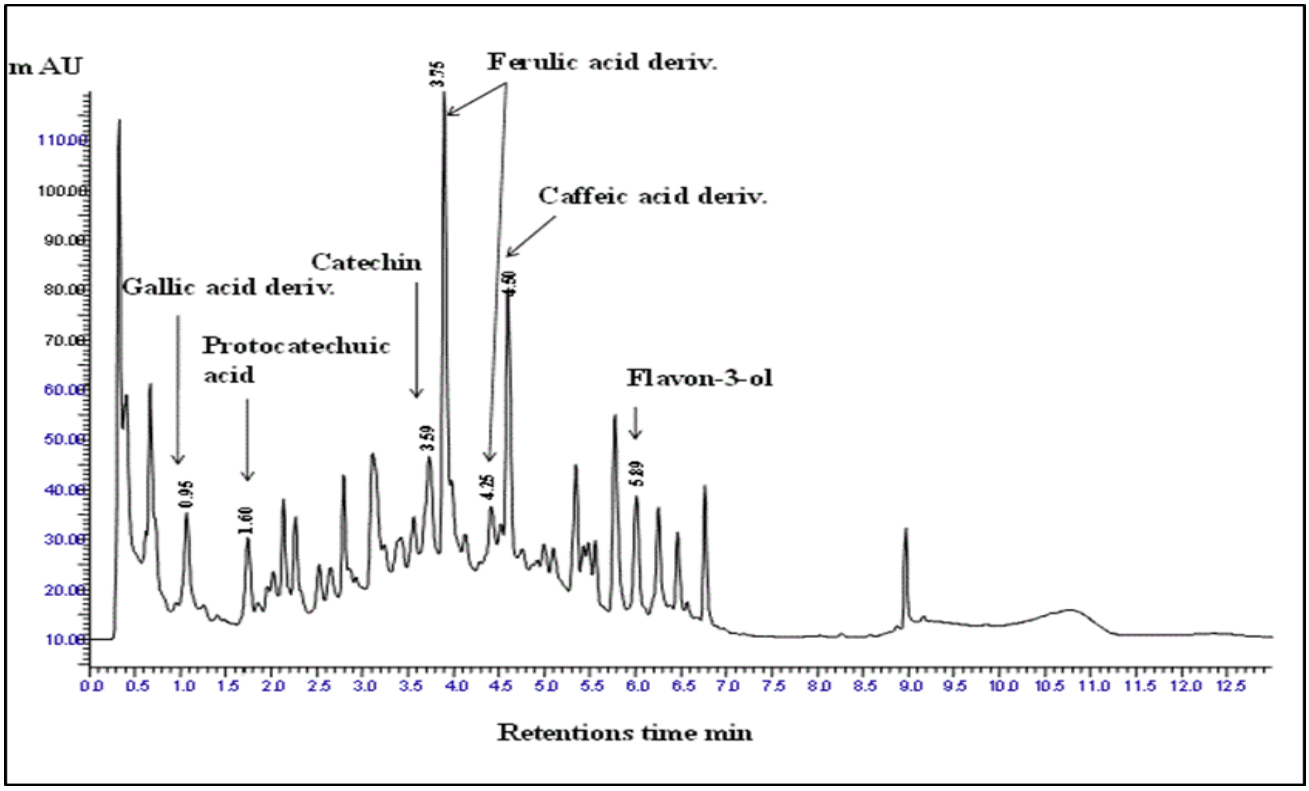


Figure 15: UPLC chromatogram of *Prunus persica* fruit methanol extract.

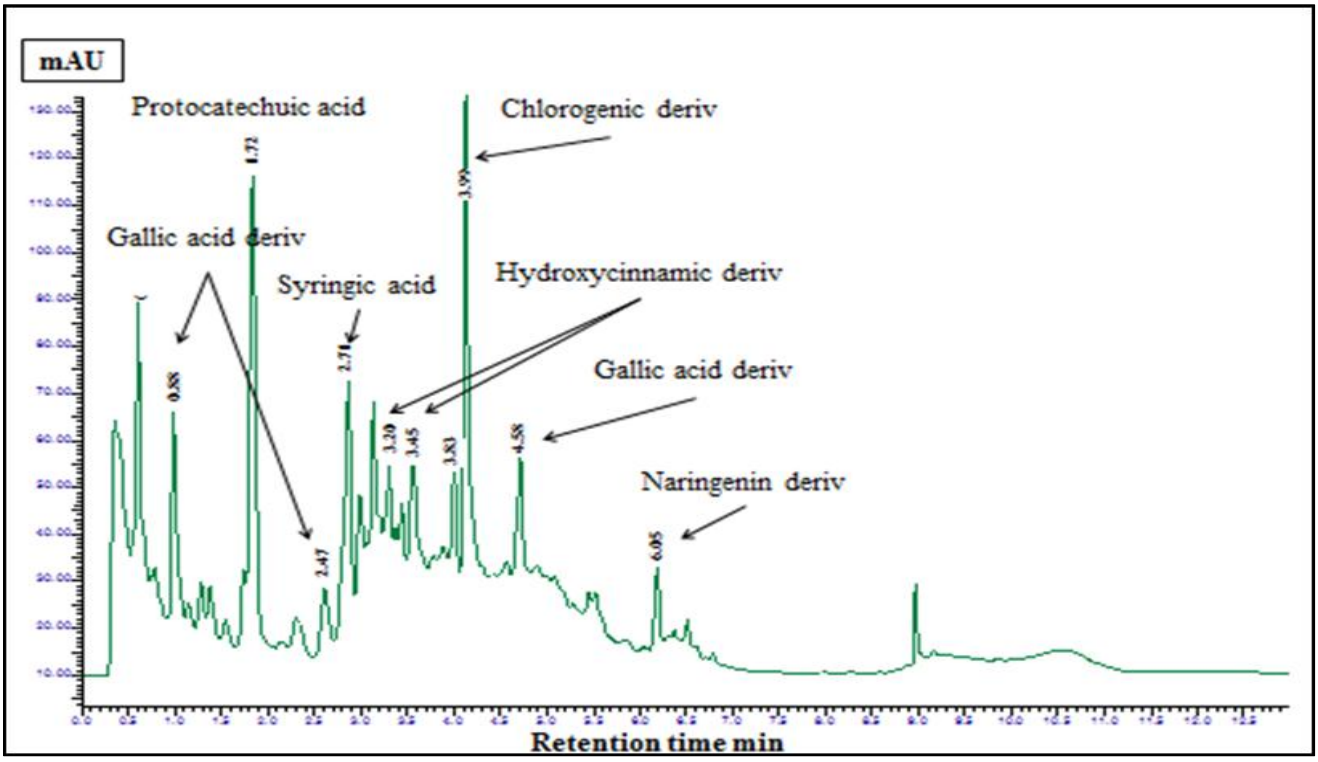


Figure 16: UPLC chromatogram of *Malus communis* fruit methanol extract.

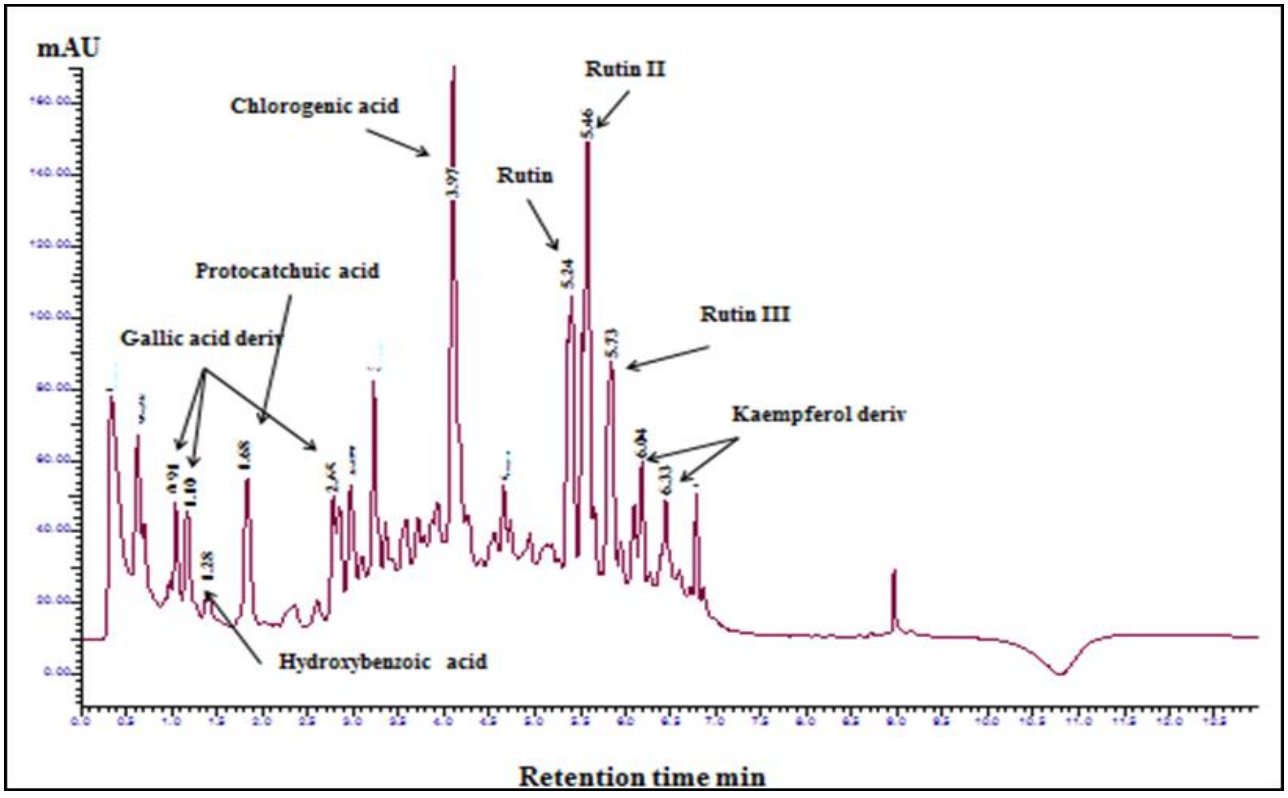


Figure 17: UPLC chromatogram of *Pyrus communis* fruit methanol extract.

The phytochemical studies on *Ficus carica* fruits are limited, the present study showed the presence of phenolic acid and some flavonoids (Fig 18). There are mainly two classes of phenolic acids based on derivative of benzoic acid such as galic acid and cinnamic acid such as hydrocynammic acid. Flavonoids are present in fig is flavonoids such as rutin, catechin, flavanols3-ol and nargenine.

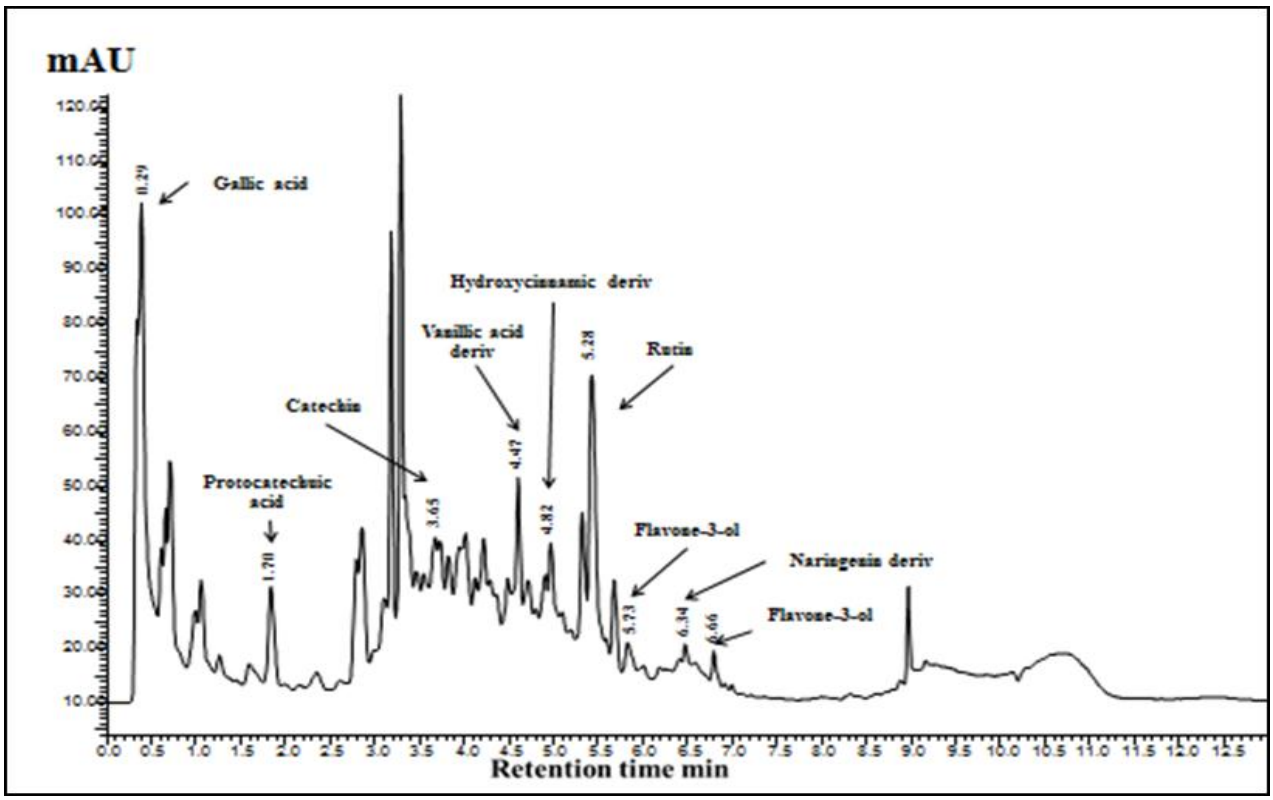


Figure 18: UPLC chromatogram of *Ficus carica* fruit methanol extract.

UPLC analysis. Showed the presence of small number of phenolics compounds in the pomegranate fruit extract. *Punica* contains syringic acid, protocatechuic acid, catechin and flavonol3-ol.

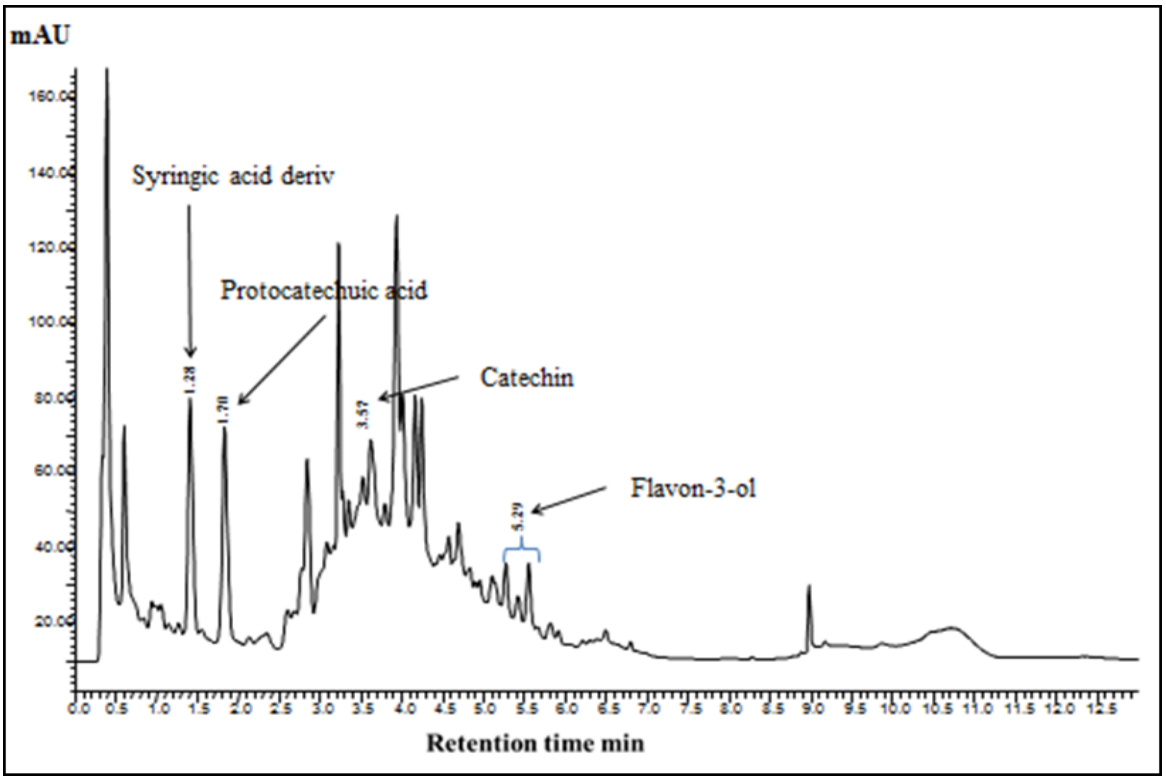


Figure 19: UPLC chromatogram of *Punica granatum* fruit methanol extract.

The UPLC chromatogram of *Vitis* fruit extract showed the presence of deferent phenolic compounds. The major constituent is catechin and rutin and their derivative. In other hand, this extract contains hydroxycinnamic acid and hydroxybenzoic acid.

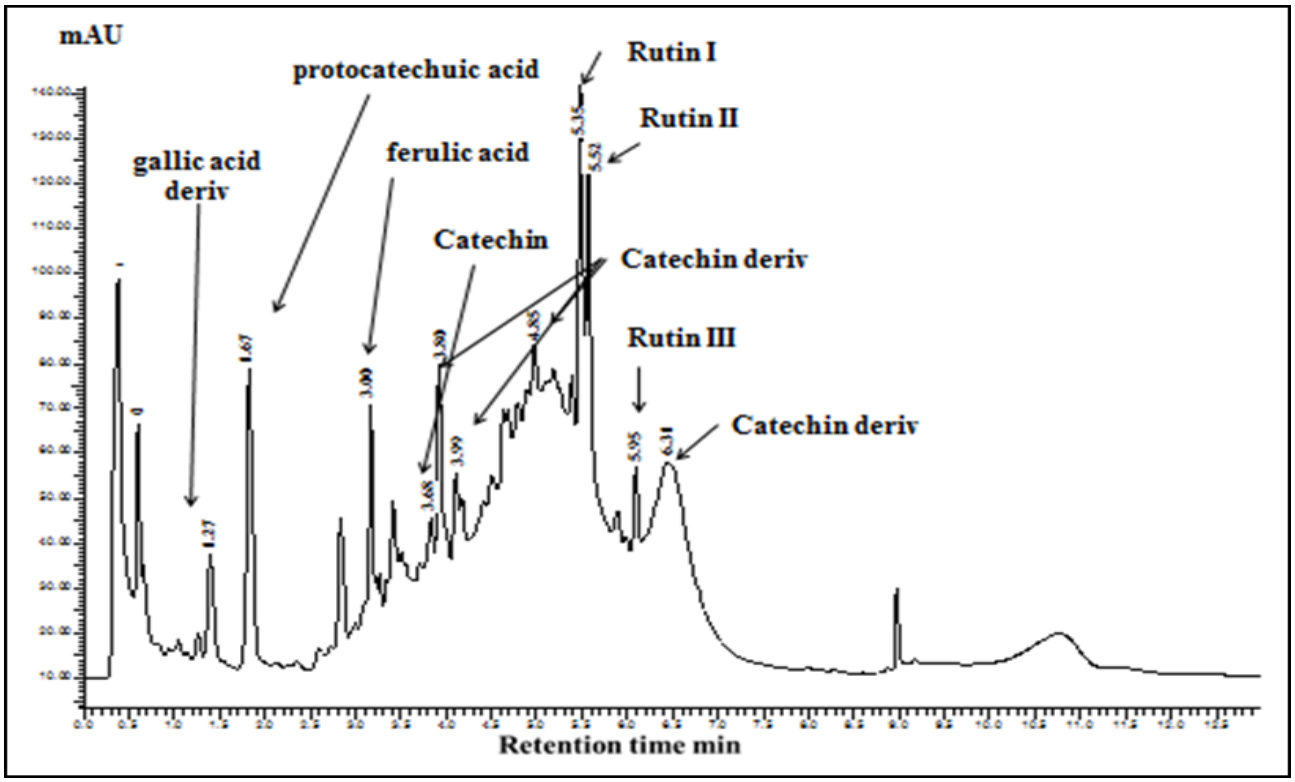


Figure 20: UPLC chromatogram of *Vitis vinifera* fruit methanol extract**.**

For banana extract the UPLC chromatogram demonstrated that the fruit is poor in the compound then other fruit. Banana contains only two major compounds gallic acid and their derivative. Also the extract revealed the presence of catechin and their derivatives.

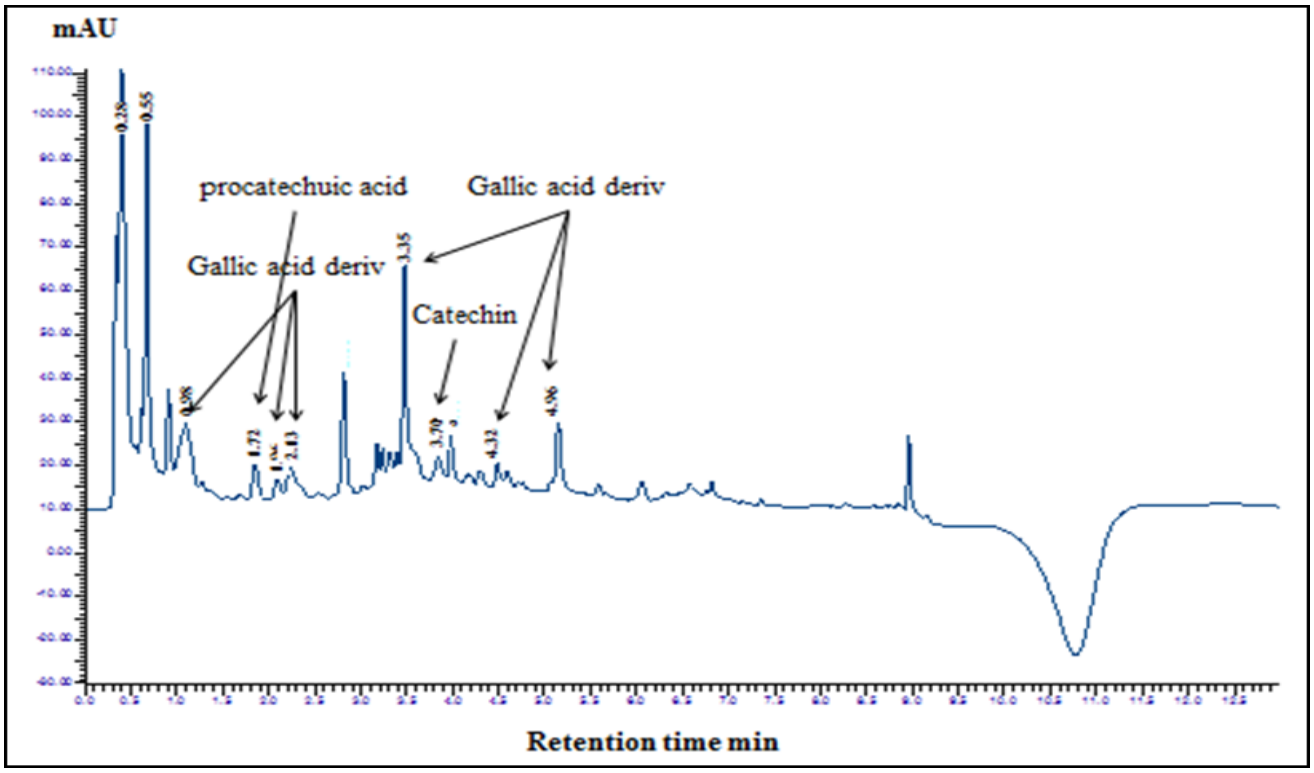


Figure 21: UPLC chromatogram of *Musa paradisiaca* fruit methanol extract.

Polyphenols and phenolic acids are also powerful antioxidants and demonstrated various health benefits by exhibiting antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilator actions (Duthie *et al*., 2000). Several studies suggest that Catechin and rutin have good effects such as antioxidant, anti-ageing and may prevent cardiovascular complications. Their beneficial effects are attributed to their ability to reduce oxidative stress, lipid peroxidation, free radical generation and low density lipoprotein (LDL) cholesterol-oxidation (Augustyniak *et al*., 2010; Ghasemzadeh *et al*., 2010). Moreover, other phenolic compounds found in the extracts such as gallic acid also possess beneficial effects on human health and deceases oxidative stress (Pandey and Rizvi, 2009).

3.2 Identification of phenolic compounds by HPLC-PDA/MS (LC/MS)

Liquid Chromatography-Mass Spectrometry (LC/MS) is a powerful technique often used in analytical chemistry due to its high sensitivity and specificity. It can also be used in polyphenol research to detect and identify specific polyphenolic compounds in the presence of other chemicals in a complex mixture in fruits. It is able to separate molecules of the same molecular weight but different atom composition, and sometimes even to differentiate stereoisomeric compounds (Pinheiro and Justino, 2012). MS detection provides information about the molecular mass and fragmentation pattern of the analyte. The combined use of

ionization in both positive and negative modes gives extra certainty to the determination of the molecular mass. The MS results, however, are hard to interpret without having any additional information about the compound (Aaby *et al*., 2007). The anthocyanins have maximum sensitivity in positive mode MS, due to their inherent positive charge. For most flavonoids, however, negative ionization mode provides the highest sensitivity (Cuyckens and Claeys, 2004).

In the present study, after the analysis of extracts by ULPC-DAD, which give only polyphenols without glycone. The most of secondary metabolites in the food are found as glycosides. For this reason use this technic is needed. The identification and the characterization of polyphenols in fruits extracts by HPLC/MS demonstrated that some of these extract contained different phenolic compounds such phenolic acid, flavonoids and anthocyanin.

HPLC/MS analysis of strawberries showed the presence of phenolic acid and anthocyanins because all compounds as related to sugars (table 6). For anthocayanins it is referred to results from positive mode MS only and with absorption maximum at 520 nm. It is responsible for red color of this fruit. Several studies demonstrated that pelargonidin is the major anthocyanins in the strawberries (Wang *et al*., 2002; Aaby et al., 2007; Cheel *et al*., 2007).

Table 6: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Fargaria ananassa*.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion [M-H] $^{-1/+1}$	Main fragment	Tentative identification
1	2,91	325	341	179 (341-162 hexose)	caffeic acid hexoside
2	3,61	325	353	191	Chlorogenic acid
3	4,27	311	325	163	*p*-coumaric acid hexoside
4	4,87	312	325	163 (325-162)	*p*-coumaric acid hexoside
5	5,98	520	(+) 447	301 (447-146)	peonidin deoxyhexose
6	30,31	520	(+) 433	271 (M-162)	pelargonidin hexoside
7	32,37	520	(+) 535	449 (M-malony); 303 (M-malonyl-pentoside)	delphinidin-malonylpentoside
8	32,47	520	(+) 435	303 (M-132)	delphinidin pentoside
9	33,09	520	(+) 611	287 (611-324 diglucoside)	cyanidin dihexoside
10	34,40	520	(+) 449	287 (449-162)	cyanidin hexoside
11	34,76	520	(+) 609	301 (609-308 rutinoside)	peonidin rutinoside

The identification of compounds in apricot allows to concluded that this fruit have different phenolic compounds such as phenolic acid like dicafeoylquinic acid. Flavonoids present are apigenin, diosmetin and isorhamnetin and also as glucosides. For anthocyanins the more observed compounds in this extract are delphinidin and peonidin. Some of these anthocyanins are bounds to sugars molecules. Hussain *et al*., 2013 demostrated that dried apricot contain various phenolic compounds. Amoung of these molecules apegenin, vanilic acid, catechin, protocatechuic acid and gallic acid.

Chlorogenic acid is the dominant phenolic compounds in apricots. Other phenolic compounds identified in apricot are: caffeic acid, p-coumaric acid, ferulic acid and catechin (Arts *et al*., 2005; Dragovic-Uzelac *et al*., 2005).

Dragovic-Uzelac *et al*., 2007 demonstrated that polyphenolic compounds in apricots change with the stage of maturity. In the case of immature apricots catechin is the major compounds present in the fruit but in mature fruits high level of chlorogenic acid was reportes.

Table 7: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Prunus armeniaca*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	2,93	325	515	353, 191	1,3-dicafeoylquinic acid
2	3,07	325	515	353, 191	1,4-dicafeoylquinic acid
3	3,31	510	463	301	peonidin glucoside
4	3,55	322	515	353, 191	1,5-dicafeoylquinic acid
5	3,66	322	515	353, 191	3,4-dicafeoylquinic acid
6	3,73	510	641	303	delphinidin ferruloylhexose
7	3,85	325	515	353, 191	3,5-dicafeoylquinic acid
8	4,19	325	515	353, 191	4,5dicafeoylquinic acid
9	4,42	330	401	269 (401-132)	apigenin pentoside
10	4,51	510	475	271	pelargonidin acetylhexose
11	4,88	265; 345	475	299	diosmetin glucuronide
12	5,04	265, 350	461	315 (461-146)	isorhamnetin deoxyhexose

13	5,95	520	465	303 (465-162 glucoside)	delphinidin hexoside I
14	6,70	520	465	303 (465-162 glucoside)	delphinidin hexoside II
15	7,27	520	475	303	delphinidin derivative
16	8,23	520	640	301	peonidin ferruloylhexose
17	9.06	520	455	303	delphinidin derivative
18	13,56	520	453	301	peonidin derivative
19	14,63	520	477	301	peonidin glucuronide
20	15,43	520	609	301	peonidin rutinoside

Six of phenolic compounds, namely caffeoyl-D-glucose, Catechin, dicafeoylquinic acid, catechin acethyl hexose, neochlorogenic acid, chlorogenic acid were identified in the extract of the peach.These compounds originate from two phenolic groups flavonoids and hydroxycenammic acid (table 8).

Two isomers of chlorogenic acid were observed chlorogenic and neochlorogenic acid. Concerning flavan-3-ol derivatives, catechin and catechin acethyl hexose were identified.

This was reported by Tomas Barberan *et al*., 2001, which identified the same compounds with some anthocyanins in peach cultivars in California.

Different phenolic compounds in peaches have been characterized in both peel and flesh tissues, such as hydroxycinnamates, flavan-3-ols, anthocyanins and flavonols (Simeone *et al*., 2012; Aubert *et al*., 2014; Zhao *et al*., 2015).

Table 8: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Prunus persica*.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	0,71	290	339		caffeoyl-D-glucose
2	2,47	279	289		Catechin
3	3,49	325	515	353, 191	dicafeoylquinic acid
4	3,64	280	493	(493-204) 289	catechin acethyl hexose
5	4,19	322	353	191	neochlorogenic acid
6	4,36	325	353	191	chlorogenic acid

Polyphenol compounds found in the investigated fig can be classified as hydroxycinammic acid and anthocyanin. The results obtained are reported in table 9. Hydroxycinnamic acids were represented by chlorogenic acid, neochlorogenic acid and cryptochlorogenic acid. All this compounds have the same λmax and mass and main fragment the difference is the retention time. Veberic *et al* (2008) demonstrated the presence of chlorogenic acid and two other phenolic compounds (Gallic and syringic acid) in fig. Other Hydroxycinnamic acids are observed such as coumaroylquinic acid, p-coumaric acid, cafeoyltartaric-p-coumaroyl acid.

In our study three anthocyanins are detected by LC/MS in the positive mode (cyanidin dervivative, cyanidin rutinoside, delphinidin benzoylhexose). The same results are reported by Solomon *et al* (2006) who found the presence of three anthocyanins in figs, namely cyanidin-3-O-rutinoside, cyanidin-3,5-O-diglucoside, and pelargonidin-3-O-rhamnoglucoside, with cyanidin-3-O-rutinoside being the predominant pigment in ripe skins. However, Ammar *et al* (2015) identified 9 compounds belonging to anthocyanidins, mainly cyanidin derivatives in black fig cultivar.

Table 9: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Ficus carica*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion [M-H] $^{-1/+1}$	Main fragment	Tentative identification
1	0,63	290	339		caffeoyl-D-glucose
2	2,46	520	379	287	cyanidin dervivative
3	3,45	325	353	191	neochlorogenic acid (3-O-cafeoylquinic acid)
5	3,5	325	353	191	cryptochlorogenic acid (4-O-cafeoylquinic acid)
6	3,63	325	353	191	chlorogenic acid (5-O-cafeoylquinic acid)
7	3,7	520	595	287	cyanidin rutinoside
8	4,03	322	337	191	coumaroylquinic acid
9	4,78	320	163		p-coumaric acid
10	5,24	320	457	163	cafeoyltartaric-p-coumaroyl acid
11	5,31	520	569	303	delphinidin benzoylhexose

The phytochemicals predominant in pomegranate fruits are polyphenols. The presence of number of phenolic compounds in different plant extracts, it is difficult to identify every compound, but it is not difficult to identify major and important phenolic compounds. For pomegranate, Noda *et al* (2002) isolated three major anthocyanidins: delphinidin, cyanidin and elargonidin.

HPLC/MS analysis was carried out to identify major phenolic compounds in pomegranate extract. Caffeoyl-D-glucose, pelargonidin glucuronide, Catechin, pelargonidin hexoside, epicatechin, procyanidin dimer and epi-catechin ethyl dimer has been identified in pomegranate extract (table10). Many other researchers demonstrated the presence of different phenolic compounds in *Punica*. Hmid *et al* (2013) reported the presence of gallic,

chlorogenic, caffeic, ferulic, ellagic acids, catechin, epicatechin, phloridzin, quercetin and rutin in pomegranate juices. The majority of the bibliographic information reported the presence of six major anthocyanins in pomegranate juice, namely the 3-mono- and 3,5-diglucoside derivatives of the anthocyanidins delphinidin, cyanidin and pelargonidin (Gil *et al*., 2000; Alighourchi *et al*., 2008; Elfalleh *et al*., 2011).

Table 10: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Punica granatum*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	0,71	290	339		caffeoyl-D-glucose
2	2,78	520	449 (+)	273 (449-176 glucuronide)	pelargonidin glucuronide
3	2,91	264	289		Catechin
4	3,05	520	435	273 (435-162 hexoside)	pelargonidin hexoside
5	3,5	266	289		Epicatechin
6	3,66	270	577 (289+289)	289	procyanidin dimer
7	4,04	270	605	289	epi-catechin ethyl dimer

The separation of polyphenols by HPLC/MS in *Vitis vinifera* detected the presence of a small number of polyphenols listed in the table 11. As can be seen the presence only one compounds of anthocyanins. Nicoletti *et al* (2008) saggesting that *Vitis* conatain various phenolic compounds such as catechin, gallic acid, protocatechuic acid and rutin. Di Lorenzo *et al* (2019) in their study reported that the skin of *vitis* is important source of anthocyanins then the pulp. They suggested that *Vitis* is rich in procyanidin dimer and trimmers in addition to other monomers like catechin and epicatechin. In our study the HPLC/MS and UPLC/DAD indicate that *Vitis* contain procyandin dimer, catechin and epicatechin.

Table 11: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Vitis vinifera*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	0,71	290	339		caffeoyl-D-glucose
2	2,91	280	289		Catechin
3	3,5	280	289		epi-catechin
4	3,63	280	577	289	procyanidin dimer

Banana pulp contains various antioxidants such as vitamins, carotenoids and phenolic compounds such as catechin, epicatechin, lignin, tannins and anthocyanins (Somya *et al*., 2002). Emaga *et al*., (2007) isolated from pulp and peel of *Musa paradisiaca* cellulose, hemicelluloses, arginine, aspartic acid, glutamic acid, leucine, valine, phenylalanine and threonine. They reported the presence of one molecules of polyphenols quercetin. The identification of phenolic compounds in this study demonstrated that banana is poor of polyphenols (table 12).

Table 12: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Musa paradisiaca*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	0,71	290	339		caffeoyl-D-glucose
2	3,71	270	577	289	procyanidin dimer

In the present study methanolic extract of peel and pulp of apples were prepared and evaluated for their chemical profile using HPLC-DAD-MS analysis. The MS analysis of apples showed the presence of phenolic acid and flavonoids. As shown in table 13 the major phenolic acid present in *Malus* are hyroxycenaamic acid such as chlorogenic acid and neochlorogenic acid. Flavonoids found in this extract are catechin and epicatechin.

Table 13: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Malus communis* (royal gala)

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	2,87	263	341	179 (341-162)	cafeic acid hexoside
2	2,93	263	289		catechin
3	3,58	325	353	191	neochlorogenic acid
4	3,6	325	353	191	chlorogenic acid
5	3,71	265	289		Epicatechin
6	3,87	316	495	191	1,5-digalloyl quinic acid
7	4,153	266; 320	337	163	p-coumaroyl quinic acid
8	4,45	326	225	208	sinapic acid

In our study, we used two varieties of apples royal gala or red apple, the second is growing in Algeria (aris or yellow apple). The chromatogram obtained from HPLC/MS of the crude extract of yellow apple showed the presence of three phenolics compounds. These compounds are isomers belonging to gallotannin family. These two varities of apple belong to the same family Rosasea but the identification and the charactrisation of polyphenols by HPLC/MS indicate strong deference between these two varieties. The cause of these results may be the deferent in the geographical region, the stage of maturity and the factors acts on the agriculture of fruits.

Giomaro *et al* (2014) found in their study that apples growing in Italy contain various phenolic compounds. They found similar compounds reported in our study and other molecules such as rutin, quercetin and their derivatives, procyanidin (dimer) and trimmers.

Table 14: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Malus communis* (aris)

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Identification
1	2,89	316	495	191	1,3-digalloyl quinic acid
2	3,03	316	495	191	1,4-digalloyl quinic acid
3	3,87	316	495	191	1,5-digalloyl quinic acid

Pear is an important source of polyphenols. Several studies indecate that the characterization of polyphenols by HPLC/MS reported presence of many classes of phenolic compounds, flavonoids (monomers and polymers of flavan-3-ols, flavonols and anthocyanins), phenolic acids (hydroxycinnamic acids derived from caffeic acid and p-coumaric acid) and simple phenolics (p-hydroquinone-glucoside: arbutin) (Öztürk *et al*., 2015; Kolniak-Ostek, 2016). Some studies suggest that caffeic acid and arbutin are the major compounds in pears (Lin and Harnly, 2008; Yim and Nam, 2016). Pérez-Jiménez *et al* (2013) reported that procyanidins of pear are not extractrable in the alcoholic or hydro-alcoholic solvent. In this study the identification of polyphenols by HPLC/MS in pear extract reported the presence of caffeic acids and isomers of caffeic acid.

Table 15: Retention time, maximum UV absorption (λmax), mass spectral data and tentative identification of the phenolic compounds in the methanol extract of *Pyrus communis*

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^{-1/+1}$	Main fragment	Tentative identification
1	0,71	290	339		caffeoyl-D-glucose
2	2,91	325	341	179 (341-162 hexose)	caffeic acid hexoside

4. *In vitro* antioxidant activity of fruit extracts

Fruits, vegetables and several foods are considered as excellent sources of phytochemicals and antioxidants. There are deferent methods used for measuring the antioxidant activity of these compounds in foods and supplements. Most of these methods are based on the coloring and discoloration of reagent in the reaction medium. Some assays are used to evaluate lipid peroxidation and others for measuring the free radical scavenging activity (Dasgupta and Klein, 2014). The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products (Gulçin *et al*., 2010). Assays based upon the use of DPPH and ABTS radicals are among the most popular spectrophotometric methods for the determination of the antioxidant capacity of food, beverages and vegetable extracts (Bendini *et al*., 2006).

4.1 Total Antioxidant Capacity (TAC) of fruit extracts

Total antioxidant activity measured by Phosphomolybdate method is a sensitive method to determine total antioxidant potential of natural antioxidants. It is one of the frequently used tests in recent years (Bunzel and Schendel, 2017). The phosphomolybdate method is quantitative. This method is based on the reduction of phosphomolybdate (Mo (VI)) to phosphomolybdenum (Mo (V)) and this reduction produced green phosphate/ Mo (V) complex in acid pH. In the presence of antioxidant sources the color is reduced which can be measured at 695 nm (Ozen *et al*., 2018). The phosphomolybdate model evaluates both water soluble and fat soluble antioxidant capacity. It increases with the increase in the concentration of extract. A high absorbance value indicated that the sample possesses high antioxidant

activity (Shailaja, 2019). Results in this study were expressed as µg Ascorbic acid equivalent per g of extract. Total antioxidant activity of fruits extracts varied from 50.72 ± 0.05 to 109 ± 0.01 µg Vit C/g extract. The result of total antioxidant capacity showed that *Malus communis* (aris) had the highest antioxidant capacity (109± 0.01) flowed by *Malus communis* (royal gala) (104 ± 0.009).

Other extracts significantly exhibited a reduction of Mo (VI) to Mo (V) with the following order straweberries (99.66 ± 0.007) > grapes (96 ± 0.009) > figs (92.66 ± 0.02) > peachs (81.66 ± 0.008) > apricots (81.33 ± 0.004) > bananas (79 ± 0.01) > pomegranate (60.5 ± 0.02) > pear (50.72 ± 0.05) (Fig 22)

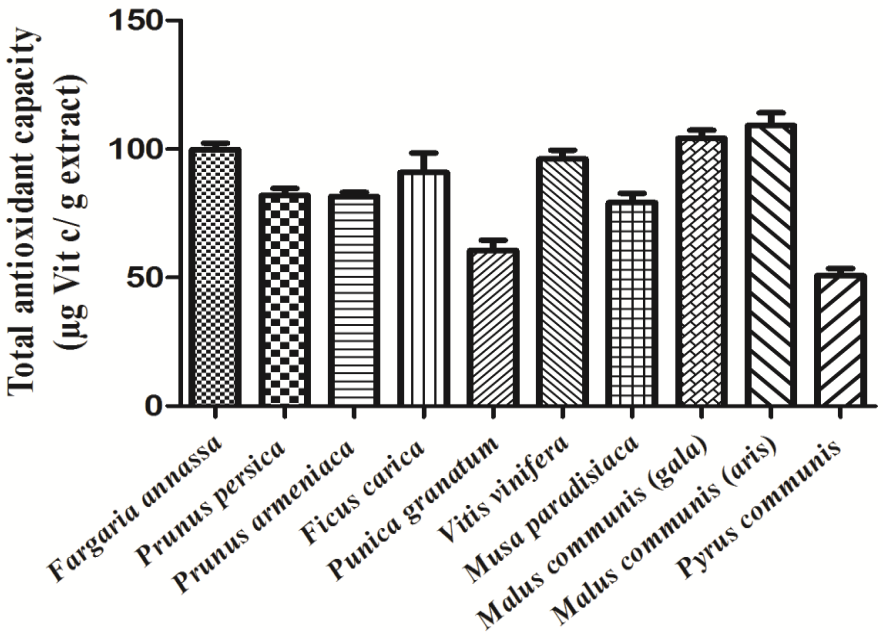


Figure 22: Total antioxidant capacity of fruits extracts. Result expressed as Mean ± SD.

Various natural product containing phenolics and flavonoids have the ability to reduce molybdenum (Ahmed *et al*., 2015; Gupta *et al*., 2017).Singh *et al*., 2018 reported that the total antioxidant capacity depend on the intrinsic levels of polyphenols (phenol, flavonoid and tannins) within the plants. However, in this study pear has high level of polyphenols but have low potential to reduce Mo (VI) to Mo (V).

4.2DPPH and ABTS Radical scavenging activity

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end point. $ABTS^{+}$ and DPPH radical scavenging methods are common spectrophotometric procedures for the evaluation of the antioxidant properties and antiradical activity of natural products. Both chromogen radical compounds can directly react with antioxidants. These chromogens (the violet DPPH radical and the blue green ABTS radical cation) are easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples (Ozcelik *et al*., 2003).

DPPH is a stable free radical with purple color. This molecule absorbs at 517 nm. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Ozcelik *et al*., 2003). In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction with the decrease of absorbance (Gupta *et al*., 2014).

Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of total antioxidant activity of pure substances solutions, aqueous mixtures and beverages (Sivakumar and Meera, 2013). A more appropriate format for the assay is a decolorization technique in that the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{\cdot+}$ described here involves the direct production of the blue/green $ABTS^{\cdot+}$ chromophore through the reaction between ABTS and potassium persulfate. All the tested compounds exhibited effectual radical cation scavenging activity antioxidant compound to become a stable diamagnetic molecule (Gulçin *et al*., 2010).

The 2,2diphényl-1-picrylhydrazyle is the first free radical used to study the relation between the structure and antioxidant activity of phenolic compounds (Popovici *et al*., 2009). It was found that the radical- scavenging activity of extract increased with increasing concentration (Ebrahimzadeh *et al*., 2009). The degree of discoloration indicates the scavenging potential of the antioxidants. The antioxidant activities obtained by the DPPH method for the fruits extracts are presented in Fig.23. Results were presented as IC_{50}. This activity was compared

with that of BHT as a synthetic antioxidant. The results revealed that methanol extract of *Fargaria* is more effective scavenger than other fruit extracts with an IC_{50} value of 0.142 ± 0.0004 mg/ml.

All extract exhibited a good scavenger lower than BHT (0.014 ± 0.001) with IC_{50} increased in the following order: red apple (0.169 ± 0.01) > grape (0.191 ± 0.0008) > permanganate (0.28 ± 0.004) > apricot (0.488 ± 0.012) > yellow apple (0.606 ± 0.033) > pear (0.617 ± 0.03) > peach (0.682 ± 0.022) only banana and fig demonstrated low activity than other fruits with IC_{50} of 2.011 ± 0.06 and 1.39 ± 0.12 respectively.

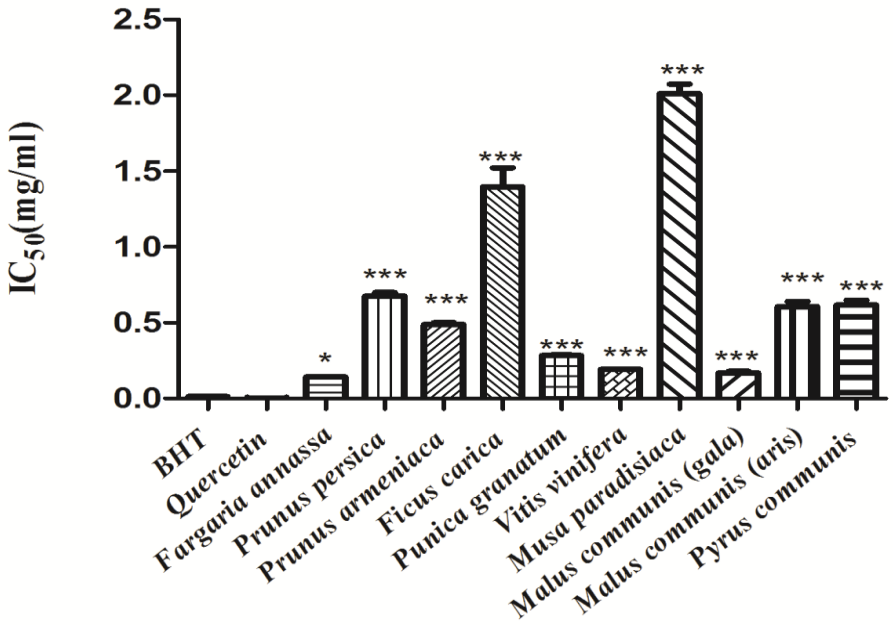


Figure 23: A comparison between different fruits extract in DPPH free radical scavenging activity. Data were presented as IC_{50} means ± SD (n = 3). (*** $p < 0.001$; * $p < 0.05$) compared to BHT as standard and Quercetin as standard polyphenol.

The $ABTS^{\cdot+}$ radical is one of various radical used for measuring the antioxidant activity of foods (Sivakumar and Meera, 2013). ABTS is a stable organic radical that has gained hydrogen; this method determines the antioxidant activity of hydrogen donating antioxidants in fruit crude extract (Le *et al*., 2007, Sivakumar and Meera, 2013). Also *Fargaria* extract has high scavenging ability of the ABTS radical with an IC_{50} value of 0.040 ± 0.003 mg/ml compared to other fruits. All fruits scavenging $ABTS^{\cdot+}$ radicals with values of IC_{50} low than that of Vit C. Red Apple, grape and permanganate had approximately the same activity with values of IC_{50}: 0.084 ± 0.004, 0.090 ± 0.001 and 0.092 ± 0.01 mg/ml respectively). *Prunus*

persica and *Prunus armeniaca* have good scavenging activity with IC_{50} values of 0.173 ± 0.003 and 0.323 ± 0.007 respectively. Banana had low ability to scavenge $ABTS^{\cdot+}$ radicals compared to other fruits extracts (Fig 24).

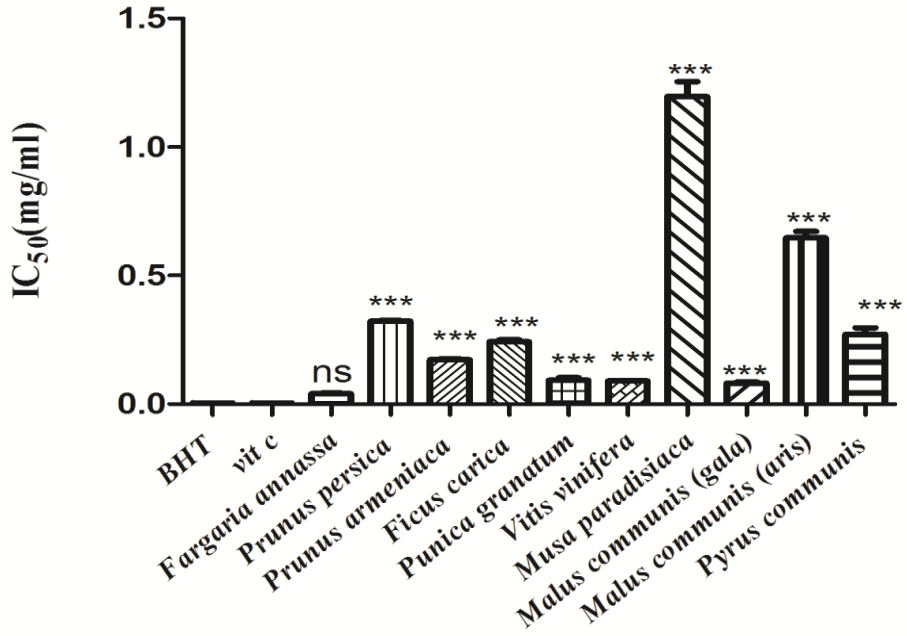


Figure 24: A comparison between different fruits extract in ABTS free radical scavenging activity. Data were presented as IC_{50} means ± SD (n = 3). (Ns: no significant*** $p < 0.001$) compared to BHT as standard.

In the present study, DPPH scavenging activity of extracts had positive relation with tannins, flavonoids and various phenolic compounds (Garg *et al*., 2012). Generally, the extract with high total phenolic contents had higher scavenging activity (Yardpiroon *et al*., 2014). It was reported that the extract of strawberries (*Fargaria*) had the highest total antioxidant activity compared with extracts of plums, orange, red grapes, kiwi fruit, pink grape fruit, white grapes, banana, apple, tomato and pears (Laura, 2010).

In the case of figs which have low activity. Similar results were reported in Tunisia by Harzallah *et al*., 2016. It is found that green figs juices have low activity with IC_{50} of 14.6 ± 0.62 mg/ml. Mujić *et al*., 2012 used dry figs and extracted in the methanol solvent. Their results also expressed an IC_{50} (0.818mg/ml) approximally close to our results. Jain *et al*., 2011 demonstrated that banana has low activity against DPPH with value of 1.9 mg/g extract wich was close to the value showed in the present study.

The scavenging effect of the $ABTS^{\cdot+}$ radical by the extracts was found to be much higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (Yu *et al*., 2002). Compounds which have $ABTS^{\cdot+}$scavenging activity did not show DPPH scavenging activity. This result is found for figs which had good effect against $ABTS^{\cdot+}$ radical and low activity in the DPPH assay.

Some researchers did not find any correlation between polyphenol content and antioxidant activity of plant extracts, while others reported a strong relationship between them (Dudonne *et al*., 2009; Floegel *et al*., 2011).

Kolniak-Ostek *et al* (2013) discussed a relationship between the $ABTS^{\cdot+}$ radical and antioxidant potential of polyphenols. They reported that the polyphenolic compounds from the group of flavan-3-ols have a greater impact on the value of antioxidant capacity than flavonols and hydrochalcones. The data presented by Eberhardt *et al* (2000) show that the polymer procyanidins have high antioxidant activity and that glycosylation and hydroxylation of the B ring had a great influence on the antioxidant properties of phenolic compounds. In this study the analysis of polyphenols by UPLC and LC/MS demonstrated the presence of flavone-3-ols in strawberries, apricot, peach, fig and permanganate. Most of compounds present in *Fargaria* are glycosylated. Epicatechin, characterized by the presence of the 3-OH group in combination with the C2-C3 double bond, is also considered as a strong antioxidant.
Liu *et al* (2010) demonstrated that the synergistic effect between polyphenols compounds have best antioxidant activity compared to pure molecules. The activity difference obtained for crude extract samples might probably be due to the extraction procedures, samples processing or drying. During the processing of samples some active volatile compounds may have been destroyed or evaporated from the dry samples (Alabri *et al*., 2013).
Antioxidant activity of flavonoids increases with increasing degree of hydroxylation. Flavonoids are able to scavenge free radicals directly by hydrogen atom donation. Blocking the hydroxy group at the C-3 position or removing the 3-OH group decreases antioxidative properties of flavonoids (Russo, 2018).

4.3 Metal Chelating activity of fruit extracts

The accumulation of metal ion was considered a source of oxidative stress and leading to occurrence of many diseases. Ferrous ion (Fe^{2+}) is commonly found in food systems. It is involved in the generation of free radicals through Fenton and Haber-Weiss reaction such as hydroxyl radical and accelerates lipid proxidation into alkoxyl radical (Özkan *et al*., 2011) and it is known as an effective prooxidant. They are responsible for DNA damage, lipid peroxidation, protein modification and other effects (Jomova and Valko, 2011). Ferric ions (Fe^{3+}) also produce radicals from peroxides, but the rate is tenfold less than that of ferrous ion. Ferrous ions (Fe^{2+}) are the most powerful pro-oxidant among the various species of metal ions (Al-Hashimi, 2012).

In the metal chelating assay, ferrozine a chelating agent reacts with ferrous chloride (Fe^{2+}) and can quantitatively form complexes. The Ferrosine-Fe^{+2} complexes produced a red chromophore which can be measured and gives maximum absorbance at 562 nm. The presence of chelating agent can disorder the formation of complexes Ferrosine-Fe^{+2} and the red color disappear (Gupta *et al*., 2014).

In this context, fruits extracts exhibited the ability to chelate metal ions. Among the different sample extracts exhibits higher metal chelating activity and inhibit the formation of ferrozine-Fe^{+2} complexes only *Pyrus communis* cannot interfer with the formation of ferrous and ferrozine complex, *Pyruscommunis* (Pear) was without chelating activity.

Figure 25 shows the chelating activity of fruits extracts under study. It shows that IC_{50} value of the methanolic extract of fruits were not significantly low than EDTA. EDTA is a strong metal chelator, hence, it is used as a standard metal chelator agent in this study.

Our results showed thatEDTA, *Purnus armeniaca* and *Ficus carica* have strong chelating activity approximally the same values of IC_{50} 0.023 ± 0.0021,0.016 ± 0.0009, 0.014 ± 0.004 respectively.

Fargaria ananassa demonstrated also higher chelating activity with IC_{50} = 0.025 ± 0.009 mg/ml and was similar to IC_{50} value of EDTA. *Punica granatum, Vitis vinifera* and *Malus communis* (red apple) have an important chelating activity in the following order: 0.089 ± 0.003, 0.24 ± 0.01, 0.40 ± 0.04 respectively low to standards.

Prunus persica and *Malus communis* have the lowest ability to chelate the ferrous iron compared to author fruits.

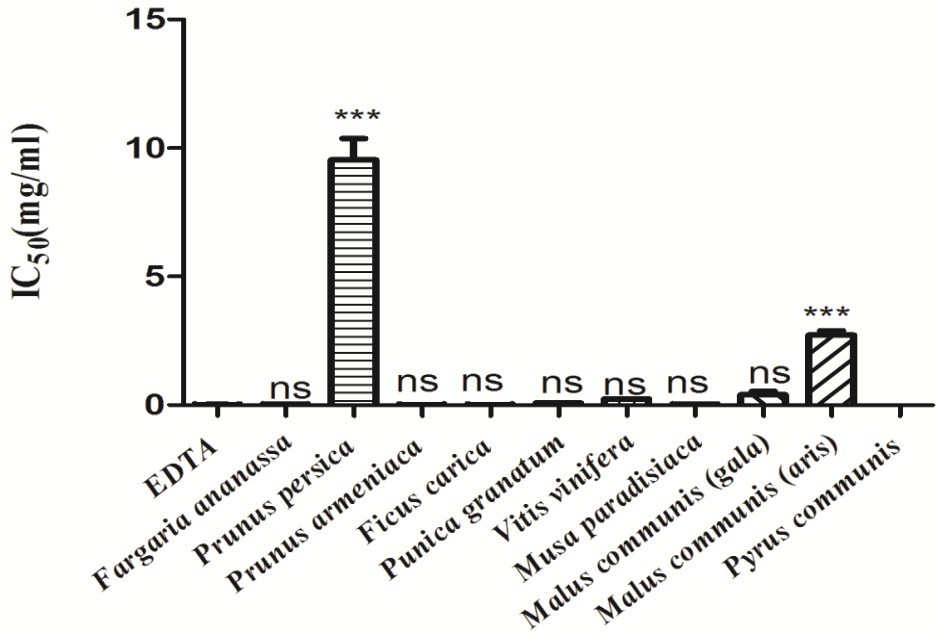


Figure 25: A comparison between different fruits extract in metal chelating activity. Data were presented as IC_{50} means ± SD (n = 3). (Ns: no significant difference; *** $p<0.001$) compared to EDTA as standard.

Overloading of iron in the body can occur and requires the consumption of exogenous chelators to prevent the build-up of free iron circulating in the body. Such exogenous chelators can be obtained from certain antioxidants in food (Le *et al*., 2007). Chelating agents stabilize transition metals and reduce their availability as catalysts, to inhibit the production of the first few free radicals and consequently suppress lipid peroxidation in biological and food systems. Chelating agent can reduce lipid peroxidation and inhibit the formation of free radicals by stabilizing the transition metals (Yu *et al*., 2002). The metal ion chelating activity was measured by the ability of some phenolic compounds to disrupt the formation of Ferrosine-Fe^{+2} complexes (Norshazila *et al*., 2010; Yumrutas and Saygideger, 2012). The high content of polyphenolic compounds present in the methanolic extracts of strawberries should be able to chelate transition metal because of the high charge density of the phenoxide group generated on deprotonation (Sivaraman *et al*., 2013).

In the case of fig which contain low amount of polyphenols in this study, but it had the better chelating activity may be due to the presence of other compounds. The secondary antioxidant compounds are efficient metal chelating agents and also prevent free radical generation (Juntachote and Berghofer, 2005). These chelating agents are found to reduce the redox potential by stabilizing the oxidized form of the metal ion through the formation of σ-bonds

with these metal ions (Gupta *et al*., 2014). Secondary antioxidants act indirectly by preventing the formation of free radicals through Fenton's reaction,while Primary antioxidants (polyphenols) prevent oxidative damage by directly scavenging free radicals (Chan *et al*., 2010). Lai and lim (2011) showed that some phenolic compounds in the plant extract are weak ion chelators. Some components such as polysaccharides, proteins or peptides in the extracts were more effective chelators of ferrous ions than phenolic compounds (Saiga *et al*., 2003; Wang *et al*., 2009).

4.4 Hydroxyl radical scavenging activity of fruit extract

Hydroxyl radical is the neutral form of hydroxide ion and is a highly reactive free radical. It is the major active oxygen species in the biological systems. Under physiological pH and temperature it is generally stable in the absence of metal ions. It is formed by deferent ways. It is formed in a Fenton reaction in the following reaction:

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + OH^{\bullet} + OH^{-}$$

On other hand, under stress conditions, an excess of $O_2^{\bullet-}$ releases free iron from ferritin and the released free iron participates in Fenton reaction to form $OH^{\bullet}$. It is also formed by the reaction between superoxide radical and H_2O_2 in a reaction called Haber– Weiss reaction by the following reaction:

$$O^{-2} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^{-}$$

Hydroxyl radicals can strongly react with both organic and inorganic molecules including DNA, proteins, lipids, and carbohydrates because they have free electron. The high concentration of hydroxyl induced damage to DNA, lipid, protein and produced carcinogenesis, mutagenesis and cytotoxicity (Saeed *et al*., 2012;Rahman*et al*., 2015). The hydroxyl and superoxide radicals play a key role in causing diseases including arthritis, ischemia, gastric problems, cancer, and acquired immune deficiency syndrome. The radicals can react rapidly with foods containing fatty acids, proteins and sugars (Debnath *et al*., 2017). The hydroxyl scavenging ability of various fruit extract was measured using a system containing $FeSO_4$ and H_2O_2, this chemicals interact between then and produce $OH^{\bullet}$, which hydroxylate salicylate. Scavenging of hydroxyl radicals is considered as a very important antioxidant activity as hydroxyl radicals are more damaging to biomolecules. The Hydroxyl

radical scavenging activity of methanol fruits extracts were determined and compared to Vit C. The results are show in Fig.26.

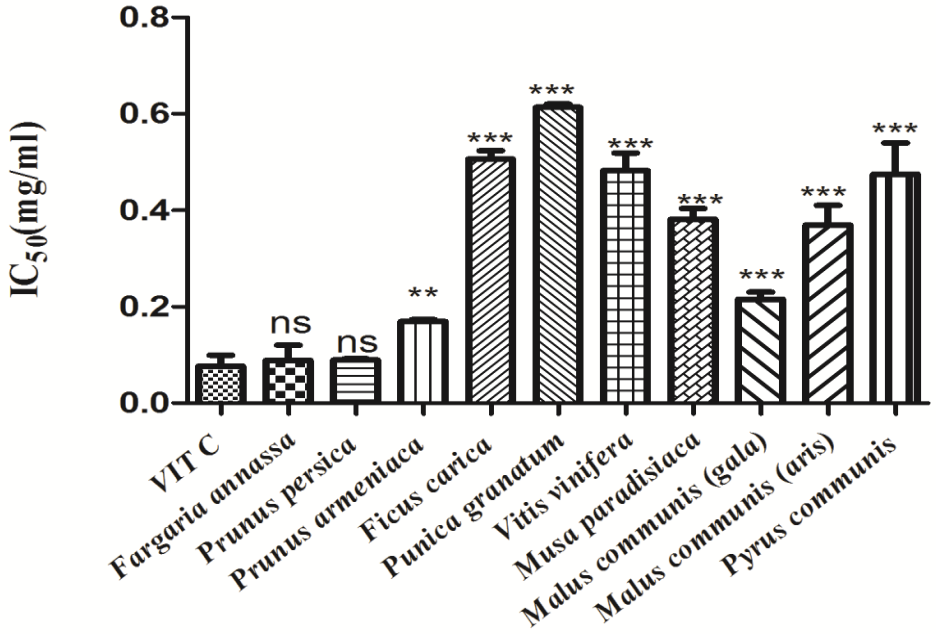


Figure 26: Comparison between different fruits extract in hydroxyl radical scavenging activity. Data were presented as IC_{50} means ± SD (n = 3). (ns: no significant difference; ** p <0.01, *** p<0.001) compared to vitamin C as standard.

In this study the fruits extracts have good ability to quench hydroxyl radicals but they were significantly lower than vitamin C (IC_{50}: 0.076 mg/ml). Except *Fargaria* and *Prunus persica* which present strong activity with IC_{50} values of 0.079 ± 0.031 and 0,089 ± 0.003 mg/ml respectively no significantly difference was notices compared to Vit C.

Prunus armeniaca showed high capacity to scavenge hydroxyl with IC_{50} value of 0.16 ± 0.008 mg/ml.

Other fruits extracts have good potential to scavange hydroxyl radical following this order: red apple 0.215 ± 0.014 > yellow apple 0.36 ± 0.04 > banana 0.38 ± 0.02 > pear 0.47 ± 0.06 > grape 0.48 ± 0.03 > fig 0.506 ± 0.01 > pomegranate 0.613 ± 0.006.

Several researchs showed a positive relation between polyphenols and scavenging radical hydroxyl capacity. These results are reported in my study for strawberries, whish is the richest fruit on polyphenols and having strong scavenging activity.

In the case of banana which have the lowest amount of polyphenols but have an important activity which may be due to other compounds.Zhu *et al* (2017) suggested other compounds

than polyphenols which are more potent to scavenging $OH^{\cdot}$ radicals. The variation in the molecular size and stereoselectivity of hydroxyl radical effect in their scavenging capacity of antoxidants compounds (Gunathilake *et al*., 2018).

Husain *et al* (1987) reported that flavonoids such as myrcetin, quercetin and rhamnetin were OH scavengers. They also noted that the effectiveness of such compounds increased with increasing of the number of hydroxyl groups attached to the aromatic B ring. As it is the case for many other free radicals, $OH^{\cdot}$ can be neutralized if it is provided with a hydrogen atom. The phenolic compounds present in the crude extract had the ability to donate a hydrogen atom to $OH^{\cdot}$. Strawberry extracts exhibited high enzymatic activity for oxygen detoxification and a high level of antioxidant capacity against free radical species including peroxyl radicals, superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Wang and Lin, 2000; Wang and Jiao, 2000).

4.5Reducing power of fruits extracts

For the measurement of the reducing power of fruits extracts, we investigated the transformation of Fe^{3+} to Fe^{2+} ion in the presence extracts which served as an indicator of their antioxidant activity (Olorunnisola *et al*., 2012).

The transformation of the yellow color of the Fe^{3+} /ferricyanide complex to Perl's Prussian blue color of the ferrous (Fe^{2+}) form was measured at 700nm. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride. Increase in the absorbance of the reaction mixture indicates the reducing power of the samples (Alam *et al*., 2013). Potassium ferricyanide has been the most popular ferric reagent used reducing power assays. In the latter case, Prussian blue is produced as the end product which is quantified spectrophotometrically and indicates the reducing power of the antioxidants tested. Production of Prussian blue may be through two different routes with the same outcome. Antioxidants can either reduce the Fe^{3+} in the solution to Fe^{2+}, which binds the ferricyanide to yield Prussian blue, or reduce the ferricyanide to ferrocyanide, which binds the free Fe^{3+} in the solution and forms Prussian blue (Shahidi and Zhong 2015).

This method is based on the principle of increase in the absorbance of the reaction mixtures. The increase in the absorbance indicates an increase in the antioxidant activity. The reducing power of fruits increased with an increase in concentration. Fig.27 showed a relationship between the increase in the absorbance, the concentration of extract and the reducing power. At 0.5mg/ml the absorbance of fruit extracts were in the flowing order *Fargariaananassa*

(1.142) >*Malus communis* (0.90) >*Punica granatum* (0.750) >*Prunus armeniaca* (0.744) >*Malus communis* (red apple (0.6) >*Prunus persica* (0.592) >*vitis vinifera* (0.5) >*Ficus carica* (0.350) >*Musa paradisiaca* (0.350) >*Pyrus communis* (0.249). Figure28 shows the RC_{50} values as reducing power of fruit extracts.

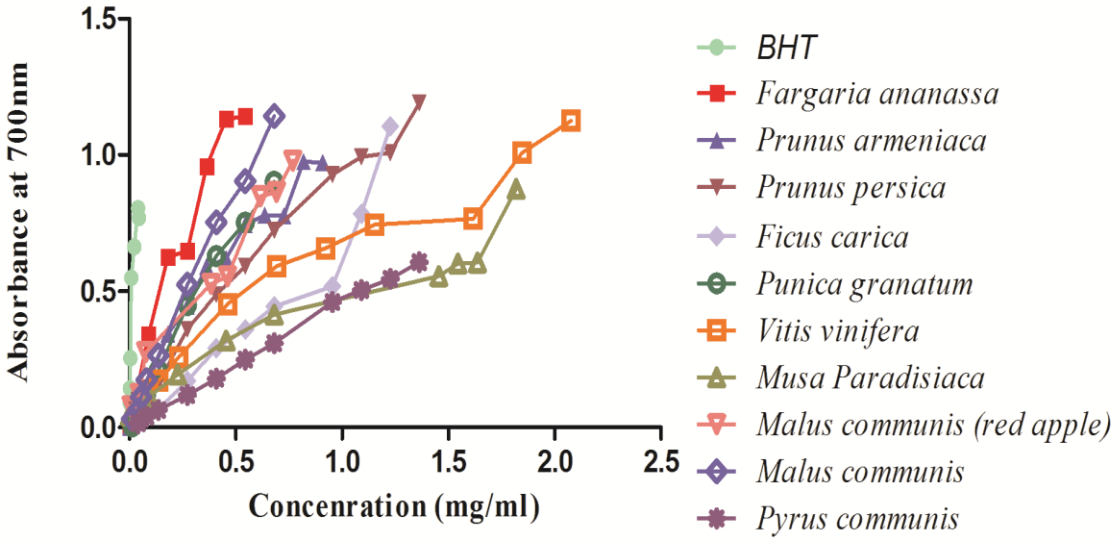


Figure 27: Reducing power of different fruits extracts.

Antioxidant activity of *Fargaria* extract was found to be strongly effective on reducing power when compared with the other extract but significantly lower than BHT used as reference drugs (Fig 28). All extracts exhibited low activity compared to BHT with RC_{50} values of: BHT (0.008mg/ml) >*Fargaria ananassa* (0.251 mg/ml) >*Malus communis* (0.280mg/ml) >*Prunus armeniaca* (0.329 mg/ml) > Malus communis (red apple (0.351 mg/ml) >*Punica granatum* (0.370 mg/ml) >*vitis vinifera* (0.6 mg/ml) >*Prunus persica* (0.779mg/ml) > Pyrus communis (1.17mg/ml) >*Ficus carica* (1.380 mg/ml) >*Musa paradisiaca* (1.98 mg/ml) >

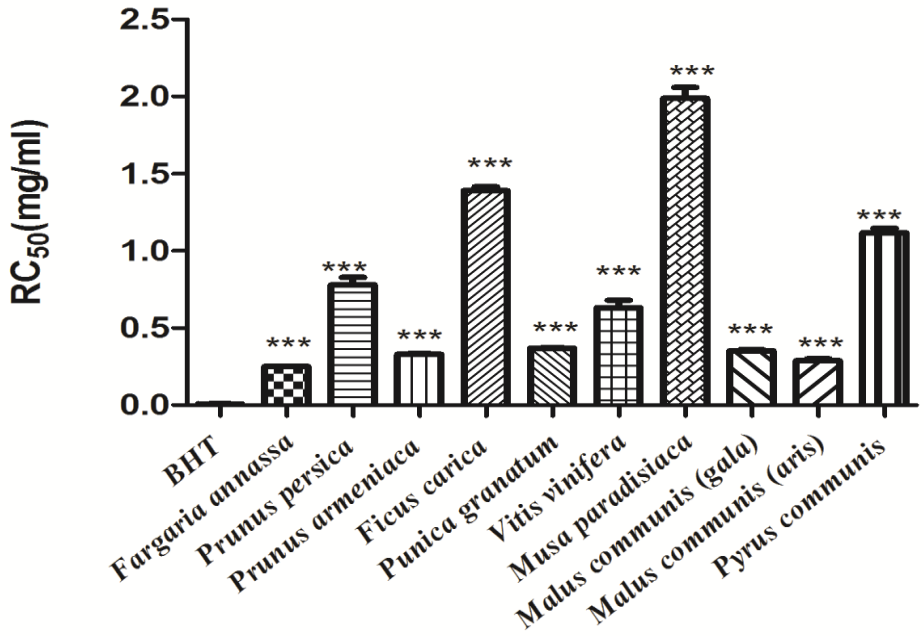


Figure 28: A comparison between different fruits extracts in reducing power assay. Data were presented as RC_{50} means ± SD (n = 3). (*** $p<0.001$) compared to BHT as standard.

In the reducing power, the transformation of Fe^{+3} to Fe^{+2} and changes of yellow color to green and blue of test solution depend on the concentration and the presence of reductants in the samples (Chung *et al*., 2002; Gulcin *et al* ., 2003; Gulcin, 2006). Compounds possessing reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they act as primary and secondary antioxidants (Sharma and Joshi, 2011).

Several studies have shown the correlations between reducing power and polyphenolic contents in the plant extracts (Pourmorad *et al*., 2006; Thirugnanasampandan *et al*., 2008; Moyo *et al*., 2012). This result found by *Fargaria ananassa* which contain high amount of polyphenol. Figs and banana contain low polyphenols content so they have low reducing power activity. For other fruits which have high level of polyphenols but have low activity, this results may be due to the presence of polyphenols with low reducing power.

Rutin, catechin and taxifolin were moderately active, and kaempherol and luteolin were poor reductants. This effect is explained by the fact that the simultaneous presence of the catechol group in the B-ring and 3-hydroxyl group in C-ring plays a crucial role in reducing the potential of flavonoids. The presence of 2, 3-double bond in conjugation with the 4-oxo group in C ring is also important for Fe^{3+} reducing capacity. Additionally, it has been determined

that flavonoids chelate iron more efficiently when the metal ion is in bivalent form, meaning that the flavonoid needs to reduce Fe^{3+} to Fe^{2+} before association. In conclusion, polyphenols with gallol and catechol groups are the most potent antioxidants because of the large iron-binding stability constants for these groups (Belščak-Cvitanović *et al*., 2018).

4.6 Antioxidant activities of fruit extracts using β-carotene-linoleate model system

The antioxidant activity of fruits extracts were measured using β-carotene bleaching assay. The absences of antioxidant induce discoloration of β-carotene because it will couple with linoleic acid. In β-carotene/linoleic acid model system, linoleic acid during incubation forms hydroperoxides free radicals (Goze *et al*., 2009). The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene group attacks the highly unsaturated β-carotene molecules. As a result, β-carotene is oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange color, which is monitored spectrophotometrically (Sarikurkcu *et al*., 2008). The rates of β-carotene blanching can be slow down in the presence of antioxidant (Kulisic *et al*., 2004). The percentage inhibition of the antioxidant in β-carotene/ linoleic acid system is proportional to the concentration. The antioxidant activity is high when the color of β-carotene does not change during the incubation period of 24 h.

In the present study, the percentage inhibition of β-carotene bleaching by fruits extracts ranged from 21.35 to 80.72 %. The highest activity was found for *Fargaria* (80.72 ± 4.57 %) and the lowest activity was noticed for *Pyrus* (21.35 ± 2.54 %).

Other fruits extracts inhibit the oxidation of β-carotene in an important level compared to BHT (98.43± 2.29 % inhibition). The best inhibitor fruits extract is *Punica* with a value of (75.04 ± 5.42 %) followed by royal gala (67.43 ± 5.13 %) then banana (62.85 ± 3.11%) then grape (55.60 ± 2.20 %) then apricot (55.22 ± 4.90 %) then aris (51.61 ± 4.49 %) then fig (36.34 ± 2.91 %). All extracts had lower activity compared to BHT.

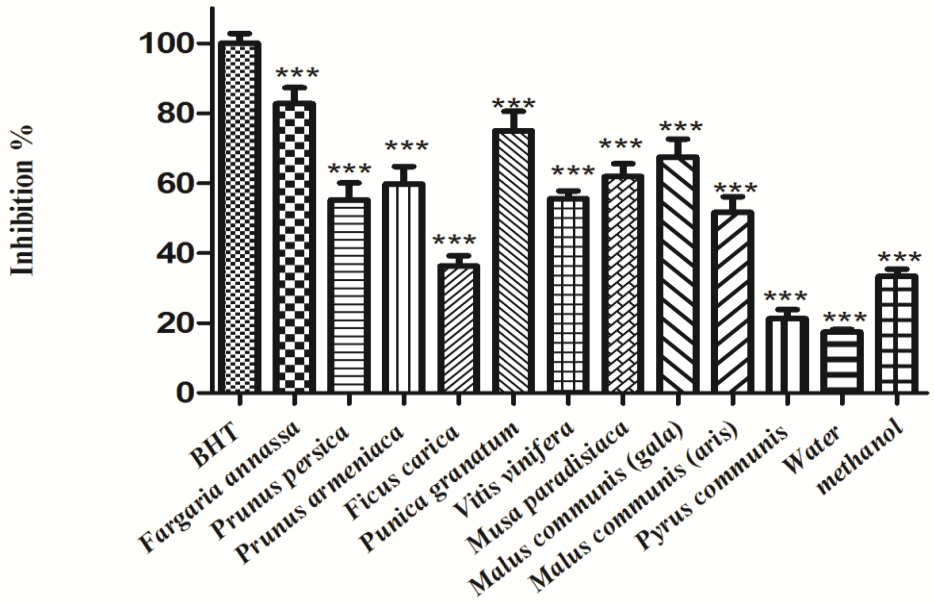


Figure 29: Antioxidant activities of different fruits extracts (2 mg/ml at 24 h of incubation) measured by β-carotene bleaching method. BHT was used as reference antioxidant. Values are means ± SD (n = 3). (*** $p < 0.001$) compared to BHT as standard.

Various studies demonstrated that the β-carotene bleaching activity is in relation to flavonoids and polyphenols contents which can inhibit oxidation of linoleic acid and the formation of hydroperoxides (Duh and Yen, 1997; Sun, 2005). In this research *Fargaria* confirm this information. However, the synergism between the antioxidants in the mixture makes the antioxidant activity, not only dependent on the concentration, but also on the structure and the interaction between the antioxidants (Conforti *et al*., 2007).

4.7 Antioxidant activity of fruit extracts on linoleic acid peroxidation

Lipid peroxidation is a major cause of food deterioration, leading to a loss of functional properties and nutritional value (Kokasal *et al*., 2009). Oxidized polyunsaturated fatty acids may induce aging and carcinogenesis. The major pathway of lipid peroxidation contains a self-catalytic free radical chain reaction. However, lipid peroxidation can be catalyzed by environmental factors, such as light, oxygen, free radicals and metal ions (Olukemi *et al*., 2005). Lipid peroxidation is thought to proceed via radical mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids, it occurs in aerobic cells and

reflects the interaction between molecular oxygen and poly-unsaturated fatty acids (Rajapakse *et al*., 2005).

The ferric thiocyanate method is used to measure the rate of peroxide formation in the first stages of lipid peroxidation (Ting *et al*., 2001). hydroperoxide produced by linoleic acid added to the reaction mixture, which has oxidized by air during the experimental period, was indirectly measured. The peroxide formed oxidizes Fe^{2+} into Fe^{3+} which reacts with thiocyanate forming a complex and gives red color (Yu *et al*., 2008). High absorbance demonstrated high concentration of peroxide during the incubation; which has a maximum absorbance at 500 nm. Low absorbance value indicated high level of inhibition of lipid peroxidation and therefore high antioxidant activity (Kalin *et al*., 2015).

As seen in Fig.30, all fruit extracts have varying percentages of inhibition on the formation of peroxides compared with BHT, which is used as a positive control. *Fargaria* exhibited good peroxidation inhibiting activity with 56.67 ± 3.34 %. On other hand, other fruits exihibited effective antioxidant activity in the linoleic acid emulsion system lower than BHT and showed the flowing order: peach (55.22 ± 2.86 %) > grape (50.33 ± 2.62 %) > apple red (46.58 ± 4.88 %) > apple yellow (46.08 ± 1.14 %) > banana (43.18 ± 5.25 %) > pomegranate (41 ± 2.69 %) > pear (40.43 ± 5.20 %) > figs (39.27 ± 4.21 %).

While *Prunus armeniaca* presents the lowest ability to inhibit the formation of peroxide during 5 days with a value of 37.55 ± 4.055 %.

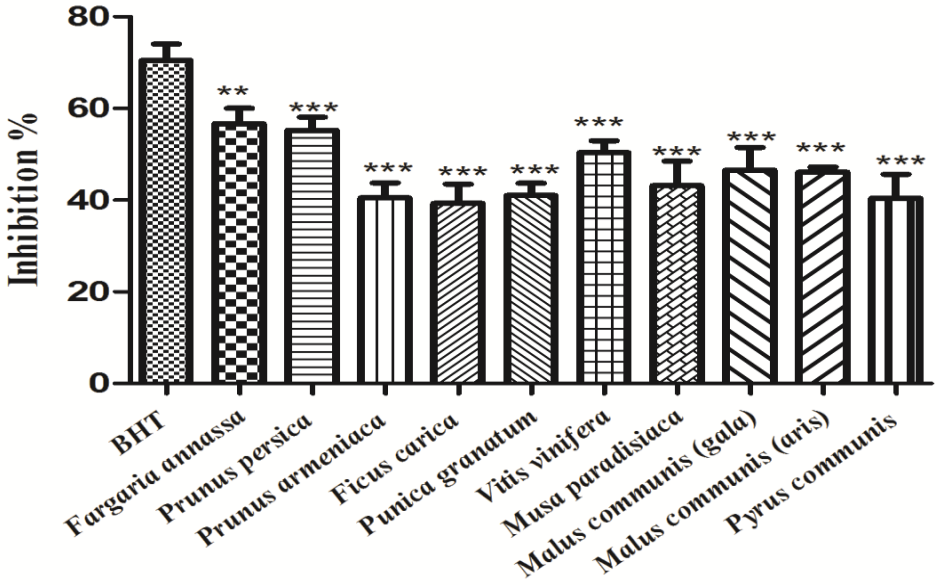


Figure 30: Antioxidant activities of different fruits extracts (2 mg/ml at 96 h of incubation) measured by FTC method. Values are % means ± SD (n = 3). ($p< 0.01$, *** $p < 0.001$) compared to BHT as standard.

The inhibition of Self-oxygenation of unsaturated fatty acids is one of the mechanisms of antioxidant activity. Initiation of a peroxidation sequence in a cell membrane or polyunsaturated fatty acid is due to the abstraction of a hydrogen atom from the double bond in the fatty acid molecule. The free radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene which then easily reacts with an oxygen molecule to give a peroxyl radical (Gorden, 1990). Peroxyl radicals can abstract hydrogen from another molecule or they can abstract hydrogen to give lipid hydoperoxide R-OOH (Anusuya *et al*., 2009). The present results indicate the relation between the amount of polyphenols and flavonoids and lipid peroxidation. The flavonoids can reduce or stop lipid peroxidation by the scavenging the peroxyl free radical. According to several studies, this activity is related to the number of hydroxyl groups present in the molecules of phenolics presents in the extracts (Heijnen *et al*., 2002).

4.8 Thiobarbituric Acid (TBA) assay

A later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by the TBA method. In this second stage, the malonaldehyde is a free radical product with high amount but is not stable for a period. Malonaldehyde has low molecular weight, it reacts with proteins, phospholipids, nucleic acid and induced cell damage (Pandey

and Rizvi, 2010). In this study, the results of the thiobarbituric acid (TBA) test were confirmed with the FTC. Based on these results, *Fargaria* extract had higher antioxidant activitywhen compared withother extracts. The antioxidant activity of the ferric thiocyanate method is higher than the antioxidant activity of the TBA assay for *Fargaria* extract. After short time, malonaldehyde a secondary product of lipid peroxidation changes into alcohol and acid. The alcohol and acid cannot be detected with a spectrophotometer (Rahmat *et al*., 2003). The FTC method indicates the amount of peroxide in the initial stages of lipid peroxidation (Rahmat *et al*., 2003; Saha *et al*., 2004). Whereas, the thiobarbituric acid method shows the amount of peroxide in the secondary stage of lipid peroxidation (Rahmat *et al*., 2003). Therefore, the higher antioxidant activity found from the ferric thiocyanate method indicated that the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage.

The inhibition percentages of the formation of malonaldehyde were in the following order: BHT (62.99 ± 0.342 %) >Strawberries (46.20 ± 3.89 %) > peach (42.95 ± 2.42 %) > red apple (40.17 ± 2.42 %) > apricot (38.30 ± 2.22 %) > pomegranate (39.02 ± 4.26 %) > banana (34.21 ± 2.14 %) > pear (37.13 ± 3.77 %) > yellow apple (28.29 ± 3.49 %) > figs (27.25 ± 3.29 %) > grape (26.04 ± 1.78 %).

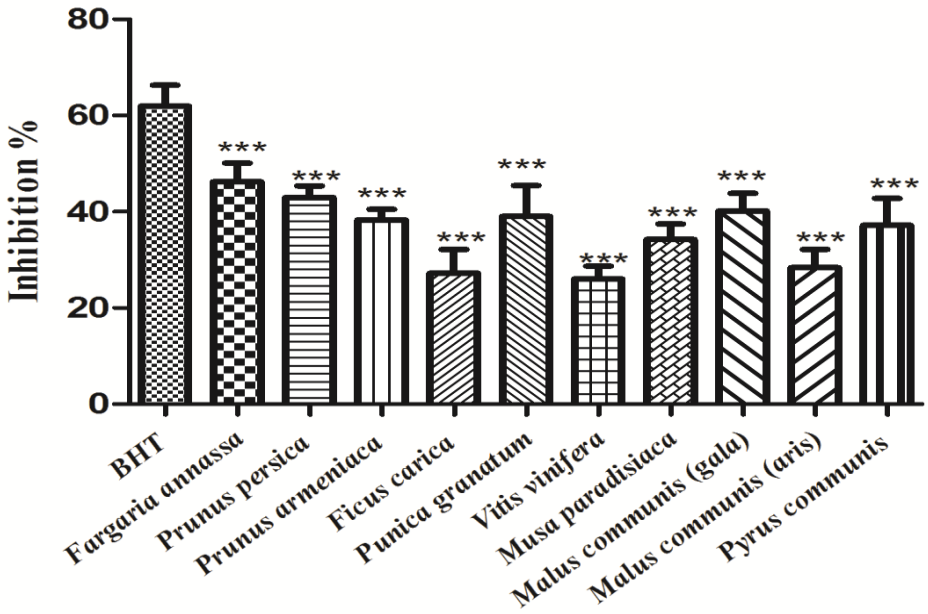


Figure 31: Antioxidant activities of different plant extracts (2 mg/ml) measured by TBA method. Values are % means ± SD (n = 3). (*** $p < 0.001$) compared to BHT as standard.

Recent studies have shown that fruits and vegetable phenols and polyphenols such as flavonoids are one of the major groups having a large spectrum of biological activities that are principally ascribed to their antioxidant property. They inhibit lipid peroxidation (Bernardi *et al*., 2008; Akhila *et al*., 2009). Polyphenols inhibit lipid peroxidation by acting as chain breaking peroxyl-radical scavengers (Sreelatha and Padma, 2009).

Caffeic acid, ascorbic and ρ-coumaric acids exhibited effective activity against the formation of hydroperoxides. Nevertheless, after the formation of these oxidation intermediates, these compounds cannot prevent the formation of the secondary oxidation products. Higher concentrations of thymol, gallic acid and ρ-coumaric acid induced higher peroxidation of triglycerides. While caffeic acid at higher concentration can inhibit lipid peroxidation (Nardini *et al*., 1998). However, gallic acid exhibit significant prooxidant effect on total oxidation in the pH 3.0 emulsions (Osborn and Akoh, 2003). In fact, in our case, when the concentration of this benzoic acid derivative was doubled the formation of hydroperoxides also increased. There are some examples reported that some phenols considered antioxidants may have pro-oxidant activity in high concentrations or in the presence of metal ions (Bouayed and Bohn, 2010; Badanai *et al*., 2015). This finding is confirmed by our result. The identification of phenols in fruits extracts demonstrated the presence of gallic acid, for this reason fruits exhibited low activity in FTC and TBA method.

5. *In vivo* antioxidant activityof fruits extracts

The beneficial effects of antioxidants *in vivo* have been assessed in various animal experiments under normal conditions and under oxidative stress. This is important because it is difficult to estimate the antioxidant capacity using the results of *in vitro* studies. Then, it is essential to examine the effects of antioxidants in animal model experiments and to elucidate the capacity and underlying mechanisms of antioxidant actions *in vivo* (Yoshida *et al*., 2013)

For all *in vivo* methods the samples that are to be tested are usually administered to the testing animals (mice, rats) with specific doses found in respective protocole. After a specified period of time, the animals are usually sacrificed and blood or tissues are used for the analysis the antioxidant and biochemical paramaters (Alam *et al*., 2013).

5.1 Plasma antioxidant capacity

Various studies reported the use of several assays for the determination of the antioxidant activity of fruit and vegetable extracts in human plasma after diet because of the difficulty in measuring each antioxidant component separately and the interactions among them (Re *et al*., 1999). These methods included ABTS, DPPH, reducing power and ORAC. These methods are based on hydrogen donation and other electron. Other parameters can be used to determine the effect of crude fruit extracts against oxidative stress *in vivo* using animal models (Katalinic *et al*., 2005)

5.1.1 Plasma antioxidant capacity using DPPH radical scavenging activity in rats

DPPH free radical was used to evaluate the antioxidant activity after animal treatment. This method is based on hydrogen or electron donation and the ability of the extract to reduce the color of DPPH to yellow color. Table 16 shows that fruit extracts can scavenge the DPPH radical. After oral administration of 200 mg/kg of fruit extracts to rats, plasma antioxidant activity was lower than that of reference group (vit c). In this dose figs can scavenge DPPH radical significantly ($p < 0.001$) higher than untreated animals group. Grape, pomegranate and peach treatement did not significantly change this activity to normal group. *Fargaria* scavenge DPPH radical higher than that of untreated group with percentage to 15.83 ± 0.94%. *Prunus armeniaca* treatement demonstrated high effect than Vit c with an inhibition value of 34.38 ± 1.82 % flowed by *Ficus* and *Vitis*.

Fargaria, *Punica* and *Prunus persica* did not increase the plasma antioxidant capacity. In this assay the effect of *Fargaria*, *Punica* and *Prunus persica* did not depend to dose but, the effect of *Vitis*, *Prunus armenaica* and *Ficus* was related to the dose administration.

5.1.2 Effect of fruits extracts on plasma reducing power in rats

Table 16 shows the effect of extracts on plasma reducing activity. The results indicate that all extracts have good reducing power. Oral administrations of 200 mg/kg of *Fargaria*, *Prunus*,*Punica* and *Vitis* had not significantly increased the reducing capacity compared to normal group. *Ficus* and *Prunus armeniaca* can reduce the Fe^{+3} to Fe^{+2} greater than that of normal group.

The oral administration of 600 mg/kg of fruits extracts resulted in a high reducing capacity in the plasma, the high value was shown for *Prunus armeniaca* (1.044 ± 0.082). The low activity is demonstrated by *Ficus* and this activity is low then the dose of 200 mg/kg. This result suggests that the antioxidants presents in the *Ficus* at high concentration becomes pro-oxidant in the presence of metal ions. *Ficus* extract demonstarted low reducing power *in vitro* and *in vivo*.

Table 16: Comparison between different fruits extracts treatement in plasma antioxidant capacity using DPPH radical. Data were presented as means ± SEM (n= 6). (Ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Tretement	Dose (mg/kg)	DPPH (inhibition %)	Reducing power (absorbance)
Normal group	0.9% (NaCl)	8.29 ± 1,53	0.34 ± 0.005
Vit C group	200	$26.05 \pm 1.28^{***}$	0.60 ± 0.1^{ns}
Fargaria ananassa	200	$15.83 \pm 0.94^{*}$	0.61 ± 0.14^{ns}
	600	17.48 ± 0.47^{ns}	$0.88 \pm 0.13^{***}$
Prunus armeniaca	200	$22.91 \pm 1.99^{***}$	0.62 ± 0.13^{ns}
	600	$34.38 \pm 1.82^{***}$	$1.044 \pm 0.08^{***}$
Ficus carica	200	$23.50 \pm 2.59^{***}$	$0.97 \pm 0.09^{***}$
	600	$27.33 \pm 1.29^{***}$	0.49 ± 0.06^{ns}
Punica granatum	200	14.49 ± 0.90^{ns}	0.50 ± 0.06^{ns}
	600	15.43 ± 1.24^{ns}	0.41 ± 0.04^{ns}
Prunus persica	200	10.28 ± 1.78^{ns}	$0.81 \pm 0.09^{**}$
	600	12.22 ± 1.28^{ns}	$0.86 \pm 0.05^{***}$
Vitis vinifera	200	10.39 ± 1.06^{ns}	0.46 ± 0.05^{ns}
	600	$22.71 \pm 3.62^{***}$	0.59 ± 0.01^{ns}

These results demonstrated that fruits have good plasma antioxidant activity using DPPH, reducing power. Human body has complex antioxidant systems counteract reactive species and reduce their damage. These antioxidant systems include enzymes such as superoxide dismutase, catalase and glutathione peroxidase; macromolecules such as albumin, and ferritin; and an array of small molecules, including ascorbic acid, alpha-tocopherol, carotenoids, polyphenols, ubiquinol-10, reduced glutathione (GSH), methionine, uric acid, and bilirubin and exogenous antioxidants from food, which can work complementary and synergistic with endogenous antioxidant to protect human health against ROS (Meziti *et al*., 2012).

5.2 Effect of extracts on lipid peroxidation in liver and kidney homogenate

Oxidative stress can be evaluated by measuring of several specific markers. In our work, we measured the MDA. MDA assay or thiobarbituric acid reactive species (TBARS) assay has been used since the 1950 for evaluate the levels of lipid peroxidation in membranes and biological systems during the oxidative stress (Potter *et al*., 2011). In our study, we used a colorimetric method, based on the fact that MDA reacts with two molecules of TBA and produce a pink-red chromogen that absorbs at maximum 532 nm. However, substances other than MDA can amplified the absorbance at 532 nm causing in overestimation of the lipid peroxidation level. Because of this interference, it is recommended to use reliable methods such as high performance liquid chromatography which is a reference method, based to measure directly the level of MDA (Haj Mouhamed *et al*., 2012). Thus, it is considered as a good biomarker of oxidative damage. MDA reacts with proteins, phospholipids, nucleic acid and induced cell damage and this damage induces various diseases associated with oxidative stress (Pandey and Rizvi, 2010).

Table 17 shows the capacity of fruit extracts to inhibit lipid peroxidation in livers and kidney of rats and minimize the formation of MDA. These results were compared with untreated group, which received NaCl (0.9%). In this assay, the relation between the rate of MDA and the concentration of extract was noticed.

The treatement of rats with fruits extracts at dose of 200 mg/kg reduced significantly ($p<0.001$) the level of MDA in the liver tissue but strawberries in this dose did not change the level of MDA compared to normal group. The administration of 600 mg/kg of all extracts reduced the level of MDA compared to untreated group. This decrease was statistically significant when compared to control group. Aydemir *et al*., 2000 reported that Vit C has antioxidant activity and protect cell membrane against damage. Vit C has strong activity with a value of 42.51 ± 2.96 nmol/g tissues.

As shown in table 17. the same result obtained in kidney homogenate of rat at 200 and 600 mg/kg. There was significant ($p < 0.001$) decrease in the level of MDA when compared to untreated animals.

5.3 Effects of fruits extracts on GSH levels in liver and kidney homogenate

Reduced glutathione (GSH) is principal soluble antioxidant present in most living cells from bacteria to mammals. GSH is a linear tripeptide of L-glutamine, L-cysteine, and glycine. GSH is an extremely important cell protectant agent. GSH acts as reducing agent and is a vital substance in detoxification. It provides antioxidant protection in the aqueous phase of cellular

systems. The central role of GSH in antioxidative defense is because it can regenerate another water-soluble antioxidant, ascorbic acid, via the ascorbate–glutathione cycle (Valko *et al*., 2007). Hence, depletion of intracellular GSH is usually regarded a measure of oxidative stress. GSH is involved in multiple roles in the liver that collectively aims to minimise oxidative stress and cataract formation. These roles include affording antioxidant protection by scavenging reactive oxygen species (Umapathy *et al*., 2017).

In this test, the GSH reacts with DTNB in the dark and forms yellow complex. Table 17 show GSH levels in the liver of rats treated with different fruit extracts.

Hepatic GSH contents were elevated significantly ($p \leq 0.001$) in *Fargaria* extract treated animals at doses of 200 and 600 mg/kg and showed non significant ($p \leq 0.05$ elevation in the levels of GSH at dose of 200 mg/kg levels of *Vitis vinifera*. Oral administrations of Prunus armenaica at two different doses (200mg/kg and 600mg/kg) increased the levels of GSH. *Punica*, *Prunus persica* and *Ficus* at two different doses have no effect on GSH production in the liver. Similarly, the administration of extracts at doses (200 and 600 mg / kg) in rats did not produce a significant increase in renal GSH levels compared to untreated group.

These results suggest that fruits extract exhibit free radical scavenging activity, which could exert a beneficial action against pathophysiological alterations, caused by the presence of superoxide and hydroxide radicals indicating the regeneration of damaged liver cells (Celep *et al*., 2013). This effect can be attributed to the antioxidant properties of polyphenols present in the extracts (Vinson *et al*., 2001), because of their strong ability to scavenge free radicals and break the reaction chain of these radicals *in vitro* and *in vivo* (Liu and Yao, 2007). Several works with extracts of various plants have reported a reduction in the oxidative stress due to the presence of high antioxidants amount such as polyphenols. Vijayakumar *et al* (2004) reported these effects for black pepper and Gladine *et al* (2007) reported these effects for rosemary, grape, citrus and calendula, whereas, Papandreou *et al* (2009) reported the same results for blue berries (*Vaccinium angustifolium*).Various studies suggest that polyphenols stimulate the gene expression of GPx . Scientific studies demonstrated that the effect of polyphenols and flavonoids *in vivo* are reduced because the ability of antioxidants *in vivo* is determined by many factors, such as bioavailability, metabolism, localization, distribution, fate of antioxidant derived radicals, and interaction with other antioxidants as well as reactivity toward free radicals. Antioxidants must be absorbed, transported, distributed and stored properly in biological fluids, cells and tissues. The bioavailability of various antioxidants and the effect of dose and duration were investigated by analysis of biological

fluids and tissues of humans and laboratory animals after taking antioxidants (Haj Mouhamed *et al*., 2012).

Table17: The effect of fruits extracts on hepatic MDA and GSH level and renal MDA and GSH level in mal Wister rats treated for 15 days. Data were presented as means ± SEM (n= 6). (Ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Treatement	Dose (mg/kg)	Hepatic GSH level (µmol/g tissue)	Hepatic MDA level (nmol/ g tissue)	Renal GSH level (µmol/g tissue)	Renal MDA level (nmol/ g tissue)
Normal group		11.28± 1.67	93.41 ± 5.82	10.44 ± 0.74	91.82 ± 3.65
Vit C group	200	$36.99 \pm 1.44^{***}$	$42.51 \pm 2.98^{***}$	$11.89 \pm 1.87^{***}$	$47.90 \pm 4.52^{***}$
Fargaria ananassa	200	$26.05 \pm 1^{***}$	85.07 ± 2.06^{ns}	10.63 ± 0.62^{ns}	$52.34 \pm 3.39^{***}$
	600	$36{,}01 \pm 2.51^{***}$	$39.51 \pm 1.11^{***}$	12.36±4.57ns	$44.38 \pm 4.80^{***}$
Prunus armeniaca	200	$19.40 \pm 0.85^{**}$	$50.78 \pm 5.74^{***}$	12.62 ± 1.87^{ns}	$60.87 \pm 3.96^{***}$
	600	$19.75 \pm 1.18^{**}$	$31.13 \pm 1.65^{***}$	12.30 ± 1.52^{ns}	$37.48 \pm 4.08^{***}$
Ficus carica	200	12.09 ± 1.43^{ns}	$62.24 \pm 1.03^{***}$	11.88 ± 0.90^{ns}	$52.35 \pm 3.07^{***}$
	600	18.32 ± 2.46^{ns}	$50.67 \pm 5.91^{***}$	11.74 ± 1.59^{ns}	$49.81 \pm 1.92^{***}$
Punica granatum	200	12.30 ± 1.35^{ns}	$56.38 \pm 3.38^{***}$	10.90 ± 1.45^{ns}	$70.68 \pm 4.93^{**}$
	600	12.73 ± 2.49^{ns}	$54.02 \pm 2.37^{***}$	12.90 ± 2.70^{ns}	$67.47 \pm 4.93^{***}$
Prunus persica	200	11.88 ± 1.43^{ns}	$58.93 \pm 0.91^{***}$	10.88 ± 1.43^{ns}	78.90 ± 3.46^{ns}
	600	14.79 ± 1.61^{ns}	$30.46 \pm 3.51^{***}$	11.79 ± 1.61^{ns}	$67.96 \pm 3.48^{***}$
Vitis vinifera	200	12.44 ± 1.11^{ns}	$62.5 \pm 3.71^{***}$	11 ± 2.50^{ns}	$65.34 \pm 1.93^{***}$
	600	23.62±2.94****	$30 \pm 3.52^{***}$	12.30 ± 3^{ns}	$53.83 \pm 2.60^{***}$

5.4 Effect of fruit extracts on Biochemical parameter and *in vivo* antioxidant activity

Biochemical parameters were used to evaluate any disorder in liver and kidney. Liver has a variety of biochemical, synthetic and excretory functions, several biochemical tests are used in the diagnosis and management of liver diseases. The parameters determined in this study are aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma – glutamyl transferase, creatinine, uric acid, urea, triglycerides and cholesterol.

5.4.1 Effect fruit extract on hepatic biochemical parameters

Table 18 shows the results of the effect of fruits extracts on hepatic parameters. The oral administration of all fruits extracts at 200 and 600 mg/kg did not significant change the serum ALT and GGT compared to normal group. Both doses of apricot, fig and grape increased significant ($p<0.001$) the level of ALP compared to untreated group. Pomegranate and peach did not change the level of ALP. While strawberries at doses of 200 and 600 mg/kg lower the level of ALP significant ($p<0.001$) compared to normal group. Pomegranate and fig significantly ($p<0.01$) increase the level of AST. While strawberries at 200 and 600 mg/kg elevated the level of AST compared to normal group.The administration of grape at 200 mg/kg decreased significantly the level of AST.

Table 18: Effect of fruits extracts (200 and 600mg/kg) on hepatic biochemical parameters in male Wistar rats treated for 15 days. Data were presented as means ± SEM (n= 6). (ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Treatement	Paremetrs	AST (UI/L)	ALT (UI/L)	ALP (UI/L)	GGT (UI/L)
Control	NaCl (0.9%)	87.16± 4.40	10.83± 0.94	129.8 ± 5.25	4.66± 1.08
Vit C	200	100± 2.49^{ns}	13.66±1.17^{ns}	171 ± 1.74***	3.44±0.65^{ns}
Fargaria ananassa	200	129± 4***	12.83±0.98^{ns}	81± 3.65***	4.16±1.94^{ns}
	600	126± 5.95***	13.66±3.01^{ns}	81± 1.18***	6.33±0.79^{ns}
Prunus armeniaca	200	92.5± 2.25^{ns}	11.16±1.40^{ns}	162± 2.69***	5.33±0.79^{ns}
	600	87.83± 2^{ns}	11.16±1.33^{ns}	166± 2.45 ***	4± 0.78^{ns}
Ficus carica	200	106.16± 2.96**	17.33±3.41^{ns}	178± 3.07***	3.83±0.49^{ns}
	600	99.4±16.85^{ns}	16.8±3.58^{ns}	166± 9.2***	3 ± 1.22^{ns}
Punica granatum	200	94.2± 3.65^{ns}	11 ± 1.80^{ns}	122± 2.88^{ns}	3.25±0.50^{ns}
	600	104 ± 1.70**	13 ± 6.40^{ns}	144± 3.87^{ns}	3.6±0.22^{ns}
Prunus persica	200	98.25±1.74^{ns}	13.2±3.89^{ns}	129 ± 7.98^{ns}	1.6± 0.40*
	600	75± 3.74^{ns}	19 ± 2.88^{ns}	144± 2.51^{ns}	3 ± 0.70^{ns}
Vitis vinifera	200	73.25± 2.38*	10± 0.95^{ns}	177± 4.34***	2.8± 0.44^{ns}
	600	87.33±2.06^{ns}	11.16±1.33^{ns}	169± 2.38***	4± 0.73^{ns}

5.4.2 Effect of fruits extracts on renal and some biochemical parameters

The oral administration of 200 and 600 mg/kg of fruits extracts did not change the cholestrol and triglycerids levels compared to untreated rats. Moreover, fruits extracts treatement did not change significantly the levels of creatinine and urea.

As seen in table 19, fruits extracts changed the level of uric acid. Results showed that boths doses of peach lowered significantly ($p<0.001$) the level of uric acid. The dose of 600 mg/kg of pomegranate and fig decreased significantly ($p<0.001$) the level of uric acid. However, the

treatment of rats with strawberry extract at 200 mg/kg changes uric acid. Oral administration of 600 mg/kg of apricot and grape extracts lowered the level of serum uric acid.

Table 19: Effect of fruits extracts (200 and 600mg/kg) on renal status and some biochemical parameters in male Wistar rats treated for 15 days. Data were presented as means ± SEM (n= 6). (Ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Treatement	Paremetrs / Doses (mg/kg)	UA (mg/L)	CREA (mg/L)	Urea (g/L)	CHOL (g/L)	TG (g/L)
Control	NaCl (0.9%)	31.66± 2.87	5 ± 1.67	0.42± 0.13	0.44± 0.09	0.78± 0.18
Vit C	200	22.16±2.13***	5.5± 0.54^{ns}	0.47± 0.04^{ns}	0.44±0.13^{ns}	0.6± 0.07^{ns}
Fargaria ananassa	200	16.83± 3.83***	5.33±1.36^{ns}	0.35± 0.07^{ns}	0.34±0.11^{ns}	0.62± 0.15^{ns}
	600	27.33± 3.82^{ns}	4.83±0.98^{ns}	0.49± 0.19^{ns}	0.46± 0.1^{ns}	0.63± 0.29^{ns}
Prunus armeniaca	200	26.33± 5.08^{ns}	5.16±0.98^{ns}	0.43± 0.03^{ns}	0.45±0.06^{ns}	0.79± 0.15^{ns}
	600	24.66± 4.41**	5.33±1.03^{ns}	0.43± 0.08	0.48±0.10^{ns}	0.68± 0.07^{ns}
Ficus carica	200	25.66± 2.25*	5.33±0.51^{ns}	0.40± 0.50^{ns}	0.47±0.03^{ns}	1.005± 0.11^{ns}
	600	15.6± 2.30***	4.6± 0.54^{ns}	0.35± 0.04^{ns}	0.38±0.04^{ns}	0.65± 0.15^{ns}
Punica granatum	200	34.2 ± 1.30^{ns}	5.2 ±2.16^{ns}	0.41± 0.02^{ns}	0.45±0.14^{ns}	0.83± 0.24^{ns}
	600	21 ± 1.58***	5.4 ± 1.14^{ns}	0.39 ± 0.05^{ns}	0.45±0.07^{ns}	0.83 ± 0.30^{ns}
	200	16.2 ± 2.38***	5.6± 0.89^{ns}	0.61± 0.06*	0.53±0.11^{ns}	0.58± 0.031^{ns}
Prunus persica	600	15.75 ± 2.87***	5.2 ± 0.44^{ns}	0.54± 0.08^{ns}	0.62±0.11^{ns}	0.56± 0.07^{ns}
	200	10.8 ± 2.04***	5.6± 0.54^{ns}	0.57± 0.02^{ns}	0.63± 0.13*	0.52± 0.21^{ns}
Vitis vinifera	600	24.66± 4.41**	5.33±1.03^{ns}	0.43± 0.08^{ns}	0.48±0.10^{ns}	0.68± 0.07^{ns}

AST, ALT and ALP are serum hepatobillary enzymes present normally in the liver in high concentrations. ALT is the more specific marker of hepatocellular injury because it occurs exclusively in the liver, whereas AST occurs to some extent also in the heart, skeletal muscle,

kidney, brain, pancreas and blood cells (Kew, 2000). The increase in the level of serum ALP indicates the presence of desoreders in the bone, liver and other diseases. High ALP levels can obstruct the bile ducts (Sharma *et al*., 2013). Reduction in the level of ALP suggests the stability of the biliary function.

The stabilization of markers of kidney indicates that fruits extracts did not caused renal damage. In the case of uric acid, the reduction in its rate suggests that fruits extracts have anti-inflammatory activity. Rock *et al* (2012) demostrated that the high level of uric acid caused gout disease after it is crystalized in joints.

6. Examination of Hepatoprotective and nephroprotective effect of fruits extracts

6.1 Plasma antioxidant capacity

Most of the metabolic and physiological processes in our body as well as the detoxification of various drugs and xenobiotic chemicals occur in the liver and kidney. During the detoxification process of these agents' liver and kidney can be damaged. There are several drugs and chemicals which produce hepatotoxicity and nephrotoxicity. Among the objectives of the present study was to evaluate the protective effect of fruits against a potential toxic substance CCl_4.

6.1.1 Effect of extracts on plasma antioxidant capacity using DPPH radical and reducing power after CCl_4 induced toxicity.

In this study, the plasma antioxidant capaticy was estimated using the major and rapid methods (DPPH and reducing power). The pretreatement of rats with CCl_4 showed that the capacity of plasma to reduce Fe^{+3} to Fe^{+2} and scavenging free radicals was very weak. Animals receiving olive oil had the same antioxidant capacity as untreated animals, olive oil had no effect on antioxidant plasma capacity.

In DPPH assay, the results showed that all fruits extracts at both doses increased significantly ($p<0.001$) plasma antioxidant capacity compared to CCl_4 treated group.

The reducing power activity of fruits extracts are presented in table 20. Both dose of figs and apricot reduced Fe^{+3} to Fe^{+2}. Moreover, oral administration of 600 mg/kg of peach and vitis increased the plasma antioxidant capacity.

Pomegranate and grape at the dose of 200 mg/kg did not change the antioxidant capacity of plasma compared to CCl_4 treated group. However, peach at 200 mg/kg and strawebberries at 600 mg/kg reduced Fe^{+3} to Fe^{+2}.

Table 20: Effect of fruits extracts on plasma antioxidant capacity. Data were presented as means ± SEM (n= 5). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to normal group.

Treatement	Doses (mg/kg)	DPPH (inhibition %)	Reducing power (absorbance)
CCl_4 group	3 ml/kg	9.42 ± 0.55	0.30 ± 0.06
Normal group	H_2O	$16.69 \pm 0.48^{***}$	0.40 ± 0.05^{ns}
Oliv oil group	3 ml/kg	$16.87 \pm 0.69^{***}$	0.45 ± 0.06^{ns}
Vit C group	200	$24.65 \pm 1.11^{***}$	0.50 ± 0.08^{ns}
Fargaria ananassa	200	$19.99 \pm 1.62^{***}$	$0.56 \pm 0.06^{*}$
	600	$24.82 \pm 1.08^{***}$	$0.60 \pm 0.03^{**}$
Prunus armeniaca	200	$15.26 \pm 1.59^{**}$	$0.80 \pm 0.04^{***}$
	600	$20.08 \pm 2.42^{***}$	$0.88 \pm 0.08^{***}$
Ficus carica	200	$19.40 \pm 0.99^{***}$	$0.80 \pm 0.05^{***}$
	600	$27.45 \pm 0.86^{***}$	$0.83 \pm 0.04^{***}$
Punica granatum	200	$14.90 \pm 1.77^{*}$	0.53 ± 0.06^{ns}
	600	$29.49 \pm 0.59^{***}$	0.48 ± 0.02^{ns}
Prunus persica	200	$17.40 \pm 0.56^{**}$	$0.69 \pm 0.04^{**}$
	600	$22.60 \pm 1.14^{***}$	$0.61 \pm 0.059^{**}$
Vitis vinifera	200	$19.54 \pm 0.44^{***}$	0.53 ± 0.12^{ns}
	600	$17.95 \pm 0.94^{***}$	$0.87 \pm 0.03^{***}$

6.2 Effect of fruits extracts on MDA and GSH level in liver and kidney.

The lipid peroxidation and antioxidant parameters (glutathione (GSH)) were estimated in the liver and kidney homogenates to determine the possible mechanisms of the hepatoprotective and nephroprotective activity of extracts.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to toxicant (CCl_4). In the present study, the results showed that intraperitoneal injection of

CCl_4induced a highly significant ($p < 0.001$) increase in the concentration of malondialdehyde. Enhanced lipid peroxidation expressed in terms of MDA (malonaldehyde) contents in CCl_4 treated rats confirmed hepatic damage.

The effect of daily oral administration for 7 days of fruits extracts on lipid peroxidation and endogenous antioxidant (GSH) in the kidneys and liver of CCl_4 induced toxicity is shown in table 21. The result showed that there was a significant ($P<0.001$) difference between the TBARS levels in CCl_4 treated group and fruits extracts treated animals. All fruits at both doses can reduce the level of MDA in liver homogenate. In the kidney the results showed that the elevation in the MDA concentration is not significant compared to normal group.The liver and kidney homogenates of groups treated with CCl_4 showed lower levels of GSH compared to that of untreated animals.

At a dose of 200 mg/kg most fruits extracts did not change the level of GSH in kidney and liver tissues compared to animals treated with CCl_4. Pretreatement with 600 mg/kg and 3ml/kg of CCl_4 and straweberries, pomegranate and grape increased the level of GSH in liver. However, both doses of fig can elevate significant ($p<0.001$) the level of GSH in both tissues compared to CCl_4 treated group.

Table 21: The effect of fruits extracts on hepatic MDA and GSH level and renal MDA and GSH level in mal Wister rats treated for 7 days. Data were presented as means ± SEM (n= 5). (Ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Treatement	Doses (mg/kg)	Hepatic GSH level (µmol/g tissue)	Hepatic MDA level (nmol/ g tissue)	Renal GSH level (µmol/g tissue)	Renal MDA level (nmol/ g tissue)
CCl_4 group	3 ml/kg	10.16 ± 0.31	159,61 ± 8.70	16.60 ± 0.63	110.20 ± 5,68
Normal group		19.60 ± 2.01^{ns}	88.45 ± 4.89***	19.11 ± 3.58^{ns}	90.41 ± 4.35^{ns}
Oliv oil group	3 ml/kg	17.11± 1.15^{ns}	89.53 ± 3.17***	20.92± 1.98^{ns}	90.45 ± 4.43^{ns}
Vit C group	200	21.68 ± 2.36^{ns}	93.41 ± 7.40***	19.41 ± 2.11^{ns}	72.09 ± 5.99^{ns}
Fargaria ananassa	200	11.16± 0.78^{ns}	129.91± 2.51*	12.21 ± 1.51^{ns}	90.15 ± 3.60^{ns}
	600	25.49 ± 2.25**	97.53± 5.05***	29.06 ± 1.51**	76.56 ± 3.65^{ns}
Prunus armeniaca	200	18.71± 4.14ns	65.31± 2.36***	23.91 ± 3.21^{ns}	90.25 ± 4.05^{ns}
	600	20.33± 1.75ns	61.59± 1.06***	24.76 ± 2.67^{ns}	82.97 ± 1.32^{ns}
Ficus carica	200	27.83± 4***	97.37± 4.71***	28.67± 1.88**	86.77 ± 2.53^{ns}
	600	28.43±2.50***	96.18± 0.94***	30.73 ± 2.67***	83.87± 4.72^{ns}
Punica granatum	200	16.93± 2.60^{ns}	119.50±3.98***	24.37 ± 1.27^{ns}	89.20 ± 2.53^{ns}
	600	26.59 ± 1.95**	79.70 ± 4.49***	21.66 ± 2.30^{ns}	87.66 ± 2.77^{ns}
Prunus persica	200	11.60± 3.14^{ns}	131 ± 6.17**	19.75 ± 1.81^{ns}	86.67± 5.94^{ns}
	600	14.52± 3.58^{ns}	86.21 ±5.74***	17.03 ± 2.43^{ns}	85.33 ± 3.13^{ns}
Vitis vinifera	200	11.51± 3.68^{ns}	106.59±3.52***	24.62 ± 1.13^{ns}	82.46 ± 5.22^{ns}
	600	23.80± 1.58*	80.63 ± 1.89***	27.60 ± 0.54*	71.95 ± 2.62^{ns}

6.3 Effect of fruits extracts on hepatic biochemical parameter after hepatotoxicity induced by CCl_4

AST, ALT, ALP and GGT, are indicators of hepatocyte damage and loss of functional integrity of the liver. Results showed that the injection of CCl_4 developed significant hepatic damage and oxidative stress as evidenced by substantial increases in the plasma activities of AST, ALT and ALP.

The serum levels of hepatic enzymes (AST and ALT) used as biochemical markers for the evaluation of hepatic injury were significantly ($p<0.001$) decreased in the fruits extracts treated animals. Figs treatement reduced three times the level of AST and two times level of ALT in liver.

Fruits extracts treatement at different doses can not prevent the alterations in the level of ALP induced by CCl_4.

In the case of GGT, in this study the treatement with fruits extracts and the injection of CCl_4 did not change the activity of serum GGT.

Table 22: Effect of fruits extracts (200 and 600mg/kg) on biochemical parameters in male Wistar rats treated for 7 days after injected by CCl_4.Data were presented as means ± SEM (n= 5). (Ns: no significant difference; * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$) compared to control group.

Treatement	**Paremetrs** / **Dose (mg/kg)**	**AST (UI/L)**	**ALT (UI/L)**	**ALP (UI/L)**	**GGT (UI/L)**
CCL_4 group	3 ml/kg	1543± 105	955± 1.31	306 ± 49	7.6± 0.92
Normal group		97± 3.08***	31± 2.25***	139 ± 24**	5.66± 1.59
Vit C	200	733± 111***	413± 91***	270± 14ns	5.88± 1.78ns
Oil group	3ml/kg	110.5±4.65***	23.5±1.76***	240 ±10.25ns	5.33±0.46ns
Fargaria ananassa	200	717± 155***	479± 103***	250± 29ns	6.4± 0.53ns
	600	623± 147***	419± 95***	236± 35ns	5.4± 0.36ns
Prunus armeniaca	200	772± 104***	389± 10***	209± 40ns	4.6± 0.62ns
	600	706± 41***	350± 50***	256± 48ns	6± 0.50ns
Ficus carica	200	559 ± 190***	395± 97***	205± 26ns	5.2± 0.73ns
	600	486 ± 43.5***	441± 44***	193± 19.44ns	3 ± 0.50*
Punica granatum	200	684 ± 71***	459 ± 50***	217± 13ns	3.8 ± 0.67ns
	600	510 ± 132***	245 ± 43***	189± 27ns	3 .6± 0.22*
Prunus persica	200	1454± 56***	936± 117ns	218 ± 32ns	9.2± 0.49ns
	600	912± 102***	395± 65***	144± 2.51ns	4± 0.28ns
Vitis vinifera	200	829± 110***	530± 90***	279± 62ns	5.6± 0.40ns
	600	844 ± 118***	432± 103***	234± 22.29ns	8.2± 2.17ns

6.4 Effect of fruits extracts on renal function and some biochemical parameter in rats after CCl_4 injection.

As a measure of renal function and status, serum urea, uric acid and creatinine are often regarded as reliable markers. The biochemical markers of kidney damage urea and creatinine were not significant changed in the fruits extracs treated groups compared the CCl_4 group.

Oral administration of fruits extracts did not change the level of uric acid. Similary, CCl_4 does not have a significant effect on uric acid, urea and creatinine compared to intoxicated group. Moreover the ingection of CCl_4 signigicantly ($p< 0.05$) elevated the level of triglyceride and cholesterol in the serum. The level of cholesterol andtriglyceride in normal group was 0.46 g/l and 0.47 g/l respectively. While in rats treated with CCl_4 are 0.67g/l and 0.73 g/l respectively. The pretreatement of rats with strawberries and grapes at both dose decreased the rate of cholesterol. At the dose of 200 mg/kg only pomegranate and figs significantly ($p<0.05$) reduced cholesterol. Grapes at both doses and peach and apricot at 600 mg/kg lowred the level of triglyceride

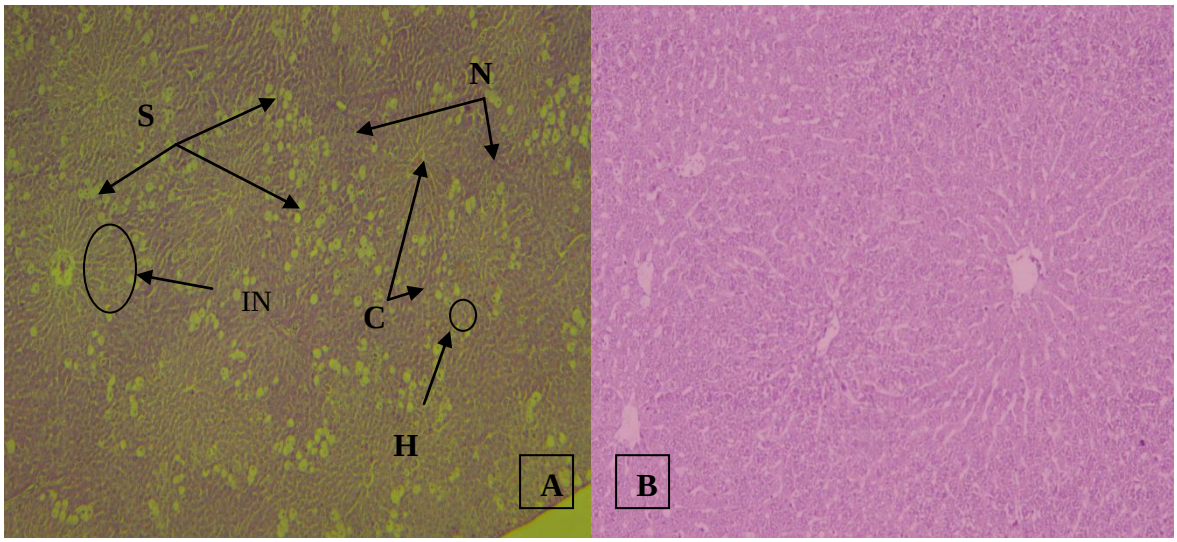
Table 23: Effect of fruits extracts (200 and 600mg/kg) on renal function and some biochemical parameters in male Wistar rats treated for 7 days after injected by CCl_4. Data were presented as means ± SEM (n= 5). (Ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

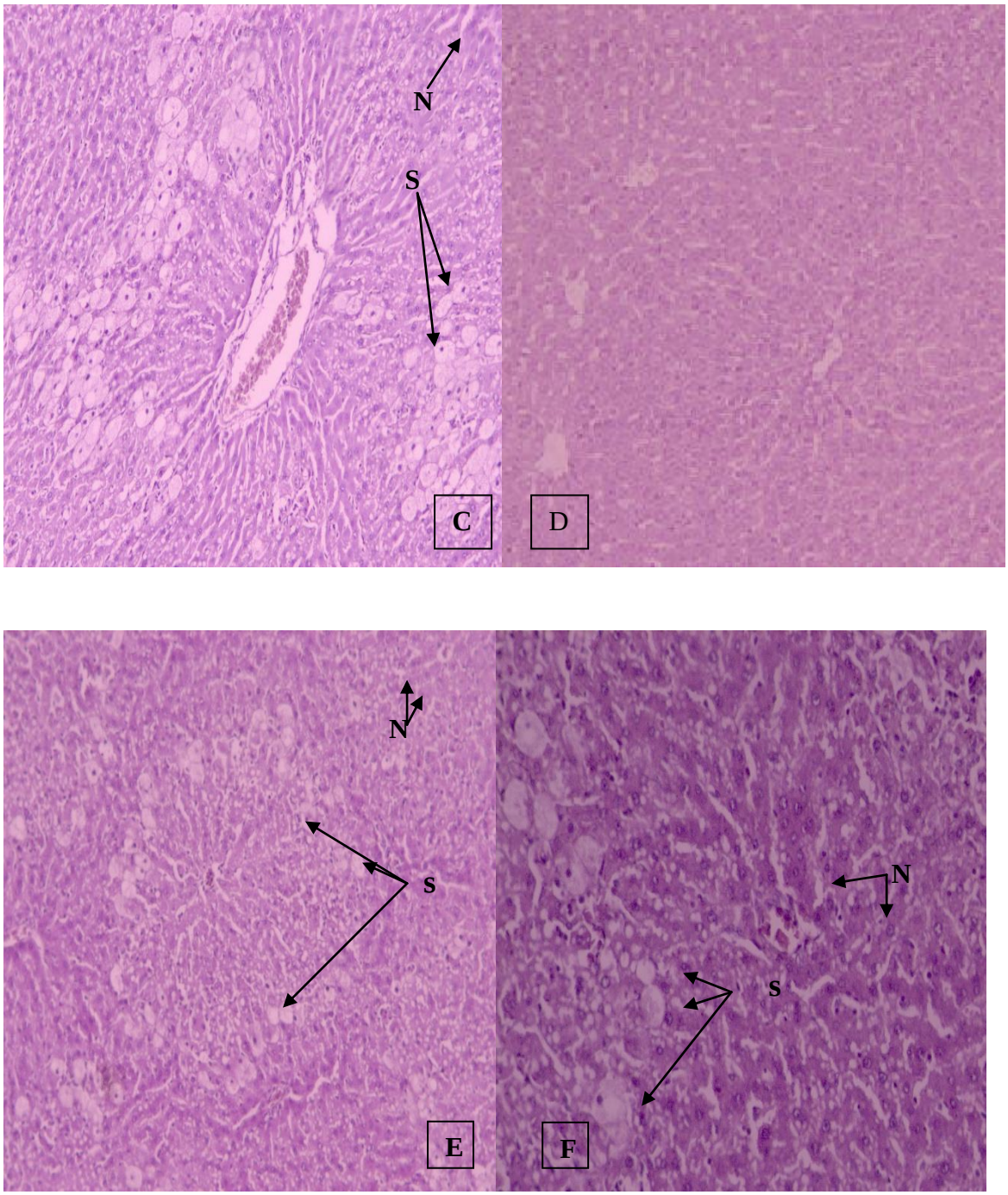
Treatement	Paremetrs / Dose (mg/kg)	UA (mg/L)	CREA (mg/L)	Urea (g/L)	CHOL (g/L)	TG (g/L)
CCl_4	3 ml/kg	16.5± 1.98	6.6 ± 0.75	0.56 ± 0.13	0.67 ± 0.08	0.73± 0.07
Normal group	(H_2O)	19.5± 2.98^{ns}	5.16±0.54^{ns}	0.38 ± 0.06^{ns}	0.46 ± 0.05*	0.47± 0.07*
Vit C	200mg/kg	13.8±2.03^{ns}	5 ± 0^{ns}	0.47± 0.02^{ns}	0.55± 0.05^{ns}	0.41±0.05**
Oil group	3ml/kg	13.66±3.32^{ns}	4.5±0.24^{ns}	0.52±0.018^{ns}	0.55±0.02^{ns}	0.49±0.04*
Fargaria ananassa	200	17.4± 2.12^{ns}	5 ± 0.2^{ns}	0.30± 0.28^{ns}	0.33± 0.03**	0.54± 0.07^{ns}
	600	14.2± 1.57^{ns}	5.6± 0.36^{ns}	0.47 ±0.06^{ns}	0.35± 0.04**	0.52± 0.10*
Prunus armeniaca	200	12 ± 1.22^{ns}	5.2 ± 0.18^{ns}	0.42± 0.04^{ns}	0.45± 0.01^{ns}	0.43± 0.07*
	600	15.4± 0.74^{ns}	5± 0.40^{ns}	0.45± 0.04^{ns}	0.50± 0.04^{ns}	0.36±0.08**
Ficus carica	200	17.4± 1.62^{ns}	5.6± 0.89^{ns}	0.24± 0.28^{ns}	0.44± 0.02*	0.52± 0.06^{ns}
	600	15.6± 0.94^{ns}	5.2 ± 0.44^{ns}	0.39± 0.03^{ns}	0.52± 0.12^{ns}	0.60± 0.04^{ns}
Punica granatum	200	18.4± 3.87^{ns}	6.8± 0.44^{ns}	0.36± 0.05^{ns}	0.44± 0.07*	0.51± 0.12^{ns}
	600	16.2 ± 2.19^{ns}	5.4 ± 0.46^{ns}	0.39 ±0.02^{ns}	0.47 ± 0.03^{ns}	0.73 ±0.12^{ns}
Prunus persica	200	11.6 ± 4^{ns}	7± 0.44^{ns}	0.34± 0.04^{ns}	0.46 ± 0.08^{ns}	0.77± 0.15^{ns}
	600	11± 0.79^{ns}	5.4 ± 0.36^{ns}	0.46± 0.02^{ns}	0.31± 0.09**	0.38±0.02**
Vitis vinifera	200	14 ± 2.12^{ns}	5.8± 0.66^{ns}	0.57±0.009^{ns}	0.37± 0.02**	0.36±0.02**
	600	18.2± 1.43^{ns}	5 ± 0.4^{ns}	0.41± 0.03^{ns}	0.30± 0.01**	0.34±0.02**

6.6 Histopatological examination of liver and kidney and the protective effect of fruit extracts

6.6.1 Histopatological of liver

To confirm our findings on the serum and liver homogenates, we investigated the pathologic changes in liver microscopically. The histological observations fundamentally supported the results obtained from serum biomarkers. The liver section in normal animals had normal hepatic cells with well preserved cytoplasm, which have clear purple color due to the presence of very large mitochondria and prominent nucleus and central vein. In the liver sections of CCl_4 intoxicated rats (Fig 32), there was disarrangement and degeneration of normal hepatic cells. The treatement with CCl_4 showed the presence of necrosis, steotosis, infiltration, congestion and histiocytes. However, histological examination of the hepatic tissue of the pretreated groups with all extracts showed a reduction in necrosis and steatosis. Indeed, the two doses studied of fruit extracts (200 and 600 mg / kg) decreases the surface of necrotic zones. Steatosis is reduced in rats pretreated with these extracts, a minimal deposit of fat vacuoles is observed.

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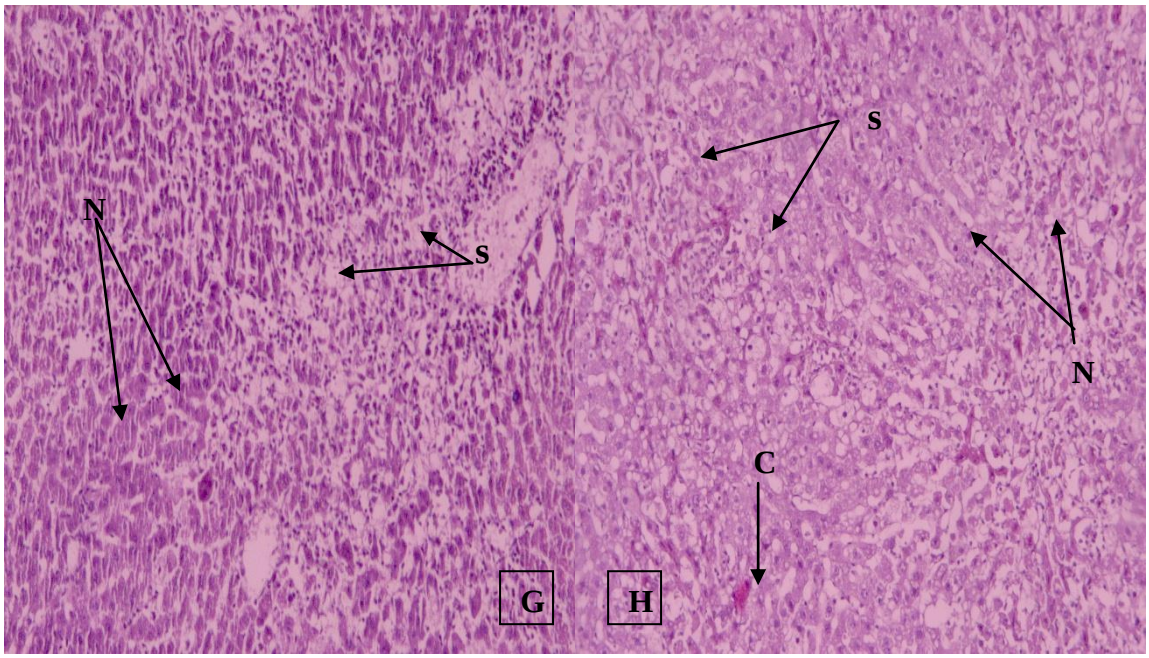
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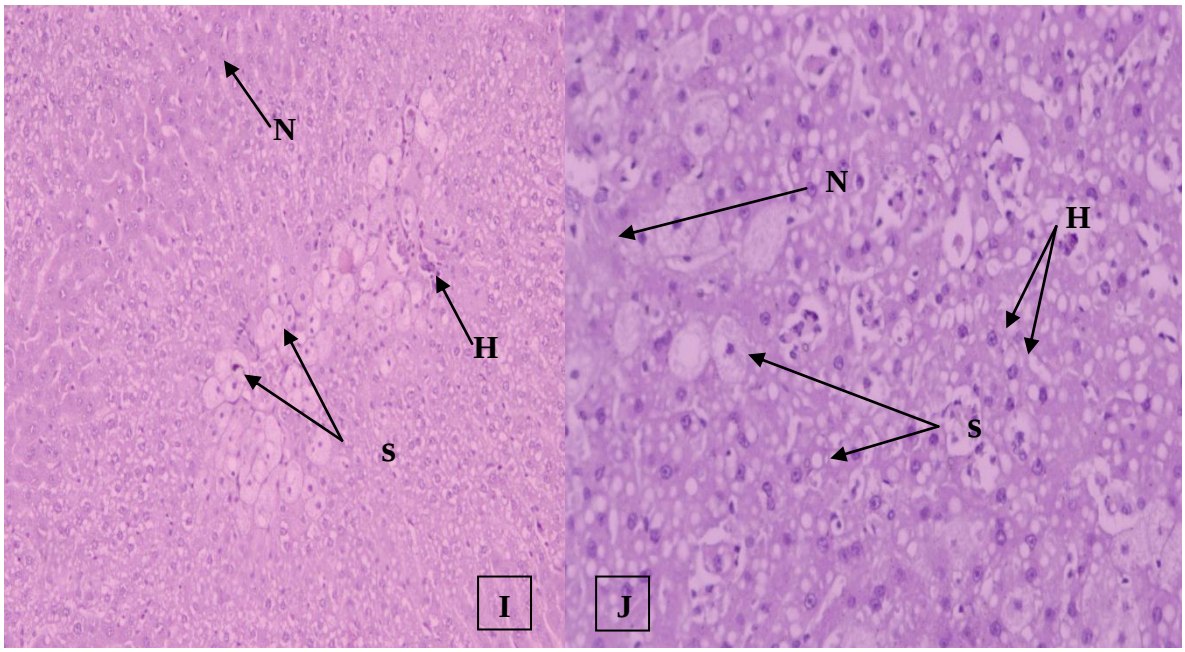
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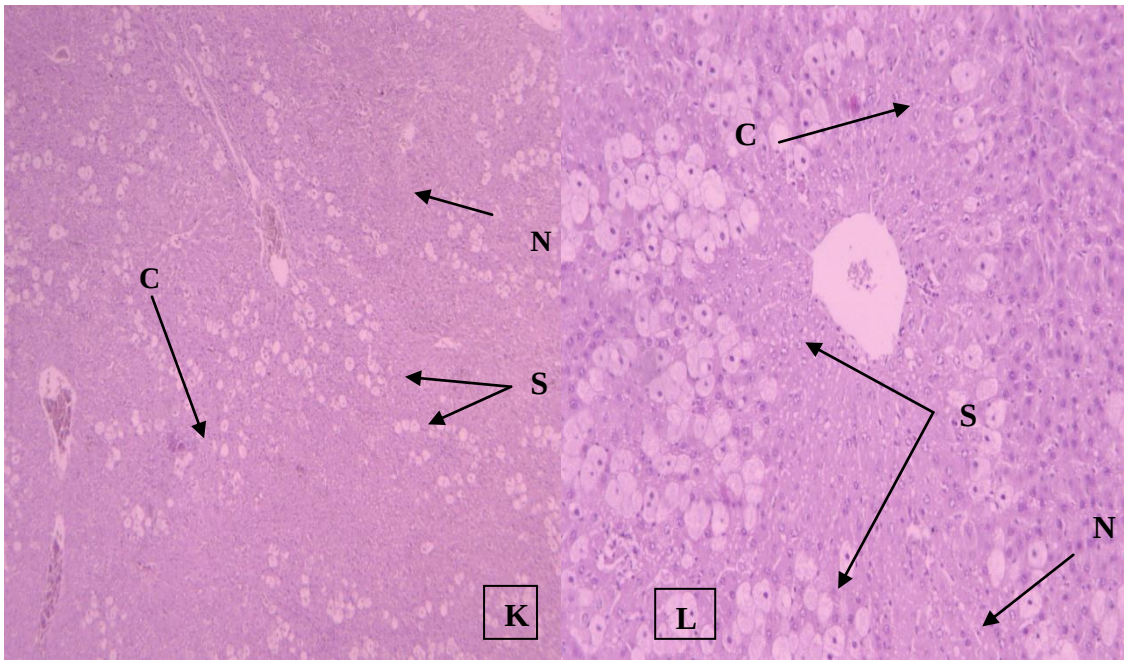
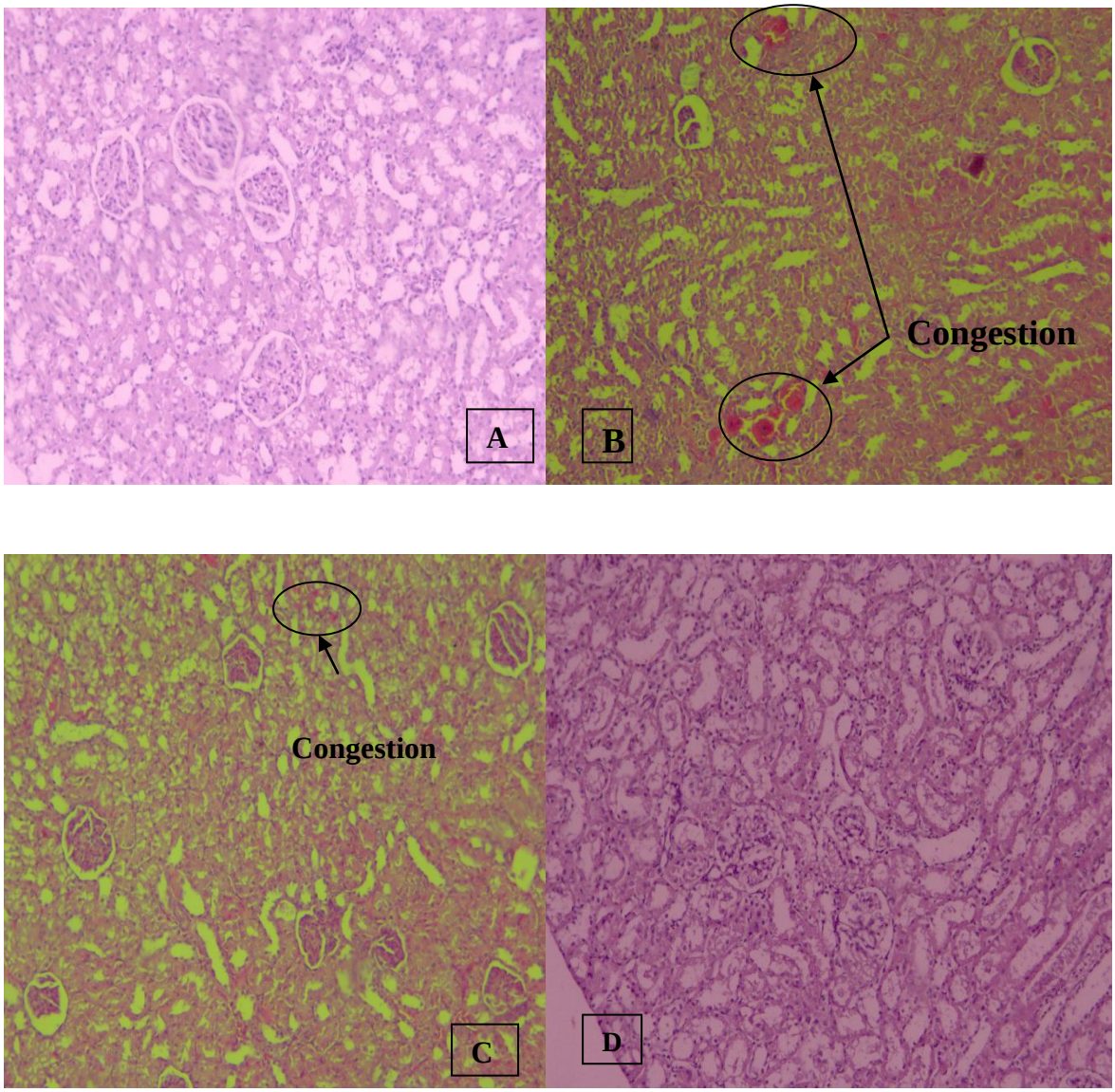


Figures 33: Histopatological of liver. A: rat treated by CCl_4. B: untreated rat. C: rat treated with Vit C and CCl_4(200 mg/kg). D: rat treated with oil olive. E: rat treated with strawberry extract and CCl_4(200 mg/kg). F:rat treated with strawberry and CCl_4(600 mg/kg). G:rat treated with apricot extract and CCl_4(200 mg/kg). H: rat treated with apricot extract and CCl_4(600 mg/kg). I:rat treated with pomegranate extract and CCl_4(200 mg/kg). J: rat treated with pomegranate extract and CCl_4(600 mg/kg). K: rat treated with peach extract and CCl_4(600 mg/kg). L: rat treated with grape extract and CCl_4(200 mg/kg). N: necrosis, S: steotosis, IN: infltration, C: congestion, H: histiocytes.

6.6.2 Histopatological of kidney

Histopathological observations of rats treated with CCl_4 revealed that the normal architecture of kidney with the absence of tissue lesions with the exception of presence of discrete congestion non-pathological in most experimental groups treated with fruits extracts in nephroprotective effect.



Figures 33: Histopatological of kidney. A: untreated rat. B: rat treated by CCl_4. C: rat treated with strawberry extract and CCl_4. D: rat treated with fig extract and CCl_4.

The liver mainly detoxifies toxic chemicals, drugs and becomes the main target organ for all possible toxic xenobiotics. CCl_4 is the most extensively used chemical agent to investigate hepatoprotective activity of compounds in various experimental animal models (Su *et al*., 2018).

The mechanism by which CCl_4 causes liver damage involves the biotransformation of CCl_4 by the cytochrome P-450 enzyme system to the toxic trichloromethyl free radical ($CCl_3^{\bullet}$), This active CCl_3 radical reacts with oxygen to produce trichloromethylperoxyl radical ($CCl_3O_2^{\cdot}$), which is then covalently binds with cellular macromolecules and biomembranes to cause lipid peroxidation in the lipid membranes of the adipose tissue. Peroxide products

finally trigger production and leakage of biomarkers like MDA. This whole cascade of biochemical events ultimately causes loss of cellular integrity and hepatic damage (Sabiu *et al*., 2017).

AST, ALT and ALP are the serum hepatobillary enzymes present normally in the liver in high concentrations. Administration of CCl_4 to rats markedly increased serum AST, ALT and ALP levels. Increase in the levels of serum aminotransferases is known to reflect the severity of liver injury. The leakage of large quantities of enzymes into the blood stream is associated with massive necrosis, congestion and inflammation in the hepatocyte cell of the liver (Singhal and Gupta, 2012). Serum ALP levels are related to the function of the hepatic cell and increase in serum level of ALP is due to increased synthesis of this enzyme. The increase in the transaminases and alkaline phosphatase is a clear indication of cellular leakage and loss of functional integrity of the membrane resulting from liver damage (Zeashan *et al*., 2008).

This study demonstrated that pre-treatment of rats with fruits extracts did not decreases ALP levels and this result suggest that there is no improvement in the secretory mechanism of the hepatic cell (Gupta *et al*., 2004).

The significant reduction in liver enzymes after treatment with fruits extracts suggests that the extracts have hepatoprotective effects (Sakr *et al*., 2011). The decrease in the serum transaminases levels provided that the treatment with fruits extracts can reduce significantly the severity of injuries caused by CCl_4 administration. The observed hepatoprotection by fruits suggests that extracts tends to prevent liver damage and destroy the seepage of enzymes into the blood stream by preventing the hepatocyte membranes (Brai *et al*., 2014).

Elevation in the levels of the end products of lipid peroxidation in the liver of rat treated with CCl_4 was observed. The increases in MDA levels in these rats suggest the occurrence of lipid peroxidation and tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (Shenoy *et al*., 2001; Wang *et al*., 2004). Treatment of rats with fruits extracts decreased MDA concentrations, suggesting that the mechanism of hepatoprotection of fruits may be due in part to its antioxidant effect. It has been suggested that the protective effect of plant extracts against CCl_4 induced liver damage may be attributed to the presence of constituents including flavonoids, tannins, triterpenoids and alkaloids (Tran *et al*., 2001; Gupta *et al*., 2004). Flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidants leading to hepatoprotection (Khalid *et al*., 2002; Al-Qarawi *et al*., 2004; Mankani *et al*., 2005).

In this study, the decrease in GSH level in CCl_4 group is due to their hepatic damage. However, the elevation of GSH in some CCl_4 -intoxicated rats agrees with the findings of

Harisch and Meyer, 1985. Increased GSH level due to increase in due to transcriptional activation of the ɣ-glutamyl cysteinyl synthetase gene (Mari and Cederbaum, 2000). The elevation of GSH in this study may be due to the presence of free radical generated by CCl_4 intoxication.

However, it was observed that hepatic GSH was not depleted in the animals. GSH plays a pivotal defensive role against oxidative insult as an endogenous scavenger of free radicals and maintenance of liver GSH under conditions of increased lipoperoxidation has been suggested as a supportive and compensatory mechanism (Spolarics and Meyenhofer, 2000). Also, CCl_4 is known to cause lipid peroxidation but do not deplete GSH (Jaeschke *et al*., 2002).

These beneficial effects may be attributed to the individual or combined action of the phytoconstituents present in the extract. The antioxidant and free radical scavenging activity of fruits could be due to its constituent flavonoids and phenolic compounds. Therefore, it is pertinent to further determine, isolate and purify the bioactive constituents with the potential hepatoprotective property (Arukwe *et al*., 2012).

CCl_4 also caused disorders in kidneys, lungs and brain. In addition, it may lead to acute and chronic renal injuries. CCl_4 is widely used in experimental models to induce liver and kidney damage (Wang *et al*., 2004).

The stabilization in creatinine, uric acid and serum urea, in this work indicated that CCl_4 do not caused renal toxicity in rats. This result was confirmed by histopatological examination of kidney tissues. The result showed no significant increase in the MDA and GSH concentration. Some studies confirmed that CCl_4 can induce nepherotoxicity (Manna *et al*., 2006; Olagunju *et al*., 2009). On other hand, CCl_4 can induce only the hepatotoxicity. Mukai *et al*., 2002 demonstrated that non significant increase in TBARS of kidney tissue as compared to liver may be as a result of the fact that CCl_4 systemically applied on rats is found to be distributed in a higher concentration in the liver when compared to the kidneys. There was no significant difference between the kidney level of TBARS in the normal control and all treated animals.

All hepatoprotective effects reported in the present work may be due to the presence of bioactive compounds in the extracts studied. Indeed, phytochemical analysis by HPLC/ MS revealed the presence of phenolic acids such as chlorogenic acid and flavonoids such as rutin and Naringenin which have hepatoprotective effects (Janbaz *et al*., 2002; Lin *et al*., 2003).

Conclusion and perspectives

Conclusion and perspectives

The present study was carried out to identify and evaluate polyphenols compounds, the antioxidant and hepatoprotective activity of fruits extracts.

The present study showed that *Fargaria ananassa*, *Prunus armeniaca*, *Prunus persica*, *Punica granatum*, *vitis venifira*, *Ficus carica*, *Malus communis*, *Pyrus communis*, *Musa padiasaca*consumed by the Algeria population and produced in Algeriacontain high amounts of polyphenols and flavonoids. All extracts have high levels of sugars and low content of proteins. Extracts of these fruits exert a good *in vitro* and *in vivo* antioxidant activity. Phytochemical secrenning revealed the presence ofseveral phenolic compounds such as gallic acid, caffeic acid, rutin, chlorogenic acid, catechin, delphinidin and procyanidin dimer. These secondary metabolites, in the presence of other compoundspresent in the extracts can improve the function and condition of the liver and kidney.

The treatment of animals with these extracts resulted in a reduction in the production of MDA in liver tissue of rats. The treatement of rats with fruits extracts after injection of CCl_4 demonstrated good protective effect.

It is concluded that Fruits are known to contain a variety of different antioxidant compounds such as ascorbic acid, tocopherol, glutathione and carotenoids, which may all contribute to protection against oxidative damage. It has been recently shown that phenolics from edible fruits are effective *in vitro* antioxidants. The antioxidative properties of phenolics arise from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their abilityto chelate transition metal ionsand their consumption can reduce the risk of several diseases associated with oxidative stress such as cancer, diabetes, aging and cardiovascular diseases.

Perspectives

Results obtained are important and interesting, but other study is important for understand the molecular and cell mechanism of these effects.

Further research needs to be carried out to:

- Identification of vitamins, sugars and carotenoids compounds in these fruits.
- Isolation of active molecules which may be responsible for antioxidant activity.
- Evaluate other oxidative stress markers (CAT, SOD).
- The use of these extracts as food additives represents one of our future aims
- Assess the anti-inflammatory activity *in vivo*.

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الملخص

تملكالانواعالأكسجينيةالنشطةقدرةعاليةعلىالحاقأضرارتمستقريباكافةالمكوناتالخلوية,ممايفسردورهافيإحداثالكثيرمنالامراض،ولهذافإنتزويدالجسمبموادخارجيةمضادةللأكسدةيبدومهماجدًاللد فاعضدهذهالانواعالأكسجينيةالضارة.تعتبر الفواكهمصدرامهمالمضاداتالأكسدةالخارجية والتيبيمكنأنتقللمنالضررالتأكسدي لهذه الجذور الحرة. تهدفهذهالدراسة إلىتقديرمحتوى عديدات الفنول والفلافونويدات و الدباغ والسكرياتو البروتيناتوكذا تعريفبعضالمركباتالفينوليةوالنشاطية المضادةللأكسدةوسميةالكبدلمستخلصالميثانوليللعديدمنالفواكهالمستهلكة في الجزائر. أظهرالتحليل الكيمائيان هناك اختلاف كبير بين الفواكه. ويعتبر حمضالغليكالمركبالرئيسي الموجود في كل المستخلصات. أظهرت النتائج ان مستخلص الفراولة يحتويكميةمعتبرة من عديدات الفنول و الفلافونويدات. بينما كمية الدباغ الأكبركانت متواجدة في مستخلص الرمان. كماوجد المحتوى العالي للسكريات في ثمار التين. تم تقدير النشاطية المضادة للأكسدة باستعمال اختبار DPPHو ABTS و استخلاب الحديد و القدرة الارجاعية ونظام β-كاروتان وتيوسيانيت الحديد و حمض التيوباربيتيك.وجد أن فاكهة الفراولة هي الافضل في القدرة على التقاط الجذور الحرة. بينما يعتبر التين الأفضل في القدرة في استخلاب الحديد. كما تملك فاكهة الفراولة قدرة عالية على تثبيط أكسدة اللبيبيدات. تم تقدير النشاطية المضادة للأكسدة حيويا بجرعات 200 مع/كغ و 600 مع/كغ عند الجرذان بعد المعاملة بالمستخلصات لمدة 14 يوما على القدرة المضادة للأكسدة في البلازما و مؤشرات الاجهاد التأكسدي في الكبد و الكلى و على بعض المؤشرات البيوكميائيةبالبلازما. أظهرت النتائج ان جميع المستخلصات عند جرعة 600 مع/كغ لها تأثيرا قويا على مؤشرات الاجهاد التأكسدي. في هذه الدراسة تم تقدير السمية الكبدية باستعمال رباعي كلوريد الكربون. اظهرت النتائج ان هذا المركب يؤثر بشكل كبير على الكبد. كما أدت المعاملة بمستخلصات كل الفواكه إلىوقاية نسيج الكبد والكلية من التأثير السام لهذا المركب. أدى اعطاء الجرعتين 200 و 600 مع/كغ من مستخلص التين إلى اظهار تأثير وقائي على الكبد وإلى التأثير على مؤشرات الإجهاد التأكسدي وعلى بعض المؤشرات البيوكميائية في البلازما. ونستخلص من نتائج هذه الدراسة أن مستخلصات الفواكه المستعملة تمتلك نشاط مضاد للأكسدة وواقي للأنشطة مرتبطا بالمحتوى الفينولي و الفلافونويدات.

الكلمات المفتاحية : مستخلصات الفواكه،عديدات الفنول، الفلافونويدات،الاجهادالتأكسدي، السمية الكبدية.

Résumé

Les espèces réactives oxygénées (ERO) ont une grande capacité d'endommager presque tous les types de constituants cellulaires. Ce qui explique leur implication dans l'induction de plusieurs pathologies. La supplémentation de l'organisme par des antioxydants exogènes s'avère très utile pour lutter contre ces espèces nocives. Les fruits sont une source importante d'antioxydant exogène qui peut minimiser les dommages oxydatifs. Le but de cette étude est d'évaluer les teneurs en polyphénols, flavonoïdes, sucres, tannin et protéines, l'identification de certains composés phénoliques, l'activité antioxydante et l'hépatotoxicité d'extraits méthanoliques de plusieurs fruits consommes en Algérie. L'identification par UPLC et HPLC / MS a montré une forte différence entre les extraits de fruits. L'acide gallique est le principal composé dans tous les extraits de fruits étudies. Les résultats ont révélé que l'extrait de *Fargariaananassa*contient une grande quantité de polyphénol, de flavonoïde et de protéine. Le contenu élevé de tannin se trouve dans l'extrait de *Punicagranatum*. Tandis que, le contenu élevé de sucres est trouvé dans le *Ficus*. L'activité antioxydante a été évaluée *in vitro*par les tests de DPPH, d l'ABTS, radical hydroxyle, pouvoir réducteur, chélation des ions ferreux, système β-carotène / acide linoléique, la méthode de thiocyanate ferrique et la méthode à l'acide thiobarburique. Les fraises présentent l'extrait le plus active dans l'activité scavenger des radicaux libres. Cependant, les figues sont le meilleur chélateur comparé aux autres fruits. *Fargaria* peut inhiber la peroxydation lipidique de différentes manières. L'activité antioxydante*in vivo*a été évaluée par la voie orale chez le rat pendant 14 jours(200 et 600 mg / kg) sur la capacité antioxydante plasmatique (PAC), les marqueurs du stress oxydatif dans le foie et les reins et certains paramètres biochimiques. Les résultats ont montré que tous les extraits à 600 mg / kg ont un bon effet contre le stress oxydatif. Dans la présente étude, l'effet hépatoprotecteur des extraits de fruits a été évalué chez des rats intoxiqués par CCl_4. Les résultats ont montré que cet agentinduisait une hépatotoxicité remarquable. Tous les extraits de fruits ont un effet protecteur important et réduisent les dommages aux tissus hépatiques et rénaux. L'administration de 200 et 600 mg / kg de *Ficus carica* a montré un effet hépatoprotecteur important, il améliore les marqueurs du stress oxydant (GSH et MDA) et les paramètres biochimiques hépatiques, les résultats sont confirmés par un examen histologique. En conclusion, les extraits de fruits examinés dans cette étude ont montré une très bonne activité antioxydante, liée à leur teneur en polyphénols et flavonoïde

Mot clés: Extraits de fruits, polyphenols, flavonoides, stress oxydant, hepatotoxicité.

Abstract

Reactive oxygen species (ROS) have high potential to damage almost all types of cellular constituents, which explains their involvement in the induction a number of pathologies. The supplementation of the body by exogenous antioxidants seems to be very helpful to fight these harmful species.Fruits are an important source of exogenous antioxidant which can minimize oxidative damage. The aim of this studyis the evaluationpolyphenols, flavonoids, tannin, sugars and protein contents, the identification of some phenolic compounds, the antioxidant and hepatotoxicity activity of methanolic extracts of the several fruits consumed in Algeria.The identification of phenolic compounds by UPLC and HPLC/MS showed deference between fruits extracts. Gallic acid is the major compounds present in all fruits extracts. The results revealed that *Fargariaananassa*contains high amount of polyphenols, flavonoids and protein. The high level of tannin was found in *Punicagranatum*.While, the high level of sugars was found in *Ficus*.The antioxidant activity was evaluated *in vitro* using the DPPH, ABTS, hydroxyl radicals,reducing power, ferrous ion chelating tests, β-carotene/ linoleic acid model system, ferric thiocyanate method and thiobarburic acid method. Strawberries extracts were the best one can scavengers of free radicals.However *Ficus*extracts was the better chelator compared to other fruits. *Fargaria*can inhibit lipid peroxidation by deferent ways. The antioxidant activity of extracts at doses of 200 and 600 mg/kg*in vivo*was assessed in rats for 14 days, on plasma antioxidant capacity (PAC), oxidative stress markers in liver and kidney and on some biochemical parameters. Results showed that all extracts at 600 mg/kg were effectives against stress oxidative. The hepatoprotective effect of fruits extracted was evaluated in rats intoxicated by CCl_4. Results showed that CCl_4induced remarkable hepatotoxicity. All fruits extracts have an important protective effect and reduce hepatic and renal tissues damage induced by this toxic agent. The administration of 200 and 600 mg/kg of *Ficuscarica*showed a significant hepatoprotective effect, it improve the oxidative stress markers (GSH and MDA) and hepatic biochemical parameters and this results confirmed by histological examination. In conclusion, Fruit extracts examined in this study showed a very high antioxidant activity and hepatoprotective properties which wererelated to their contents in polyphenols and flavonoids.

Key words: Fruits extracts, polyphenols, flavonoïds, oxidative-stress, hepatotoxicity.