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Original article

Phytochemical screening and *in vivo* and *in vitro* evaluation antioxidant capacity of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* fruits growing in Algeria

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Summary. *Purpose:* Fruits are important source of phytochemicals such as polyphenols, flavonoïds, tannins, carteneoides and vitamins which poss antioxidant activity. The aim of this study is the screening of polyphenols and the evaluation of antioxidant activity *in vitro* and *in vivo* of *Fargaria ananassa*, *Prunus armeniaca* and *Prunus persica* (Rosacea family) fruits extracts. *Methods:* The antioxidant activity of hydromethanolic extracts were estimated by methods of the scavenging activity against DPPH, ABTS, hydroxyl radicals (HO.), β-carotene/linoleic acid model system, lipid peroxidation, reducing power and chelating activity. Total phenolics and flavonoids content in these extracts were determined using Folin–Ciocalteu's reagent and Aluminum chloride colorimetric methods respectively. In vivo antioxidant activity was conducted by biological study using male wistar rats. *Result:* The results showed that *Fargaria ananassa* contains high amount of polyphenols and flavonoids (310 ± 0.003µg gallic acid /mg extract and 14.78 ± 0.001µg quercetin equivalent/mg extract respectively), followed by *Prunus armeniaca* and *Prunus persica,* but the highest levels of tannin were found in *Prunus armeniaca* (127.9±0.003 µg tannic acid equivalent/ mg extract). *Fargaria* extract have a high antiradical effect towards DPPH radical, however, *Prunus persica* have a good effect in the inhibition of lipid peroxidation. The administrations of *Fargaria* extract (200 mg/kg and 600 mg/kg) elevates the plasma antioxidant activity and reduced the level of MDA with 85.07 ± 2.06% and 39.54 ± 1.11% respectively and increased the levels of GSH in the liver of rats. The UPLC analysis of extracts demonstrated the presence of various phenolic acids (gallic acid, cinnamic acid and hydrocinnamic acid) and flavonoids (rutin and flavons3-ols) in all extracts. *Conclusion:* These results support the idea that the consumption of fruits can reduce the risk of disease related to free radicals such as cancer, cardiovascular diseases, hypertension, diabetes and stroke. This effect can be attributed at least in part to the antioxidant properties of polyphenols present in the extracts.

Key words: *Fargaria ananassa*, *Prunus armeniaca*, *Prunus persica,* polyphenols, flavonoids, tannin, antioxidant activity.

Introduction

The oxidative stress is the imbalance between antioxidants and the free radicals (1). Free radicals are defined as the molecules with an unpaired electron (2). These molecules are highly reactive and have an important role in cell physiology, such as life cycle regulation, development, migration, induction of signaling pathways, activation of second messengers, and triggering of antioxidant responses (3). The oxidative stress is associated with various diseases such as hypertension, cardiovascular disease, atherosclerosis, diabetes, cancer and arthritis (3, 4). Fruits and vegetables are important source of antioxidants such as phenolic acid, fla-

vonoids, cartonoids, vitamin C. These compounds are important functional food. Fruits can be considered as natural materials to prevent human from various pathologies as they may help to reduce the risk of many age related degenerative diseases (5). Polyphenols can maintain the health by several mechanisms including the elimination of free radicals. The protection and regeneration of other dietary antioxidant and the chelation of pro-oxidant metals (6-8). In this study fruits of *Fargaria ananassa* (Strawberry), *Prunus armeniaca* (apricot) and *Prunus persica* (peach) were used. These fruits belong to the same family *Rosaceae* growing in Algeria and are widely consumed by the Algerian population. Apricot, strawberry and peach are considered as rich sources of phytomolecules such as vitamins, polyphenols, flavonoids, carotenoids, fatty acids and proteins (9, 10). The aim of the present study was to examine the *in vitro* and *in vivo* antioxidant properties and the polyphenols content of these fruits in order to establish a relation between the consumption of these fruits and prevention from pathologies where oxidation stress is implicated.

Materials and methods

Plant material

In this study, fruits of *Fargaria ananassa and Prunus persica* were purchased from commercial market in Amoucha, Setif (Algeria) on April and July 2016. *Purnus armeniaca* fruits were harvested from Tizi Nbachar in Setif region on May 2016. Fresh Fruits were used for the present study.

Animals

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

Preparation of extract

The extraction of phenolic compounds was carried out according to method used by Markham (11). Hundred gram of the consumed parts of the fruits were washed with water, homogenized and mixed with 1 liter of methanol (85:15 v/v for the first extraction and then in 50:50 v/v for the second step) and kept at room temperature for 5 days to allow maximum extraction of bioactive molecules. The resulting solution was then filtered and the supernatant was evaporated using vacuum rotary evaporator at 40° C to obtain crude methanol extract. The crude extract was dried and stored at 4° C until use.

Determination of total polyphenols

The amount of total phenolic content in fruit samples was estimated using the Folin–Ciocalteu reagent as described by Li (12) with slight modification. In brief 200µl of samples solution were mixed with 1000µl of Folin –Ciocalteu reagent (1:10diluted with distilled water). The mixture was allowed to stand for 5 min, and then 800µL of sodium carbonate (7.5%) was added to the mixture. The mixture 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.

Determination of flavonoids content

Aluminum chloride colorimetric method adapted from Bahromun (13) was used for the determination of total flavonoids. One 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.

Determination of tannin content

The amount of tannins was determined using the method described by Gharzouli (14). 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 read at 576nm. Results were expressed as mg equivalent tannic acid per gram dried weight (mg TAE/g DW).

Determination of protein in the extracts

The amount of proteins in the extracts was estimated by Commasie bleu method described by Bardford (15). 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 mixed with vortex, and then 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 BSA equivalent per g extract.

Total soluble sugars content

Total soluble sugars were determined using the method described by Dubois (16). In brief, 1 ml of samples was treated with 1 ml of 5% phenol and 5 ml 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.

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.

Determination of the in vitro Antioxidant activity of extracts

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 (17). 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.

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 (18). 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 the sample). 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}.

Free radical scavenging ability by ABTS

The free-radical-scavenging activity was determined by ABTS radical cation decolorization assay Re (19). Briefly, $ABTS^{+}$ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potas-

sium 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.

Reducing power of extracts

The reducing power of the fruit extract was estimated according to the method described by Ebrahimzadeh (20) .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 [K3Fe (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.

Ferrous ion chelating activity of the extracts

The method described by Decker and Welch (21) 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 $FeCl_2$ (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.

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 (22). 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} ×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.

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 (23). 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 folowing equation: Inhibition (%) = [Ac – As / Ac × 100].

Where, A_s 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.

Thiobarbituric Acid (TBA) assay

According the method of Kikuzaki and Nakatani (24), 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.

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 (25) with slight modifications. The reaction mixture consists of100 µL of varying concentration of samples or standard antioxidants, 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (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 = $[A_{control} - A_{sample}] / A_{control} \times 100$.

Where $A_{control}$ was the absorbance of the control (without sample) and A_{sample} was the absorbance in the presence of the sample.

Determination of the in vivo Antioxidant activity of fruits extracts

Experimental design

Rats were divided into 08 groups having 6rats in each group: Group 01: received 0.9% of NaCl. Group 02: received vitamin C (200 mg/kg); Group 03: received the dose 200mg /kg of *Fargaria ananassa* extract. Group 04: received the dose 600mg /kg of *Fargaria ananassa* extract. Group 05: received the dose 200mg / kg of *Purnus armeniaca* extract. Group 06: received the dose 600mg /kg of *Purnus armeniaca* extract. Group 07: received the dose 200mg /kg of *Purnus persica* extract. Group 08: received the dose 600mg /kg of *Purnus persica* 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.

In vivo Antioxidant activity of plant extracts

Effect of extracts on plasma antioxidant capacity using DPPH radical

In this assay, the ability of plasma to scavenging the DPPH radical was measured by the method of Burists and Bucars (26) with slight modifications. Briefly, 50 µl of plasma was mixed with DPPH solution (0.004%). the mixture was incubated for 30 min, 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 = $[(Abs_{control}-Abs_{sample})/Abs_{control}]$ X 100

A control: is the absorbance of the blank solution

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

Effect of extracts on plasma reducing power

According the method of Chung (27) 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. Then, the mixture was 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.

Preparation of liver 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 4C and the supernatant was used for the determination of (GSH) activity and lipid peroxidation (MDA).

Assessment of reduced glutathione concentration

GSH was measured using a previously described procedure by Ellman (28). GSH can react with 5, 50-dithio-bis (2-nitrobenzoic acid) (DTNB) and formed 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. It was expressed as µmol/ g tissue.

Assessment of lipid peroxidation

According the method of Okhawa (29) lipid peroxidation rate was determined by malondialdehyde level (MDA). This assay was based on the reaction between TBA and MDA. 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 535 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.

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

Total polyphenols, flavonoids and tannins contents in the extracts

Fruits are major source of biologically active components. Most of these compounds have antioxidants effects such as polyphenols, flavonoids, protein and vitamins. These substances have an important role in the prevention of various diseases (30, 31). Total phenolic, flavonoids and tannins contents in different extracts are shown in Table 1. Total phenolic compounds ranged between 112.5 and 310 µg GAE /mg DW, and the results showed that *Fargaia* is rich in polyphenols followed by *Prunus armeniaca* and *Prunus persica.* Total flavonoids contents were expressed as mg quercetin equivalents per gram of dry weight (µg QE/mg). *Fargaria* extract exhibited the highest flavonoids content. The quantification of tannins contents showed that *Prunus armeniaca* extract contained the highest tannins concentration with the value of 127.9 ± 0.0003 µg TAE /mg extract. The lowest tannins content was noticed for *Prunus persica* extract with a value of 62.83 ± 0.03 µg TAE /mg extract.

Table 1. Total polyphenols, flavonoids and tannins contents in fruits extracts

Extract	Total phenolic content (µg GAE/mg)	Total flavonoids (µg QE/mg)	Total tannins (µg TAE/mg)
Fargaria ananassa	310±0.003	14.78±0.001	81.5±0.01
Prunus armeniaca	232.5±0.02	5.68±0.002	127.9±0.003
Prunus persica.	112.5±0.02	6.02±0.003	62.83±0.03

GAE : Gallic Acid Equivalent QE : Quercetin Equivalent TAE : Tannic Acid Equivalent. Results expressed as means ± SD

Protein and Sugar content in the extracts

Fruits are important source of bioactive molecules such as protein, carbohydrate, amino acid, phenolic compounds and minerals (32). As shown in table 2, *Fargaria* contains the highest amount of sugars with a value of (958± 0.06 mg D-glucose/ g) the lowest amount was observed in *Prunus armeniaca* (285 ±0.06 mg D-glucose/g). Total protein content was high in *Fargaria* (2.73 ±0.04 g/l) and low in *Prunus persica* (1.55±0.01mg/g).

Identification of different phenolic acids, flavonoids and ascorbic acid in fruits extracts

Phenolic compounds are very important fruits constituents because of their scavenging ability due to their hydroxyl groups (33). The UPLC chromatogram of *Fargaria ananassa, Prunus persica* and *Prunus armeniaca* fruits extracts revealed the presence of various phenolic acid and flavonoids such as gallic acid, protocachuic acid, caffeic acid, flavon-3-ols. *Fargaria* extract showed the presence of some compounds which are absent in the *Prunus persica* and *Prunus armeniaca* such as hydroxynammic acid, p-coumaric, cinamic acid as presented in the Fig1. Catechin was detectable in the *Fargaria ananassa, Prunus persica. Prunus persica* and *armeniaca* contained ferulic acid but only rutin and vanillic acid and chlorogenic acid was found in *Prunus armeniaca* (Fig.2).

In vitro antioxidant activity of fruit extracts

Total Antioxidant Capacity (TAC) of fruit extracts

The total antioxidant was estimated using phosphomolybdate assay. It is based on the reduction of molybdenum (VI) to molybdentum (V) in the presence of antioxidant, and this reduction produced green phosphate/ Mo (V) complex in acid pH, which can measured at 695 nm (34, 35). The phosphomolybdate model evaluates both water soluble and fat soluble antioxidant capacity. It increases with the increase in the concentration of extract. The result was expressed as µg Ascorbic acid equivalent per g of extract. The result of total antioxidant capacity showed that *Fargaria* had the highest antioxidant capacity (99.6 ± 0.007) flowed by *Prunus persica* and *Prunus armeniaca* (81.33 ± 0.04 and 81.66 ± 0.008), respectively.

Table 2. Total sugars and proteins in fruits extracts

Extract	Total sugar (mg d-glucose E/g)	Total protein (mg/g)
Fargaria ananassa	958 ±0.06	2.73 ±0.04
Prunus persica	610 ±0.008	1.55 ±0.01
Prunus armeniaca	285 ±0.06	2.12 ±0.02

Results are expressed as Mean ± SD (n=3)

DPPH radical scavenging activity of fruits extracts

DPPH is widely used to assess the radical scavenging activity of antioxidant compounds (20, 36, 37). The 2,2diphényl-1-picrylhydrazyle is the first free radical used to study the relation between the structure

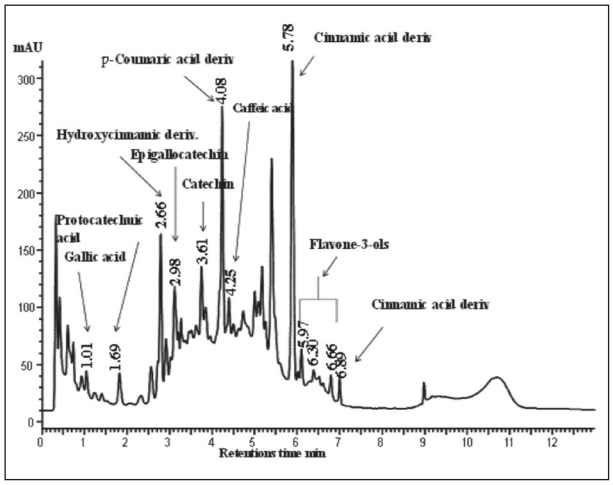

Fig 1. UPLC chromatogram of *Fargaria ananassa* fruit methanol extract

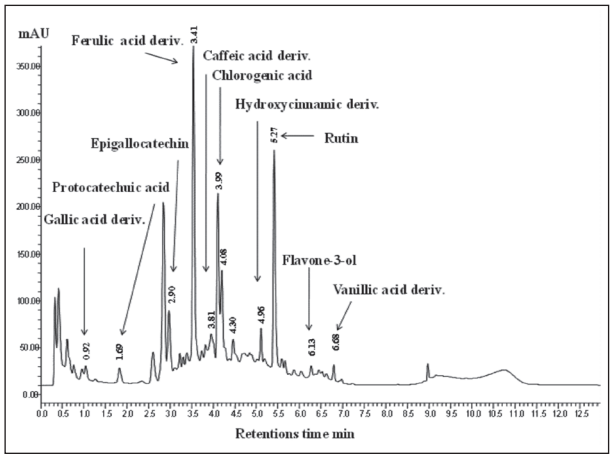

Fig 2. UPLC chromatogram of *Prunus armeniaca* fruit methanol extract

and antioxidant activity of phenolic compounds (38). It was found that the radical- scavenging activity of extract increased with increasing concentration (39). 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.4. 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 the *Prunus armeniaca* and *Prunus persica* with IC_{50}values 0.142 ± 0.0004, 0.488 ±0.012, 0.673 ±0.02 mg/ml for the three extracts respectively.

Free radical scavenging ability using ABTS

The ABTS radical is one of various radical used for measuring the antioxidant activity in foods (40). ABTS is a stable organic radical that has gained hydrogen; this method determines the antioxidant activity of hydrogen donating antioxidants in fruit crude extract (41, 40). *Fargaria* extract has high scavenging ability of the ABTS radical with an IC_{50} value of 0.040 ± 0.003 mg/ml. *Prunus persica* and *Prunus armeniaca* have good scavenging activity with IC_{50} values of 0.173±0.003 and 0.323± 0.007 respectively.

Reducing power of fruits extracts

Various studies have revealed that the electron donation ability reflects the reducing power of the bioactive compound. The amount of the Fe^{+2} complex was determined by measuring the formation of perls Prussian blue at 700 nm. Results are shows in fig 6 and7. Fig.6 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 *Fargaria* (1.142) >*Prunus armeniaca* (0.744) >*Prunus persica* (0.592). Figure7 shows the reducing power of fruit extracts. All extracts exhibited low activity compared to BHT with RC_{50} values of: BHT (0.008mg/ml) >*Fargaria* (0.251 mg/ml) >*Prunus armeniaca* (0.329 mg/ml) >*Prunus persica* (0.779 mg/ml).

Metal Chelating activity of extracts

The Ferrosine-Fe^{+2} complex produced a red chromophore which can be measured at 562 nm (42). In this study, the chelating activity of fruits extracts is

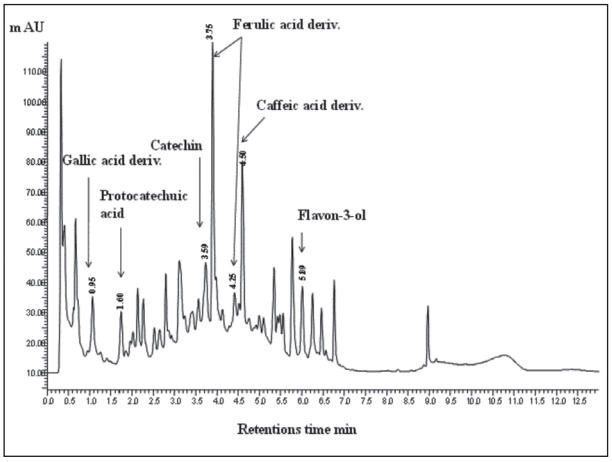

Fig 3. UPLC chromatogram of *Prunus persica* fruit methanol extract.

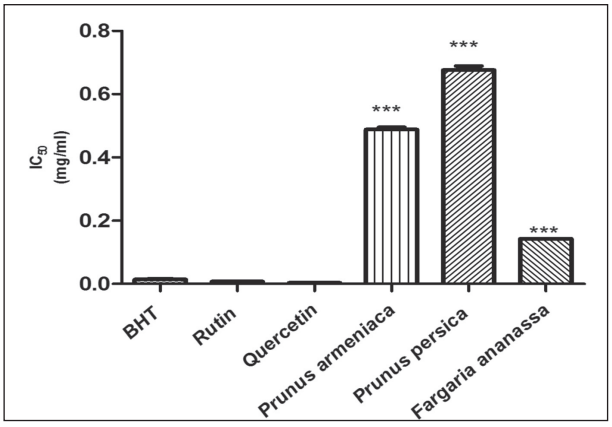

Fig. 4. DPPH free radical scavenging activity of different fruit extracts. Data were presented as IC_{50} means± SD (n = 3). *** ($p < 0.001$) compared to BHT as standard.

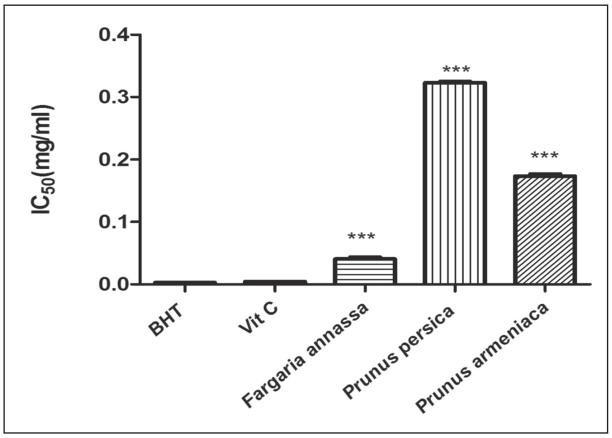

Fig.5. ABTS radical scavenging activity of different fruits extracts. Data were presented as IC_{50} means ± SD (n = 3). (*** $p<0.001$) compared to BHT and vit C as standards

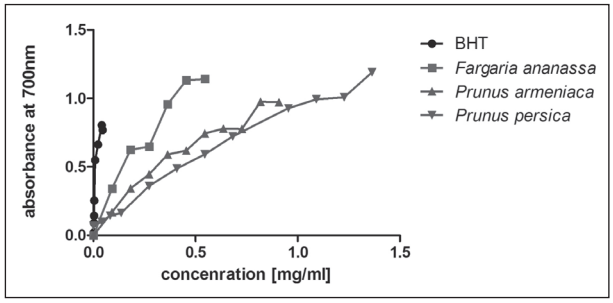

Fig 6. Antioxidant activity of fruits extracts expressed as reducing power. Values are means ± SD (n = 3).

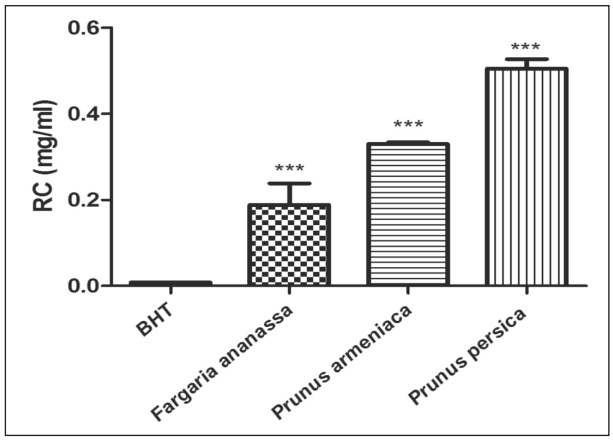

Fig 7. Reducing power of different fruits extract. Data were presented as RC_{50} means ±SD (n=3). (*** P < 0.001) compared to BHT as a standard

presented in Fig 5. *Prunus aremeniaca* had the highest ferrous ion chelating activity compared to *Fargaria*, and both extract had low activity than EDTA. EDTA is a strong metal chelator, hence, it is used as a standard metal chelator agent in this study. The low metal chelating activity was found for *Prunus persica* with EC_{50} value of 9.01 mg/ml.

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

The antioxidant activity of fruits extracts was also estimated by β-carotene bleaching assay. In β-carotene/linoleic acid model system, linoleic acid during incubation forms hydroperoxides free radicals (43). 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 55.22 to 80.72 %. The highest activity was found for

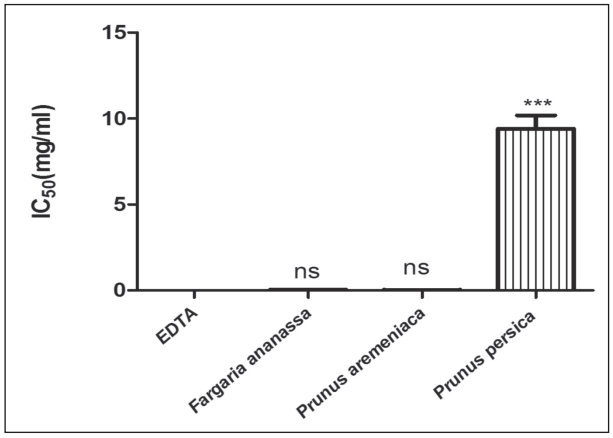

Fig 8. Metal chelating activity of different fruits extracts. Data were expressed as EC50 means ± SD (n = 3).(ns: no significant difference, *** p<0.001) compared to EDTA as a standard chelating agent.

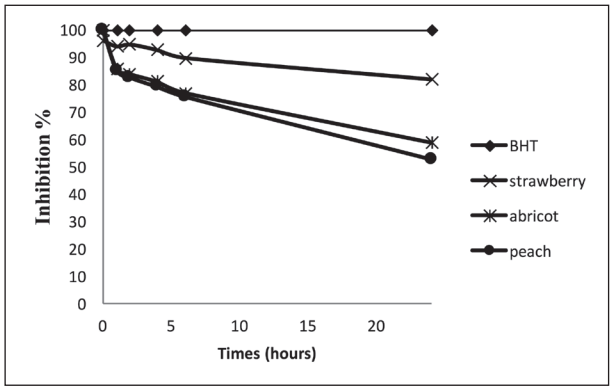

Fig 9. Changes in the percentage of the inhibition ratios of linoleic acid oxidation of different fruit extracts (2mg/ml) using β-carotene bleaching method, compared to BHT as a positive control during 24 h.

Fargaria (80.72 ± 4.57%) and the lowest activity was noticed for *Prunus* (55.22 ± 4.90 %). All extracts had lower activity compared to BHT.

Antioxidant activity of fruit extracts on linoleic acid peroxidation

The ferric thiocyanate method is used to measure the rate of peroxide formation in the first stages of lipid peroxidation (44). During linoleic-acid oxidation, the peroxide formed reacts and oxidize Fe^{2+} into Fe^{3+} to give red color (45). High absorbance demonstrated high concentration of peroxide during the incubation; which has a maximum absorbance at 500 nm. Low absorbance value indicates high level of antioxidant

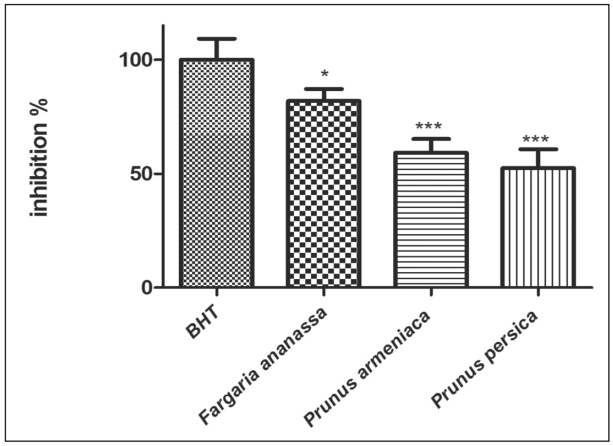

Fig.10. Antioxidant activities of different fruits extracts measured by β-carotene bleaching method (2 mg/ml at 24 h of incubation). BHT was used as standards antioxidant. Values are means ± SD (n = 3). (*p < 0.05, *** p < 0.001) compared to BHT as standard.

activity. Fig.11 shows the results of thiocyanate method. All fruit extracts have varying percentages of inhibition in the formation of peroxides compared with vit C, which is used as positive control. *Fargaria* and *Prunus* exhibited good peroxidation inhibiting activity, with 56.67 ± 3.34 % and 55.22 ± 2.86% respectively. While *Prunus armeniaca* presents the lowest ability to inhibit the formation of peroxide during 5 days with a value of 37.55 ± 4.055 %.

Thiobarbituric Acid (TBA) assay

FTC and TBA are the important methods used to measure the amount of peroxide radicals. TBA is used to indicate the amount of peroxide radicals in the second stage. Fig.11 showed the antioxidant activity of fruits extracts in TBA method. Results showed that *Fargaria* had high antioxidant effect than other fruits extracts. The inhibitions of the formation of Malonaldehyde were in the following order: vit C (62.19 ± 0.342%) >*Fargaria ananassa* (46.20± 3.89%) >*Prunus persica* (42.95 ±2.42%) > *Prunus armeniaca* (38.30 ± 2.22%) Fig 11.

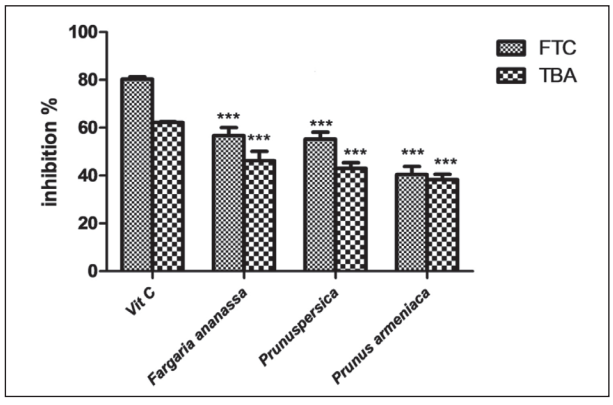

Fig 11. A comparison between total antioxidant activities of fruits extracts using the FTC and TBA methods. Vit C: Positive standards. Values are means ± SD (n=3)

Hydroxyl radical scavenging activity of fruit extracts

Hydroxyl radicals are the major active oxygen species in the biological systems; it reacts with fatty acids of cell membrane phospholipids (46, 47). The high concentration of hydroxyl induced damage to DNA, lipid, protein and produced carcinogenesis, mutagenesis and cytotoxicity (48, 49). The scavenging of hydroxyl is important to prevent cells from oxidative damage. The reaction of Fenton is the first way to produce $OH^{\cdot}$, in this reaction the transition of metal can degrade the hydrogen peroxide and generate hydroxyl radical. The hydroxyl scavenging ability of various fruit extract was measured using a system containing $FeSO_4$ and H_2O_2, this chemicals produced $OH^{\cdot}$, which hydroxylate salicylate . The Hydroxyl radical scavenging activity of methanol fruits extracts were determined and compared to vit C. The results of fruit extract scavenging activity were show in Fig.12. All extract can reduce the formation of hydroxyl radical with different IC_{50} values. *Fargaria* and *Prunus* present strong activity with IC_{50} values of (0.079 ± 0.031 and 0,089 ± 0.003 mg/ml) respectively,

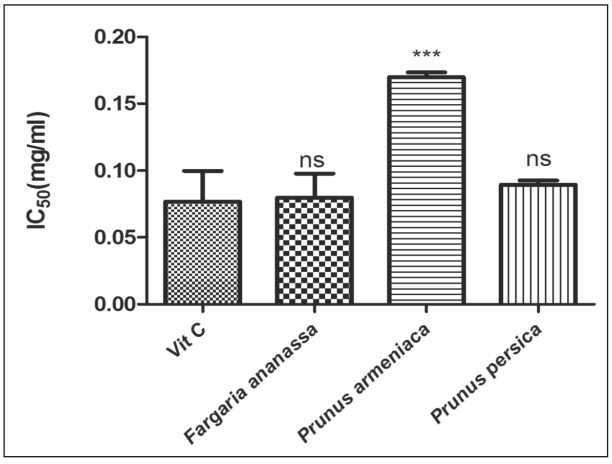

Fig 12. Hydroxyl radical scavenging activity of different fruits extracts. Data are presented as IC_{50} means ± SD (n = 3).(ns: no significant difference, *** p<0.001) compared to vitamin C as standard

but were significantly ($p< 0.05$) lower than vitamin C (IC_{50} = 0,076 ±0.007 mg/ml). *Prunus armeniaca* had an IC_{50} value of (0.16 ± 0.008 mg/ml) and possess good scavenging activity against $OH^{\cdot}$ radicals.

In vivo antioxidant activity of fruit extracts

Plasma antioxidant capacity using DPPH radical scavenging activity

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. Fig.13 shows that fruit extracts can scavenge the DPPH radical. After the oral administration of 200 mg/kg of fruit extracts to rats , plasma antioxidant activity was lower than that of reference group (vit c) with the following order vit C 26.05 ± 1.28% >*Prunus armenaica* 22.91 ±1.99% >*Fargaria* 15.84 ± 0.94% >*Prunus persica* 10.78 ± 0.72%. For the dose of 600 mg/kg, *Prunus armeniaca* demonstrated high effect than Vit c with an inhibition value of 34.38 ± 1.82%.

Effect of fruits extracts on plasma reducing power in rats

Figure 14 shows the effect of extracts on plasma reducing activity. The results indicate that all extracts have good reducing power. At 200 mg/kg *Fargaria ananassa* and *Prunus armeniaca* have reducing capacity of 0.619 ± 0.14 and 0.625 ± 0.13 respectively approximate to the value of reference group (0.6 ± 0.10). *Prunus persica* show high activity with value of 0.865 ± 0.08. The oral administration of 600 mg/kg of fruit extract resulted in a high reducing capacity in the plasma, the high value was shown for *Prunus armeniaca* (1.044 ± 0.082).

MDA levels

In the present study, TBARS method was used to evaluate the levels of lipid peroxidation during the oxidative stress, Thiobarbituric acid reactive substances were produced and induced lipid peroxidation (50). In lipid peroxidation, MDA is one of the major aldehydes produced. Thus, it is considered as a good biomarker of oxidative damage. MDA reacts with proteins, phospholipids, nucleic acid and induced cell damage (51) and this damage induces various diseases associated with oxidative stress (52). Fig.15 show the capacity of fruit extracts to inhibit lipid peroxidation in the liver of rats and minimize the formation of MDA. The results were compared with reference group, which received Vit C (200 mg/kg). In this assay, the relation between

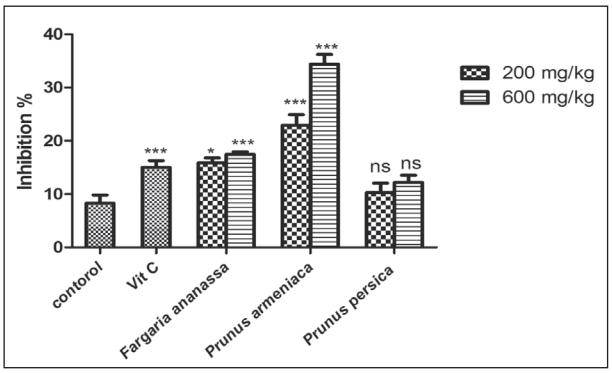

Fig.13. Effect of fruit extracts and vitamin C. on DPPH scavenging activity in plasma of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; * $p < 0.05$; *** $p < 0.001$) compared to control group

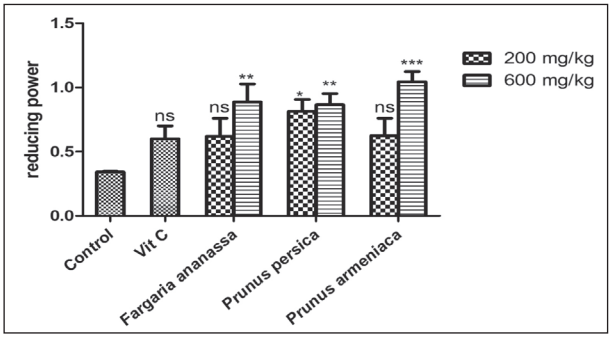

Fig.14. Effect of fruit extracts and vitamin C. on reducing activity in plasma of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) compared to control group.

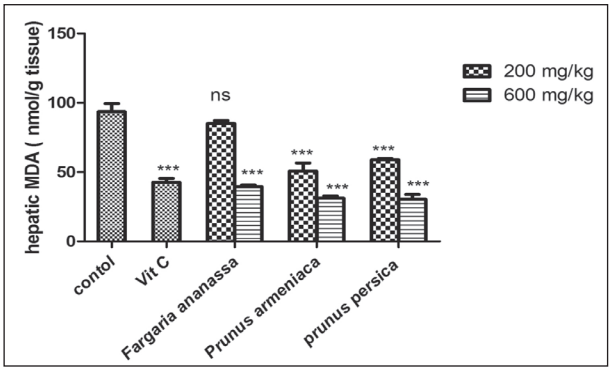

Fig 15. Effect of fruit extracts and vitamin C on MDA level in liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; *** $p < 0.001$) compared to control group

the rate of MDA and the concentration of extract was noticed. Extracts of *Fargaria, Prunus and Prunus* at 200 mg/kg had a good effect with values of 85.07±2.06, 50±5.70 and 58.93±0.91 nnomol/g tissue respectively. However, the dose of (600 mg/kg) decreased the MDA levels and this decrease was statistically significant when compared to control group. Aydemir (53) reported that Vit C has antioxidant activity and protect cell membrane against damage. Vit C has strong activity with value of 42.51 ± 2.96 nmol/g tissue.

Effects of fruits extracts on GSH levels in liver homogenate

Reduced glutathione (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 (54). Hence, depletion of intracellular GSH is usually regarded a measure of oxidative stress. In this test, the GSH reacts with DTNB in the dark and forms yellow complex. Fig.16 show GSH levels in the liver of rats treated different extracts. *Prunus persica and Prunus armeniaca* at 200 mg/kg and 600 mg/ kg did not change the levels of GSH in the liver. *Fargaria* treatment caused an increase in the levels of GSH at dose of 600 mg/kg with a value of 36.01 ± 2.51 µmol/g tissue.

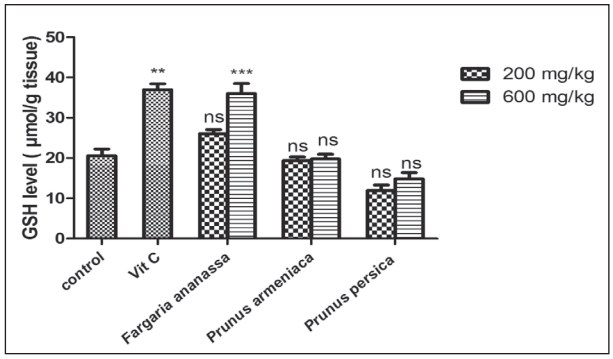

Fig 16. Effect of fruits extract and vitamin C on GSH level in the liver of rats. Values are given as means ± SEM (n=6). (ns: no significant difference; ** p < 0.01; *** p < 0.001) compared to control group.

Discussion

Fruits and vegetables contain phytochemicals with antioxidant activity. These antioxidants have multifunction. Their activity and mode of action in a particular test system may depend on the oxidation conditions, which may in turn affect both the kinetics of oxidation and the composition of the system .Therefore, a multi-dimensional assay protocol would be an advantage by reducing these limitations (55). The chemistry behind the antioxidant capacity assays has been reviewed by Huang (42). Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered the major contributors to the antioxidant activity of fruits and vegetables (36). The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and free radicals quenchers, as well as their metal chelating abilities (36). Therefore, multiple methods were used in this study to evaluate the antioxidant properties of fruit extracts.

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 (56, 57). During the second stage of maturation, the fruit have a high phenolic content, which might be associated with an amplified polyphenol oxidase activity (58). In addition, both genetic and agronomic or environmental factors play a role on the phytochemical composition and nutritional quality of the crops (59). Climate has an important role on 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 (60, 61). Flavonoids have high antioxidant effect, which depends on the environment condition. Various factors may change the action of flavonoids and product alteration in their efficacy as antioxidant (62). In this study, fruits were originated from a semi-arid region of Sétif, Algeria with changing climate (wet and cold in winter and dry and hot in summer).

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 in their chemical nature, the solvent used and the experimental condition (63).

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. 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 (64). The sugars and protein have antioxidant effect (65).

Polyphenols and phenolic acids are also powerful antioxidants and demonstrated various health benefits by exhibiting antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilator actions (66). 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 (67, 68). Moreover, other phenolic compounds found in the extracts such as gallic acid also possess beneficial effects on human health and deceases oxidative stress (69). Various natural product containing phenolics and flavonoids have the ability to reduce molybdenum (35, 70, 71).

In the present study, DPPH scavenging activity of extracts is correlated with tannins, flavonoids and various phenolic compounds (37). Generally, the extract with high total phenolic contents had higher scaving-ing activity (18). 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 (8).

The scavenging effect of the ABTS+ radical by the extracts was found to be much higher than that of DPPH radical. Factors like stereo selectivity 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 (72). Compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. This is not the case in this study.

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 (73, 74, 75). In this study this activity was also related to the amount of polyphenols and flavonoids in the samples.

Ferrous ion has an important role in food systems (76). It is well known as an effective pro-oxidant. Transition metals, like ion can stimulate lipid peroxidation, the formation of free radical such as hydroxyl radical and accelerates lipid proxidation into alkoxyl radical (77). Chelating agent can reduce lipid peroxidation and inhibit the formation of free radicals by stabilizing the transition metals (72). The metal ion chelating activity was measured by the ability of some phenolic compounds to disrupt the formation of Ferrosine-Fe+2 complex (78, 79).

The absences of antioxidant induce discoloration of β-carotene because it will couple with linoleic acid and generates free radicals. The rates of β-carotene blanching can be slow down in the presence of antioxidant (80).Various studies demonstrated that the β-carotene bleaching activity is in relation to flavonoids and polyphenols compounds which can inhibit oxidation of linoleic acid and the formation of hydroperoxides (81, 82).

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 (83). Peroxyl radicals can abstract hydrogen from another molecule or they can abstract hydrogen to give lipid hydoperoxide R-OOH (84). These 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 in the extracts.

When compared the FTC method and TBA the result of the present study indicate that the product of peroxide in the first stage is high compared to the second stage. 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 (51).

Husain (85) reported that flavonoids such as myrcetin, quercetin and rhamnetin were OH scavengers. They also noted that the effectiveness of such compounds increases with increasing the number of hydroxyl groups attached to the aromatic B ring. As is the case for many other free radicals, OH 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. 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 (86, 87).

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. These methods included ABTS, DPPH, reducing power and ORAC. These methods are based on hydrogen donation and other electron (42, 88). Other parameters can be used to determine the effect of crude fruit extracts against oxidative stress in vivo using animal models.

These results demonstrated that fruits have good plasma antioxidant activity using DPPH, reducing power. The plasma contains albumin, bilirubin, reduced glutathione and uric acid endogenous antioxidant and may be take exogenous antioxidants from food. Which can work complementary and synergistic with endogenous antioxidant to protect human health against ROS (89).

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 (90). This effect can be attributed to the antioxidant properties of polyphenols present in the extracts (91), because of their strong ability to scavenge free radicals and break the reaction chain of these radicals *in vitro* and *in vivo* (92). 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 (93), found these effects for black pepper and Gladine (94) reported these effects for rosemary, grape, citrus, and calendula; whereas, Papandreou (95) reported the same results for blue berries (Vaccinium angustifolium).Various studies suggest that polyphenols stimulate the gene expression of SOD, and GPx (96).

The present study showed that *Fargaria ananassa*, *Prunus armeniaca and Prunus persica* consumed by the population and produced in Sétif region, Algeria contain high amounts of polyphenols and flavonoids. The extracts of these fruits exert a good *in vitro* and *in vivo* antioxidant activity; bioactive compounds such as polyphenols are important contributors to the antioxidant activity of fruits. The treatment of animals with these extracts resulted in a reduction in the production of MDA in liver tissue of rats. It is concluded that *Fargaria ananassa*, *Prunus armeniaca and Prunus persica* are important source of phenolic acid and flavonoids and their consumption can reduce the risk of several diseases associated with oxidative stress such as cancer, diabetes, aging and cardiovascular diseases.

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Refreces

1. Meda NTR, Bangou MJ, Bakasso S, Millogo-Rasolodimby J, Nacoulma OG. Antioxidant activity of phenolic and flavonoid fractions of Cleome gynandra and Maerua angolensis of Burkina Faso. Journal of Applied Pharmaceutical Science, 2013; 3: 36-42.
2. Das Sarma A, Rahaman Mallick AR, Ghosh AK. Free Radicals and Their Role in Different Clinical Conditions: An Overview. International Journal of Pharma Sciences and Research (IJPSR), 2010; 1:185-192.
3. Duran-Bedolla J, Rodriguez MH, Navor VS, Arancibia SR, Cerbon M, Rodriguez MC. Oxidative stress: production in several processes and organelles during Plasmodium sp development. Oxidants and Antioxidants in Medical Science, 2013; 2: 93-100.
4. Ahmed D, Munim F, Saeed S. Phenolic and flavonoid contents and anti-oxidative potential of epicarp and mesocarp of Lagenaria sicerariafruit: a comparative study. Asian Pac. J. Trop. Med, 2014; 7: 249-255.
5. Singh JP, Kaur A, Singh N, Nim L, Shevkani K, Kaur H, Arora DS. In vitro antioxidant and antimicrobial properties of jambolan (Syzygium cumini) fruit polyphenols. LWT-Food Science and Technology, 2016; 65: 1025-1030.

6. Amin I,Tan, SH. Antioxidant activity of selected commercial seaweeds. Mal J Nutr, 2002; 8: 167–177.
7. Lee WY, Emmy Hainid KI, Abbe Maleyki MJ, Amin I. Antioxidantcapacity and phenolic content of selected commercially available cruciferous vegetables. Mal J Nutr, 2007; 13: 71– 80.
8. Laura A, Alvarez-Parrilla E, Gustavo A, Alez-Aguilar G. Fruit and Vegetable Phytochemicals. Chemistry, nutritional value and stability, 2010; Pp 3-36.
9. Campbell OE, Padilla-Zakour OI. Phenolic and carotenoid composition of canned peaches (Prunus persica) and apricots (Prunus armeniaca) as affected by variety and peeling, Food Research International, 2013; 54: 448–455.
10. Garrigaa M, Munoz CA, Caligari PDS, Retamales JB. Effect of salt stress on genotypes of commercial (Fragaria x ananassa) and Chilean strawberry (F. chiloensis). Scientia Horticulturae, 2015; 195: 37-47.
11. Markham KR. Techniques of Flavonoid Identification (Chap.1 and 2). London: Academic Press, 1982; Pp.1- 113.
12. Li HB, Cheng KWC, Fan KWF, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fraction of selected microalgae.Food chem,2007; 102: 771-776.
13. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Luyckx M, Cazin M, Pinkas M. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparations .Arzneim Forsch/Drug Res, 1996; 46: 1086-1089.
14. Gharzouli K, Khennouf S, Amira S, Gharzouli A. Effects of aqueous extracts from Quercus ilex L. root bark, Punica granatum L. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. Phytother Res, 1999; 13: 42-45.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 7: 248-54.
16. Dubois M, Gilis K, Hamilton J, Rebers P, Smith F. Colorimetric method for determination of sugars and related substances. Anal chem, 1956; 28: 350-356.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem, 1999; 269: 337-341.
18. Yardpiroon B, Aphidech S, Prasong S. Phytochemical and Biological Activities of the Wild Grape Fruit Extracts Using Different Solvents. British J of Pharmaceutical Res, 2014; 4: 23-36.
19. Re R. Antioxidant activity applying an improved ABTS radical cation decolorization assays. Free Radic Biol Med 1999; 26: 1231–1237.
20. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B, Asgarirad H. In vitro antioxidant and free radical scavenging activity of Leonurus cardiaca subsp. Persicus, Grammosciadium platycarpum and Onosma demawendicum. African J Biotechnol, 2010; 9: 8865-8871.
21. Decker EA, Welch B. Role of ferritin as lipid oxidation catalyst in muscle food. J Agr Food Chem, 1990; 36: 674-677.
22. Gursoy N, Tepe B, Akpulat AH. Chemical Composition and Antioxidant Activity of the Essential Oils of Salvia palaestina (Bentham) and S. ceratophylla (L). J Records of Natural Product, 2012; 6: 278-287.
23. Yen GC, Chang YC, Su SW. Antioxidant activity and active compounds of rice koji fermented with Aspergillus Candidus. Food Chem, 2003; 83: 49–54.
24. Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. J Food Sci, 1993; 58: 1407–1410.
25. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. J Phytochemrstry, 1989; 28: 1057 -1060.
26. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res, 2000; 14: 323–328.
27. Chung YC, Chen SJ, Hsu CK, Chang CT, Chou ST. Studies on the antioxidative activity of Graptopetalum paraguayense. Food Chem, 2005; 91: 419–424.
28. Ellman G. Tissue sulfhydryl groups. Arch biochem biophis, 1959; 82: 70-77.
29. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid. Anal Chem 1979; 95: 351–358.
30. Oomah BD, Mazza G. Functional Foods. In: The Wiley Encyclopedia of Science & Technology, 2nd edition, V2 (edited by F.J. Francis). 2000; Pp. 1176- 1182. NewYork, NY: Wiley
31. Kaur Ch, Kapoor HC. Antioxidants in fruits and vegetables - the millennium's health. J food sci and technology, 2001; 36: 703- 725.
32. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. Scientific World Journal, 2013: 1-16.
33. Uddin R, Saha MR, Subhan N, Hossain H, Jahan I A, Akter R, Alam A. HPLC-analysis of polyphenolic compounds in gardenia jasminoidesand determination of antioxidant activity by using free radical scavenging assays. Advanced Pharmaceutical Bulletin, 2014; 4: 273-281.
34. Aliyu AB, Ibrahim MA, Ibrahim H, Musa AM, Lawal AY, Oshanimi JA, Usman M, Abdulkadir IE, Oyewale AO, Amupitan JO. Free radical scavenging and total antioxidant capacity of methanol extract of Ethulia Conyzoides growing in Nigeria. Romanian Biotechnological Letters, 2012; 17: 7458-7465.
35. Ravisankar N, Sivaraj CH, Seeni S, Joseph J, Raaman N. Antioxidant activites and phytochemical analysis ofmethanol extract of leaves of hypericum hookerianum. Int J of Pharmacy and Pharmaceutical Sci, 2014; 6: 0975-1491.
36. Vladimir-KneževićS, Biljana BlažekovićB, Štefan MB, Alegro A, Kőzegi, T, Petrik J. Antioxidant Activities and Polyphenolic Contents of Three selected Micromeria Species from Croatia. J molecules, 2011; 16: 1454-1470.
37. Garg D, Muley A, Khare N, Marar Th. Comparative Analysis of Phytochemical Profile and Antioxidant Activity of Some Indian Culinary Herbs. Res J of Pharmaceutical, Biological and Chem Sci, 2012; 3: 845-854.
38. Popovici C, Saykova I, Tylkowski B. Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. Génie Industriel, 2009; 4: 25-39.
39. Ebrahimzadeh MA, Nabavi SF, and Nabavi SM. Antihemolytic and antioxydant activity of Hibiscus esculentusleaves.

Pharmacologyonline, 2009; 2: 1097-1105.
40. Sivakumar ChV, Meera I. Antioxidant and Biological Activities of Three Morphotypes of Murraya koenigii L.from Uttarakhand. Food Processing & Technology, 2013; 4: 2-7.
41. Le K, Chiu F, Ken NG. Identification and quantification of antioxidants in Fructus lycii. Food chem, 2007; 105: 353-363.
42. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J of Agricultural Food Chem, 2005; 53: 1841-1856.
43. Goze I, Alim A, Tepe AS, Sokmen M, Sevgi K Tepe B. Screening of the antioxidant activity of essential oil and various extracts of Origanum rotundifolium Boiss. From Turkey. Journal of Medicinal Plants Research, 2009; 3: 246-254.
44. Ting HCh, Hsu WY, Tsai ChF, Lu FJ, Ming-Chih MCh, Chen WK. Thein vitro andin vivo antioxidant properties of seabuckthorn (Hippophae rhamnoides L.) seed oil. Food chem, 2001; 125: 652-659.
45. Yu L, Zhao M, Wang JSh, Cui Ch, Yang B, Jiang Y, Zhao Q. Antioxidant, immunomodulatory and anti-breast cancer activities of phenolic extract from pine (Pinus massoniana Lamb) bark. Innovative Food Science and Emerging Technologies, 2008; 9: 122–128.
46. Padmanabhan P, Jangle SN. In-vitro antioxidant potential of an herbal preparation containing four selected medicinal plants. Journal of Krishna Institute of Medical Sciences University, 2012; 1: 2231-4261.
47. Archana CM, Showmya J, Harini K, Pradeepa M, Geetha N. Radical scavenging and antioxidant activity of methanolic leaf extract of Chromolaena odorata (l) king and robinson by in vitro assays. International Journal of Pharmaceutical Sciences and Business Management, 2015; 3: 1-12.
48. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 2012; 12: 2-12.
49. Rahman MdM, Badrul Islam MdB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallidagrowing in Bangladesh. BMC Res Notes, 2015; 8: 2-9.
50. Behuliak M, Palfy R, Gardlik R, Hodosy J, Halcak L, Celec P. Variability of thiobarbituric acid reacting substances in saliva. Disease Markers, 2009; 26: 49-53.
51. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative Medicine and Cellular Longevity, 2010; 3: 2-12.
52. Mateos R, Lecumberri E, Ramos S, Goya L, Bravo L. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress: Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidants from fruits. Journal of Chromatography, 2005; 827: 76-82.
53. Aydemir T, Ozturk R, Bozkaya LA, Tarhan L. Effects of antioxidant vitamins A, C, E and trace elements Cu, Se on Cu Zn SOD, GSH-Px, CAT and LPO levels in chicken erythrocytes. Cell Biochemistry and Clinical Function, 2000; 18: 109-115.
54. Valko M, Leibfritz D Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol, 2007; 39: 44–84.
55. Lako J, Trenerry VC Rochfort S (2008) Routine analytical methods for use in South Pacific regional laboratories for determining naturally occurring antioxidants in food. International Food Research Journal 15: 313-323
56. Hind AB, Abu-Bakr A, Abu G. Compositional changes during guava fruit ripening. Food Chemistry, 2003; 80: 557-563.
57. Liu H, Cao J, Jiang W. Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. J Food Science and Technology, 2015; 1-7.
58. Parr AJ, Bolwell GP. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture, 2000; 80: 985–1012.
59. Chuang Ch, Mcintosh M. Potential Mechanisms by Which Polyphenol Rich Grapes Prevent Obesity-Mediated Inflammation and Metabolic Diseases. Annu Rev Nutr, 2011; 31: 155–176.
60. Dragovic-Uzelac V, Levaj B, Mrkic V, Bursac D, Boras M. The content of polyphenols and cartenoids in three apricot cultivars depending on stage of maturity and geographical. Food chem, 2007; 102: 966-975.
61. Pék Z, Szuvandzseiv P, Daood H, Neményi A, Helyes L. Effect of irrigation on yield parameters and antioxidant profiles of processing cherry tomato.Cent Eur J Biol, 2014; 9: 383-395.
62. Chen HY, Yen GCh. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L) leaves. Food Chem, 2007; 10: 686-694.
63. Mahmoudi S, Khali M, Mahmoudi N. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (Cynara scolymus L). Nature and Technologie, 2012; 09: 35 -40.
64. Ali S, Masud T, Abbasi KS. Physico-chemical characteristics of apricot (Prunus armeniacaL.) grown in Northern Areas of Pakistan. Scientia Horticulturae, 2011; 130: 386-392.
65. Wong SP, Leong LP, William Koh JH. Antioxidant activities of aqueous extracts of selected plants. Food Chem, 2006; 99: 775-783.
66. Duthie GG, Duthie SJ, Kyle JAM. plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev, 2000; 13: 79-106.
67. Augustyniak A, Bartosz G, Cipak A, Duburs G, Horakova L, Luczaj W, Majekova M, Odysseos AD, Rackova L, Skrzydlewska E, Stefek M, Strosova M, Tirzitis G, Venskutonis PR, Viskupicoca J, Vraka PS, Zarkovic N. Natural and synthetic antioxidants: an updated overview. Free Radic Res, 2010; 44: 1216-62.
68. Ghasemzadeh A, Jaafar HZE Rahmat A. Antioxidant activities, total phenolics and flavonoidscontent in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules 2010; 15: 4324-4333.
69. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants

in human health and disease. Oxid Med Cell Longev, 2009; 2: 270-8.
70. Ahmed D, Khan MM, Saeed R. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. Antioxidant, 2015; 4: 394-409.
71. Gupta M, Karmakar N, Sasmal S. in vitro antioxidant activity of aqueous and alcoholic extracts of polyherbal formulation onsisting of Ficus glomerata Roxb and Symplocos racemosa Roxb. Stem Bark Assessed in Free Radical Scavenging Assays. Int J of Pharmacognosy and Phytochemical Res, 2017; 9: 181-189.
72. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. J. Agric. Food Chem, 2002; 50: 1619–1624.
73. Chung,YC, Chang, CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR–NK1. J Agr Food Chem, 2002; 50: 2454–2458.
74. Gülçin I, Oktay M, Kireçci E, Küfrevioglu ÖI. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chem, 2003; 83: 371–382.
75. Gülçin Ï. Antioxidant and antiradical activities of L-carnitine. Life Sci, 2006; 78: 803–81.
76. Al-Hashimi AG. Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts. African J of Food Sci, 2012; 6: 506-511.
77. Özkan A, Yumrutaş Ö, Saygideğer SD, Kulak M. (2011) Evaluation of Antioxidant Activities and Phenolic Contents of Some Edible and Medicinal Plants from Turkey's Flora. Advances in Environmental Biology, 2011; 5: 231-236.
78. Norshazila S, Syed Zahir I, Suleiman KM, Aisyah MR, Kamarul RK. Antioxidant Levels and Activities of Selected Seeds of Malaysian Tropical Fruits. Mal J Nutr, 2010; 16: 149-159.
79. Yumrutas O, Saygideger SSD. Determination of antioxidant and antimutagenic activities ofPhlomis armeniacaandMentha pulegium. J of Applied Pharmaceutical Science, 2012; 02: 36-40.
80. Kulisic T, Radoni A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem, 2004; 85: 633–640
81. Duh PD, Yen GC. Antioxidative activity of three herbal water extracts. Food Chem, 1997; 60: 639–45.
82. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chemistry, 2005; 90:743-9.
83. Gorden MH. The mechanism of antioxidant action in vitro. Food Antioxidant, 1990; Pp1-8.
84. Anusuya N, Anusaya S, Manian R, Siddhuraju P, Manian S. Antioxidant and free radical scavenging activity of certain dietary and medicinal plant extracts. Food global science books, 2009; 3: 47-52.
85. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry, 1987; 26: 2489-2491.
86. Wang SY, Lin KS. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. Journal of agricultural and food chemistry, 2000; 48: 140-146.
87. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J of Agricultural and Food Chem, 2000; 48: 5677–5684.
88. Katalinic V, Modun D, Music TI, Boban M. Gender differences in antioxidant capacity of rat tissues determined by 2,2V-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comp Biochem Physiol, 2005; 140: 47-52.
89. Meziti A, Meziti H, Boudiaf K, Benboubetra M, Bouriche H. Polyphenolic Profile and antioxidant Activities of Nigella Sativa Seed Extracts in vitro and in vivo. World Academy of Science, Engineering and Technology, 2012; 6: 04-24.
90. Celep E, Ahmet Aydin A, Kirmızıbekmez H, Yesilada E. Appraisal ofin vitroandin vivo antioxidant activity potential of Cornelian cherry leaves. Food and Chemical Toxicology, 2013; 62: 448–455.
91. Vinson J A Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis, 2001;1567-72.
92. Liu Q, Yao H. Antioxidant activities of barley seeds extracts. Food Chemistry, 2007; 102: 732-737.
93. Vijayakumar RS, Surya D. Nalini N. Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high fat diet induced oxidative stress. Redox Report, 2004; 9: 105-110.
94. Gladine C, Morand Ch, Rock E, Guffat D, Bouchart D, Durand D. The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. Animal Feed Science and Technology, 2007; 139: 257-272.
95. Papandreou MA, Dimakopoulou A, Linardaki ZI Cordopatis P Klimis-Zacas D Margarity M, Lamari FN. Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behavioural Brain Research, 2009;198: 352-358.
96. Sánchez-Reus MI, Del Rio MAG, Iglesias I, Elorza M, Slowing K, Benedí J. Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. Neuropharmacol, 2007; 52: 606-516.

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