

Antioxidant activity and phenolic content of commonly consumed fruits and vegetables in Algeria

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Summary. The purpose of this study was to evaluate the phenolic content and the antioxidant activity of some commonly foods consumed in Algeria. 22 vegetables and 14 fruits extracts were evaluated for their polyphenolic content and antioxidant potential using different methods. Results showed that beans, cauliflower and courgette were rich in total polyphenols. However, Jew's mallow was the richest in flavonoids and the highest content of tannins was noticed in the pomegranate. The antioxidant activity of fruits and vegetables extracts using β -carotene bleaching assay showed that lettuce, courgette, cauliflower, artichoke, mallow, bean, green bean, green pea, peach and apricot were the most effective with antioxidant activity percentage greater than 70%. However, eggplant, lettuce, courgette, artichoke, mallow, chard, green bean, green pea, black grapes and pomegranate showed the highest antioxidant activity against DPPH radical with $IC_{50} \leq 0.8$ mg/ml. Also, potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea showed the highest chelating activity with $IC_{50} \leq 0.8$ mg / ml and onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea showed the highest reducing power ($IC_{50} \leq 5$ mg / ml). Finally, these selected consumed fruits and vegetables are natural source of polyphenols and have an important antioxidant activity and their consumption may reduce the risk of pathologies induced by oxidative stress.

Keywords: antioxidant activity, phenolic content, fruits, vegetables, DPPH, chelating activity, reducing power, β -carotene bleaching

Introduction

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms, leading to damage to lipids, proteins and nucleic acids (1), which are involved in several diseases such as cancer, aging skin, inflammatory, cardiovascular and neurodegenerative diseases (2).

Antioxidants which can neutralize free radicals are of great importance in preventing the development of these diseases (3). Thus, to avoid the toxic effect of synthetic antioxidants, many studies have investigated new antioxidants of natural origin as polyphenols (4).

Polyphenols are bioactive compounds usually found in fruits, vegetables, legumes, grains, chocolate,

and beverages such as fruit juices, tea, coffee and red wine (5). These secondary metabolites can act as scavengers of free radicals which are responsible for the initiation of oxidation, as well as chain breaking antioxidants, singlet oxygen deactivators, reducing agents, metal chelating agents and inhibitors of specific oxidative enzymes (6).

Many studies showed that diets rich in fruits and vegetables are good antioxidants and can reduce the risk of development of many diseases associated with oxidative stress as cancers, atherosclerosis, aging, inflammatory, cardiovascular and neurodegenerative diseases (7, 8).

The health effects of polyphenols and their properties, especially when these compounds are present in

large quantities in food, are important to consumers, which requires the evaluation of their antioxidant activity. Thus, the objective of this study was to evaluate the total polyphenols content and *in vitro* antioxidant activity of some consumed vegetables and fruits in Algeria.

Material and methods

Plants materials

All fresh vegetables and fruits used in this study (Table 1) were bought from the local market in Biskra (south eastern of Algeria) and Setif (north eastern of Algeria) regions at the time of their most frequent consumption during 2012-2013. At least 1 kg of the good quality produces without bruises and damage were purchased.

Extraction of phenolics from food samples

The extraction was carried out according to the method described by Hossain et al (9). 1 kg of consumed part of fresh fruits and vegetables previously cleaned and washed with distilled water, except for Jew's mallow which was used in dry form were cut into small pieces and crushed. Then 100 g of each crushed material were macerated in 625 ml of methanol / water mixture (80/20: V / V) for 3 days at 4 °C. The macerate was filtered and the filtrate was subjected to a rotary evaporation under reduced pressure at 45 °C. The extract obtained was dried and stored at 4 °C until use.

Determination of total phenolic content

Total polyphenol content was assayed by Folin-Ciocalteu reagent described by Li et al (10). 100 µl of each extract were added to 500 µl of Folin-Ciocalteu reagent (10 times diluted in distilled water). After 4 min of incubation, 400µl of 7.5% sodium carbonate were added and the solution mixtures were kept in the dark for 1 h and 30 min at room temperature. Then, the absorbance of each solution was read at 765 nm against a blank by a spectrophotometer.

The concentration of the total polyphenols was calculated from the regression equation of the calibration curve of gallic acid at different concentrations (12.5 to 100 µg/ml) and expressed in micrograms of

gallic acid equivalent per milligram of dry extract (µg of GAE / mg of extract).

Determination of total flavonoids content

The flavonoids evaluation was assayed by the method of Quettier-Deleu et al (11) using aluminum trichloride (AlCl₃). 500µl of each extract was added to an equal volume of a solution of AlCl₃ (2% in methanol). The mixture was vigorously stirred and after 10 minutes of incubation, the absorbance was read at 430 nm by a spectrophotometer. The quantification of flavonoids was evaluated from the calibration curve of quercetin at different concentrations (1.25 to 40 µg /ml). The results were expressed in micrograms of quercetin equivalent per milligram of dry extract (µg of QE / mg of extract).

Determination of total tannins content

Tannins were assayed by the method described by Bate-Smith (12). A volume of 500 µl of fresh bovine blood (which had an absorbance equal to 1.6 at a wavelength of 576 nm) was added to 500 µl of the extract. After stirring and centrifugation for 10 min at 4000 rpm, the absorbance of the supernatant was read at 576 nm. The quantification of tannins was carried out using the calibration curve of tannic acid at different concentrations (200 to 600 µg/ml).

The results were expressed in micrograms of equivalent tannic acid per milligram of dry extract (µg of TAE / mg of extract).

In vitro antioxidant activity

The diversity of nature and the structure of plant compounds require the development of many methods to evaluate their antioxidant activity. Thus, different methods are used to measure the antioxidant activity of the extracts. Each method uses or generates a different radical that is involved in the oxidation process. Only one method is insufficient to represent the total antioxidant capacity of the extracts, and for this purpose four different tests were used to evaluate the antioxidant activities of the extracts which are DPPH radical scavenging assay, ferrous ion chelating, ferric reducing power and β-carotene/ linoleic acid bleaching assay.

Table 1. Common name, region of purchase, scientific name and used part of fruits and vegetables

	Common name	Region of purchase	Scientific name	Used part
Vegetables	Artichoke (flower)	Setif	<i>Cynara cardunculus</i> L.var. <i>scolymus</i>	Flower
	Artichoke (stem)	Setif	<i>Cynara cardunculus</i> L.var. <i>scolymus</i>	Stem
	Bean	Setif	<i>Vicia faba</i> L.	Seed
	Beetroot	Biskra	<i>Beta vulgaris</i> L.var. <i>rapacea</i> Koch	Root
	Cabbage	Setif	<i>Brassica oleracea</i> L.	Leaves
	Carrot	Biskra	<i>Daucus carota</i> ssp. <i>sativus</i>	Tuber
	Cauli flower	Biskra	<i>Brassica oleracea</i> L.	Flower
	Chard	Setif	<i>Beta vulgaris</i> L.var. <i>cicla</i> Pers	Leaves
	Courgette	Biskra	<i>Cucurbita pepo</i> L.	Fruit
	Cucumber	Biskra	<i>Cucumis sativus</i> L.	Fruit
	Eggplant	Biskra	<i>Solanum melongena</i> L.	Fruit
	Fennel	Biskra	<i>Foeniculum dulce</i> Mill.	Leaves
	Green bean	Setif	<i>Phaseolus vulgaris</i> L.	Fruit (clove)
	Green pea	Setif	<i>Pisum sativum</i>	Seed
	Jew's mallow	Biskra	<i>Corchorus olitorius</i> L.	Leaves
	Lettuce	Setif	<i>Lactuca sativa</i> L.	Leaves
	Onion	Biskra	<i>Allium cepa</i> L.	Bulb
	Pepper	Biskra	<i>Capsicum annum</i> L.	Fruit
	Potato	Biskra	<i>Solanum tuberosum</i> L.	Tuber
	Pumpkin	Setif	<i>Cucurbita maxima</i> Duch.	Fruit
Tomato	Biskra	<i>Solanum lycopersicum</i> L.	Fruit	
Turnip	Biskra	<i>Brassica napus</i> L.	Root	
Fruits	Apple	Biskra	<i>Malus communis</i> Poir.	Fruit
	Apricot	Setif	<i>Prunus armeniaca</i> L.	Fruit
	Banana	Setif	<i>Musa sapientum</i> L.	Fruit
	Dates "Deglat-Nour"	Biskra	<i>Phoenix dactylifera</i> L.	Fruit
	Dates "Ghars"	Biskra	<i>Phoenix dactylifera</i> L.	Fruit
	Dates "Mech-Degla"	Biskra	<i>Phoenix dactylifera</i> L.	Fruit
	Grapes (black)	Biskra	<i>Vitis venifera</i> L.	Fruit
	Grapes (white)	Biskra	<i>Vitis venifera</i> L.	Fruit
	Mandarin	Setif	<i>Citrus reticulata</i>	Fruit
	Medlar of Japan	Setif	<i>Eryobotrya japonica</i> Lindl.	Fruit
	Orange	Setif	<i>Citrus sinensis</i> Osb.	Fruit
	Peach	Setif	<i>Prunus persica</i> L.	Fruit
	Pear	Biskra	<i>Pyrus communis</i> L.	Fruit
	Pomegranate	Setif	<i>Punica granatum</i> L.	Fruit

DPPH radical scavenging assay

The DPPH radical scavenging method is a spectrophotometric procedure used to determine the antioxidant capacity of the components. It is based on the ability of the DPPH radical to discolor from purple to

yellow color in the presence of antioxidants by accepting an electron or hydrogen atom given by an antioxidant compound (13).

The antiradical activity of the extracts in this study was measured by the 2,2'-diphenyl-1-picrylhydrazyl

(DPPH) test according to the method of Brand-Williams et al (14) with slight modification. A range of extract concentrations and quercetin as antioxidant reference were prepared. A volume of 50 μ L of each extract solution was mixed with 1.25 ml of DPPH (0.04 mg / ml) prepared in methanol. After stirring, the mixture was incubated for 30 minutes in the darkness at room temperature and the absorbance was read at 517 nm against a blank. The inhibition of free radical activity was calculated according to the following equation: **The antiradical activity (%) = [(Abs_{control} - Abs_{sample}) / Abs_{control}] x 100.**

The IC₅₀, which is the concentration of extract or quercetin responsible for 50% of inhibition of DPPH radical was determined from the plot of inhibition percentage against extract or quercetin concentration.

Ferrous ions chelating activity

Transition metal ions such as copper and iron are important for the generation of highly reactive hydroxyl radicals via the Fenton reaction in *in vivo* and *in vitro* systems. Compounds that bind to metal ions can alter the redox potential of these ions, making them catalytically silent. Therefore, compounds that can act as effective chelators for the sequestration of copper and iron ions are considered antioxidants by intercepting and / or suppressing radicals (15).

The chelating activity of the extracts was measured following the inhibition of the formation of the Fe (II) -Ferrozine complex after incubation of the samples with divalent iron according to the method described by Le et al (16). The sample solutions (250 μ l) were initially mixed with 50 μ l FeCl₂ (0.6 mM in distilled water) and 450 μ l of methanol. After 5 min, 50 μ l of ferrozine (5 mM in methanol) were added to the reaction medium and the mixture was stirred well and then left to react for 10 min at room temperature. The red chromophore (Fe (II) -Ferrozine) had maximum absorption at 562 nm and the chelation activity was calculated according to the following equation:

$$\text{The chelating activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

Where IC₅₀ is the concentration of extract responsible for chelate 50 % of iron ions.

Ferric reduction power

The reducing power of the extracts can provide a significant indication of the potential antioxidant

activity of the plant. The presence of antioxidants in the extracts would result in the reduction of Fe³⁺ to Fe²⁺ by giving an electron. The amount of Fe²⁺ complex can be monitored by measuring the absorbance at 700 nm (13).

The reducing capacity of the various vegetables and fruits extracts was evaluated using the method of Ozsoy et al (17). 100 μ l of each extract was mixed with 100 μ l of phosphate buffer solution (0.2 M, pH 6.6) and 100 μ l of 1% potassium hexacyanoferrate solution. The mixture was incubated for 20 minutes at 50 ° C in a water bath. After that, 250 μ l of 1% trichloroacetic acid was added and the mixture was centrifuged for 10 minutes. Then, 250 μ l of the supernatant were taken and mixed with 250 μ l of distilled water and 500 μ l of 0.1% aqueous solution of FeCl₃. The absorbance was read at 700 nm. A calibration curve was plotted from the line obtained with the BHT used as reference at different concentrations. The IC₅₀ value was defined as the effective concentration of the extract or standard which had the absorbance of 0.5.

β -carotene/ linoleic acid bleaching assay

The β -carotene bleaching test measures the ability of antioxidants to retard the β -carotene decolorization induced by conjugated diene hydroperoxides resulting from the oxidative degradation of linoleic acid (13).

In this test, the antioxidant capacity of the extracts is determined according to the method of Kartal et al (18). 25 μ l of linoleic acid and 200 mg of tween 40 were added to 0.5 mg of β -carotene dissolved in 1 ml of chloroform. After evaporation of chloroform by a rotavapor, 100 ml of distilled water saturated with oxygen were added with vigorous stirring. 2.5 ml of this emulsion were transferred into tubes and 350 μ l of each extract or BHT as reference antioxidant with a concentration of 2 mg / ml were added. The kinetics of discoloration of the emulsion in the presence and the absence of antioxidant (negative control in which the sample was replaced by 350 μ l of methanol) was monitored at 490 nm at regular intervals of time for 24 hours. The relative antioxidant activity of the extracts was calculated according to the following equation:

$$\text{Antioxidant Activity (\%)} = \frac{(\text{Abs}_{\text{sample}} / \text{Abs}_{\text{BHT}})}{\text{Abs}_{\text{BHT}}} \times 100$$

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism software (version 5.01 for Windows). The results were presented as mean \pm standard deviation (SD) and were analyzed by the one way analysis of variance (ANOVA) followed by Dunnett's test. For the comparison of the results, the $P \leq 0.05$ values were considered statistically significant.

Results

Total phenolic content

Phenolic compounds are widely distributed in the plant kingdom and have significant antioxidant activity because of their ability to yield hydrogen and to form stable intermediate radicals. The total polyphenols, flavonoids and tannins contents of vegetables and fruits was presented in Table 2 and 3.

Table 2. Polyphenols, flavonoids, tannins content in vegetables

Common name	Polyphenols content in extract ($\mu\text{g GAE/mg}$)	Flavonoids content in extract ($\mu\text{g QE/mg}$)	Tannins content in extract ($\mu\text{g TAE/mg}$)
Artichoke (flower)	108 \pm 5.34	0.89 \pm 0.02	2.74 \pm 0.17
Artichoke (stem)	34.51 \pm 4.35	1.46 \pm 0.03	3.47 \pm 0.07
Bean	360.2 \pm 8.20	11.07 \pm 0.56	7.89 \pm 0.04
Beetroot	87.11 \pm 3.79	2.37 \pm 0.06	17.91 \pm 0.74
Cabbage	62.22 \pm 1.35	0.73 \pm 0.09	4.48 \pm 0.03
Carrot	43.88 \pm 1.72	1.43 \pm 0.05	7.35 \pm 0.04
Cauliflower	340.22 \pm 4.66	2.78 \pm 0.28	5.26 \pm 0.01
Chard	255.88 \pm 4.87	32.13 \pm 0.99	19.59 \pm 0.12
Courgette	305.85 \pm 3.79	19.93 \pm 0.35	21.59 \pm 0.12
Cucumber	69.11 \pm 2.78	0.34 \pm 0.00	5.53 \pm 0.02
Eggplant	292.96 \pm 4.29	4.50 \pm 0.16	8.68 \pm 0.18
Fennel	43.77 \pm 9.23	0.53 \pm 0.01	2.70 \pm 0.21
Green bean	91.92 \pm 9.00	2.53 \pm 0.06	8.47 \pm 0.52
Green pea	259.2 \pm 3.95	10.07 \pm 0.80	9.06 \pm 0.06
Jew's mallow	276.37 \pm 5.30	34.8 \pm 0.80	20.91 \pm 1.24
Lettuce	300.66 \pm 5.97	12.28 \pm 0.25	8.81 \pm 0.25
Onion	245.88 \pm 7.07	4.38 \pm 0.07	6.54 \pm 0.91
Pepper	294.07 \pm 5.62	2.49 \pm 0.20	6.51 \pm 0.01
Potato	167.22 \pm 3.61	2.72 \pm 0.23	5.88 \pm 0.24
Pumpkin	80 \pm 3.14	2.90 \pm 0.00	2.98 \pm 0.51
Tomato	197.40 \pm 9.06	2.08 \pm 0.04	8.04 \pm 0.54

Results were expressed as mean \pm SD, n=3

Total phenolic content

As seen in Table 2, the total polyphenols content in vegetables ranged from 34.51 to 360.2 $\mu\text{g GAE/mg}$ of extract. Bean, cauliflower, courgette and lettuce showed the highest total polyphenol contents with values of 360.2 \pm 8.20, 340.22 \pm 4.66, 305.85 \pm 3.79 and 300.66 \pm 5.97 $\mu\text{g GAE/mg}$ of extract, respectively, followed by pepper (294.07 \pm 5.62 $\mu\text{g GAE/mg}$ extract), eggplant (292.96 \pm 4.29 $\mu\text{g GAE/mg}$ of extract) and Jew's mallow (276.37 \pm 5.30 $\mu\text{g GAE/mg}$ of extract).

However, artichoke stems (34.51 \pm 4.35 $\mu\text{g GAE/mg}$ of extract), fennel (43.77 \pm 9.23 $\mu\text{g GAE/mg}$ of extract), cabbage (62.22 \pm 1.35 $\mu\text{g GAE/mg}$ extract) and cucumber (69.11 \pm 2.78 $\mu\text{g GAE/mg}$ extract) contained low phenolics content.

Concerning the quantification of phenolics in fruits, it was found that the pomegranate contained the highest total polyphenols content (200.51 \pm 1.26 $\mu\text{g GAE/mg}$ of extract) followed by pear (151.55 \pm 1.25 $\mu\text{g GAE/mg}$ of extract), apple (115.77 \pm 0.00 $\mu\text{g GAE/mg}$ of extract).

Table 3. Polyphenols, flavonoids, tannins content in fruits

Common name	Polyphenols content in extract ($\mu\text{g GAE/mg}$)	Flavonoids content in extract ($\mu\text{g QE/mg}$)	Tannins content in extract ($\mu\text{g TAE/mg}$)
Apple	115.77 \pm 0.00	1.19 \pm 0.01	5.33 \pm 0.09
Apricot	48.3 \pm 4.76	0.66 \pm 0.13	2.26 \pm 0.01
Banana	41.55 \pm 2.19	0.04 \pm 0.00	3.65 \pm 0.02
Dates "Deglat-Nour" cultivar	84.15 \pm 3.14	0.22 \pm 0.01	1.86 \pm 0.00
Dates "Ghars" cultivar	56.77 \pm 1.72	0.20 \pm 0.06	2.25 \pm 0.10
Dates "Mech-Degla" cultivar	29.48 \pm 7.18	2.94 \pm 0.05	1.77 \pm 0.06
Grapes (black)	91 \pm 2.98	0.63 \pm 0.02	4.99 \pm 0.07
Grapes (white)	92.11 \pm 3.45	0.41 \pm 0.05	5.17 \pm 0.01
Mandarine orange	115.25 \pm 0.12	0.08 \pm 0.00	10.91 \pm 0.29
Medlar of Japan	80.36 \pm 1.40	0.83 \pm 0.09	1.84 \pm 0.02
Orange	65.22 \pm 1.72	1.14 \pm 0.00	7.50 \pm 0.19
Peach	46.86 \pm 8.30	1.05 \pm 0.11	2.27 \pm 0.01
Pear	151.55 \pm 1.25	1.03 \pm 0.03	5.15 \pm 0.10
Pomegranate	200.51 \pm 1.26	0.86 \pm 0.16	39.44 \pm 0.83

Results were expressed as mean \pm SD, n=3

GAE / mg of extract) and mandarin ($115.25 \pm 0.12 \mu\text{g}$ of GAE / mg of extract) (Table 3).

Total flavonoids content

As shown in Table 2, flavonoids content in vegetables ranged from 0.34 ± 0.00 to $34.8 \pm 0.80 \mu\text{g}$ of QE / mg of extract. Cucumber, turnip and fennel contained the lowest content, while Jew's mallow, chard, courgette and lettuce contained the highest flavonoids content with values of 34.8 ± 0.80 , 32.13 ± 0.99 , 19.93 ± 0.35 and $12.28 \pm 0.25 \mu\text{g}$ of QE / mg of extract, respectively. However, the dates "Mech-Degla", apple, orange, contained the highest content of flavonoids (2.94 ± 0.05 , 1.19 ± 0.01 , $1.14 \pm 0.00 \mu\text{g}$ of QE / mg of extract respectively) (Table 3).

Total tannins content

As shown in Table 2, tannins content in the vegetables was found to be ranged from 2.70 ± 0.21 to $21.59 \pm 1.24 \mu\text{g}$ of TAE / mg of extract. Courgette contained the highest content ($21.59 \pm 0.12 \mu\text{g}$ of TAE / mg of extract) followed by Jew's mallow ($20.91 \pm 1.24 \mu\text{g}$ of TAE / mg of extract), chard ($19.59 \pm 0.12 \mu\text{g}$ of TAE / mg of extract) and beetroot ($17.91 \pm 0.74 \mu\text{g}$ of TAE / mg of extract). Whereas, the pomegranate was the richest fruit in tannins with a content of $39.44 \pm 0.83 \mu\text{g}$ of TAE / mg of extract (Table 3).

In vitro antioxidant activity

The antioxidant activity of 22 vegetables and 14 fruits extracts assessed by DPPH radical scavenging assay, ferrous ion chelating assay, reducing power and β -carotene/linoleic acid bleaching assay were presented in Table 4 and 5.

DPPH radical scavenging activity

In this study (Table 4 and 5), green bean, Jew's mallow, lettuce, eggplant, artichoke (flower), courgette, green pea, chard, pomegranate and black grapes showed high antioxidant activity against DPPH radical with $\text{IC}_{50} \leq 0.8 \text{ mg/ml}$. Pepper, tomato, onion, potato, beetroot, pumpkin, cauliflower, cabbage, bean, white grapes, apple, peach, medlar of Japan and apricot had IC_{50} value ranged between 0.8 and 2 mg / ml. Whereas, carrot, turnip, cucumber, fennel, artichoke (stem), pear, banana, mandarin, orange and dates had low antiradical activity with $\text{IC}_{50} \geq 2 \text{ mg / ml}$.

Ferrous ion chelating activity

As shown in Table 4 and 5, vegetables and fruits can be classified into four groups according to their chelating capacity (high, medium, low chelating capacity).

The chelating potential of the extracts was inversely proportional to the IC_{50} value. Vegetables and fruits with high chelating activity included potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea with $\text{IC}_{50} \leq 0.8 \text{ mg / ml}$. Pepper, onion, eggplant, white grapes and dates "Ghars" showed IC_{50} between 0.8 and 2 mg / ml. Tomato, beetroot, artichoke (stem), pear, black grapes, apple, pomegranate, banana, mandarin, orange, peach, medlar of Japan, apricot, dates "Mech-Degla" and "Deglat-Nour" had low chelating activity ($\text{IC}_{50} \geq 2 \text{ mg / ml}$).

Ferric reducing power

In the same manner, vegetables and fruits have been classified into four groups according to their reducing capacity (high, medium, low reducing capacity).

Table 4 and 5 showed that extracts with high antioxidant activity ($\text{IC}_{50} \leq 5 \text{ mg / ml}$) included onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea. Tomato, eggplant, pumpkin, turnip, cabbage, artichoke (flower), artichoke (stem), and pomegranate had the medium activity with IC_{50} ranged between 5 and 10 mg / ml. However, extracts with low reducing power ($\text{IC}_{50} \geq 10 \text{ mg / ml}$) included pepper, lettuce, carrot, beetroot, cucumber, fennel, cauliflower, pear, black grapes, white grapes, apple, banana, mandarin, orange, peach, apricot, medlar of Japan and dates.

β -carotene/ linoleic acid bleaching assay

According to our results, the tested fruits and vegetables can be classified also into four groups according to their antioxidant activity which is ranging from 13 % to 92% (high, medium, low and very low).

From the 22 vegetable and 14 fruit extracts which were tested for their inhibition of linoleic acid oxidation, 7 vegetables and 2 fruits were found in the group that had high antioxidant activity (> 70%) including lettuce, courgette, artichoke (flower), artichoke (stem),

Jew's mallow, green bean, bean, green peas, peach and apricot. The group having a medium activity (50-70%) was represented by pepper, beetroot, cabbage, chard, mandarin, orange, medlar of Japan. However, tomato, onion, eggplant, potato, cucumber, fennel, black grapes, pomegranate and banana represented a group with a low antioxidant activity (<50%). Carrot, pumpkin, turnip, pear, white grapes, apple and dates showed a very low antioxidant activity percent (<40%) (Table 4 and 5).

Discussion

In the present study, the total phenolic, flavonoids and tannins contents of 14 fruits and 22 vegetables consumed commonly in Algeria were evaluated and their antioxidant activity using four different antioxidant assays were also assessed.

Phenolic compounds such as flavonoids and tannins are widely distributed in fruits and vegetables and have gained much attention due to their antioxidant

Table 4: Antioxidant activities of vegetables extracts

Common name	DPPH radical scavenging assay (IC ₅₀ mg/ml)	Ferrous ion chelating activity (IC ₅₀ mg/ml)	Ferric reducing power (EC ₅₀ mg/ml)	β-carotene / linoleic acid assay bleaching assay (% of inhibition after 24 h of incubation)
Artichoke (flower)	0.36±0.02 ^(d)	0.04±0.01 ^(a)	7.73±0.076 ^(d)	72.12±2.53 ^(d)
Artichoke (stem)	4.02±0.28 ^(d)	2.21±0.16 ^(d)	7.68±0.57 ^(d)	78.73±1.43 ^(d)
Bean	1.58±0.04 ^(d)	0.09±0.00 ^(a)	4.08±0.20 ^(d)	88.42±2.97 ^(a)
Beetroot	1.76±0.01 ^(d)	5.23±0.17 ^(d)	37.30±1.27 ^(d)	51.11±5.37 ^(d)
Cabbage	1.79±0.06 ^(d)	0.22±0.01 ^(d)	9.07±0.18 ^(d)	66.06±3.64 ^(d)
Carrot	3.77±0.06 ^(d)	0.32±0.01 ^(d)	76.25±1.90 ^(d)	35.45±5.38 ^(d)
Cauliflower	1.67±0.03 ^(d)	0.72±0.02 ^(d)	16.75±0.66 ^(d)	72.07±1.55 ^(d)
Chard	0.77±0.01 ^(d)	0.07±0.01 ^(a)	4.95±0.18 ^(d)	64.31±7.69 ^(d)
Courgette	0.48±0.02 ^(d)	0.18±0.01 ^(d)	3.32±0.01 ^(d)	84.89±2.66 ^(c)
Cucumber	7.21±0.24 ^(d)	0.17±0.00 ^(d)	60.91±3.46 ^(d)	47.17±9.22 ^(d)
Eggplant	0.36±0.00 ^(d)	1.27±0.06 ^(d)	7.13±0.09 ^(d)	49.14±5.28 ^(d)
Fennel	3.29±0.07 ^(d)	0.29±0.02 ^(d)	53.28±2.28 ^(d)	47.72±1.20 ^(d)
Green bean	0.04±0.00 ^(b)	0.23±0.00 ^(d)	3.44±0.31 ^(d)	91.70±3.37 ^(a)
Green pea	0.65±0.01 ^(d)	0.69±0.01 ^(d)	3.40±0.11 ^(d)	92.31±7.64 ^(a)
Jew's mallow	0.06±0.00 ^(d)	0.35±0.03 ^(d)	2.30±0.03 ^(d)	76.92±2.93 ^(d)
Lettuce	0.22±0.00 ^(d)	0.19±0.00 ^(d)	12.19±0.48 ^(d)	77.17±5.38 ^(d)
Onion	1.14±0.16 ^(d)	0.97±0.08 ^(d)	4.02±0.18 ^(d)	41.36±5.47 ^(d)
Pepper	1.71±0.01 ^(d)	1.63±0.01 ^(d)	26.02±0.57 ^(d)	53.78±11.54 ^(d)
Potato	1.02±0.11 ^(d)	0.32±0.02 ^(d)	4.32±0.05 ^(d)	48.93±0.46 ^(d)
Pumpkin	1.69±0.03 ^(d)	0.16±0.00 ^(c)	6.59±0.54 ^(d)	21.13±2.92 ^(d)
Tomato	0.96±0.01 ^(d)	10.17±0.83 ^(d)	5.22±0.60 ^(d)	44.39±8.18 ^(d)
Turnip	2.15±0.03 ^(d)	0.18±0.01 ^(d)	5.35±0.44 ^(d)	20.25±3.81 ^(d)
Rutin	0.0072±0.00			
EDTA		0.0064±0.00		
BHT			0.32±0.00	94.94±3.69
H ₂ O				6,41±0.38
MeOH				9.59±0.74

^(a) : No significant difference, ^(b): * (P<0.05), ^(c):** (P<0.01), ^(d): *** (P<0.001) compared to standards
Results were expressed as mean ±SD, n=3

Table 5: Antioxidant activities of fruits extracts

Common name	DPPH radical scavenging assay (IC ₅₀ mg/ml)	Ferrous ion chelating activity (IC ₅₀ mg/ml)	Ferric reducing power (EC ₅₀ mg/ml)	β-carotene bleaching / linoleic acid assay (% of inhibition after 24 h of incubation)
Apple	1.65±0.04 ^(d)	9.18±0.11 ^(d)	19.68±0.48 ^(d)	13.38±0.31 ^(d)
Apricot	1.67±0.03 ^(d)	7.94±1.33 ^(d)	30.00±1.47 ^(d)	75.76±2.00 ^(d)
Banana	9.20±0.87 ^(d)	5.48±0.38 ^(d)	52.30±3.01 ^(d)	45.15±5.21 ^(d)
Dates “Deglat-Nour” cultivar	3.72±0.08 ^(d)	2.04±0.04 ^(d)	108.53±15.98 ^(d)	15.93±2.19 ^(d)
Dates “Ghars” cultivar	4.15±0.13 ^(d)	1.60±0.07 ^(d)	31.73±0.54 ^(d)	13.58±0.08 ^(d)
Dates “Mech-Degla” cultivar	4.59±0.10 ^(d)	2.97±0.04 ^(d)	195.25±10.92 ^(d)	34.69±16.11 ^(d)
Grapes (black)	0.74±0.00 ^(d)	3.29±0.03 ^(d)	16.08±0.74 ^(c)	46.76±0.71 ^(d)
Grapes (white)	1.40±0.11 ^(d)	1.07±0.08 ^(d)	16.12±2.54 ^(c)	22.32±2.59 ^(d)
Mandarin	4.92±0.09 ^(d)	23.41±0.60 ^(d)	52.07±0.02 ^(d)	58.68±3.84 ^(d)
Medlar of Japan	0.95±0.02 ^(d)	18.81±0.06 ^(d)	18.43±0.34 ^(d)	61.01±0.80 ^(d)
Orange	2.48±0.04 ^(d)	6.66±0.18 ^(d)	23.64±2.18 ^(d)	53.18±5.50 ^(d)
Peach	0.98±0.02 ^(d)	21.47±1.44 ^(d)	14.62±0.66 ^(c)	80.97±1.30 ^(d)
Pear	3.16±0.04 ^(d)	11.08±0.89 ^(d)	28.28±0.30 ^(d)	17.17±1.00 ^(d)
Pomegranate	0.32±0.01 ^(d)	2.78±0.36 ^(d)	9.58±0.22 ^(d)	42.02±4.97 ^(d)
Rutin	0.0072±0.00 ^(d)			
EDTA		0.0064±0.00		
BHT			0.32±0.00	94.94±3.69
H ₂ O				6.41±0.38
MeOH				9.59±0.74

^(a) : No significant difference, ^(b): * (P<0.05), ^(c):** (P<0.01), ^(d): *** (P<0.001) compared to standards.

Results were expressed as mean ±SD, n=3

activities and free radical scavenging abilities, which potentially have benefit for human health. Thus, many reports had evaluated the phenolic content of fruits and vegetables (7).

Results obtained in the present study revealed that the level of these phenolic compounds in beans, cauliflower and courgette extract were considerable. In comparison with other studies, the total polyphenols content in tomato, onion, courgette, white grapes, orange, bean, lettuce, eggplant and pepper have been found in this study were higher than those of Cie lik et al (19), Liu et al (20), Baginsky et al (21) and Mokhtar et al (22). However, phenolic content in cauliflower, carrot and pea were lower than those of Dos Reis et al (23) and Kähkönen et al (24). The total phenolic content estimated in pomegranate (200.51 ± 1.26 µg of GAE / mg of extract) was higher than that of Derakhshan et al (25) which was equal to 23.8 ± 6.74 µg GAE/mg of juice extract.

Flavonoids are plant polyphenols found frequently in fruits, vegetables, and grains and are divided into several subclasses including anthocyanins, flavanols (catechins), flavones, flavanones, and flavonols (26).

The flavonoids content in this study was higher in Jew's mallow and chard which were higher than those of Oboh (27) and Sacan and Yanardag (28). However, the flavonoids content in three varieties of dates and courgette were higher than those of other authors (29, 30).

The orange, red, and blue or violet coloration in vegetables, fruits, flowers, and plant storage tissue are due to water-soluble anthocyanins, which are natural pigments reduced from the yellow flavonoids due to loss of oxygen (26). Thus, the anthocyanins detected in eggplant, onion, apple, black grapes and pomegranate may be considered as responsible for the high phenolic content in this study.

Tannins are a group of polyphenols present in various concentrations in many fruits and vegetables

consumed by human. Studies revealed that the phyto-constituents belonging to tannins class possess potent antioxidant activity; some exhibit radical scavenging activity as well (31).

The obtained results showed that the highest level of tannins was detected in the pomegranate which was higher than that of Orak et al (32) who estimated the tannin content in Turkish pomegranate ($16.38 \pm 0.35 \mu\text{g TAE/mg}$ of juice extract). Several classes of pomegranate tannins include ellagitannin such as ellagic acid, punicalagin and punicalin that are found in pomegranate juice and peel showed a great antioxidant activity as reported by Zarfeshany et al (33). Also, many vegetables and fruits have shown to be a rich source of polyphenols, flavonoids and tannins as ferulic, chlorogenic, coumaric and syringic acids, luteolin, quercetin, kaempferol, and catechins which showed marked antioxidant activities (34, 35). However, the comparison the presented results with those of bibliography remains difficult because each study uses a different extraction method.

In this study, the extraction of the polyphenols was carried out by maceration in a hydro-methanol mixture (80% methanol) which is frequently used for the extraction of phenolic compounds, where the solubility of the phenolic compounds was influenced by the degrees of polarity of solvent, the degree of polymerization of the phenolic compounds, and the interaction of the phenolic compounds with other food constituents and the formation of insoluble complexes. Thus, there is no uniform or completely satisfactory procedure suitable for the extraction of all phenols or a specific class of phenolic compounds in plant materials. (36). As mentioned by Pérez-Jiménez et al (37), a procedure for the extraction of antioxidants from plant foods should combine at least two extraction cycles performed with aqueous-organic solvents with different polarities in order to extract antioxidant compounds with different chemical structures. Also, several factors may influence the phenolic content of food plants such as the geographic region where they were cultivated (38, 39), altitude, environmental factors as soil, irrigation, temperature range, light quality, exposure to diseases and pests, the harvest season, industrial processing, the way of drying, storage and method of extraction and quantification (34). Furthermore, many studies showed that the highest polyphenol con-

tent and antioxidant activity has been reported for fruits and vegetables grown in arid zones which is explained by the fact that fruits and vegetables increase their phytochemicals to adapt with abiotic stress (38, 39).

The total antioxidant properties of plants cannot be evaluated by single method because of complex nature of their phytochemicals which may act through different mechanisms. Therefore, two or more methods should always be employed in order to evaluate the total anti-oxidative effects of fruits and vegetables extracts (40). Of these, DPPH scavenging, ferrous ion chelating, reducing power test and β -carotene bleaching assay are used for the evaluation of the antioxidant activities of the extracts.

The radical scavenging activity of extracts of fruits and vegetables was evaluated using DPPH assay which is a commonly used for its rapidity and effectiveness (41). In this assay, the lowest IC_{50} value indicated the more potent antioxidant activity of the extract in terms of hydrogen atom or electron donating capacity.

Results showed that fruits and vegetables extracts exhibited a good DPPH radical scavenging effect that may be related to their higher polyphenols contents. Hence, polyphenol-rich foods found in vegetables and fruits can serve as free radical scavenger. Furthermore, the obtained results corroborate with findings of Jiang et al (42), Lui et al (20), Oboh (27) and Marathe et al (43) who studied the correlation between the antioxidant activity and the polyphenolic content of different varieties of vegetables and fruits. Pincemail et al (44) reported also that fruits and vegetables rich in anthocyanins generally have a greater total antioxidant capacity than those rich in flavanones and flavonols and this may be explain the high antioxidant activity of eggplant, pomegranate and black grapes.

The metal chelating assay is based on the ability of extract to chelate transition metals by binding them to ferrous (Fe^{2+}) ion catalyzing oxidation and disrupting the formation of Fe^{2+} -ferrozine complex (intense red purplish in color). This chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation through the inhibition of lipid peroxides to peroxy and alkoxy radicals via the Fenton reaction (13).

The obtained results showed that the vegetables and fruits extracts exhibited appreciable chelating ef-

fect with the highest antioxidant capacity noticed by potato, lettuce, carrot, courgette, pumpkin, turnip, cucumber, fennel, cauliflower, cabbage, artichoke (flower), Jew's mallow, chard, green bean, bean and green pea. This iron chelating activity of extracts shown in this study could be related to their amount of total phenolic and flavonoids contents. Similar correlations between polyphenols and iron-chelating ability were also noted by Nathan and Brumaghim (45) whose reported that the strong iron-binding properties of polyphenols, whether the iron chelating ability of catechol or gallol containing polyphenols are actually plays a key role in their antioxidant activity and anti-lipid peroxidation by blocking the Fenton reaction. Gebhardt and Fausel (46) mentioned also that artichoke extract have a marked chelating potential which can be due, at least, to some ubiquitous and artichoke-specific polyphenolic and flavonoid compounds.

Reducing power assay is also widely used in evaluating antioxidant activity of plant polyphenols. The samples with higher reducing power show higher absorbance. The presence of reductants like antioxidants in the fruits and vegetables extracts causes the reduction of the ferric to the ferrous form, indicating that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process (41).

Onion, potato, courgette, Jew's mallow, chard, green bean, bean and green pea showed the a considerable reducing power indicating that they can act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. These obtained results agree with other reports on reducing power of plants food who reported that the reducing power of polyphenolics is probably due to the presence of hydroxyl group, which might act as electron donors (47, 48).

β -carotene bleaching test is based on the oxidation of linoleic acid generates peroxide radicals, following the abstraction of hydrogen atoms from diallyl methylene groups of linoleic acid. These free radicals will subsequently oxidize the highly unsaturated β -carotene, thus causing the disappearance of its red color. However, in the presence of an antioxidant compound, this degradation process is prevented. It also reflects the ability to inhibit the lipid peroxidation *in vitro* (13).

In this study, the vegetables and fruits extracts found to hinder the extent of β -carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system which is in agreement with results of many studies (49) who studied the lipid peroxidation of food rich vegetables and fruits. The antioxidant activity percentage of peroxidation inhibition of the presented vegetables and fruits extracts was similar to that reported by Ismail et al (50). Also, Karadeniz et al (51) found close results for pears, grapes and apples. But, pomegranate showed a lower antioxidant activity (42.02%) than that found in the study of Singh et al (52).

The statistical analysis indicated that legumes (green bean, bean and green pea) and courgette had significantly high antioxidant activity (91.70%, 88.42%, 92.31%, 84.89%) compared with BHT as a reference antioxidant and the same results were reported by Amrowicz and Pegg (53). Also, previous studies indicated the good correlation between the antioxidant capacity of the fruits and vegetables and their phenolic content (54). However, many studies have found no correlation between total polyphenols content and antioxidant activity of plant extracts (50, 55) and this may be explained by the fact that the molecular antioxidant response of the phenolic compounds varies considerably according to their chemical structure (56). Thus, the antioxidant activity of fruits and vegetables depends on not only to its content of phenolic compounds but also on the type of phenolics and their relative distribution (57) and the interactions between antioxidants (58).

Conclusion

This study showed that consumed fruits and vegetables in Algeria contain polyphenols, flavonoids and tannins which are affected mainly by the geographical region and harvesting time and showed a good antioxidant activity in relation to their phenolic content and their consumption may deliver greater health benefits thought the supply of natural antioxidant. So, the use of a balanced diet containing enough fruits and vegetables as a source of natural antioxidants could be much more effective and economical than artificial supplementation with antioxidants such as ascorbic acid

or -tocopherol for body protection against oxidative stress. Also, this work highlighted that it is important to use different free radicals and oxidation systems to evaluate the antioxidant activity of fruit and vegetable extracts because the extracts didn't present the same results in all methodologies and this could be due to difference in chemical composition of extracts and different mediums and principals of technics.

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