

Received:  
3 August 2018  
Revised:  
20 March 2019  
Accepted:  
23 April 2019

Cite as: Nadia Zeghad,  
Ejaz Ahmed,  
Abdelmalik Belkhiri,  
Yvan Vander Heyden,  
Kristiaan Demeyer.  
Antioxidant activity of *Vitis  
vinifera*, *Punica granatum*,  
*Citrus aurantium* and *Opuntia  
ficus indica* fruits cultivated in  
Algeria.  
Heliyon 5 (2019) e01575.  
doi: 10.1016/j.heliyon.2019.  
e01575



# Antioxidant activity of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* fruits cultivated in Algeria

Nadia Zeghad<sup>a,\*</sup>, Ejaz Ahmed<sup>b,c</sup>, Abdelmalik Belkhiri<sup>d</sup>, Yvan Vander Heyden<sup>c</sup>,  
Kristiaan Demeyer<sup>c</sup>

<sup>a</sup> *Laboratory of Pharmacology and Toxicology, Faculty of Sciences of Nature and Life, University Constantine 1, Algeria*

<sup>b</sup> *Department of Botany, Faculty of Sciences, PMAS-Arid Agriculture University Rawalpindi, Pakistan*

<sup>c</sup> *Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Vrije Universiteit Brussel, VUB, Belgium*

<sup>d</sup> *Laboratory of Pharmacognosy, Faculty of Medicine, University Constantine 3, Algeria*

<sup>e</sup> *Department of Toxicology, Dermato-Cosmetology and Pharmacognosy, Vrije Universiteit Brussels, VUB Brussels, Belgium*

\* Corresponding author.

E-mail address: [zeghadnadia@umc.edu.dz](mailto:zeghadnadia@umc.edu.dz) (N. Zeghad).

## Abstract

Four edible fruits from *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* from Algeria were analyzed in order to determine the total polyphenol, total flavonoid and flavonol contents using classical spectrophotometric methods. The antioxidant activity was established by three different single-electron-transfer-based assays (ABTS radical cation, FRAP, and DPPH assays) and one hydrogen-atom-transfer based assay (ORAC). Among the four fruits tested, the *Vitis vinifera* hydroalcoholic extract showed the highest antioxidant capacity with all methods. The results indicated that the antioxidant activity and the total phenolic content of the selected plants are significantly different ( $p < 0.001$ ).

Keywords: Food analysis, Analytical chemistry

## 1. Introduction

Plant-based foods are not only important sources of macronutrients (lipids, sugars, carbohydrates and proteins), but also of secondary metabolites. The latter are becoming increasingly popular for their beneficial effects on consumer health, for instance, by providing antioxidants. In the recent years the focus on natural antioxidants, especially from plant material as like vegetables and fruits, has tremendously increased (Bunea et al., 2011). An inverse association has been perceived between age-related disorders and the consumption of fruits and vegetables. This property may be due to the fact that antioxidant agents, especially phenolic compounds, which are commonly occurring antioxidants in fruits and vegetables, commonly consumed (Dudonne et al., 2009). Some authors indicate that most frequently the antioxidant activities of a fruit or vegetable may be due to the presence of polyphenolic compounds (Karadeniz et al., 2005). Epidemiological studies indicate that a considerably higher amount of natural antioxidants in the human diet may protect us against various life threatening diseases, like hepatitis and aging, cancer (Bunea et al., 2011). The development of these disorders is mainly due to the presence of reactive oxygen species (ROS) in the human body (Ma et al., 2011). By stimulating and sustaining cellular defense mechanisms, antioxidants can help preventing cellular components against oxidative damage caused by free-radical species (Kumaran and Karunakaran, 2007). Therefore a sufficient amount of diet-based antioxidants are required to counter the ROS. Antioxidants are now a days commercially used in food products to increase the shelf life. A large number of these antioxidants is of synthetic origin, which poses considerable health risks. Therefore a growing concern to replace these synthetic antioxidants by natural is observed (Dudonne et al., 2009). For these reasons the antioxidant activity is the most commonly determined among the different potential biological activities of plant extracts. Several studies on antioxidant capacity of fruit extracts have been recently reviewed (Maria et al., 2000; Kriengsak et al., 2006). Results showed that some fruits extracts from pineapple, mango, papaya, apple, berries and others exhibited high level of antioxidant power (Maria et al., 2000; Kriengsak et al., 2006).

The fruits of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* from the north of Africa developed in the Mediterranean climate, were selected for the present study because they are widely consumed in Algeria and thus play an ample role in the diet nutraceutical value, more generally in all countries of the Maghreb. To the best of our knowledge, no studies have been carried out yet

on these Algerian fruits, regarding their antioxidant activities. Our present research aims to quantify the antioxidant capacity of the hydroalcoholic fruit extracts of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica*. The results obtained by the ORAC, DPPH, ABTS and FRAP assays were compared and correlated with the total polyphenols, flavonoids and flavonols contents. The principal flavonoids present in the selected fruit extracts were also determined by an HPLC method.

## 2. Material and methods

### 2.1. Plant material

Four different fruits were purchased during their maturation periods from the local market of Constantine (North-East Algeria). These included *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* (Table 1). One sample of each fruit was properly washed and air dried. The edible parts of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* were freeze-dried, grinded and stored at  $-25\text{ }^{\circ}\text{C}$  until analysis.

### 2.2. Sample preparation

The samples were extracted following the methods of Babero et al. (2008) and Ma et al. (2009). Samples of 25g lyophilized edible fruits of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* were macerated at room temperature for 24 h with 500 mL methanol/water (70/30, v/v) along with ultrasound extraction for 30 min (Fisher scientific fb 15046, Leicestershire, England) ( $>20\text{ kHz}$  in frequency). After filtration, the remaining vegetal material was re-extracted twice applying same procedure. The filtrates were combined and concentrated in a rotavap (Buchi, Switzerland) under vacuum at low temperature ( $<40\text{ }^{\circ}\text{C}$ ). The extracts were lyophilized (Christ Alpha 2–4 LD plus, Osterode am Harz, Germany) and stored at under low temperature ( $-25\text{ }^{\circ}\text{C}$ ) for future use.

**Table 1.** Characterization of the plant material.

Species	Vegetative part	Vegetative stage	Used parts
<i>Vitis vinifera</i> L (Grapes)	Berry of grapes	Mature fruit (September 2013)	Whole fruit with seeds and envelope
<i>Punica granatum</i> L (Pomegranate)	Berry of pomegranate	Mature fruit (September 2013)	Fruit without bark
<i>Citrus aurantium</i> L (Bitter orange)	Berry of bitter orange	Mature fruit (January 2014)	Fruit without bark
<i>Opuntia ficus-indica</i> L (Prickly pear)	Berry of prickly pear	Mature fruit (September 2013)	Fruit without bark

### 2.3. Total phenolic content determination

The total phenolic content (TPC) of the plant samples was determined by the Folin Ciocalteu assay, described by [Adesegun et al. \(2007\)](#). 1.0 mL of extract dissolved in distilled water was mixed with 5.0 mL Folin-Ciocalteu reagent (10%) and 4.0 mL sodium carbonate (0.7M). After 2 h incubation at room temperature, the absorbance was recorded at 760 nm (Spectroscan 60DV UV/Vis spectrophotometer, [Biotech Engineering Management Co. Ltd](#), Cyprus) against water blank. A calibration curve was constructed with gallic acid standards in water (0–0.1 mg/mL). All assays were repeated three times ( $n=3$ ). Total phenolic content was expressed as mg GAE/g dry fruit extract (mean  $\pm$  standard deviation) (GAE: gallic acid equivalent).

### 2.4. Total flavonoid content determination

The total flavonoid content of the samples was determined applying a colorimetric method ([Ayoola et al., 2008](#)). 2.0 mL extract, dissolved in ethanol, was added to 2.0 mL  $\text{AlCl}_3$  reagent (2%) in ethanol. Absorbance was recorded at 420 nm after 30 min incubation at room temperature against an ethanol blank. A calibration curve with quercetin standards in ethanol (0–0.1 mg/mL) was developed. All assays were repeated three times ( $n=3$ ). Total flavonoid content was expressed as mg QE  $\text{g}^{-1}$  dry fruit extract (mean  $\pm$  standard deviation) (QE: quercetin equivalents).

### 2.5. Flavonol content determination

The flavonol content of the samples was determined since flavonols represent the main fraction of compounds responsible for the antioxidant activity ([Tapan, 2011](#)). The content was estimated according to [Oyedemi et al. \(2010\)](#). 2.0 mL of the sample dissolved in ethanol was mixed with 2.0 mL  $\text{AlCl}_3$  (2%) prepared in ethanol (96%) and 3.0 mL sodium acetate solution (50 g/l). The absorbance at 440 nm was recorded after 2.5 h incubation at 20 °C. A calibration curve with quercetin standards in ethanol (0–0.1 mg/mL) was constructed. All assays were repeated three times ( $n=3$ ). Total flavonol content was expressed as mg QE  $\text{g}^{-1}$  dry fruit extract (mean  $\pm$  standard deviation).

### 2.6. Antioxidant activities

#### 2.6.1. DPPH antioxidant assay

The free radical scavenging activity was determined spectrophotometrically against the stable DPPH radical. 2.0 mL of each concentration of fruit samples (0.1–1 mg/mL), prepared in methanol, was mixed with an equal volume of a methanolic DPPH solution (0.004 %). The absorbance of each sample was determined at 517 nm after

incubation for 30 min at room temperature. Trolox was used as positive control. Scavenging activity (%) was calculated from absorbance values using the following equation:

$$\text{Percentage Scavenging activity} = [(A - B)/A] \times 100$$

**With A:** the absorbance of the control sample (DPPH solution)

**B:** the absorbance of each sample dilution

All assays were repeated three times ( $n=3$ ). The antioxidant activities of the fruits and control were estimated by determining the  $IC_{50}$  (mg/mL) values, i.e. the concentration required to scavenge 50% DPPH radicals, expressed as mean  $\pm$  standard deviation (Talukder et al., 2013).

### 2.6.2. ABTS radical scavenging assay

The assay utilizes ABTS radical produced by the oxidation of  $ABTS^{2-}$  with potassium persulfate. The ABTS radical scavenging assay was performed following the method described in Re et al. (1999). 7mM ABTS solution at pH 7.4 (5mM  $NaH_2PO_4$ , 5mM  $Na_2HPO_4$ , 154mM NaCl) was mixed with 2.5mM potassium persulfate (v/v) to generate the  $ABTS^\circ$  radical. The solution was allowed to stand for 16 hrs of storage in the dark before use. The mixture was diluted with ethanol to adjust the absorbance at  $0.70 \pm 0.02$  at 734 nm. For each sample, 100  $\mu$ l of various concentrations (0.1–1 mg/mL), dissolved in buffer (5mM  $NaH_2PO_4$ , 5mM  $Na_2HPO_4$ , 154mM NaCl) was allowed to react with freshly prepared ABTS solution (900 $\mu$ l) at room temperature. After six minutes the absorbance was measured. A standard curve was prepared based on different Trolox concentrations in water (0.01–0.1 mg/mL). All assays are repeated three times ( $n=3$ ).  $IC_{50}$  value (mg/L), which represents the concentration of a sample required to scavenge 50% of the ABTS radicals, expressed as mean  $\pm$  standard deviation was calculated. The same equation as in the DPPH assay was used here.

### 2.6.3. Ferric reducing antioxidant power (FRAP)

The reducing power of *Vitis vinifera*, *Punica granatum*, *Opuntia ficus indica*, and *Citrus aurantium* was determined by the method of Hseu et al. (2008). 1.0 mL of each sample diluted in buffer (0.1–1 mg/mL) was mixed with 1.0 mL phosphate buffer (0.2M, pH 6.6) and 1.0 mL potassium ferricyanide [ $K_3Fe(CN)_6$ ] (1%). Incubation at 50 °C was performed for 20 min. The 1.0 mL trichloroacetic acid (TCA) (10%) was added to the solution, followed by centrifugation at 3000 rpm for 10 min. 1.5 mL distilled water and 150 $\mu$ l  $FeCl_3$  (0.1%) were then mixed with the supernatant and the absorbance was recorded at 700 nm. Rutin was used as reference material. All assays of *Vitis vinifera* L, *Punica granatum* L, *Citrus aurantium* L or

*Opuntia ficus indica* L are repeated three times ( $n=3$ ). The  $EC_{50}$  (mg/mL) i.e. the effective concentration of an extract that reduces 50% of the ferric iron  $Fe^{3+}$  to ferrous iron  $Fe^{2+}$  expressed as mean  $\pm$  standard deviation, was estimated.

#### 2.6.4. Oxygen radical absorbance capacity (ORAC) assay

A 96-well black-walled plate was used to perform the ORAC assay. Each sample, Trolox standard or blank (25  $\mu$ l) were diluted in phosphate buffer (75 mM, pH 7.4) in 2.5% DMSO, and 150  $\mu$ l of fluorescein ( $8.16 \cdot 10^{-5}$  mM). The plate was covered and incubated for 10 min in a preheated FL 600 microplate reader (37  $^{\circ}$ C) with 3 min of gentle shaking. Consecutively, 25  $\mu$ L AAHP (153 mM) was added and the plate was then immediately shifted to the plate reader. The fluorescence was measured every minute for 35 min, using an excitation filter set at 485/20 nm and an emission filter at 528/20 nm (VICTOR<sup>3</sup> 1420: Perkin-Elmer, USA). For each sample and standard the net area under the fluorescence curve (AUC) was calculated using the Eq. (1). Then using the regression line equation final ORAC values were calculated, Eq. (2). Final results are expressed as Trolox equivalents  $\mu$ M/gram of dry fruit extract samples (Dejian et al., 2002). All tests are repeated three times ( $n=3$ ).

$$AUC = 0.5 + f_1/f_0 + \dots\dots\dots f_i/f_0 + \dots\dots\dots f_{34}/f_0 + (f_{40}/f_0) \quad (1)$$

$$ORAC(\mu MTE) = (AUC_{sample} - AUC_{blank}) / (AUC_{Trolox} - AUC_{blank}) \times C_{Trolox} * k \quad (2)$$

With  $f_0$  the initial fluorescence reading at 0 min and  $f_i$  the fluorescence reading at time 1,  $C_{Trolox}$  the concentration ( $\mu$ M) of Trolox (25  $\mu$ M) and  $k$  the sample's dilution factor.

## 2.7. HPLC identification of phenolic compounds and flavonoids

Chromatographic analysis for the identification of phenolic acids and flavonoids was carried out using four coupled Chromolith Performance RP-18e columns (100 mm  $\times$  4.6mm I.D) from Merck (Darmstadt, Germany). The mobile phase used was trifluoroacetic acid 0.05% (v/v) in water to which methanol was added in a linear gradient from 95:5 to 5:95 (v/v) within 90 min and added an isocratic part till 100 min, with a flow rate of 1.0 mL/min. UV detection was performed at 280 nm and 325 nm using a Lachrom Merck Hitachi L-7400 UV Detector (Alaerts et al., 2014).

Phenolic acids and flavonoids found in the fruit extracts were identified by comparison with the retention times and UV-spectra of authentic reference standards.

### 3. Results and discussion

#### 3.1. Total polyphenols, flavonoids, and flavonols

Results are given in the Table 2 and reveal that the total polyphenol contents ranged from  $1.95 \pm 0.07$  to  $23.03 \pm 0.17$  mg GAE  $g^{-1}$ . *Vitis vinifera* extracts showed the highest content followed by *Punica granatum*, whereas *Opuntia ficus indica* had the lowest polyphenolic content. Total flavonoid and total flavonol contents varied from  $1.35 \pm 0.02$  to  $14.37 \pm 0.08$  mg QE  $g^{-1}$  and from  $2.24 \pm 0.04$  to  $5.34 \pm 0.02$  mg QE  $g^{-1}$  respectively. These results suggest that the selected fruits especially *Vitis vinifera*, *Punica granatum* and *Citrus aurantium* are rich in polyphenols and flavonoids, which are well known for their free radical scavenging and antioxidant activities (Faccim de Braum et al., 2013; Nivedhini et al., 2014). These results are in accordance with earlier findings. For example, for the phenolic content, Baydar et al. (2004), Shams Ardekani et al. (2009), Moulehi et al. (2012) and Chougui et al. (2013) reported comparable results for *Vitis vinifera* ( $29.55 \pm 3.2$  mg GAE  $g^{-1}$ ), *Punica granatum* ( $14.94 \pm 1.6$  mg GAE  $g^{-1}$ ), *Citrus aurantium* ( $16.80 \pm 0.25$  mg GAE  $g^{-1}$ ) and *Opuntia ficus indica* ( $2.45$  mg GAE  $g^{-1}$ ) fruits respectively.

#### 3.2. Antioxidant activities

The edible parts of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* fruits were selected because they are the most frequent seasonal fruits found in the Algerian alimentary market and play an important role in the diet of local people as they are widely consumed. To the best of our knowledge no studies have been carried on the Algerian *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* edible fruits parts regarding their antioxidant activities and chemical composition responsible for these activities. Information on these activities

**Table 2.** Total phenolic (TPC), flavonoid (TFC) and flavonol (TFLC) contents of the selected fruits and their antioxidant activities.

Sample	TPC (mg/g) GAE $g^{-1}$	TFC (mg/g) QE $g^{-1}$	TFLC (mg/g) QE $g^{-1}$	DPPH IC <sub>50</sub> (mg/mL)	ABTS IC <sub>50</sub> (mg/mL)	FRAP IC <sub>50</sub> (mg/mL)	ORAC IC <sub>50</sub> ( $\mu$ M TE/g)
VV	$23.06 \pm 0.17$	$14.37 \pm 0.04$	$5.34 \pm 0.02$	$0.270 \pm 0.001$	$0.040 \pm 0.003$	$0.98 \pm 0.01$	$2036 \pm 46$
PG	$15.39 \pm 0.08$	$12.95 \pm 0.07$	$4.00 \pm 0.06$	$0.600 \pm 0.003$	$0.58 \pm 0.03$	$2.61 \pm 0.02$	$1328 \pm 11$
CA	$14.34 \pm 0.30$	$7.27 \pm 0.04$	$3.99 \pm 0.04$	$0.810 \pm 0.005$	$0.49 \pm 0.03$	$3.82 \pm 0.01$	$842 \pm 20$
OFI	$1.95 \pm 0.07$	$1.35 \pm 0.02$	$2.24 \pm 0.04$	$3.52 \pm 0.03$	$0.80 \pm 0.05$	$8.04 \pm 0.02$	$1043 \pm 24$

**Abbreviations:** VV: *Vitis vinifera*, PG: *Punica granatum*, CA: *Citrus aurantium*, OFI: *Opuntia ficus-indica*. GAE: gallic acid equivalent. QE: quercetin equivalent.  $\mu$ M TE/g:  $\mu$ M Trolox equivalent/g dry extract. Values are expressed as Mean  $\pm$  SD ( $n=3$ ).

is also limited. This study was therefore undertaken to obtain some data in relation to this aspect.

The most commonly employed antioxidant assays are divided in two major categories: methods based on single electron transfer (ET), including the ABTS, FRAP and DPPH assays, and on hydrogen-atom transfer (HAT) including the ORAC assay. The DPPH assay is a simple inexpensive assay which utilizes a stable, nitrogen containing, purple colored radical, which is reduced by natural antioxidants or reducing compounds to a pale yellow colored hydrazine (Mohd Azman et al., 2013). The FRAP method is also a quick bioassay in which yellow colored ferric tripyridyltriazine complex (Fe(III)-TPTZ) is reduced by phenolic compounds to a blue colored ferrous complex (Fe(II)-TPTZ) due to the electron donating abilities of its compounds (Mohd Azman et al., 2013). The ORAC assay measures the scavenging of peroxy radicals, which are formed from AAHP radicals by spontaneous decomposition. ORAC is a widely used assay for flavonoids and phenolic acids.

The electron transfer (ET) based assays utilize the ability of an active compound (antioxidant) to reduce an oxidant, which upon reduction changes color. The degree of color change is then correlated with the antioxidant activity of the sample. The HAT-based assays involve the utilization of a competitive environment between antioxidant compound and thermally generated peroxy radicals (Dudonne et al., 2009). Because of the complexity and heterogeneity of the chemical compounds responsible for the total antioxidant capacity of botanical products, this capacity cannot accurately be determined by applying only one single assay. Different tests, involving multiple-reaction characteristics and mechanisms, must be used in order to obtain a reliable picture.

The different extracts were assayed for their capacity to neutralize free radicals. A smaller IC<sub>50</sub> value indicates a higher antioxidant activity. For the DPPH, ABTS and FRAP (hydrogen atom transfer based) assays, the lowest IC<sub>50</sub>-values (highest antioxidant capacity) were found for *Vitis vinifera* regardless of the type of assay applied (Table 2). Highest IC<sub>50</sub> values were obtained with *Opuntia ficus-indica*. In case of the ORAC, highest value was shown by *Vitis vinifera*.

Finding a high correlation coefficient ( $r$ ) can provide information on the relationship between variables. The value of ' $r$ ' was considered positively high when  $0.68 \leq r$  (Fidrianny et al., 2013). The scavenging values of DPPH, ABTS and FRAP are all highly correlated with total polyphenol contents ( $r^2 = 0.90, 0.85, 0.98$  respectively), but the latter was not correlated with the ORAC-assay ( $r^2 = 0.50$ ).

Different active phytochemicals may synergistically increase the antioxidant capacity based upon the nature of their phenolic groups, upon their mechanisms of working and upon the experimental conditions used for antioxidant activity

measurements (Faccim de Braum et al., 2013). Antioxidant capacity may also be reduced by interference from non-antioxidant compounds (Bhourri Mnari et al., 2016). A single assay will therefore never reflect the entire potential antioxidant capacity of a sample. Hence, only by combining the results of different assays, more reliable information about the potential activity of the compounds present in the samples can be achieved (Bunea et al., 2011; Elfalleh et al., 2012).

In view of the good correlation found for the flavonoids content in the fruit extracts, it may be assumed that these compounds exert a significant antioxidant capacity. It is well known that this activity is strongly influenced by the specific flavonoid structure (Amić et al., 2003). Higher antioxidant activity is associated with OH-groups in ortho C3'4', OH-function in C3, oxo function in C4 and a double bond between C2 and C3.

These results are in good agreement with previous findings where high correlation was found between different methods used for determining antioxidant activity and the total phenolic content in different plant samples, especially fruits (Dudonne et al., 2009; Floegel et al., 2011). Phenolic compounds are abundantly present secondary metabolites in plants, especially fruits, and impart antioxidant activity (Macheix et al., 1990). A strong correlation between total phenolic content and antioxidant activity estimated by FRAP assay is also reported for fruit juices (Gardner et al., 2000). Based upon the DPPH and ABTS assays, Gorinstein et al. (2010) reported a strong correlation between the polyphenol content and antioxidant activity in three exotic fruits.

The results show that the edible parts of the fruits investigated appear to play a significant role in their antioxidant activity. These edible parts present a reservoir of important bioactive compounds that may be helpful in sustaining good health. HPLC analysis was employed to determine the composition in phenolic acids and flavonoids of the four fruit species tested. Because of the great diversity of phenolic compounds in extracts it is difficult to identify all. Some specific compounds can however be identified based on the retention times obtained with specific reference substances. In Table 3 the retention times obtained for these reference compounds are given.

The HPLC profiles for our four fruit extracts are shown in Figs. 1, 2, 3, and 4. Based on their retention times, phenolic acids (cafeic acid, cinnamic acid, benzoic acid) and four flavonoids (catechin, epicatechin gallate, esculetin and quercetin) were identified in all selected fruit species. Coumaric acid, hesperidin, quercitrin, kaempferol, and rhamnetin were found in *Vitis vinifera* and *Citrus aurantium*. Rutin, epicatechin, gallic acid and chlorogenic acid were only detected in the fruit extract of *Punica granatum*. Naringenin was only found in *Citrus aurantium*. The results showed that apigenin was absent in all four fruit extracts. Grapes are the fruits that contain

**Table 3.** Retention times obtained with reference compounds.

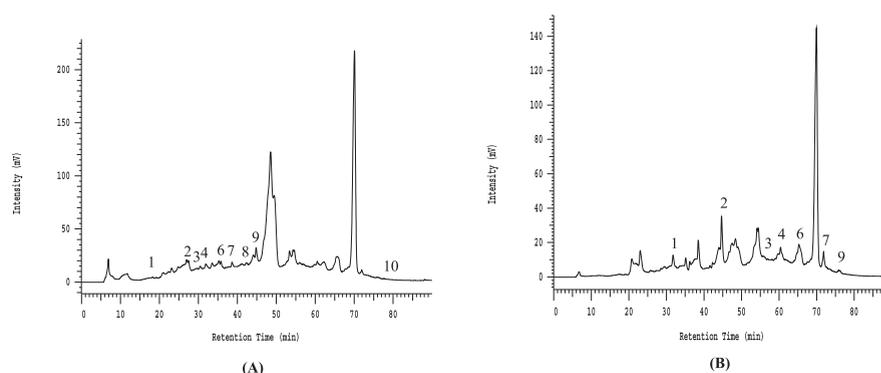
Authentic standards	Retention time (min)	Authentic standards	Retention time (min)
Gallic acid*	11.95	Esculetin**	30.11
Catechin*	27.17	Hesperedin**	53.49
Chlorogenic acid*	31.17	Rutin**	55.45
Cafeic acid*	31.87	Quercetin**	59.44
Vanillic acid*	32.6	Naringenin**	64.27
Epicatechin gallate*	34.41	Quercitrin**	66.11
Epicatechin*	36.08	Kaempferol**	71.95
Cinnamic acid*	43.12	Apigenin**	72.99
Benzoic acid*	46.12	Rhamnetin**	76
Coumaric acid	76.61	/	/

\*: detected at 280 nm; \*\*: detected at 325 nm.

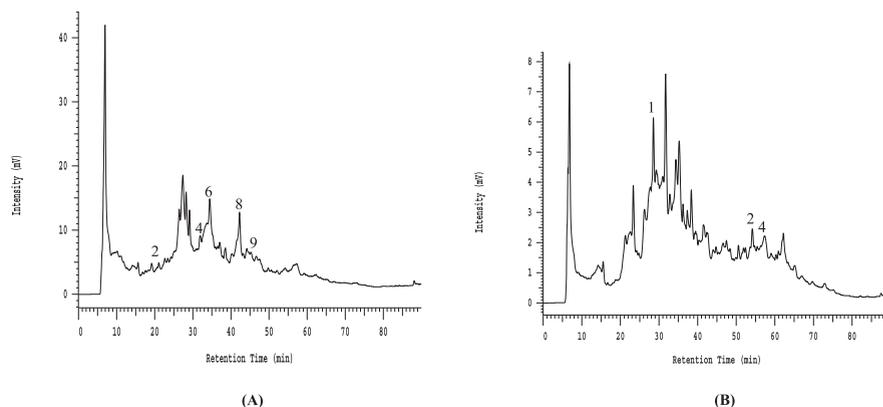
the highest amount of phenolic compounds (Macheix et al., 1990). Their phenolic compounds are mainly found in peels, pulps, and seeds.

Godevac et al. (2010) divided the phenolic compounds of grape fruits into two main categories: phenolic acids (mainly present in pulp and peel) and flavonoids. The condensed tannins, constituted by the flavan-3-ols (monomeric and polymeric), are localized principally in the outer seed coat (about 3–5 times more than in the endosperm).

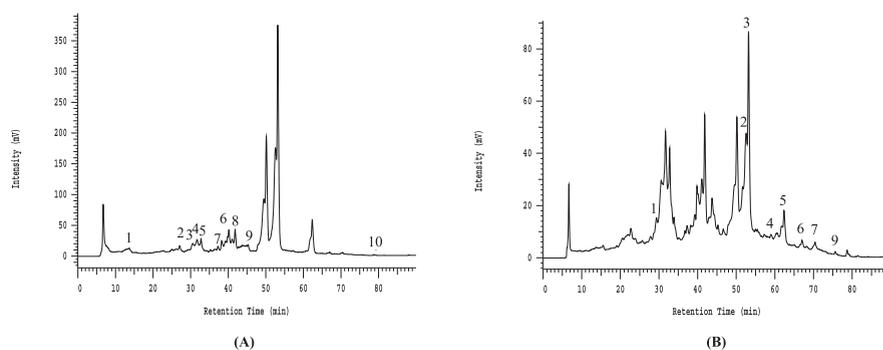
In the present study, the HPLC results of the hydroalcoholic extract of *Vitis vinifera* fruit show the presence of well-known phenolic acids and flavonoids (Fig. 1). These include gallic acid, chlorogenic acid, caffeic acid, cinnamic acid, benzoic acid, catechin, epicatechin gallate, coumarin, esculetin, hesperidin, rutin, quercetin, quercitrin,



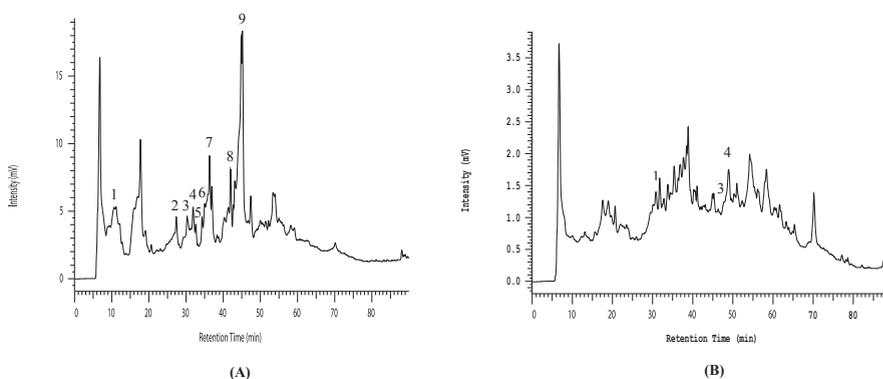
**Fig. 1.** HPLC profile at 280 nm (A) and 325 nm (B) of the fruit extract of *Vitis vinifera*. Identified compounds: (A): 1: chlorogenic acid, 2: catechin, 3: gallic acid, 4: caffeic acid, 6: epicatechin gallate, 7: epicatechin, 8: cinnamic acid, 9: benzoic acid, 10: coumaric acid. (B): 1: esculetin, 2: hesperidin, 3: rutin, 4: quercetin, 6: quercitrin, 7: kaempferol, 9: rhamnetin.



**Fig. 2.** HPLC profile at 280 nm (A) and 325 nm (B) of the fruit extract of *Punica granatum*. Identified compounds: (A) 2: catechin, 4: caffeic acid, 6: epicatechin gallate, 8: cinnamic acid, 9: benzoic acid. (B): 1: esculetin, 2: hesperidin, 4: quercetin.



**Fig. 3.** HPLC profile at 280 nm (A) and 325 nm (B) of the fruit extract of *Citrus aurantium*. Identified compounds: (A): 1: gallic acid, 2: catechin, 3: chlorogenic acid, 4: caffeic acid, 5: vanillic acid, 6: epicatechin gallate, 7: epicatechin, 8: cinnamic acid, 9: benzoic acid, 10: coumaric acid. (B): 1: esculetin, 2: hesperidin, 3: rutin, 4: quercetin, 5: naringenin, 6: quercitrin, 7: kaempferol, 9: rhamnetin.



**Fig. 4.** HPLC profile at 280 nm (A) and 325 nm (B) of the fruit extract of *Opuntia ficus indica*. Identified compounds: (A): 1: gallic acid, 2: catechin, 3: chlorogenic acid, 4: caffeic acid, 5: vanillic acid, 6: epicatechin gallate, 7: epicatechin, 8: cinnamic acid, 9: benzoic acid. (B): 1: esculetin, 3: rutin, 4: quercetin.

kaempferol and rhamnetin. These results are partly similar to the findings of [Gursoy \(2012\)](#) who found as major phenolic compounds in *Vitis vinifera* fruit benzoic acid, *p*-coumaric acid, gallic acid, caffeic acid, and cinnamic acid. [Godevac et al. \(2010\)](#) also described as most commonly found phenolic acids in grapes gallic acid, benzoic acid and cinnamic acid. The flavonoids found in grapes include red and blue anthocyanins, flavonols and colorless flavan-3-ols. Flavan-3-ols, including catechin and epicatechin, are the flavonoids abundantly found and isolated from grape seeds and peels.

The results presented in [Fig. 2](#) demonstrate that *Punica granatum* fruit extracts exhibit a number of antioxidant compounds. Some HPLC-based reports claim the presence of various phenolic compounds in pomegranate fruit. The present study reveals also different phenolic compounds, like caffeic, cinnamic and benzoic acids, catechin, epicatechin gallate, esculetin, hesperidin and quercetin. [Hmid et al. \(2013\)](#) reported also the presence of caffeic acid, chlorogenic acid, gallic acid, quercetin, catechin and epicatechin in pomegranate. In addition, they also reported on the presence of rutin, ferulic acid, chlorogenic acid and ellagic acid. The absence of the latter compounds in our sample may be due to the fact that the polyphenolic content differs qualitatively and quantitatively from one plant to another and from one organ to another of the same species. This may be due to several factors: biotic conditions, harvesting conditions, cultivation practice, ripening stage, growth location, seasonality, drying temperature, extraction solvent polarity and the extraction method ([Aganga and Mosase, 2001](#); [Bhourri Mnari et al., 2016](#)):

**\*Biotic conditions (external factors):** plants have developed diverse protection strategies against stressful factors; on the one hand, the development of structures like thorns, glandular hairs and foliar trichomes; on the other, the production of secondary metabolites. The latter act as defense against predators and pathogens, inhibiting insect growth and development, acting as allelopathic agents, enhancing the attraction of pollinators, the resistance to viruses, the protection against ultraviolet radiation, even acting as signal molecules for nodulation in vegetable-rhizobium symbiosis and as antioxidant agents.

Secondary metabolites vary qualitatively and quantitatively between species, but also between populations of the same species, between individuals of those populations and between the organs of a certain individual, to such an extent that some authors defend phenolic quantification as an approach method to assess the level of chemical defense of a plant. The synthesis of secondary metabolites varies and is induced by ecological factors such as UV-light, hydric stress and temperature. These compounds have been shown to function as UV filters, as protectors against herbivores, and as allelopathic agents ([Valares Masa et al., 2016](#)).

**\* Development stage of the plant, harvesting conditions and ripening stage:** The lowest total phenolic compounds is seen at the beginning of maturation and

gradually increases with the pulp ripening stage. Such differences might be related to changes in the secondary metabolism (Tomsone and Kruma, 2017).

\* **Extraction method:** the extraction yield and consequently, the biological activity of vegetal extracts can be strongly affected by the solvent applied. Recent studies showed that the total phenolic content extracted from plants was affected by the solvent polarity; for example, solvents with low polarity, such as ethyl acetate, are less efficient than more polar solvents. Water and ethanol or methanol are often recommended for extract preparation because of their polarity. The use of organic solvents for extractions has several disadvantages, such as: (a) solvent residue in the product, (b) worker exposure, (c) disposal of waste solvents, and (d) environmental pollution. Several studies have shown that ethanol, methanol and water are effective for polyphenol extraction (Abarca-Vargas et al., 2016).

\* **Drying temperature:** drying methods that use high temperatures to dehydrate plant extracts cause a dramatic loss of phenolic compounds and antioxidant activity, which reaches up to 60%. Other drying methods that remove moisture at low temperatures avoid degradation of the active constituents. Many drying methods, such as convection oven drying, freeze-drying and microwave drying, are also necessary used to preserve medicinal herbs (Abdullah et al., 2018).

Ali et al. (2014) reported the presence of phenolic compounds, such as ellagic acid and gallic acid, along with punicalagin, in *Punica granatum* fruit peel. Kaempferol was not detected in our extracts. This finding is in good agreement with a previous report of Kulkarni and Aradhya (2005), where no detectable amounts of kaempferol could be found in eight Iranian pomegranate fruit.

The *Citrus* plant also contains a large variety of flavonoid derivatives, including naringin, hesperidin and polymethoxylated flavones, which are unique to it, while compounds, such as rutin and quercetin are much common in the plant kingdom (Nogata et al., 2006). Mostly, *Citrus* species deposit ample amount of various flavonoids during their organ development (Mata Bilbao et al., 2007). Flavonoids found in *Citrus sp* can be grouped into three groups; i.e. flavones, flavonols and flavanones (Mata Bilbao et al., 2007). In our present study, 19 different standards are detected in the *Citrus aurantium* fruit extracts, except apigenin (Fig. 3). These results are partly similar to Nogata et al. (2006), where it was found that *Citrus aurantium* is extremely rich in flavonoids, principally in naringenin, hesperidin and rutin. Previous reports also showed that *Citrus aurantium* accumulates many types of phenolic compounds in their fruits, with hesperidin and neohesperidin in smaller amounts and naringin as principal flavanone glycoside (Gattuso et al., 2007; Mata Bilbao et al., 2007). Berhow et al. (1998) indicated also that *Citrus* fruits are rich in flavonoids. They have also quantified a number of specific flavonoids in the fruit tissue like hesperidin, naringenin and luteolin among others using sensitive HPLC technology.

In the fruit extracts of *Opuntia ficus indica*, compounds eluting at retention times for caffeic acid, vanillic acid, gallic acid, cinnamic acid, chlorogenic acid, benzoic acid and epicatechin gallate, epicatechin, catechin, esculetin, rutin, and quercetin were retrieved (Fig. 4). Fernández Lopez et al. (2010) reported the presence of significant amounts of flavonoids, including kaempferol, luteolin, isorhamnetin and quercetin, from which quercetin was the predominant compound.

It can be noted from the Table 2, a good correlation between the low content of polyphenols and flavonoids in the fruit extracts with a relatively reduced scavenging effect, this supports the fact that this types of compounds play a major role in the antioxidant potential.

In the present qualitative study, some phenolic acids and flavonoids were identified by comparison of their peaks and their retention times to those produced by standards. Further investigations need to be done to make a quantitative analysis of these compounds.

#### 4. Conclusion

The hydroalcoholic extracts of the edible parts of the fruits of *Vitis vinifera*, *Punica granatum*, *Citrus aurantium* and *Opuntia ficus indica* were analyzed for their antioxidant activity using three assays based upon single electron transfer (DPPH, ABTS, FRAP) and one hydrogen atom transfer assay (ORAC). HPLC analysis unveiled the presence of numerous phenolics and flavonoids in the fruit extracts. All fruit extracts demonstrated high positive correlation between total phenolics, flavonoids or flavonols contents with the DPPH, ABTS, FRAP and ORAC capacities. Consequently, compound-activity relationships for the antioxidant activity can be built as a fonction of HPLC-DAD, HPLC-MS or NMR information on the conditions that each different samples are analyzed. Samples of Algerian fruits collected at different regions and seasons can then be compared in order to analyze how climate and soil affects the antioxidant capacity.

#### Declarations

#### Author contribution statement

Nadia Zeghad: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Ejaz Ahmed: Analyzed and interpreted the data.

Abdelmalik Belkhiri: Conceived and designed the experiments.

Yvan Vander Heyden: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kristiaan Demeyer: Conceived and designed the experiments; Wrote the paper.

## Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Competing interest statement

The authors declare no conflict of interest.

## Additional information

No additional information is available for this paper.

## Acknowledgements

Authors are thankful to the laboratory of, analytical chemistry, applied chemometrics and molecular modeling, Vrije Universiteit Brussels, for their help for the realization of the present work.

## References

- Abarca-Vargas, R., Pena Malacara, C.F., Petricevic, V.L., 2016. Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea butiana* Holtum and Standl, (var. Rose) extracts. *Antioxidants* 5 (45), 1–11.
- Abdullah, S., Shaari, A.R., Rukunudin, I.H., Syarhabil Ahmed, M., 2018. Effect of drying temperature on rosmarinic acid and sinensetin concentration in *Orthosiphon stamineus* herbal leaves. *Mater. Sci. Eng.* 318, 1–6.
- Adesegun, S.A., Fajana, A., Orabueze, C.I., Coker, H.A.B., 2007. Evaluation of antioxidant properties of *Phaulopsis fascispala* C B Cl (Acanthaceae). *Evidence based Complementary and Alternative Medicine* 6 (2), 227–231.
- Aganga, A.A., Mosase, K.W., 2001. Tannins content, nutritive value and dry matter digestibility of *Lonchocarpus capussa*, *Ziziphus mucropata*, *Sclerocarya birrea*, *Kirkia acuminata* and *Rhus lancea* seeds. *Anim. Feed Sci. Technol.* 91, 107–113.
- Alaerts, G., Pieters, S., Logie, H., Van Erps, J., Merino-Arévalo, M., Dejaegher, B., Smeyers-Verbeke, J., Vander Heyden, Y., 2014. Exploration and classification of chromatographic fingerprints as additional tool for identification and quality control of several *Artemisia* species. *J. Pharm. Biomed. Anal.* 95, 34–46.
- Ali, S.I., El Baz, F.K., El Emary, G.A.E., Khan, E.A., Mohamed, A.A., 2014. HPLC-analysis of polyphenolic compounds and free radical scavenging activity

of pomegranate fruit (*Punica granatum L.*). *Int. J. Pharm. Chem. Res.* 6 (4), 348–355.

Amić, D., Dadidović-Amić, D., Bešlo, D., Trinajstić, N., 2003. Structure-radical scavenging activity relationships of flavonoids. *Croat. Chem. Acta* 76 (1), 55–61.

Ayoola, G.A., Ipav, S.S., Solidiya, M.O., Adepoju-Bello, A.A., Coker, H.A.B., Odugbemi, T.O., 2008. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* olive (Guttiferae). *International Journal of Health Research* 1 (2), 81–93.

Babero, G.F., Liazid, A., Palma, M., Barroso, C.G., 2008. Ultrasound assisted extraction of capsaicinoids from peppers. *Talanta* 75 (5), 1332–1337.

Baydar, N.G., Özkan, G., Saçdıç, O., 2004. Total phenolic contents and antibacterial activities of grape (*Vitis vinifera L.*) extracts. *Food Control* 15, 335–339.

Berhow, M., Tisserat, B., Kanés, K., Vandercook, C., 1998. Survey of Phenolic Compounds. Technical Bulletin Number, p. 1856.

Bhourri Mnari, A., Harzallah, A., Amri, Z., Dhaou Aguir, S., Hammami, M., 2016. Phytochemical content, antioxidant properties, and phenolic profile of Tunisian raisin varieties (*Vitis vinifera L.*). *International Journal of Food Properties* 19, 578–590.

Bunea, A., Rugină, D.O., Zotita, A.M.P., 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blue berries from Romania. *Notulae Botanicae Horti Agrobotanici* 39 (2), 70–76.

Chougui, N., Sahi, Y., Belkacemi, M., 2013. Comparative study between the different compartments of *Opuntia ficus indica L.* In: *Inside Food Symposium*. Leuven-Belgium.

Dejian, H., Boxin, O., Maureen, H.W., Judith, A.F., Ronald, L.P., 2002. High-Throughput assay of oxygen radical absorbance capacity (ORAC) using a multi-channel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric. Food Chem.* 50, 4437–4444.

Dudonne, S., Vitrac, X., Coutie, P., Woillez, M., Merillon, J.M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, and ORAC assays. *J. Agric. Food Chem.* 57 (5), 1768–1774.

Elfalleh, W., Yahia, Y., Ferchichi, A., 2012. Main pomegranate phytochemicals and their contribution to the antioxidant potencies of pomegranate juice. In: *International Symposium on the Pomegranate*. Madrid, Spain.

Faccim de Braum, T., Zadra, M., Piana, M., Boligon, A.A., Fröhlich, J.K., Borba de Freitas, R., Stefanello, S.T., Frobrig Froeder, A.L., Belke, B.V., Teixeira Nune, L., Jesus, R.D.S., Machado, M.M., Teixeira, D.R., Antunes Soares, F.A., Athayde, M.L., 2013. HPLC analysis of phenolics compounds and antioxidant capacity of leaves of *Vitex megapotamica* (Sprengel) Moldenke. *Molecules* 18, 8342–8357.

Fernández-López, J.A., Almekla, L., Obón, J.M., Castellar, R., 2010. Determination of antioxidant constituents in cactus pear fruits. *Plant Foods Hum. Nutr.* 65, 253–259.

Fidrianny, I., Rahmiyani, I., Wirasutisna, K.R., 2013. Antioxidant capacities from various leaves extracts of four varieties mangoes using DPPH, ABTS assays and correlation with total phenolic, flavonoid, carotenoid. *Int. J. Pharm. Pharm. Sci.* 5 (4), 189–194.

Floegel, A., Kim, D.O., Chung, S.J., Koo, S.I., Chuun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* 24, 1043–1048.

Gardner, P.T., White, T.A.C., McPhail, D.B., Duthie, G.G., 2000. The relative contributions of vitamin C, carotenoids and phenolic to the antioxidant potential of fruit juices. *Food Chem.* 50, 471–474.

Gattuso, G., Barrera, D., Gargiulli, C., Leuzzi, U., Caristi, C., 2007. Flavonoid composition of *Citrus* juices. *Molecules* 12, 1641–1673.

Godevac, D., Tešević, V., Veličlović, M., Vujišić, L., Vajs, V., Milosavljević, S., 2010. Polyphenolic compounds in seeds from some grape cultivars grown in Serbia. *J. Serb. Chem. Soc.* 75 (12), 1641–1652.

Gorinstein, S., Haruenkit, R., Poovarodom, S., Vearasilp, S., Ruamsuke, P., Namiesnik, J., Leontowicz, M., Leontowicz, H., Suhaj, M., Sheng, G.P., 2010. Some analytical assays for the determination of bioactivity of exotic fruits. *Phytochem. Anal.* 21, 355–362.

Gursoy, N., 2012. Influence of the seasonal conditions on phenolic composition and antioxidant activity of *Vitis vinifera* L. *Afr. J. Microbiol. Res.* 6 (43), 7059–7067.

Hmid, I., Elothmani, D., Hanine, H., Oukabli, A., Mehinagic, E., 2013. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L) cultivars grown in Morocco. *Arabian Journal of Chemistry* 10, 2675–2684.

Hseu, Y.C., Chang, W.H., Chen, C.S., Liao, J.W., Huang, C.J., Lu, F.J., Hia, Y.C., Hsu, H.K., Wu, J.J., Yang, H.L., 2008. Antioxidant activities of *Toona sinensis*

leaves extract using different antioxidant models. *Food Chem. Toxicol.* 46, 105–114.

Karadeniz, F., Burdurlu, H.S., Koca, N., Soyer, Y., 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture* 23, 297–303.

Kriengsak, T., Unaroj, B., Kevin, C., Luis, C., David, H.B., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extract. *J. Food Compos. Anal.* 19, 669–675.

Kulkarni, A.P., Aradhya, S.M., 2005. Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chem.* 93, 319–324.

Kumaran, A., Karunakaran, R.J., 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Science and Technology* 40, 344–352.

Ma, Y.Q., Chen, J.C., Liu, D.H., Ye, X.Q., 2009. Simultaneous extraction of phenolic compounds of *Citrus* peel extracts: effect of ultrasounds. *Ultrason. Sonochem.* 16 (1), 57–62.

Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S., Zhan, R., Xiug, S., Zhou, Y., 2011. Polyphenolic compounds and antioxidant properties in mango fruits. *Sci. Hortic.* 129, 102–107.

Macheix, J.J., Fleuriet, A., Billot, J., 1990. *Fruit Phenolics*. CRD Press, Boca Raton, FL.

Maria, I.G., Fransisco, A.T., Betty, H., Deirdre, M.H., Adel, A.K., 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* 48, 4581–4589.

Mata Bilbao, M.L., Andrés-Lacueva, C., Jàuregui, O., Lamuela-Raventós, R.M., 2007. Determination of flavonoids in a *Citrus* fruit extract by LC-DAD and LC-MS. *Food Chem.* 101, 1742–1747.

Mohd Azman, A.N., Husni, S., Almajano, P.M., Gallego, G.M., 2013. Solvent effect on antioxidant activity and total phenolic content of *Betula alba* and *Convolvulus arvensis*. *International Journal of Biological, Veterinary, Agricultural and Food Engineering* 7 (5), 152–157.

Moulehi, I., Bourgou, S., Ourghemmi, I., Saidani Tounsi, M., 2012. Variety and ripening impact on phenolic composition and antioxidant activity of Mandarin (*Citrus reticulata blanco*) and bitter orange (*Citrus aurantium L*) seeds extracts. *Ind. Crops Prod.* 39, 74–80.

Nivedhini, V., Chandran, R., Parimelazhagan, T., 2014. Chemical composition and antioxidant activity of *Cucumis dipsaceus Ehrenb. Ex Spach* fruit. *International Food Research Journal* 21 (4), 1465–1472.

Nogata, Y., Sakamoto, K., Shiratsuchi, H., 2006. Flavonoid composition of fruit tissues of *Citrus* species. *Biosci Biotechnol Biochem* 70 (1), 178–192.

Oyedemi, S.O., Bradley, G., Afolayan, A.J., 2010. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychnos henningsii gilk.* *African Journal of Pharmacy and Pharmacology* 4 (2), 70–78.

Re, R., Pellegrini, N., Pannala, A., Yang, M., Rice Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay free radical. *Biology Medicinal* 26, 1231–1237.

Shams Ardekani, M.R., Hajimahmoodi, M., Oveisi, M.R., Sadeghi, N., Jannat, B., Ranjbar, A.M., Gholam, N., Moridi, T., 2009. Comparative antioxidant activity and total flavonoid content of Persian pomegranate (*Punica granaum L*) cultivars. *Iran. J. Pharm. Res. (IJPR)* 10 (3), 519–524.

Talukder, M.E.U., Aklima, J., Emrar, T.B., Islam, S., Rahman, A., Bhuiya, R.H., 2013. *In vitro* antioxidant potential of *Momordica charanta* fruit extracts. *Br. J. Pharmaceut. Res.* 3 (4), 963–971.

Tapan, S., 2011. Antioxidant activity of some wild edible plants of *Meghalya state* of India: a comparison using two solvents extraction systems. *International Journal of Nutrition and Metabolism* 4 (3), 51–56.

Tomsone, L., Kruma, Z., 2017. Influence of harvest time on the phenolic content of horseradish leaves. *Baltic Conference on Food Science and Technology* 45–50.

Valares Masa, C., Sosa Diaz, T., Alias Galleo, J.C., Chaves Lobon, N., 2016. Quantitative variation of flavonoids and diterpenes in leaves and stems of *Cistus ladanifer L* at different ages. *Molecules* 21 (275), 1–14.