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HPLC-DAD-MS/MS profiling of phenolics from different varieties of peach leaves and evaluation of their antioxidant activity: A comparative study

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ABSTRACT

Peach (*Prunus persica* L.) leaves have been traditionally used in folk medicine for their several biological activities that are correlated to the presence of polyphenolic compounds. The aim of the present study is to characterize phenolic compounds present in foliar extracts of seven peach varieties cultivated in Algeria by HPLC–MS and the study of their antioxidant potential. Antioxidant capacity of the foliar extracts was assessed by several tests acting by different mechanisms: Oxygen Radical Absorbance Capacity (ORAC), 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH), 2,-Azinobis(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) and Iron Chelating Activity (ICA). Fourteen phenolic compounds were identified in the peach leaf extracts including cinnamic acids and flavonols. Flavonols represent the main class of phenolic compounds accounting for an average percentage higher than 95% of the overall phenolics. Kaempferol 3-glucoside is the main phenolic compound in all peach leaf extracts with an average percentage higher than 32% followed by quercetin 3-glucoside (17.9%), quercetin 3-galactoside (17.1%) and kaempferol 3-galactoside (15.4%). Results showed that variety significantly affected the phenolic content of peach leaves. Romea and Red Top varieties present the higher concentration in phenolic compounds, Dixired, Flavorcrest and Tebana a moderate one and, Cardinal and Spring Belle the lowest content. The data obtained with DPPH, ABTS, ORAC and PFRAP assays showed that polyphenols present in the all foliar peach extracts were potent antioxidative agents. Except for ICA assay, good positive correlations were found between phenolic concentration and the different measured antioxidant capacities. That means that phenolic compounds present in peach leaf cultivars were major contributors of reducing power and scavenging radicals capacities (DPPH, ABTS and ORAC). All these results allowed us to conclude that peach leaves are a good source of phenolics with active properties, as antioxidant ones.

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1. Introduction

Prunus persica (L.) belongs to the Rosaceae family. This tree is cultivated throughout the world for its fruits. In 2015, peach production was accounted as more than twenty million tons in worldwide. The three major producers are China, European Union and United States with 13,600, 4027 and 903, respectively (Foreign Agricultural Service/USDA, sept 2015). Many studies have been done on peach fruits [1–6] to analyze their phenolic content and, their nutritional and pharmacological values are well recognized. However very scarce data were available on by-products of *Prunus persica* L. trees, as their leaves. Even so peach leaves are traditionally used for their antihelmentic, laxative and sedative properties [7]. Several studies have already been carried out on peach leaves to characterize their antibacterial, antimalarial, antiasthmatic, anticoagulant, hepatoprotective and spasmogenic properties [8]. In

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Algeria, fresh or dried peach leaves were consumed as herbal infusion to treat gastritis, whooping cough, chronic bronchitis and get rid of intestinal worms. Some of these activities can be correlated to the presence of phenolic compounds such as caffeic acid, chlorogenic acid, *p*-coumaric acid, kaempferol, quercetin and quercetin 3-*O*-glucoside [9,10] and can be related to the antioxidant properties of these molecules.

In this study, our objective was to determine the chemical composition and the content of different peach leaves extracts in term of phenolic compounds and to assess their antioxidant capacity. The studied leaves were the ones of seven peach varieties grown in Algeria; cultivars differing at least from their fruit characteristic (fruit size, skin and pulp color, time of harvest): four free stone cultivars (Cardinal, Flavorcrest, Red Top, Spring Belle), a semi-free stone cultivars (Dixired) and two clingstone cultivars (Romea and Tebana). We have already published a work on the phenolic analysis of the fruits of these seven varieties [3] and in this paper our interest was assigned to the leaves. It is important to not address our question on a unique cultivar but on several as it has been reported that among the numerous factors influencing the phytochemical content of a plant organ the genotype has to be taken in consideration. To our knowledge, this is the first time that such a comparison of *P. persica* leaves was carried out. In addition to the quantification and the identification of phenolics by high-performance liquid chromatography coupled to mass spectrometry (HPLC–MS), we evaluated the antioxidant capacity of the foliar extracts by several assays involving different mechanisms [11,12] in order to approach all the antioxidant aspects. We performed Oxygen Radical Absorbance Capacity (ORAC), 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH), 2,-Azinobis(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) and Iron Chelating Activity (ICA).

2. Materials and methods

2.1. Reagents and standards

Chlorogenic acid and quercetin 3-*O*-glucoside were supplied by Extrasynthese (France). Folin-Ciocalteu reagent, catechin, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium ferricyanure, trichloroactetic acid, ferric chloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid), 3′,6′-dihydroxyspiro[2-benzofuran-3,9′-xanthene]-1-one (fluorescein), phosphate buffer (0.1 M, pH 7.4), L-ascorbic acid were purchased from Sigma (France). Chlorhydric acid and formic acid were obtained from Fisher chemical and acetonitrile by Fisher scientific. Ethylenediaminetetraaceticacid (EDTA) and sodium hydroxide were obtained from Fluka. Acetone, methanol, ferrous sulfate, sodium nitrate, potassium persulfate were supplied by Prolabo (France).

2.2. Plant material

The leaves of peach (*Prunus persica* L.) used in this study were collected from the Technical Institute of Fruit Tree Cultivation and Vine (Institut Technique de l'Arboriculture Fruitière et de la Vigne, ITAFV, Tessala El Merdja, Birtouta, Algiers, Algeria) during September 2011. The leaves of seven peach varieties: Cardinal, Dixired, Flavorcrest, Red Top, Romea, Spring Belle and Tebana were washed with tap water, drained and kept to dry at room temperature under subdued light. Dried leaves were homogenized in a blender before being sifted through a 200 μm sifter. The obtained powders were kept at 4 °C until extraction.

2.3. Sample preparation

A sample of 20 g of peach leaf powder were extracted in 200 mL of acetone/water (60/40, v/v) at room temperature (2 h and under agitation). Samples were centrifuged for 20 min at 4000 rpm (Sigma Laboratory Centrifuges, Bioblock Scientific). The supernatant was collected and the pellet re-extracted with 60% acetone (200 mL) as above. Then, the two supernatants were mixed and evaporated to dryness at 40 °C with a rotary evaporator before being freeze-dried (Gamma 1–16 LSC, Martin Christ, Osterode am Harz, Germany). The extracts were reconstituted in 20 mL methanol/water (30/70, v/v) and each extract was purified on a Supelclean™ LC-18 solid phase extraction (SPE) tubes (Supelco, Bellefonte, Pennsylvania, USA) to remove chlorophylls. Compounds of interest (phenolic compounds) were eluted with 90% methanol, dried in vacuum rotary evaporator (Laborota 4000-efficient, Heidolph Instruments, Schwabach, Germany) at 40 °C and then freeze-dried. The freeze-dried extracts were kept at 4 °C until analysis.

2.4. Chromatographic separation

Chromatographic separation was performed using an LC Agilent Series 1200 system (Agilent Technologies, Santa Clara, CA, USA) equipped with an automatic injector, a degasser, a binary pump, a column heater/selector and a UV–visible-DAD detector from the same supplier. The column was a Prontosil C_{18} (5 μm, 250 mm × 4.6 mm), Bischoff (Leonberg, Germany). Lyophilized peach leaf extract was diluted in 50% methanol containing 1% formic acid and filtered through 0.45 μm PTFE filter. The elution method used a binary gradient, solvent A (water/formic acid, 95/5, v/v) and solvent B (acetonitrile/formic acid, 95/5, v/v). The run was set up with the following gradient: 0 min 90% A 10% B, 85 min 50% A 50% B, 95 min 100% B linear for 10 min, followed by 10 min for re-equilibration. The flow rate was 0.8 mL/min and the sample injection volume was 20 μL. Identification was achieved by matching retention time and spectra of the peaks with those of commercially available standards. Quantification was performed by external calibration at 280 and 360 nm with a diode array detector (DAD) in accordance with the maximum absorbance of each phenolic family. Calibration curves were obtained by injecting standards diluted at five different concentrations (R^2: 0.9988–0.9998).

2.5. ESI-MS and MS/MS analysis

Additionally, the identification was achieved by ESI-MS and MS/MS analysis. MS/MS experiments were performed on an Esquire 3000 + ion trap mass spectrometer using an ESI source from Bruker Daltonics (Billerica, MA, USA). The HPLC output flow was split with a passive splitter at an average 1:100 ratio depending on the flow solvent, viscosity and rate. Drying gas was set at 9.0 L/min and 350 °C, nebulizer pressure was set to 27 psi. ESI-MS parameters (positive mode): HV capillary − 4100 V, end plate offset − 500 V, capillary exit 134.3 V, skimmer 40 V, trap drive 59.3, scan 25,000 μs, rolling average 2 and trap averages 5.

Individual polyphenols were quantified by means of calibration curve using external standards: Hydroxycinnamic acids as chlorogenic acid at 280 nm and flavonols as quercetin 3-*O*-glucoside at 360 nm. Concentrations were calculated in mg per g of dry weight extract (mg/g DWE). Mean values of each peach variety were calculated from three technical replicates.

2.6. Total phenolic content (TPC) determination

The content of total phenolic compounds was analyzed spectrophotometrically using the Folin-Ciocalteu (F–C) colorimetric

method adapted to 96-wells plate [13]. Twenty μL of the standard chlorogenic acid solution, diluted leaf extract or methanol (blank) was mixed with 100 μL of Folin-Ciocalteu's reagent (diluted 10 times with water). The samples were allowed to stand for 2−3 min before 80 μL of a 7.5% sodium carbonate aqueous solution was added. Samples were allowed to stand for 60 min at room temperature in dark before the absorbance was measured at 765 nm versus blank using a plate reader spectrophotometer (Fluostar Optima; BMG Labtech). Absorbance values were compared with those of standards prepared similarly with known chlorogenic acid concentrations. The total phenolic content was expressed as milligrams of chlorogenic acid equivalent per g of dry weight extract (mg CAE/g DWE). Measurements were done in three replications. All samples were analyzed in triplicate.

2.7. Total flavonoid content (TFC) determination

The amount of total flavonoids was determined by the aluminum chloride colorimetric assay [14]. Fourty μL of an appropriate dilution of extract in methanol were added by 60 μL of distilled water and 6 μL of 5% $NaNO_2$. Six μL of 10% $AlCl_3$ was added 5 min later. After 6 min, 40 μL of 1 M NaOH was added and the total was made up to 200 μL with distilled water. The absorbance was measured against a blank at 510 nm. The flavonoids content was determined using calibration curve prepared with rutin as standard and expressed as mg rutin equivalent per g of dry weight extract (mg rutin/g DWE). All samples were analyzed in triplicate in at least three independent experiments.

2.8. Antioxidant activities

2.8.1. DPPH assay

In the 2,2-DiPhenyl-PicrylHydrazyl (DPPH) assay, the antioxidants were able to reduce the stable radical $DPPH^{o}$ to the yellow colored DiPhenyl-PicrylHydrazine (DPPH-H). The DPPH radical-scavenging activity (DPPH-RSA) of the peach leaf extracts was estimated according to the method of Blois [15]. To the methanol solution (50 μL) of the sample extracts, 150 μL of 200 μM methanolic solution of DPPH was added in a 96-wells plate. Then the plate was allowed to stand for 20 min at room temperature in dark. The decrease in absorbance was determined at 520 nm against methanol as a blank. The remaining concentration of DPPH in the reaction medium was calculated from a calibration curve obtained with Trolox and results were expressed as mg of Trolox equivalent per g of dry weight extract (mg TE/g DWE). All measurements were carried out in three replications. All samples were analyzed in triplicate.

2.8.2. ORAC assay

The ORAC assay measures the antioxidant scavenging capacity against peroxyl radical generated by thermal decomposition of AAPH at 37 °C. Fluorescein (FL) was used as the fluorescent probe. The loss of fluorescence of FL was an indication of the extent of damage from its reaction with the peroxyl radical. The protective effect of an antioxidant was measured by assessing the area under the fluorescence decay curve (AUC) relative to that of a blank in which no antioxidant was present [16]. The ORAC assay was performed according to the method of Ou et al. [17]. All working solutions of AAPH, fluorescein and peach leaf extracts were diluted in 75 mM phosphate buffer (pH 7.4) and the final reaction mixture was 200 μL. Extract (30 μL) and fluorescein (180 μL; 117 nmol/L final concentration) solutions were placed in the well of black 96-wells microplate. The mixture was preincubated for 5 min at 37 °C prior AAPH (90 μL; 40 mmol/L final concentration) addition. The plate was immediately placed in the reader (Fluostar Optima; BMG Labtech) and the fluorescence recorded every minute for 70 min. Fluorescence measures were carried out at 37 °C. Excitation and emission filters were 485 and 520 nm, respectively. A blank (phosphate buffer) and five calibration solutions (Trolox) were also carried out in the same run. The antioxidant capacities of the peach leaf extracts were expressed as mg of Trolox equivalent per g of dry weight extract (mg TE/g DWE). All reaction mixtures were prepared by quadruplicate and at least three independent runs were performed for each sample.

2.8.3. ABTS assay

This technique was carried out as reported by Re et al. [18]. ABTS [2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] radical cation ($ABTS^{+}$) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand at room temperature in the dark for 12−16 h before use. Prior to assay, the solution was diluted in methanol to get an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 10 μL of plant extract (or Trolox) to 250 μL of the diluted $ABTS^{+}$ solution, absorbance at 734 nm at 30 °C was measured using microplate reader exactly after 6 min after initial mixing. The positive control contained 10 μL of methanol instead of test sample. A standard curve was prepared by measuring the reduction in absorbance of $ABTS^{+}$ solution at different concentrations of Trolox. Results were expressed as mg of Trolox equivalent per g of dry weight extract (mg TE/g DWE). All measurements were carried out in three replications. All samples were analyzed in triplicate.

2.8.4. PFRAP assay

The reducing power of peach leaf extracts was assessed by the method of Oyaizu [19] adapted for 96-wells microplate. This method is based on the reduction of Fe^{3+} to Fe^{2+}, which is recorded by measuring the formation of Perl's Prussian blue. Ten μL of methanolic dilution of extract or ascorbic acid (as standard) were mixed with 30 μL of phosphate buffer (0.2 M, pH 6.6) and 30 μL of 1% potassium ferricyanide, then incubated at 50 °C for 20 min. After incubation, 30 μL of 10% trichloroacetic acid, 100 μL of distilled water and 20 μL of 0.1% ferric chloride were added to each well. Absorbance was measured at 700 nm and results were expressed as mg ascorbic acid equivalent per g of dry weight extract (mg AA/g DWE). All measurements were carried out in three replications. All samples were analyzed in triplicate.

2.8.5. ICA assay

The capacity of peach leaf extracts to chelate $Fe^{2+}$was determined by measuring the formation of the Fe^{2+}-ferrozine complex according to the method of Dinis et al. [20]. To 40 μL of methanolic dilution of extract or EDTA (as standard), 80 μL of deionized water and 40 μL of $FeSO_4$ (0.2 mM) were added and mixed in 96-wells microplate. The reaction was initiated by adding 40 μL of ferrozine (2 mM). After 10 min at room temperature, the absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. Methanol was used as positive control instead of foliar peach extracts. Results were expressed as mg EDTA equivalent per gram of dry weight extract (mg EDTA/g DWE). All measurements were carried out in three replications. All samples were analyzed in triplicate.

2.9. Statistical analysis

All samples were analyzed at least in triplicates. Data were expressed as means ± standard deviation (SD). Concerning the antioxidant measurements, after a Kolmogorov-Smirnov test to confirm the normality of the data, parametric tests were used for analysis of variance and correlation. Differences were evaluated by one-way analysis of variance (ANOVA) completed by Tukey's test.

Different values of antioxidant activities obtained in our extracts were compared using the Pearson correlation test. Differences were considered statistically significant at $p < 0.05$. GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA) was used to perform these analyses.

3. Results and discussion

3.1. Individual phenolic compound identification

In this study, phenolic compounds were identified by HPLC-MS/MS analysis. Liquid chromatography coupled with mass spectrometry (LC/MS) is a potent technique for the analysis of complex botanical extracts. HPLC is efficient in separating chemical compounds in a mixture and MS provides abundant information for structural elucidation of the compounds when tandem mass spectrometry is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal plants, especially when a pure standard is unavailable [21].

Phenolic compounds present in foliar peach extracts were identified by the examination of the mass spectra in negative mode (MS and MS/MS). To confirm the identification of the phenolic compounds, several markers were used: chlorogenic acid (hydroxycinnamic acid), quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside (flavonols). Using this procedure fourteen phenolic compounds were characterized in all peach leaf extracts (Table 1). The identified compounds derivate from two phenolic groups: hydroxycinnamic acids and flavonols. Flavonols present maximum absorption beside 360 whereas hydroxycinnamic acids present maximum absorptions in the range 280–340 nm (Fig. 1).

Our findings are in agreement with the work of Geissman [22] who found that caffeic acid, chlorogenic acid, quercetin and kaempferol were the major components present in peach leaf extracts.

Among cinnamic acids, two hydroxycinnamic acids were noted. The assignment of chlorogenic acid was based on the observation of the ion fragments *m/z* 353 $[M - H]^-$ and 191 corresponding to the deprotonated caffeic acid. Whereas, dicaffeoylquinic acid was assigned on the observation of the ion fragments *m/z* 515 $[M - H]^-$, 353 and 191 corresponding to the deprotonated molecules, the deprotonated chlorogenic acid and the deprotonated caffeic acid, respectively. Chlorogenic acid (**1**) was identified by chromatographic comparison with authentic marker. Dicaffeoylquinic acid (**6**) was tentatively identified by comparison with literature since this compound was already identified in the leaves of lettuce and escarole [23] and berry leaves [24].

All the identified flavonols were conjugated with a glycosidic unit. Mass spectra allowed the identification of each flavonol aglycone by the observation of characteristic fragmentation *m/z* values (301 quercetin; 285 kaempferol; 315 isorhamnetin) due to the loss of the sugar moiety. The assignment of the sugar was based on the characteristic loss of a fragment of *m/z* 162 (glucoside or galactoside), as well as the characteristic loss of a fragment of *m/z* 308 (rhamnoglucoside). The identification of flavonol derivatives was completed by comparison with pure standards and/or comparison with data literature.

Our findings are in agreement with previously published studies in which similar compounds were identified in other plants belonging to the genus *Prunus*. Olszewska [25] has isolated seven flavonol glycosides from the leaves of *Prunus serotina* Ehrh. These flavonoids were identified as three quercetin monoosides: hyperoside (quercetin 3-galactoside), avicularin (quercetin 3-arabinoside) andreynoutrin (quercetin 3-xyloside); three quercetin diosides: (3-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside and 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside) as well isorhamnetindioside: (3-*O*-(6″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside). Olszewska and Kwapisz [26] reported the presence of chlorogenic acid, quercetin 3-galactoside (hyperoside) and kaempferol 3-glucoside (astragalin) in *Prunus padus* L. leaves.

3.2. Phenolic contents

The seven peach leaf extracts can be classified into two groups based on their phenolic contents (Table 2). In the first group, Romea and Red Top present the higher concentration in phenolic compounds with values ranging from 386.5 to 392.2 mg/g DWE (dry weight extract). Dixired, Flavorcrest and Tebana showed moderate concentrations ranging from 320.6 to 346.6 mg/g DWE. The second group consisted of Cardinal and Spring Belle, which displayed the lowest content with values of 140 and 146 mg/g DWE, at least half less of the content of the varieties found in group 1.

In the phenolic peach leaf extracts, we have identified cinnamates and flavonols. Considering cinnamates, they only represented 1.3–9.1% of total phenolics and we found chlorogenic and dicaffeoylquinic acids. Chlorogenic acid was present 10 to 60-fold more than dicaffeoylquinic acids. The maximum level of chlorogenic acid was found in Spring Belle extract (12.9 mg/g) followed by

Table 1
Chromatographic (peak number, retention time and UV_{max}) and mass data (molecular ions, fragment ions and relative abundances, in negative mode) of peach leaf phenolic compounds.

Compound	No	t_R (min)	UV_{max} (nm)	$[M - H]^-$ (*m/z*)	MS/MS (*m/z*)
Hydroxycinnamates					
chlorogenic acid	**1**	6.9	325	353	191(100)
dicaffeoylquinic acid	**6**	25.4	325	515	353(100), 191(23)
Flavonols					
kaempferol dihexoside	**2**	13.6	345	609	447(100), 285(18)
quercetin 3-galactoside	**3**	22.6	355	463	301(100)
quercetin 3-rutinoside	**4**	23.1	355	609	301(100)
quercetin 3-glucoside	**5**	23.5	355	463	301(100)
kaempferol 3-galactoside	**7**	26.2	345	447	285(100)
kaempferol 3-rutinoside	**8**	27.5	345	593	285(100)
kaempferol 3-glucoside	**9**	28.1	345	447	285(100)
Isorhamnetin 3-glucoside	**10**	29.9	335	447	315(100)
quercetinacetyl-hexoside	**11**	30.5	355	505	463(27), 301(100)
kaempferol acetyl-hexoside	**12**	34.6	345	489	327(27), 285(100)
quercetin(*p*-coumaroyl)-hexoside	**13**	37.8	355	609	463(100), 301(21)
kaempferol(*p*-coumaroyl)-hexoside	**14**	41.9	345	593	447(100), 285(17)

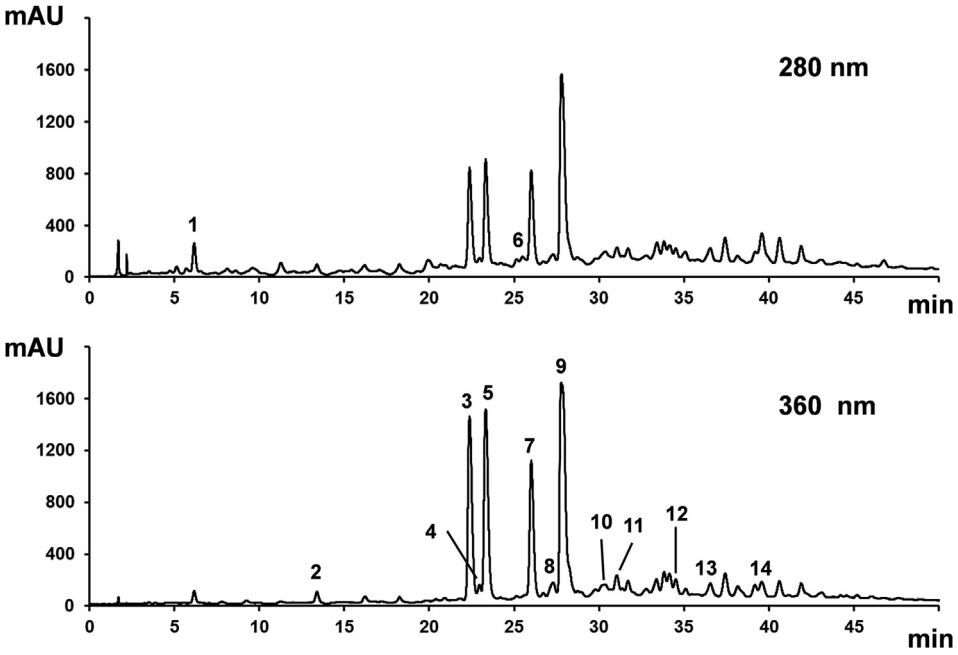

Fig. 1. HPLC chromatogramsof peach leaf extract at 280 nm (A) and 360 nm (B). (**1**) chlorogenic acid; (**2**) kaempferol dihexoside; (**3**) quercetin3-galactoside; (**4**) quercetin3-rutinoside; (**5**) quercetin-3 glucoside; (**6**) dicaffeoylquinic acid; (**7**) kaempferol3-galactoside; (**8**) kaempferol3-rutinoside; (**9**) kaempferol3-glucoside; (**10**) isorhamnetin3-glucoside; (**11**) quercetin-acetyl-hexoside; (**12**) kaempferol acetyl-hexoside; (**13**) quercetin(p-coumaroyl)-hexoside; (**14**) kaempferol(p-coumaroyl)-hexoside.

Table 2
Content of phenolic compounds (mg/g of extract) in peach leaves cultivars.

Compound	Cardinal	%	Dixired	%	Flavorcrest	%	Red Top	%	Romea	%	Spring Belle	%	Tebana	%
Cinnamates														
chlorogenic acid	9.3 ± 0.2^{c}	6.7	11.8 ± 0.3^{b}	3.7	8.3 ± 0.1^{d}	2.6	11.8 ± 0.3^{b}	3.0	4.4 ± 0.1^{e}	1.2	12.9 ± 0.5^{a}	8.9	8.6 ± 0.1^{cd}	2.5
dicaffeoylquinic acid	0.3 ± 0.1^{e}	0.2	0.7 ± 0.1^{a}	0.2	0.6 ± 0.1^{b}	0.2	0.2 ± 0.1^{e}	0.1	0.4 ± 0.1^{c}	0.1	0.3 ± 0.1^{d}	0.2	0.4 ± 0.1^{d}	0.1
Total	**9.6**	**6.9**	**12.5**	**3.9**	**8.9**	**2.8**	**12.0**	**3.1**	**4.8**	**1.3**	**13.2**	**9.1**	**9.0**	**2.6**
Flavonols														
kaempferol dihexoside	1.9 ± 0.1^{e}	1.4	3.9 ± 0.2^{b}	1.2	3.2 ± 0.1^{c}	1	3.0 ± 0.2^{cd}	0.8	5.3 ± 0.2^{a}	1.4	2.5 ± 0.1^{de}	1.7	3.5 ± 0.1^{bc}	1.0
quercetin 3-galactoside	21.3 ± 0.5^{c}	15.4	54.5 ± 4.5^{b}	17.2	65.2 ± 1.8^{ab}	20.4	72.7 ± 6.1^{a}	18.8	52.5 ± 2.9^{b}	13.8	26.4 ± 1.8^{c}	18.2	60.0 ± 3.7^{ab}	17.5
quercetin 3-rutinoside	1.0 ± 0.1^{e}	0.7	2.2 ± 0.2^{cd}	0.7	2.6 ± 0.1^{bc}	0.8	3.1 ± 0.2^{ab}	0.8	2.4 ± 0.1^{cd}	0.6	1.9 ± 0.1^{d}	1.3	3.2 ± 0.2^{a}	0.9
quercetin 3-glucoside	23.3 ± 0.6^{c}	16.8	56.6 ± 5.4^{b}	17.9	62.4 ± 1.7^{ab}	19.5	76.3 ± 6.1^{a}	19.7	63.8 ± 3.3^{ab}	16.7	26.4 ± 1.6^{c}	18.2	60.2 ± 3.4^{b}	17.6
kaempferol 3-galactoside	17.9 ± 0.6^{d}	12.9	50.5 ± 4.4^{bc}	15.9	46.9 ± 1.4^{c}	14.7	54.7 ± 2.6^{bc}	14.2	75.5 ± 4.2^{a}	19.8	20.3 ± 0.4^{d}	14.0	61.2 ± 3.9^{b}	17.9
kaempferol 3-rutinoside	1.4 ± 0.1^{c}	1.0	4.2 ± 0.1^{b}	1.3	4.4 ± 0.2^{b}	1.4	7.8 ± 0.1^{a}	2.0	5.3 ± 0.6^{b}	1.4	1.7 ± 0.1^{c}	1.2	5.4 ± 0.5^{b}	1.6
kaempferol 3-glucoside	50.0 ± 1.0^{d}	36.1	109.3 ± 6.8^{bc}	34.5	98.5 ± 2.5^{c}	30.8	122.4 ± 8.0^{ab}	31.7	137.9 ± 8.2^{a}	36.2	42.1 ± 3.1^{d}	29.1	112.4 ± 4.8^{bc}	32.8
isorhamnetinglucoside	1.9 ± 0.1^{d}	1.4	4.9 ± 0.5^{c}	1.5	5.8 ± 0.1^{bc}	1.8	7.8 ± 0.1^{a}	2.0	7.2 ± 0.7^{ab}	1.9	2.4 ± 0.1^{d}	1.7	6.7 ± 0.4^{ab}	2.0
quercetinacetyl-hexoside	2.6 ± 0.1^{c}	1.8	7.1 ± 0.3^{b}	2.2	7.8 ± 0.3^{b}	2.4	12.9 ± 2.1^{a}	3.3	13.2 ± 1.0^{a}	3.5	2.4 ± 0.1^{c}	1.7	8.7 ± 0.5^{b}	2.5
kaempferol acetyl-hexoside	1.2 ± 0.1^{c}	0.9	3.3 ± 0.4^{b}	1.0	2.9 ± 0.1^{b}	0.9	6.3 ± 0.6^{a}	1.6	3.0 ± 0.3^{b}	0.8	1.1 ± 0.1^{c}	0.8	3.9 ± 0.4^{b}	1.1
quercetin *p*-coumaroyl-glucoside	1.5 ± 0.1^{b}	1.1	4.0 ± 0.3^{a}	1.3	4.4 ± 0.0^{a}	1.4	3.7 ± 0.4^{a}	1.0	4.1 ± 0.4^{a}	1.1	1.5 ± 0.1^{b}	1.0	4.4 ± 0.4^{a}	1.3
kaempferol *p*-coumaroyl-glucoside	5.0 ± 0.8^{bc}	3.6	4.0 ± 0.3^{cd}	1.3	7.1 ± 0.3^{a}	2.2	3.6 ± 0.4^{cd}	0.9	6.3 ± 0.5^{ab}	1.7	2.8 ± 0.3^{d}	1.9	4.0 ± 0.4^{cd}	1.2
Total	**129.0**	**93.1**	**304.5**	**96.1**	**311.2**	**97.2**	**374.4**	**96.9**	**376.5**	**98.7**	**131.5**	**90.9**	**333.6**	**97.4**
total phenolic	**138.6**		**317.0**		**320.1**		**386.4**		**381.3**		**144.7**		**342.6**	

Different letters indicate significant differences at $p < 0.05$.

Dixired and Red Top (11.8 mg/g), Cardinal (9.3 mg/g), Tebana (8.6 mg/g) and Flavorcrest (8.3 mg/g). Romea has the lowest level (4.4 mg/g).

Twelve flavonols were identified and quantified in all peach leaf extracts: six kaempferol derivates (kaempferol dihexoside, 3-galactoside, 3-rutinoside, 3-glucoside, acetylhexoside and kaempferol glucoside acylated by *p*-coumaric acid); five quercetin derivates (quercetin 3-galactoside, 3-rutinoside, 3-glucoside, acetylhexoside and quercetin-glucoside acylated by *p*-coumaric acid) and one derivative of isorhamnetin (isorhamnetin 3-glucoside). Flavonols represent the main class of phenolic compounds accounting for an average percentage higher than 95% of the overall phenolics. These results are in accordance with those of Olszewska and Kwapisz [26] who reported that flavonoid compounds are main chemical components in *Prunus serotina* leaves. They also characterized and identified fourteen flavonoids as quercetin, kaempferol, and isorhamnetin monosides and diosides containing the D-galactopyranosyl, D-glucopyranosyl, L-arabinofuranosyl, L-arabinopyranosyl, L-rhamnopyranosyl, and/or D-xylopyranosyl units as the sugar residues.

Kaempferol 3-glucoside is the main phenolic compound in all peach leaf extracts with an average percentage higher than 32% followed by quercetin 3-glucoside (17.9%), quercetin 3-galactoside (17.1%) and kaempferol 3-galactoside (15.4%). A previous study showed that kaempferol 3-glucoside (astragalin) was the prominent flavonol glycoside in peach leaves [22]. Our results confirm that kaempferol 3-glucoside is the main flavonol of peach leaves.

LianSen et al. [27] compared flavonoids and other phenolic

compounds present in leaves of 62 peach cultivars. Of the 92 detected components, 11 were identified: gallic acid, 5,7-dimethoxycoumarin, quercetin, quercetin derivatives (3-rutinoside, 3-rhamnoside, 3-glucoside, 3-galactoside, 3-sophoriside); kaempferol and kaempferol derivatives (3-rutionoside, 3,7-dirhamnoside). LianSen et al. (1994) found that quercetin 3-glucoside exhibited the highest absolute amount of the identified compounds, followed by quercetin 3-rhamnoside and quercetin 3-galactoside. These differences between their results and ours may be attributed to the peach cultivar itself (the seven varieties that we studied were not encountered in 62 cultivars studied by LianSen and its colleagues) and/or to environmental conditions such as soil composition, sun light exposition, sampling period and cultural practices; as well as the extraction conditions (solvent polarity) and the sensibility of the analytical method used.

3.3. TPC, TFC and antioxidant activities

We performed the following assays: total phenolic content (TPC) with Folin-Ciocalteu (F–C) assay, total flavonoid content (TFC), Oxygen Radical Absorbance Capacity (ORAC), 2,2-DiPhenyl-PicrylHydrazyl radical (DPPH), 2,2′-Azinobis(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), Potassium Ferricyanide Reducing Antioxidant Power (PFRAP) and Iron Chelating Activity (ICA).

Total phenolic compounds (F–C), total flavonoid compounds and antioxidant activities of leaf extracts of the seven peach cultivars are presented in Table 3. As different methods were applied to evaluate antioxidant activities, for each assay the rank of the obtained antioxidant capacity value was specified in order to compare more easily peach cultivars with one another.

The total phenolic content (TPC) based on Folin-Ciocalteu assay (F–C) significantly varied between 221.4 and 406.3 mg CAE/g DWE. No significant differences were found between Romea, Red Top, Tebana, Flavorcrest and Dixired which represent cultivars having the highest content of TPC with values of 406.3, 384, 327.8, 322.4 and 320.3 mg CAE/g, respectively. Cardinal and Spring Belle have the lowest content of TPC with values of 226.4 and 221.4 mg CAE/g, respectively. These results are in accordance with those previously determined with HPLC analysis (Table 2). Data of the literature on *Prunus persica* L. leaf polyphenolic content reported values of 38 and of 109.3 mg/g DW [28,29], values which are 3 to 10-fold less than the ones we obtained. However if we considered studies on other plants of the same family, the Rosaceae, as *Pyrus pachia* or *Crataegus azarolus*, these plants displayed a total phenolic content of leaf extracts of 325 and 396 mg/g DW, respectively [30,31]. Such values are in the same order of the values we obtained for polyphenol peach leaf extracts.

The total flavonoid content (TFC) based on the aluminum chloride complex formation varied between 149.4 and 286.4 mg RE/g peach leaf extracts. Tebana was the richest cultivar (286.4 mg RE/g). Then, in the order of the highest value, we found Red Top (239.8 mg RE/g), Romea (224.9 mg RE/g), Dixired (224.0 mg RE/g) and Flavorcrest (209.4 mg RE/g) but these values were not significantly different ($p < 0.05$). Spring Belle and Cardinal had the lowest content of TFC (168.9 and 149.4 mg RE/g, respectively). The content of flavonoids in leaves of other Rosaceae plants is equivalent to our values, as instance 150 mg/g was found in *Pyrus pachia* [31].

In PFRAP assay, substances which have reduction capacity, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which subsequently reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm [32]. The antioxidant activity of the foliar extracts evaluated with the PFRAP assay was higher in Tebana and Dixired cultivars with a values of 94.1 and 84.9 mg AA/g. Romea, Flavorcrest and Red Top showed and intermediate activities (69.1, 63.2 and 62.7 mg AA/g, respectively). In Spring Belle and Cardinal, we measured the lowest values (38.8 and 33.4 mg AA/g, respectively). Such a reducing power form leaf extract of *Prunus persica* L. has already been pointed out by Deb and its colleagues [33].

Scavenging the stable DPPH radical model is another commonly used method to evaluate antioxidant activity. Antioxidants react with DPPH, which is a stable free radical with characteristic absorption at 517 nm, and convert it to 2,2-diphenyl-1-picrylhydrazine. The degree of discoloration indicates the scavenging capacity of the extract, which is due to the hydrogen donating ability [34]. With the DPPH assay, Red Top extract exhibited the highest scavenging activity (152.2 mg TE/g) followed by Romea (135.2 mg TE/g). Flavorcrest, Dixired and Tebana displayed intermediate values of 115.1, 111.9 and 104.9 mg TE/g, respectively. Spring Belle obtained a more moderate value (74.7 mg TE/g). Cardinal exhibited the lowest DPPH antioxidant capacity (57.2 mg TE/g). It has been reported that leaf aqueous extracts of *Prunus persica* L. inhibited generation of superoxide and hydroxyl radicals [35] and exhibited a DPPH radical scavenging activity [33]. The values that we obtained are relevant to the ones displayed by ethanolic extracts of apple leaves (around 86 mg rutin equivalent/g [36]) and by water extracts of different Rosaceae

Table 3
TPC, TFC andantioxidant activities of the seven studied peach leaves cultivars.

Parameter	Cardinal	Dixired	Flavorcrest	Red Top	Romea	Spring Belle	Tebana
Phenolic compounds							
F–C (mg of CA/g)	226.4 ± 40.3^{bc}	320.3 ± 25.4^{ab}	322.4 ± 31.3^{a}	384.0 ± 50^{a}	406.3 ± 26.5^{a}	221.4 ± 36.27^{c}	327.8 ± 15.1^{a}
Rank	*6*	*5*	*4*	*2*	*1*	*7*	*3*
TFC (mg of RE/g)	149.4 ± 5.7^{c}	224.0 ± 6.0^{b}	209.4 ± 9.1^{b}	239.8 ± 16.2^{b}	224.9 ± 3.4^{b}	168.9 ± 3.5^{c}	286.4 ± 9.6^{a}
Rank	*7*	*4*	*5*	*2*	*3*	*6*	*1*
Antioxidant activities							
FRAP (mg of AA/g)	33.4 ± 9.3^{d}	84.9 ± 3.4^{ab}	63.2 ± 6.6^{c}	62.7 ± 10.0^{c}	69.1 ± 7.4^{bc}	38.8 ± 9.4^{d}	94.1 ± 8.1^{a}
Rank	*7*	*2*	*4*	*5*	*3*	*6*	*1*
DPPH (mg of TE/g)	57.2 ± 11.6^{d}	111.9 ± 7.2^{b}	115.1 ± 7.9^{b}	152.2 ± 12.9^{a}	135.2 ± 10.7^{ab}	$74.7 \pm 13.5^{c,d}$	104.9 ± 14.1^{bc}
Rank	*7*	*4*	*3*	*1*	*2*	*6*	*5*
ABTS (mg of TE/g)	102.0 ± 4.3^{f}	148.5 ± 4.2^{cd}	158.3 ± 2.4^{c}	189.0 ± 6.4^{a}	169.9 ± 1.5^{b}	119.3 ± 0.7^{e}	144.0 ± 4.4^{d}
Rank	*7*	*4*	*3*	*1*	*2*	*6*	*5*
ORAC (mg of TE/g)	425.8 ± 39.2^{d}	652.1 ± 48.8^{bc}	517.3 ± 81.0^{cd}	735.2 ± 54.8^{ab}	859.6 ± 23.5^{a}	406.4 ± 59.8^{d}	615.0 ± 54.8^{bc}
Rank	*6*	*3*	*5*	*2*	*1*	*7*	*4*
ICA (mg of EDTA/g)	47.2 ± 1.3^{a}	37.5 ± 2.1^{b}	33.2 ± 1.1^{bc}	34.0 ± 1.0^{bc}	24.3 ± 1.3^{d}	50.6 ± 2.6^{a}	31.7 ± 2.8^{c}
Rank	*2*	*3*	*5*	*4*	*7*	*1*	*6*

Different letters indicate significant differences at $p < 0.05$.
F–C = Folin-Ciocalteu.

leaves (110.1; 125.2 and 105.2 mg ascorbic acid/g DW for strawberry, blackberry and raspberry, respectively, [36]. Moreover, Sivaci and Duman [37] reported that there were variations in phenolic contents and antioxidant activities according to the variety as they carried out a study on leaves and stems of three almond (*Prunus amygdalus* L.) varieties.

Considering results of the ABTS assay, expressed in mg TE/g, Red Top was the variety with the greatest antioxidant capacity (189 mg TE/g) followed by Romea, Flavorcrest, Dixired and Tebana (169.9,158, 148.5 and 144 mg TE/g, respectively). Spring Belle obtained more moderate value (119.3 mg TE/g, respectively) and Cardinal exhibited the lowest value (102 mg TE/g). As for the other antioxidant assays about peach foliar extract, data are very scarce and we can just estimate our values face to the ones of other Rosaceae plants. To maintain our comparison, we took in note the value of the ethanolic extracts of apple leaves: it is around 171 mg rutin equivalent/g, so in the same order of what we measured in peach leaves [36].

When the antioxidant activity was measured by the ORAC assay, the highest antioxidant activity corresponded to the one of Romea (859.6 mg TE/g) followed by Red Top (735.2 mg TE/g). More moderate values were obtained for Dixired and Tebana (652.1 and 615 mg TE/g, respectively), and then the lowest ones were measured for Cardinal and Spring Belle (425.8 and 406.4 mg TE/g, respectively). Buricova et al. [38] reported ORAC values of water extracts of different Rosaceae leaves and these ones are in accordance to the values that we obtained on peach (around 265; 326 and 222 mg TE/g DW for strawberry, blackberry and raspberry, respectively). Moreover, an ethanol:water extract of *Prunus azorica* displayed a value of 125 mg TE/g DW [39].

The data obtained with F—C, TFC, PFRAP, DPPH, ABTS and ORAC assays showed that polyphenols present in the all foliar peach extracts were potent antioxidative agents. That means that phenolic compounds present in these peach leaf cultivars were major contributors of reducing power and scavenging radicals capacities (DPPH, ABTS and ORAC). Besides, we have generally found antioxidant values stronger than the ones reported in the literature. This point can be explained by differences between used extraction protocols. Indeed we have carried out an acetone extraction whereas in the cited papers on Rosaceae plants, leaves were extracted with water and/or ethanol. And Kratchanova et al. [16] reported that ORAC values and total phenolic content ones were higher for acetone extraction than as instance water extraction. As instance, for raspberry, a polyphenol content of 78 and 49 mg/g was noted after a 80% acetone extraction and a water extraction, respectively; and ORAC values of 289.3 mg TE/g with 80% acetone and 152.2 with water.

Iron chelating activity of leaf extracts of the seven peach varieties was measured. Spring Belle and Cardinal extracts had the strongest ICA values (50.6 and 47.2 mg EDTA/g, respectively). Dixired, Red Top and Flavorcrest showed intermediate values (37.5, 34 and 33.2 mg EDTA/g, respectively). Tebana exhibited a more moderate value with 31.7 mg EDTA/g, whereas Romea displayed the weakest one (24.3 mg EDTA/g). These data suggested that the variety influenced the iron chelating activity of peach leaves. To our knowledge, it is the first time that values of ICA are reported on *Prunus persica* leaves. Nevertheless, Bouaziz et al. [30] have already indicated an ICA activity of leaves extracts of *Crataegus azarolus* L., a Rosaceae plant.

3.4. Pearson correlation analysis

To investigate relationships between all the antioxidant assays, a regression analysis was used. Table 4 shows the Pearson correlation coefficients between the antioxidant capacities values obtained with the different methods for quantifying antioxidant activity.

Table 4
Correlations betweenTPC, TFC and the antioxidant values of the seven studied peach leaves cultivars obtained by different assays (PFRAP, DPPH, ABTS, ORAC, ICA)[a].

	TFC	PFRAP	DPPH	ABTS	ORAC	ICA
F—C	0.6**	0.56**	0.81***	0.82***	0.86***	−0.83***
TFC		0.83***	0.59**	0.55**	0.53*	−0.70***
PFRAP			0.46*	0.51*	0.60**	−0.67***
DPPH				0.93***	0.74***	−0.76***
ABTS					0.79***	−0.77***
ORAC						−0.82***

*, **and *** indicate significant differencesat $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Correlations between F—C, TFC, PFRAP, DPPH, ABTS and ORAC were positively high ($0.46 < r < 0.86$). We can suggest that the hydrogen and electron donating abilities of peach leaf extracts were directly proportional to the concentration of total phenolics.

However, ICA values negatively correlated with F—C, TFC, PFRAP, DPPH, ABTS and ORAC (r ranging from − 0.67 to − 0.83). This weak correlation between the ICA values and, other antioxidant values and the phenolic content, indicate that polyphenols might not be the main iron chelator compounds. These results are in accordance with those obtained by several authors [30,40]. For that reason, it is essential to evaluate antioxidant activities by different methods using different mechanisms of radical-scavenging activity measurement.

4. Conclusion

A combination between liquid chromatography with DAD detector (HPLC-DAD) coupled with mass spectrometry (LC-ESI/MS) and information from the literature have been used to identify and quantify the polyphenols present in the leaves of seven peach varieties. The study of polyphenol profiles by HPLC-DAD-ESI-MS/MS allowed the identification of fourteen phenolic compounds. Flavonols represent the main class of phenolic compounds accounting for an average percentage higher than 95% of the overall phenolics. Kaempferol 3-glucoside is the main phenolic compound in all peach leaf extracts with an average percentage higher than 32% followed by quercetin 3-glucoside (17.9%), quercetin 3-galactoside (17.1%) and kaempferol 3-galactoside (15.4%).

Results showed that variety significantly affected the phenolic content of peach leaves. Romea and Red Top varieties present the higher concentration in phenolic compounds, Dixired, Flavorcrest and Tebana a moderate one and, Cardinal and Spring Belle the lowest content. This high level in phenolics is correlated to antioxidant effects, at least for the ones measured by PFRAP, DPPH, ABTS and ORAC methods. These effective correlations pointed out that, in these cases, phenolic molecules found in peach leaves mainly contribute to the antioxidant potential. To the best of our knowledge, this is the first time that such a comparison of peach leaves was carried out.

All the findings of our study indicate that peach leaves could represent a reliable source of natural antioxidant compounds and that these foliar extracts might be considered for the development of potential pharmaceutical drugs targeting diseases related to oxidative stress. Nevertheless, we planned to go further in our work in order to accurately identify which phenolic molecule(s) contribute(s) to the biological properties of peach leaves as well as their mode of action.

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Appendix A. Supplementary data

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References

[1] C. Andreotti, D. Ravaglia, A. Ragaini, G. Costa, Phenolic compounds in peach (Prunus persica) cultivars at harvest and during fruit maturation, Ann. Appl. Biol. 153 (2008) 11–23.
[2] C.M. Cantin, M.A. Moreno, Y. Gogorcena, Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [Prunus persica (L.) Batsch] breeding progenies, J. Agric. Food Chem. 57 (2009) 4586–4592.
[3] A. Mokrani, S. Krisa, S. Cluzet, G. Da Costa, H. Temsamani, E. Renouf, J.-M. Mérillon, K. Madani, M. Mesnil, A. Monvoisin, Phenolic contents and bioactive potential of peach fruit extracts, Food Chem. 202 (2016) 212–220.
[4] S.B. Rossato, C. Haas, M.d.C.B. Raseira, J.C.F. Moreira, J.Â.S. Zuanazzi, Antioxidant potential of peels and fleshes of peaches from different cultivars, J. Med. Food 12 (2009) 1119–1126.
[5] E. Tsantili, Y. Shin, J.F. Nock, C.B. Watkins, Antioxidant concentrations during chilling injury development in peaches, Postharvest Biol. Technol. 57 (2010) 27–34.
[6] M. Vizzotto, L. Cisneros-Zevallos, D.H. Byrne, D.W. Ramming, W. Okie, Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm, J. Am. Soc. Hortic. Sci. 132 (2007) 334–340.
[7] A. Nadkarni, Indian Materia Medica Popular Prakashan Private Ltd, in, Bombay, 1976.
[8] A.H. Gilani, N. Aziz, S.M. Ali, M. Saeed, Pharmacological basis for the use of peach leaves in constipation, J. Ethnopharmacol. 73 (2000) 87–93.
[9] J.A. Duke, Handbook of Phytochemical Constituent Grass, Herbs and Other Economic Plants, CRC press, 1992.
[10] G. Liakopoulos, S. Stavrianakou, G. Karabourniotis, Analysis of epicuticular phenolics of Prunus persica and Olea europaea leaves: evidence for the chemical origin of the UV-induced blue fluorescence of stomata, Ann. Bot. 87 (2001) 641–648.
[11] S. Dudonné, P. Poupard, P. Coutiere, M. Woillez, T. Richard, J.-M. Mérillon, X. Vitrac, Phenolic composition and antioxidant properties of poplar bud (Populus nigra) extract: individual antioxidant contribution of phenolics and transcriptional effect on skin aging, J. Agric. Food Chem. 59 (2011) 4527–4536.
[12] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (2005) 4290–4302.
[13] V. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[14] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64 (1999) 555–559.
[15] M.S. Blois, Antioxidant Determinations by the Use of a Stable Free Radical, 1958.
[16] M. Kratchanova, P. Denev, M. Ciz, A. Lojek, A. Mihailov, Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems, Acta Biochim. Pol. 57 (2010) 229–234.
[17] B. Ou, M. Hampsch-Woodill, R.L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, J. Agric. Food Chem. 49 (2001) 4619–4626.
[18] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237.
[19] M. Oyaizu, Studies on products of browning reaction–antioxidative activities of products of browning reaction prepared from glucosamine, Eiyogaku Zasshi = Jpn. J. Nutr. 44 (1986) 307–315.
[20] T.C.P. Dinis, V.M.C. Madeira, L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch. Biochem. Biophys. 315 (1994) 161–169.
[21] M. Ye, J. Han, H. Chen, J. Zheng, D. Guo, Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry, J. Am. Soc. Mass Spectrom. 18 (2007) 82–91.
[22] T. Geissman, The flavonoid constituents of normal and virus-infected peach and cherry leaves, Arch. Biochem. Biophys. 60 (1956) 21–26.
[23] R. Llorach, A. Martínez-Sánchez, F.A. Tomás-Barberán, M.I. Gil, F. Ferreres, Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole, Food Chem. 108 (2008) 1028–1038.
[24] J. Oszmiański, A. Wojdylo, J. Gorzelany, I. Kapusta, Identification and characterization of low molecular weight polyphenols in berry leaf extracts by HPLC-DAD and LC-ESI/MS, J. Agric. Food Chem. 59 (2011) 12830–12835.
[25] M. Olszewska, Flavonoids from prunus Serotina ehrh, Acta Pol. Pharm. 62 (2005) 127–133.
[26] M.A. Olszewska, A. Kwapisz, Metabolite profiling and antioxidant activity of Prunus padus L. flowers and leaves, Nat. Prod. Res. 25 (2011) 1115–1131.
[27] L. LianSen, J. HakYoon, N. Crowe, Z. TianHong, P. DiFei, Z. JianTao, G. Dong, L. Meng, Phylogeny of peach (Prunus persica (L.) Batsch) cultivars revealed by HPLC analysis of leaf flavonoids and other phenolics, Adv. Hortic. (1994) 481–486.
[28] A. Kazan, H. Koyu, I.C. Turu, O. Yesil-Celiktas, Supercritical fluid extraction of Prunus persica leaves and utilization possibilities as a source of phenolic compounds, J. Supercrit. Fluids 92 (2014) 55–59.
[29] N.M. Kiyani, D.-N.R. Salamat, M.-S. Naser, S.H. Aghdam, d. Akbar, Estimation the nutritional value and chemical composition of peach tree leaves using in vitro gas production and in situ techniques, Ann. Biol. Res. 2 (2011) 319–324.
[30] A. Bouaziz, S. Khennouf, S. Abdalla, S. Djidel, M. Abu Zarga, A. Bentahar, Phytochemical analysis, antioxidant activity and hypotensive effect of Algerian azarole (Crataegus azarolus L.) leaves extracts, Res. J. Pharm. Biol. Chem. Sci. 5 (2014) 286–305.
[31] P. Rawat, N. Saroj, P. Rawat, P. Kumar, T.D. Singh, M. Pal, Evaluation for total phenolic, total flavonoid and antioxidant activity of leaves and roots of Pyrus pashia, Int. J. Med. Pharm. Res. 1 (2015) 193–196.
[32] P. Jayanthi, P. Lalitha, Reducing power of the solvent extracts of Eichhornia crassipes (Mart.) Solms, Int. J. Pharm. Pharm. Sci. 3 (2011) 126–128.
[33] L. Deb, R. Gupta, A.S. Dutta, A. Yadav, D. Bhowmik, K.S. Kumar, Evaluation of antioxidant activity of aqueous fraction of Prunus persica L aqueous extract, Der Pharm. Sin. 1 (2010) 157–164.
[34] C. Ajila, K. Naidu, S. Bhat, U.P. Rao, Bioactive compounds and antioxidant potential of mango peel extract, Food Chem. 105 (2007) 982–988.
[35] F. Liu, T. Ng, Antioxidative and free radical scavenging activities of selected medicinal herbs, Life Sci. 66 (2000) 725–735.
[36] M. Liaudanskas, P. Viškelis, R. Raudonis, D. Kviklys, N. Uselis, V. Janulis, Phenolic composition and antioxidant activity of Malus domestica leaves, Sci. World J. (2014) 2014.
[37] A. Sivaci, S. Duman, Evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of some almond (Prunus amygdalus L.) varieties, Biol. Res. 47 (2014) 1.
[38] L. Buricova, M. Andjelkovic, A. Cermakova, Z. Reblova, O. Jurcek, E. Kolehmainen, R. Verhe, F. Kvasnicka, Antioxidant Capacities and Antioxidants of Strawberry, Blackberry and Raspberry Leaves, 2011.
[39] L. Tavares, D. Carrilho, M. Tyagi, D. Barata, A.T. Serra, C.M.M. Duarte, R.O. Duarte, R.P. Feliciano, M.R. Bronze, P. Chicau, Antioxidant capacity of Macaronesian traditional medicinal plants, Molecules 15 (2010) 2576–2592.
[40] F. Shahidi, M. Naczk, Phenolics in Food and Nutraceuticals, CRC press, 2003.

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