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In vitro antioxidant activities and phenolic content in crop residues of Tunisian globe artichoke



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ABSTRACT

The phenolic content and *in vitro* antioxidant activity of the bracts, leaves and floral stems of two Tunisian globe artichoke cultivars ('Violet d'Hyères' and 'Blanc d'Oran') were assessed; the tests used to assay antioxidant activity were based on ABTS [2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid] and DPPH [2,2-diphenyl-1-picrylhydrazyl]. In addition reducing power was measured and the phosphomolybdenum total antioxidant activity assay was included. The bracts of 'Violet d'Hyères' possessed, on average, twice the quantity of phenolic compounds than those of 'Blanc d'Oran'. They also had a higher content of 3,5-*O*-dicafeoylquinic acid. The bracts and floral stems contained high levels of total caffeoylquinic acid, whereas the leaves provided a potentially exploitable source of luteolin. Ethanolic extracts of both cultivars exhibited a high level of antioxidant activity. The ABTS EC₅₀ value was high in extracts from the leaves of both cultivars, whereas the DPPH test showed no variation between the bracts, floral stems and leaves. The bracts were associated with strong reducing power and total antioxidant activity. Overall, the leaves were associated with more antioxidant activity than the bracts or the floral stems. The implication is that globe artichoke crop residues could provide a useful source of antioxidant compounds.

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1. Introduction

The increasing demand for high-quality bio-products, economically and environmentally friendly technologies, as well as restrictive legislative actions, has stimulated scientific research on the extraction, purification and identification of bioactive compounds from natural sources. Indeed, the growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on vegetable sources and, as consequence, on the screening of raw materials in order to identify new antioxidants (Moure et al., 2001). Replacing synthetic antioxidants with natural ones may have benefits for health implications and industrial applications, such as the solubility in both oil and water. Phenolic compounds, ubiquitous in plants, are an essential part of the human diet and are of considerable interest due to their antioxidant

properties (Balasundram et al., 2006). In particular, several researchers have investigated the possibility of extracting natural antioxidants from agricultural and industrial residues such as potato peel waste (Rodriguez de Sotillo et al., 1994), olive oil waste waters (Visioli et al., 1999), grape seeds (Yamaguchi et al., 1999), mango peels (Berardini et al., 2005) and apple pomace (Schieber et al., 2003).

The globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is native to the Mediterranean area, where its commercial production makes a significant contribution to the agricultural economy (Mauromicale and Ierna, 2000). About 65% of its global production is concentrated in the Mediterranean Basin (FAOstat, 2012), a region where autochthonous landraces have been grown (Mauro et al., 2009). This crop is grown for its immature inflorescence (also referred as capitulum or head), which is widely consumed as fresh or conserved vegetable, due to its good sensory properties and content of health-promoting compounds (Lombardo et al., 2010, 2012, 2013; Schütz et al., 2006). Globe artichoke is commonly used in traditional medicine due

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to its pharmacological properties such as its anti-inflammatory, antihepatotoxicity, antioxidative, anti-lipoperoxidation, antidiabetic, anti-cancer, glycaemia reduction and antimicrobial activities (Coinu et al., 2007; Fantini et al., 2011; Rondanelli et al., 2011; Saénz-Rodríguez et al., 2002). The use of residues (mostly external bracts, leaves and floral stem) as sources of bioactive compounds has emerged as an economically viable solution to the problem of solid waste treatment. These crop residues represent 80–85% of the above-ground biomass and have a high content of flavones luteolin and apigenin and caffeoylquinic acids (Llorach et al., 2002; Pandino et al., 2013a, 2013b; Romani et al., 2006; Wang et al., 2003). The two main phenolic compounds are 5-O-caffeoylquinic acid and 1,5-di-O-caffeoylquinic acid (Schütz et al., 2004; Wang et al., 2003; Yoo et al., 2012), which have a strong antioxidant capacity (Vinson et al., 1995).

A comparison of the phenolic content of globe artichoke part of plants showed an extremely low content of hydroxycinnamic acids and flavonoids in the leaves versus the content in receptacle and flower bracts (Fратиanni et al., 2007). The high phenolic content in globe artichoke crop residues may vary according to plant parts and cultivars (Farag et al., 2013; Pandino et al., 2012a), head maturity, storage and processing techniques (Lutz et al., 2011; Pandino et al., 2012b; Wang et al., 2003).

In Tunisia, even if the globe artichoke production has increased during last years, only papers on the characterization of wild cardoon (*C. cardunculus* var. *sylvestris*) have been published (Falleh et al., 2008; Khaldi et al., 2013). To the best of our knowledge, this is the first study to report the phenolic profile and antioxidant activities from waste material (bracts, leaves and stems) of Tunisian cultivars of globe artichoke. The aim of this research was to quantify the extracted phenolic contents in the capitula residues (bracts, floral stem and leaves) of the two Tunisian globe artichoke cultivars and to evaluate the antioxidant activities of the extracts, so as to identify the plant's materials of major interest for pharmaceutical and related industries.

2. Materials and methods

2.1. Plant material, experimental field and management practices

Two cultivars ('Violet d'Hyères' and 'Blanc d'Oran'), native in northern Tunisia and producing green heads from April to May, were grown in 2011–2012 season at the experimental field of Technical Center of Potato and Artichoke of Tunisia located in Jdaïda-Mannouba (latitude 36°49'25.24" N, longitude 9°57'55.09" W, altitude 595 m). This is a typical area for globe artichoke cultivation in the Mediterranean Basin, with mild winter and hot rainless summer. Plants were spaced by 1.2 m × 0.6 m with 13,888 plants/ha for 'Blanc d'Oran' and by 1.2 m × 0.8 m with 10,416 plants/ha for 'Violet d'Hyères'. Crop management practices (irrigation, fertilization, pest management, weeds control, etc.) were subsequently performed according to local practices.

2.2. Reagents and solvents

Reagents and solvents were purchased from VWR (Leighton Buzzard, UK) and were of analytical or HPLC grade. Apigenin-7-O-glucoside, apigenin, luteolin-7-O-glucoside, luteolin, 5-O-caffeoylquinic acid (chlorogenic acid) were obtained from Extrasynthese (Lyon, France), 1,3-O-di-caffeoylquinic acid (cynarin) was from Roth (Karlsruhe, Germany).

2.3. Extraction procedure

Plant residue parts of globe artichoke (bracts, leaves and floral stem) were collected separately at the usual marketing stage

regardless of their size when the central global flower buds of the capitula were shorter than 2 mm (this is the normal marketing stage, Mauromicale and Ierna, 2005). They were washed with running water to remove impurities, air-dried in an oven at a temperature of 37 °C and, finally, crushed and sieved through mesh cloth to get the fine powder. About 50 g of the powdered plant materials (bracts, leaves and floral stem) were extracted with 500 mL of 95% ethanol by maceration under stirring at room temperature for 7 days (Harikrishnan and Balasundaram, 2005). After filtration, the solvent was evaporated under vacuum and all the resulting extracts obtained were then transferred to vials and stored in the dark at 4 °C to preserve them from photo-oxidation.

2.4. HPLC analysis

The extraction procedure, performed for samples under study, was carried out as described previously by Pandino et al. (2010). Each extract was analysed using a series 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA) equipped with ChemStation software (B.03.01) and a diode array detection system. Separations were achieved on a Zorbax Eclipse XDB-C18 (4.6 mm × 150 mm; 5.0 µm particle size), operated at 30 °C, with a 0.2 µm stainless steel inline filter. The method was adapted from Pandino et al. (2010): the mobile phase was 1% formic acid in water (solvent A) and in acetonitrile (solvent B) at a flow rate of 0.5 mL/min. The gradient started with 5% B to reach 10% B at 10 min, 40% B at 30 min, 90% B at 50 min, 90% B at 58 min. Chromatograms were recorded at 280, 310, and 350 nm from diode array and data collected between 200 and 600 nm. Each compound was identified based on retention time, UV spectrum and already published identification on compounds from globe artichoke (Schütz et al., 2004; Wang et al., 2003). Quantification was performed by calibration curve using the available standards. In particular, mono- and dicaffeoylquinic acids were calculated using 5-O-caffeoylquinic acid and 1,3-O-dicaffeoylquinic acid as reference, respectively. Here, the caffeoylquinic acids are presented according to Lattanzio et al. (2009). Apigenin and luteolin conjugates were quantified as apigenin-7-O-glucoside and luteolin-7-O-glucoside, respectively. All data presented are mean values ± standard deviation of three independent experiments ($n=3$) and expressed as mg g^{-1} of dry matter (DM).

2.5. Antioxidant activity

To determine antioxidant activity, different concentrations of ethanolic extracts were prepared in methanol. Four common tests for measuring antioxidant activity were applied to globe artichoke waste material extracts: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid antioxidant capacity assay (ABTS assay), 2,2-diphenyl-1-picrylhydrazyl assay (DPPH assay), reducing power assay and total antioxidant activity (Phosphomolybdenum assay). The four methods presented can be divided into two groups depending on the oxidizing reagent. Two methods use organic radical producers (ABTS and DPPH assays) and the others work with metal ions for oxidation (Phosphomolybdenum and reducing power assays). The ABTS and DPPH tests acting by radical reduction use preformed radicals and determine the decrease in absorbance, while the reducing power and phosphomolybdenum assays measure the formed ferrous ions by increased absorbance.

2.6. DPPH radical scavenging assay

An aliquot (20 µL) from the stock solution of each extract was dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol) (Kontogiorgis

and Hadjipavlou-Litina, 2003). The reaction mixture was kept at room temperature and the absorbance of the solution was measured at 517 nm after 20 min. The absorbance of the samples in the absence of DPPH were subtracted from the corresponding absorbance with DPPH. The % inhibition (I) of the free radical DPPH was calculated using the following equation:

$$I\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the tested compound), and A_{sample} is the absorbance of the tested compound. A_{blank} was read against absolute ethanol. The concentration of the sample, required to scavenge 50% of the DPPH free radical, was determined by the plot between % inhibition and concentration and labelled as EC_{50} . A lower EC_{50} value corresponds to a higher antioxidant activity of the plant extract.

2.7. ABTS+ radical cation scavenging

For the determination of the antiradical activity, a protocol based on the ABTS+ free radical discoloration assay was used as described previously (Rice-Evans, 1999). A stock solution of 7 mM of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) aqueous solution was prepared. ABTS radical cations (ABTS^{•+}) were produced by the reaction of the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) (Re et al., 1999; Rice-Evans et al., 1996). The stock solution was kept in the dark at room temperature for 16 h, allowing it to form the ABTS radicals (ABTS^{•+}). The radicals were stable in this form for more than 2 days when stored under these conditions. Finally, the stock solution was diluted with ethanol (approx. 1/88) to obtain an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30 °C. A reagent blank reading was taken (A0). For the spectrophotometric assay, 3.9 mL of the ABTS^{•+} diluted solution was mixed with 100 mL of ethanolic extract. Mixtures were mixed vigorously for 30 s and allowed to stand for 5 min in the dark at room temperature. Subsequently, the absorbance for each sample (ABTS^{•+} solution plus compound, At) was measured at 734 nm and corrected for the absorbance of a control (ABTS^{•+} solution without test sample, A0). The absorbance reading was taken at 30 °C exactly 5 min after the initial mixing. The % inhibition and the concentration of the sample, required to scavenge 50% of the ABTS free radical, was determined as in DPPH radical scavenging activity.

2.8. Reducing power assay

The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, Fe^{2+} concentration can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The capacity of extracts to reduce Fe^{3+} was assessed by the method of Oyaizu (1986). An aliquot of each sample (250 μ L) was mixed with 250 μ L of sodium phosphate buffer (0.2 M, pH 6.6) and 250 μ L of 1% $K_3Fe(CN)_6$ incubated at 50 °C for 20 min. After adding 250 μ L of 10% trichloroacetic acid, the mixture was centrifuged at $3750 \times g$ for 10 min. The supernatant (100 μ L) was then taken out and immediately mixed with 100 μ L of methanol and 25 μ L of 0.1% ferric chloride. After incubation for 10 min, the absorbance against blank was determined at 700 nm. Increased absorbance indicates increased reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated by the graph of absorbance at 700 nm against extract concentration.

2.9. Phosphomolybdenum assay

Briefly, 200 μ L of extract samples were mixed with 2 mL of the phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) (Prieto et al., 1999). The test tubes were incubated for 90 min at 95 °C and cooled to room temperature. Subsequently the absorbance was measured at 695 nm. A standard curve was constructed for ascorbic acid in order to estimate the percentage of molybdenum reduced by tested extracts. Results were expressed as μ g ascorbic acid/mL extract. EC_{50} (mg/mL) is the effective concentration at which the total antioxidant activity (TAA) was 50% and was obtained by interpolation from linear regression analysis.

3. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and means were separated by Duncan test ($p < 0.05$) for bracts, leaves and floral stem from the same globe artichoke cultivar, by Student test for cultivars and correlations analysis by Pearson test, using the SPSS program, release 11.0 for Windows (SPSS, Chicago, IL, USA). All data presented represent mean values of three independent experiments ($n = 3$). Samples were also discriminated by multivariate parametric methods where the principal component analysis (PCA) was carried out using XLStat-Pro 7.5 (2007) for Windows (Addinsoft, New York, NY, USA).

4. Results and discussion

4.1. Phenolic profile

The phenolic profiles of the residues of two Tunisian globe artichoke cultivars ('Violet d'Hyères' and 'Blanc d'Oran') was obtained by chromatographic separation, UV and mass spectrometry (Table 1). The analysis identified the presence of 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid (chlorogenic acid), 3,5-O-dicaffeoylquinic acid, 1,5-O-dicaffeoylquinic acid and monosuccinylcaffeoylquinic acid, along with the flavonoids luteolin-7-O-glucoside, luteolin-7-O-glucuronide, luteolin-7-O-malonylglucoside, luteolin, apigenin-7-O-glucoside and apigenin. The profiles varied both qualitatively and quantitatively between the bracts, the leaves and the floral stems. The bracts of 'Violet d'Hyères' contained a higher content of polyphenols than those of 'Blanc d'Oran' (Table 1). 'Violet d'Hyères', which is much appreciated in Tunisia for the culinary flavor of its capitulum, can in effect be regarded as a functional food, and also as a potential source of antioxidant phenolics. The indications were that specific phenolic compounds accumulated in particular parts of the plant, as also noted by previous works (Lombardo et al., 2010; Fratianni et al., 2007; Pandino et al., 2011). It is well understood that globe artichoke leaves contain appreciable amounts of both mono- and di-caffeoylquinic acids (Gouveia and Castilho, 2012; Llorach et al., 2002; Wang et al., 2003). On a dry weight basis, artichoke leaves can contain from 1 to 6% of each of these two compounds (Hammouda et al., 1993; Slanina et al., 1993). Here, the caffeoylquinic acids were well represented, particularly in the bracts and the floral stems (Table 1), but they were present in only very low amounts in the leaves. One possible explanation for this potentially aberrant result is that whereas young leaves were analysed in the present experiments, other researchers have concentrated on the analysis of mature leaves (Negro et al., 2012; Wang et al., 2003). The predominant phenolic compound present in the leaves of 'Violet d'Hyères' was 5-O-caffeoylquinic acid, while in 'Blanc d'Oran' it was 1,5-O-dicaffeoylquinic acid, just as is the case in the leaves of the Italian cultivar 'Violetto di Toscana' (Coinu et al., 2007; Romani

Table 1

Phenolic profile of ethanolic extracts (mg/g dry matter) from bracts, leaves and floral stem of two globe artichoke cultivars grown in Tunisia.

Compound	'Violet d'Hyères'			'Blanc d'Oran'		
	Bracts	Leaves	Floral stem	Bracts	Leaves	Floral stem
3-O-caffeoylquinic acid	nd	nd	0.21 ± 0.06	nd	nd	nd
5-O-caffeoylquinic acid	3.18 ± 1.54	3.82 ± 0.87	3.17 ± 0.47	2.99 ± 0.52	2.13 ± 0.49	2.17 ± 0.35
3,5-O-dicaffeoylquinic acid	7.57 ± 0.54 ^a	1.26 ± 0.13 ^b	2.20 ± 0.68 ^b	1.14 ± 0.86 ^y	0.41 ± 0.04 ^y	2.73 ± 0.79 ^x
1,5-O-dicaffeoylquinic acid	3.38 ± 0.34 ^a	0.85 ± 0.20 ^b	4.03 ± 1.02 ^a	2.39 ± 1.20 ^y	0.79 ± 0.07 ^y	5.39 ± 1.40 ^x
monosuccinylcaffeoylquinic acid	0.62 ± 0.13 ^a	0.24 ± 0.07 ^b	0.71 ± 0.17 ^a	0.37 ± 0.04 ^x	0.15 ± 0.01 ^y	0.29 ± 0.06 ^x
Total caffeoylquinic acid	14.74 ± 2.39^a	6.16 ± 1.13^c	10.32 ± 2.23^b	6.90 ± 1.90^y	3.48 ± 0.55^z	10.59 ± 2.30^x
apigenin-7-O-glucoside	0.42 ± 0.13 ^a	0.16 ± 0.14 ^b	nd	0.35 ± 0.11 ^x	0.18 ± 0.03 ^y	nd
apigenin	0.13 ± 0.01	nd	nd	0.04 ± 0.01	nd	nd
Total apigenin	0.55 ± 0.12^a	0.16 ± 0.14^b	nd	0.39 ± 0.10^x	0.18 ± 0.03^y	nd
luteolin-7-O-glucoside	nd	2.47 ± 0.71	nd	nd	2.79 ± 0.42	nd
luteolin-7-O-glucuronide	nd	1.45 ± 0.09	nd	nd	1.73 ± 0.37	nd
luteolin-7-O-malonylglucoside	nd	0.25 ± 0.06	nd	nd	0.23 ± 0.01	nd
luteolin	0.04 ± 0.03	0.01 ± 0.01	nd	nd	0.03 ± 0.02	nd
Total luteolin	0.04 ± 0.07^b	4.18 ± 0.82^a	nd	nd	4.79 ± 0.80^x	nd
Total polyphenols identified	15.34 ± 2.47^a	10.51 ± 2.07^b	10.32 ± 2.23^b	7.29 ± 1.91^x	8.45 ± 1.26^x	10.59 ± 2.30^x

Mean composition of sampled globe artichoke from three replications ± standard deviation. Different letters (a–c) and (x–z), for the same compound, within each row indicate significant differences ($p < 0.05$) among plant parts of each cultivar.

et al., 2006). This variation may reflect a real genetic effect, but there remains a possibility that it reflects an artefact associated with either incomplete HPLC separation and/or imperfect extraction of the phenolic compounds. Globe artichoke bracts and leaves tend to accumulate flavonoids, a class of compound completely absent from the floral stems (Romani et al., 2006). The flavonoid apigenin-7-O-glucoside was present in small concentrations in the leaves of both cultivars (0.16 mg/g dry matter in 'Violet d'Hyères' and 0.18 mg/g in 'Blanc d'Oran') (Table 1), levels which are similar to those found in the leaves of 'Violetto di Sicilia' (Lombardo et al., 2010). While apigenin also is accumulated in 'Violetto di Sicilia' leaves, this flavonoid was undetectable in either the leaves or the floral stems of either of the Tunisian cultivars. Luteolin was also absent from the floral stems of both cultivars and the 'Blanc d'Oran' bracts, while its derivatives luteolin-7-O-glucoside, luteolin-7-O-glucuronide and luteolin-7-O-malonylglucoside were all abundant in the leaves of both cultivars.

4.2. Antioxidant activity

The principle of antioxidant activity is based on the availability of electrons to neutralize any free radicals. In this study, the antioxidant activity of the extracts of globe artichoke by-products have been evaluated applying DPPH, ABTS+ radical-scavenging and phosphomolybdenum and reducing power assays.

4.3. ABTS assay

The antioxidant activity exhibited by ethanolic extracts of the bracts, leaves and floral stems of the two cultivars was evaluated using the ABTS assay. The highest activity was concentrated in the bracts in both cultivars (Fig. 1), while that present in the floral stems was higher than that in the leaves. The assay suggested the possibility of a cultivar effect on antioxidant activity. Overall, the Tunisian cultivars appeared to represent a promising source of antioxidant compounds.

4.4. DPPH assay

DPPH has been widely accepted as the basis for quantifying free radical scavenging activity (Sanchez-Moreno, 2002). This assay did not reveal any differences between the various parts of the 'Violet d'Hyères' plant (Fig. 2), while extracts of the leaves and floral stems of 'Blanc d'Oran' produced a significantly higher EC50 estimate than that of its bracts (Table 2). The bracts of 'Blanc d'Oran' contained the least phenolics, but were associated with the highest measured antioxidant activity. Thus the data suggested that free radical scavenging activity was not influenced by the phenolic content, while most of the variation for antioxidant activity could be accounted for by variation in the phenolic profile.

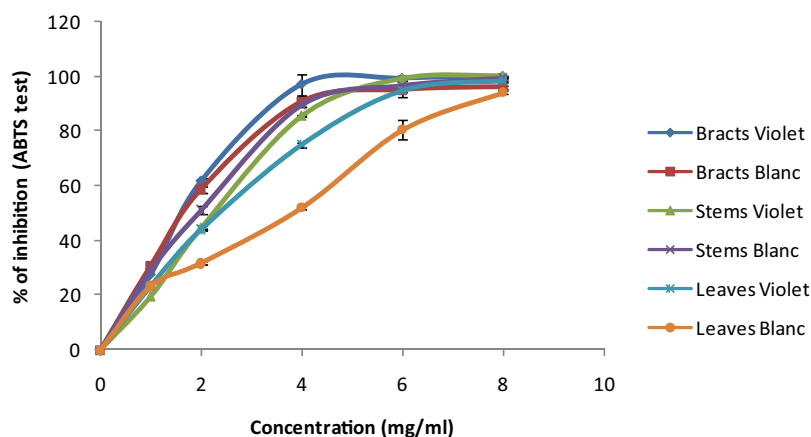


Fig. 1. Radical scavenging activity determined by ABTS assay in the presence of different concentrations in globe artichoke waste materials. Vertical bars represent the standard deviation.

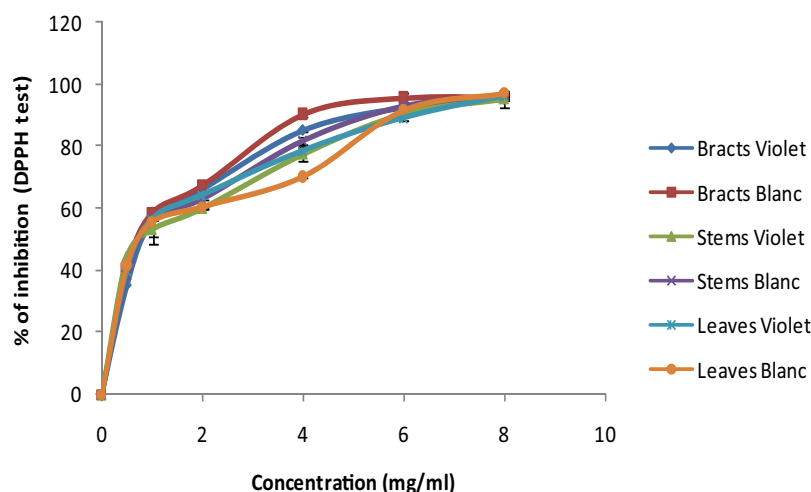


Fig. 2. Radical scavenging activity determined by DPPH assay in the presence of different concentrations in globe artichoke waste materials. Vertical bars represent the standard deviation.

4.5. Phosphomolybdenum assay

The antioxidant capacity shown by the ethanolic extracts of the globe artichoke residues was measured spectrophotometrically using the phosphomolybdenum method (Prieto et al., 1999). At 1000 μg residue per mL ethanol, the extract from 'Blanc d'Oran' bracts displayed a high level of antioxidant capacity, whereas a similar extract from the bracts of 'Violet d'Hyères' showed a lower capacity (Fig. 3). At both 200 and 1000 $\mu\text{g}/\text{mL}$, extracts of 'Blanc d'Oran' leaves proved to be less active than those produced from the leaves of 'Violet d'Hyères'. Extracts of the 'Violet d'Hyères' floral stems had a lower capacity with increase of dose. Variation in the antioxidant capacity of these extracts may be attributable to differences in the tissues' phenolic content, especially with respect to other polyphenols such as ellagic tannins, ellagic acid and gallic acid (Gil et al., 2000).

4.6. Reducing power assay

To measure the reductive capacity of the artichoke residues, the transformation induced by ethanolic extracts of Fe^{3+} to Fe^{2+} was investigated following the Oyaizu (1986) method. In the concentration range investigated, the reducing power of each extract increased linearly with concentration. The bracts of 'Blanc d'Oran' possessed the most reducing power. At 500 $\mu\text{g}/\text{mL}$, the reducing power of both cultivars was >0.5 , and the extracts were ranked in the order 'Blanc d'Oran' bracts, 'Violet d'Hyères' bracts, 'Blanc d'Oran' floral stem, 'Violet d'Hyères' floral stem, 'Violet d'Hyères' leaves, 'Blanc d'Oran' leaves (Fig. 4). In a similar study, Vamanu et al. (2011) has shown that increasing the concentration of the

extract raises the measured reducing power, and that a direct relationship between antioxidant activity and reducing power is to be found. A highly positive correlation has been established in many plant species between phenolic content and antioxidant activity (Oktay et al., 2003), while there is also a direct correlation between antioxidant activity and reducing power, at least in some plant extracts (Tanaka et al., 1988). Reducing activity is generally associated with the presence of reductones (Duh et al., 1999), which exert their antioxidant action by disrupting free radical chains through the transfer of a proton (Gordon, 1990). Reductones have also been reported to react with peroxide precursors, thereby inhibiting peroxide formation. The implication is that globe artichoke residues contain high amounts of reductone available for the stabilization and termination of radical chain reactions.

4.7. EC_{50} in antioxidant properties

Table 2 summarizes the antioxidant properties of the full set of materials, as measured by their reducing power, phosphomolybdenum and DPPH and ABTS assays. The data have been normalized and expressed in the form of EC_{50} values (mg ethanolic extract per mL) for comparison. On this basis, residues of 'Blanc d'Oran' performed better than those of 'Violet d'Hyères', although both cultivars performed well with respect to the ABTS assay. Overall, bract extracts were associated with a lower EC_{50} than either the floral stems or the leaves. The levels of antioxidant present in the globe artichoke residues were sufficiently high to warrant their exploitation in the formulation of nutraceuticals and functional foods.

Table 2

EC_{50} values (mg/mL) of ethanolic extracts from bracts, leaves and floral stem of the two globe artichoke cultivars grown in Tunisia.

Assay	'Violet d'Hyères'			'Blanc d'Oran'		
	Bracts	Leaves	Floral stem	Bracts	Leaves	Floral stem
*ABTS	1.56 $\pm$ 0.08 ^b	2.11 $\pm$ 0.04 ^a	2.05 $\pm$ 0.01 ^a	1.58 $\pm$ 0.06 ^z	2.73 $\pm$ 0.09 ^x	1.74 $\pm$ 0.04 ^y
*DPPH	0.89 $\pm$ 0.07	0.82 $\pm$ 0.01	0.82 $\pm$ 0.07	0.74 $\pm$ 0.01 ^y	0.87 $\pm$ 0.04 ^x	0.85 $\pm$ 0.01 ^x
**TAA	0.38 $\pm$ 0.01 ^c	0.40 $\pm$ 0.01 ^b	0.60 $\pm$ 0.01 ^a	0.31 $\pm$ 0.01 ^z	0.40 $\pm$ 0.01 ^y	0.60 $\pm$ 0.01 ^x
***Reducing power	0.27 $\pm$ 0.01 ^b	0.34 $\pm$ 0.01 ^a	0.34 $\pm$ 0.01 ^a	0.22 $\pm$ 0.01 ^z	0.38 $\pm$ 0.01 ^x	0.31 $\pm$ 0.01 ^y

Mean composition of sampled globe artichoke from three replications $\pm$ standard deviation. Different letters (a–c) and (x–z), for the same compound, within each row indicate significant differences ($p < 0.05$) among plant parts of each cultivar.

* EC_{50} (mg/mL): effective concentration at which 50% of DPPH or ABTS radicals are scavenged.

** EC_{50} (mg/mL): effective concentration at which the total antioxidant activity (TAA) was 50%.

*** EC_{50} (mg/mL): effective concentration at which the absorbance is 0.5.

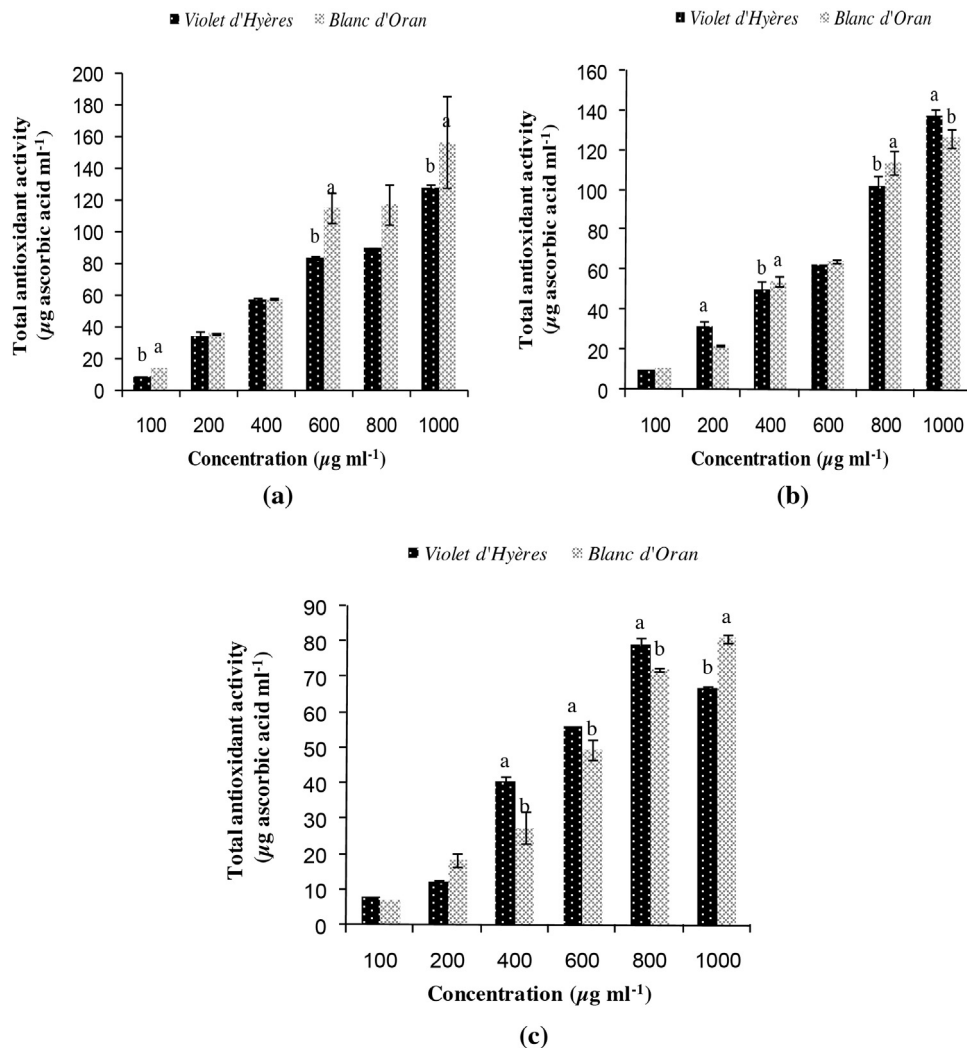


Fig. 3. Total antioxidant activity determined by Phosphomolybdenum assay as vitamin C equivalents in the presence of different concentrations from bracts (a), leaves (b) and floral stem (c) of two globe artichoke cultivars grown in Tunisia. Vertical bars represent the standard deviation.

4.8. Multivariate analysis

A multivariate PCA was applied to the phenolic content and antioxidant activity present in the residues of each cultivar (Fig. 5a and b). The first axis, which accounted for 42.1% of the overall variance, distinguished the floral stems, while the

second (23.4% of the variance) differentiated the bracts and leaves. A comparison between the two PCA plots indicated that the EC50s associated with the phosphomolybdenum assay and the contents of 3-*O*-caffeoylquinic acid, 1,5-*O*-dicafeoylquinic acid and monosuccinylcaffeoylquinic acid largely explained the discrimination of the floral stems, whereas the EC50s associated

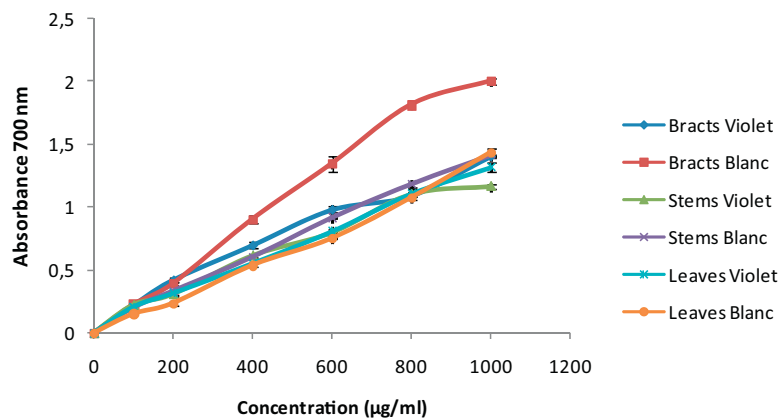


Fig. 4. Reducing power assay: absorbance changes at 700 nm in the presence of different concentrations in globe artichoke waste materials. Vertical bars represent the standard deviation.

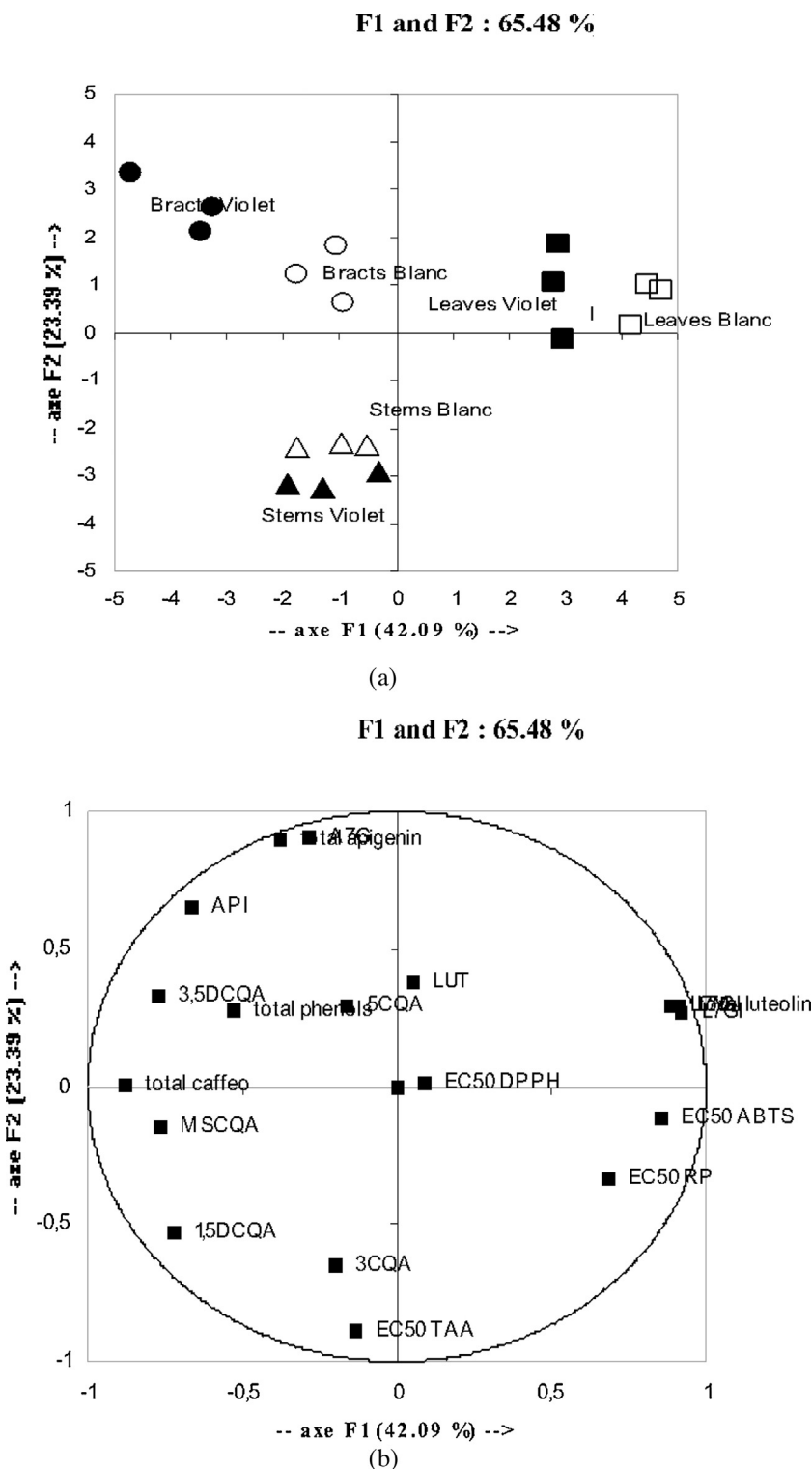


Fig. 5. Score plot (a) and loading plot (b) of principal component analysis applied to the data set of phenolic profile and antioxidants activities of globe artichoke waste materials from two cultivars. The two cultivars were presented as follows: 'Violet d'Hyères' (filled symbols) and 'Blanc d'Oran' (empty symbols), while their waste materials as: bracts (circles), leaves (squares) and floral stem (triangles).

3CQA=3-*O*-caffeoylquinic acid; 5CQA=5-*O*-caffeoylquinic acid; 3,5DCQA=3,5-*O*-dicaffeoylquinic acid; 1,5DCQA=1,5-*O*-dicaffeoylquinic acid; MSCQA=monosuccinyl-caffeoylquinic acid; A7G=apigenin-7-*O*-glucoside; API=apigenin; L7G=luteolin-7-*O*-glucoside; L7Gl=luteolin-7-*O*-glucuronide; L7M=luteolin-7-*O*-malonylglucoside; LUT=luteolin; total caffeo=total caffeoylquinic acids.

with the ABTS and DPPH assays, along with the total luteolin, luteolin-7-*O*-malonylglucoside, luteolin-7-*O*-glucoside and luteolin-7-*O*-glucuronide contents, were the major contributors to the separation of the leaf material from the rest of the plant residue.

The contents of caffeoylquinic acid, 3,5-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, phenolics, apigenin, luteolin, apigenin and apigenin-7-*O*-glucoside differentiated the bracts. Consequently, the data obtained by the multivariate analysis confirm those

Table 3
Matrix of correlation of selected parameters of waste materials from the two globe artichoke cultivars.

	3CQA	5CQA	3,5DCQA	1,5DCQA	MSCQA	A7G	API	L7G	L7GI	L7M	LUT	Total caffeoylquinic acid	Total apigenin	Total luteolin	Total phenols	EC ₅₀ ABTS	EC ₅₀ TAA	EC ₅₀ RP	EC ₅₀ DPPH
3CQA	1																		
5CQA	ns	1																	
3,5DCQA	ns	ns	1																
1,5DCQA	ns	ns	ns	1															
MSCQA	0.672**	ns	0.594**	0.512*	1														
A7G	ns	ns	ns	ns	ns	1													
API	ns	ns	0.851**	ns	0.712**	0.712**	1												
L7G	ns	ns	0.851**	-0.494**	-0.748**	-0.637**	ns	1											
L7GI	ns	ns	-0.503**	-0.759**	-0.662**	-0.662**	ns	0.983**	1										
L7M	ns	ns	-0.486**	-0.753**	-0.628**	-0.628**	ns	0.985**	0.958**	1									
LUT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1								
Total caffeoylquinic acid	ns	ns	0.893**	0.748**	0.759**	0.759**	0.617**	-0.669**	-0.694**	-0.650**	ns	1							
Total apigenin	ns	ns	0.535**	ns	ns	0.988**	0.812**	ns	ns	ns	ns	ns	1						
Total luteolin	ns	ns	-0.494**	-0.756**	-0.648**	ns	0.574**	0.998**	0.992**	0.980**	ns	-0.678**	ns	1					
Total phenols	ns	ns	0.851**	ns	0.545**	ns	ns	ns	ns	ns	ns	0.844**	ns	ns	1				
EC ₅₀ ABTS	ns	ns	ns	-0.718**	-0.522**	ns	ns	0.807**	0.837**	0.746**	ns	-0.495**	ns	0.822**	ns	1			
EC ₅₀ TAA	ns	ns	ns	ns	0.863**	0.795**	ns	ns	ns	ns	ns	ns	0.892**	ns	ns	ns	1		
EC ₅₀ RP	ns	ns	ns	-0.800**	ns	-0.538**	ns	0.664**	0.672**	0.647**	ns	ns	-0.504**	0.673**	ns	0.866**	ns	1	
EC ₅₀ DPPH	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1

3CQA = 3-O-caffeoylquinic acid; 5CQA = 5-O-caffeoylquinic acid; 3,5DCQA = 3,5-O-dicaffeoylquinic acid; 1,5DCQA = 1,5-O-dicaffeoylquinic acid; MSCQA = monosuccinyl/caffeoylquinic acid; A7G = apigenin-7-O-glucoside; API = apigenin; L7G = luteolin-7-O-glucoside; L7GI = luteolin-7-O-glucuronide; L7M = luteolin-7-O-malonylglucoside; LUT = luteolin; total caffeoylquinic acid; total apigenin; total luteolin; total phenols; EC₅₀ ABTS; EC₅₀ TAA; EC₅₀ RP; EC₅₀ DPPH.

* Significant correlation (0.05).

** Significant correlation (0.01).

obtained previously, showing that each cultivar behaves differently with respect to the plant part (Pandino et al., 2013a).

4.9. Pearson correlation analysis

The correlation between the content of phenolics and antioxidant activity is well founded, but little consideration has been given to the existence of qualitative and/or quantitative variation in the phenolics profile. Furthermore, total phenolic compounds have been reported to be responsible for the antioxidant activities of globe artichoke as well as their by-products (Alghazeer et al., 2012; Lombardo et al., 2013; Pandino et al., 2011). Thus, an evaluation of the correlation between antioxidant activity and phenolic profile was attempted here (Table 3). Analysis of the data using a Pearson correlation approach indicated that the major phenolics affecting radical scavenging activity were luteolin-7-O-glucuronide ($r = +0.84$, $p < 0.01$) followed by total luteolin ($+0.82$, $p < 0.01$) and luteolin-7-O-glucoside ($+0.81$, $p < 0.01$). The most convincing correlations were revealed where the antioxidant data was obtained using the phospholybdenum method ($r = +0.89$ for total apigenin, $r = +0.86$ for apigenin-7-O-glucoside). The results suggest that the flavonoids, which constitute the bulk of the phenolics present in artichoke residues (Pandino et al., 2013b), provide the majority of the antioxidant activity, because they are highly reactive as hydrogen and electron donors (Cotelle, 2001). A negative, and only moderate low correlation was established between the content of caffeoylquinic acid and the ABTS assay (-0.49 , $p < 0.05$).

5. Conclusions

Overall, there were significant cultivar differences for the total phenolic content and species profile in the artichoke residues. The bracts and floral stem contained high levels of caffeoylquinic acid, whereas luteolin derivatives featured strongly in the leaves. The multivariate analysis implied that the content of phenolics and the level of antioxidant activity were more strongly influenced by the plant organ than by cultivar. The bracts, leaves and floral stem, generally considered as a waste product, should rather be regarded as a potential source of income derived via the extraction of natural antioxidants. Their presence explains why the plant is exploited in many herbal medicine formulations. A potential market for these products lies in the supplementation of various foodstuffs or as raw material for the pharmaceutical industry.

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