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Phenolic, non-phenolic compounds and antioxidant activity of pomegranate flower, leaf and bark extracts of four Tunisian cultivars

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The aim of this work was to investigate phenolic and non-phenolic content and to determine the antioxidant activity in pomegranate flowers, leaves and barks among four Tunisian cultivars. The highest amounts of poylphenol compounds were recorded in Tounsi flower and Gabsi leaf extracts (218.64 and 131.91 mg/g of dry weight (DW), respectively). However, bark extracts showed relatively low proportions of phenolics but exhibited high concentrations of carotenoïds. By measuring the antioxidant activity of the studied samples, the reducing power was correlated with phenolic compounds (r = 0.760; p < 0.01). The highest concentrations were recorded in Gabsi leaves and Chelfi flowers (254.25 and 140.63 mg/ml, respectively). 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was correlated with total carotenoïds content (r = 0.733; p < 0.01). These results suggest that the reducing power of our samples is mainly provided by phenolic compounds whereas non phenolic compounds are mainly contributing to the scavenging activity in the flowering period of pomegranate tree.

Key words: Pomegranate tree, antioxidant activity, phenolic compounds, non phenolic compounds, flowering period.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belonging to the Punicaceae family, is a deciduous small tree distributed originally in the region from Iran to the Himalayas in northern India (Elfalleh et al., 2011). In Tunisia, pomegranate has been cultivated traditionally under diverse agro-climatic conditions. It is well known typically in the coastal regions and in many regions inside the country (Mars and Marrakchi, 1998). Tunisia is considered as a micro-gene center of this species, with more than 60 local ecotypes already collected (Mars, 2001). The region of Gabes which presents the first production department pomegranate in Tunisia contributes to 35% of the total production of these fruits, with 25,000 tons in the year 2011 (General Directorate of Agricultural Production (GDAP), 2011). The region of Testour (governorate of Béja) is an important producer of pomegranates. It has large amount of pomegranate trees in the country, with 400 thousand trees. Testour produced 11.900 tons of pomegranates in 2012. The relationship between quality of foods and health has been recognized for thousands of years.

Recently, consumption of fresh fruit and natural juice increased widely, not only nutritional value, but also therapeutic effect. Pomegranates are popularly consumed

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as fresh fruit, as beverages, as food products and as extracts wherein they are used as botanical ingredients in herbal medicines and dietary supplements. The major source of dietary pomegranate phytochemicals is the fruit (peel, seeds and juice) (Elfalleh et al., 2011).

Pomegranate leaf extract exhibited antioxidant effects through free radical scavenging activity *in vitro*. Antioxidants from leaf tissue have been isolated and identified (Hussein et al., 1997). Pomegranate leaf extract have shown many pharmacological activities, such as antioxidant, antibacterial, astringent, antitumor, and antidiarrheal activity (Zhang et al., 2010). In Ayurvedic medicine, the pomegranate is considered "a pharmacy unto itself," with the bark and roots believed to have anthelmintic and vermifuge properties (Naovi et al., 1991). In Unani medicine, a Middle Eastern traditional medical system that later took root in India (Izhar, 1989), pomegranate flowers serve as a remedy for diabetes mellitus (Saxena and Vikram, 2004). In Tunisia, researchers showed a great interest to this mysterious fruit which have been widely investigated through several previous studies conducted to investigate the phyto-chemical composition of Tunisian pomegranate cultivars.

In this field, Elfalleh et al. (2009) identified phyto-chemicals from various parts of the pomegranate fruit: peel, juice and seeds, and showed that the named parts exhibited a strong antioxidant effect. Hasnaoui et al. (2011) have also characterized seeds and juice contents of sugars and organic acids of sour and sweet Tunisian pomegranate cultivars. Moreover, Zaouay et al. (2012) compared the physico-chemical characteristics as well as the antioxidant activity of 13 pomegranate cultivars grown in southern Tunisia. Additionally, the molecular genetics of the pomegranate have been also investigated by Tunisian research groups.

Jbir et al. (2008) have characterized some Tunisian pomegranate cultivars using amplified fragment length polymorphism analysis. Hassnaoui et al. (2010) also revealed some molecular polymorphisms in Tunisian Pomegranate by random amplified polymorphic DNA (RAPD) fingerprints. The aim of this research is to quantify the phenolic antioxidant (such as total phenols, O-diphenols, flavonoids, anthocyanin and condensed tannins) and non-phenolic antioxidant (carotenoids) pomegranate extracts from flowers, leaves and barks in four different Tunisian cultivars. The samples are also evaluated for their antioxidant capacity.

MATERIALS AND METHODS

Fresh flowers (Fl), leaves (L) and barks (B) from "Tounsi, Nabli, Gabsi and Chelfi" cultivars were manually picked from Testour (Governorate of Beja) in the northwest region of Tunisia (36.55°N, 9.45°E) on the flowering period (May, 2011) of pomegranate trees aged over 20 years. Samples were collected from the same orchards. Samples were washed and dried at 40°C in a hot air oven until constant weight, then ground (Moulinex, France), sieved to finally obtain fine powders and transferred to darkness for further use.

Methanolic extraction

The fine powders where extracted with methanol in the dark for 24 h in a shaker (Eyela Model MMS-300, Tokyo Rikakikai Co., Ltd., Japan) at room temperature and then the solvents were evaporated by vacuum rotary evaporator (EYELA N1000, Japan). After filtration (0.45 µm), all extracts obtained were then transferred to vials and kept in the dark at -20°C prior to use.

Determination of total phenols and o-diphenols

Total phenolic contents and o-diphenols of methanolic fractions were determined according to the method of Montedoro et al. (1992) with minor modifications. For total phenols, 0.4 ml of each fraction and 10 ml of diluted Folin-Ciocalteu reagent were mixed. After 1 min incubation, 8 ml of sodium carbonate (75 g/L) was added and the mixture was incubated for 2 h. The absorbance was measured at 765 nm. The same extract was used to determine total o-diphenols. Then, 1 ml of a solution of HCl (0.5 N), 1 ml of a solution of a mixture of $NaNO_2$ (10 g) and $NaMoO_4 \cdot 2H_2O$ (10 g) in 100 ml H_2O, and finally 1 ml of a solution of NaOH (1 N) were added to 100 µl of the methanolic extracts. After 30 min, o-diphenols were read at 500 nm. The total phenols and o-diphenols were expressed on a dry weight basis as mg tyrosol equivalents/g of sample.

Determination of total flavonoids

Total flavonoid contents of the extracts were determined according to the colorimetric assay developed by Zhishen et al. (1999). One ml of properly diluted extracts was mixed with 4 ml of distilled water. At zero time, 0.3 ml of (5%, w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH was added. Finally, the volume was made up to 10 ml immediately by the addition of 2.4 ml of distilled water. The mixture was shaken vigorously and the absorbance was read at 510 nm. The results were also expressed on a dry weight basis as mg catechin equivalents (CEQ)/g of sample.

Determination of total anthocyanins

Anthocyanin content of dried samples was analyzed according to Giusti and Wrolstad (2000) with minor modifications. One gram of each sample was extracted with 25 ml of acidified methanol (1% HCl) for 24 h at room temperature in the dark, and then centrifuged at 1000 × g for 15 min. Anthocyanin levels were calculated from the methanolic extract as A530 – (0.24 × A653). Total anthocyanin content was determined as mg cyanidin 3-glucoside equivalents (CyE) per g of fresh weight, using an extinction coefficient of 26.900 L mol^{-1} cm^{-1} at 530 nm and a molar mass (MW) of 449.2 g mol^{-1}.

$$\text{Total anthocyanin} = Ab \times MW \times V \times 100 / \varepsilon \times G$$

Determination of total condensed tannins

Determination of condensed tannins was based on the procedure

reported by Sun et al. (1998) with slight modification. A volume of 50 µl of extract solution was mixed with 1.5 ml of 4% vanillin-methanol solution and 750 µl of sulfuric acid H_2SO_4, and the mixture was allowed to stand in the dark for 15 min. The absorbance was measured at 500 nm and the amount of tannins was calculated from the calibration curve of tannic acid standard solutions, and expressed as mg tannic acid/g of dry weight.

Determination of total carotenoids

Total carotenoids were extracted according to the method of Talcott and Howard (1999), with slight modifications. Two grams of sample where extracted using 25 ml of acetone/ethanol (1:1, v/v) with 200 mg/L butylated hydroxytoluene (BHT) added. All manipulations were carried out under a yellow fluorescent light (Thorn) to avoid light-induced changes. After extraction, sample was centrifuged at 1500 × *g* for 15 min at 4 to 5°C. The supernatant was collected, and the remaining residue was re-extracted using the same method until the residue was colorless. Finally, the supernatants were brought to 100 ml with the extraction solvent, and the absorbance at 470 nm was measured using a spectrophotometer. Total carotenoids were calculated as follows and expressed as milligrams per g of dry weight (DW).

$$\text{Total carotenoids} = Ab \times V \times 10^6 / A^{1\%} \times 100G$$

Ab is the absorbance at 470 nm, *V* is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a 1% mixture of carotenoids at 2500, and *G* is sample weight (g).

DPPH radical scavenging activity

The DPPH method (Kontogiorgis and Hadjipavlou-Litina, 2005) was used to determine antioxidant activity of studied samples extracts. 10 µl from the stock solution of the sample were dissolved in absolute methanol to a final volume of 100 µl and then added to 500 µl DPPH (0.1 mM in absolute methanol) and 400 µl Tris Hcl (0.1 M). The reaction mixture was kept at room temperature. The absorbance of the solution was measured at 517 nm after 30 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding with DPPH. The reduction (%) values were determined and compared to appropriate standards. Inhibition of the free radical DPPH in percent (I %) was calculated using the following equation:

$$I\% = (A_{blank} - A_{sample/} A_{blank}) \times 100$$

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.

Reducing power

The reducing powers of the extracts from pomegranate flowers, leaves and barks were determined according to the method described by Chung et al. (2005). A 0.25 ml aliquot of each extract were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50°C for 20 min. 0.25 ml of 1% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 3750 rpm for 10 min. The supernatant (0.5 ml) was mixed with 0.5 ml distilled water and 0.1% $FeCl_3$ (0.125 ml) and then the absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values and expressed as mg/ml of ascorbic acid.

Statistical analyses

All data were subjected to analyses of variance (ANOVA one-way) using statistical package for social sciences (SPSS) 11.0 software (SPSS, Chicago, IL, USA). The data shown are mean values (n = 3) and the significance of the differences was compared using Duncan multiple range test at $p < 0.05$ probability level. Simple associations between variables were calculated as the Pearson correlation. Principal component analysis (PCA) was carried out and all parameters analyzed were used as variables in PCA using XLStat-Pro version 7.5.2 for Windows (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

Phenolic compounds content

Total phenols and O-diphenols content

The total phenolic content (TPC) of pomegranate flowers, leaves and barks are presented in Table 1 ($p < 0.05$). The highest amounts were recorded in flower extracts (218.64 mg/g of DW in Tounsi flowers (TNFl)) and in leaf extracts (131.91 mg/g of DW in Gabsi leaves (GL)). However, bark extracts presented lower TPC than did flower and leaf samples (23.64 mg/g in Gabsi barks (GB)). This result is in accordance with those found by Zhang et al. (2010) who reported that both flowers and leaves possessed high concentrations of phenolics, but more abundant in flower extracts. Also, phenolic levels of their Chinese samples were higher than did ours. However, our results are far higher than those found by Elfalleh et al. (2011), who studied the total phenolic composition of Gabsi flower and leaf extract. This difference can be attributed to the variation in geological regions and changes in climate and ecotypes as well as cultural practice such as fertilization, irrigation, etc (Mars and Marrakchi, 1998). O-diphenol levels were also studied and differences were found in their content in the analyzed samples. In fact, as illustrated for total phenols, similar results were reported for the O-diphenol contents. Flowers and leaves monopolized the highest amounts (from 1.93 to 3.47 mg/g DW). Meanwhile, bark extracts presented a relatively small fraction of O-phenols compared to flower and leaf extracts. Their amounts ranged from 0.66 in Gabsi barks (GB) to 0.86 mg/g DW in Chelfi barks (CHB).

Total flavonoid content

Total flavonoid contents are summarized in Table 1. Levels ranged from 0.19 to 1.53 (mg CEQ/g DW). The most

Table 1. Total phenols, O-diphenols, flavonoids, anthocyanins, tannins and total carotenoids content (mg/g DW) of flower (Fl), leaf (L) and bark (B) extracts in four different Tunisian pomegranate cultivars.

Cultivar	Phenol	O-diphenol	Flavonoid	Anthocyanin	Tannin	Carotenoid
Tounsi (Fl)	218.64±11.84^{e}	2.25±0.05^{d}	0.77±0.02^{c}	0.58±0.03^{g}	6.62±0.37^{a}	0.26±0.01^{d}
Nabli (Fl)	139.85±7.99^{d}	1.93±0.06^{c}	1.36±0.01^{g}	0.53±0.02^{f}	9.12±0.28^{f}	0.19±0.01^{b}
Gabsi (Fl)	103.51±13.71^{c}	2.03±0.001^{c}	0.92±0.11^{d}	0.61±0.03$^{g.h}$	9.04±0.17^{f}	0.33±0.01^{f}
Chelfi (Fl)	139.4±7.02^{d}	3.47±0.17^{f}	1.36±0.00^{g}	0.66±0.05^{h}	8.71±0.8$^{e.f}$	0.19±0.01^{b}
Tounsi (L)	97.2±2.01^{c}	1.93±0.03^{c}	0.73±0.00^{c}	0.13±0.03$^{a.b}$	7.24±0.14$^{a.b.c}$	0.11±0.00^{a}
Nabli (L)	108.73±13.99^{c}	2.43±0.04^{e}	1.05±0.08^{e}	0.13±0.01$^{b.c}$	8.4±0.78$^{d.e.f}$	0.1±0.07^{a}
Gabsi (L)	131.91±7.48^{d}	2.49±0.02^{e}	1.53±0.04^{h}	0.18±0.02$^{c.d}$	7.87±0.47$^{c.d.e}$	0.11±0.00^{a}
Chelfi (L)	94.5±5.17^{c}	2.21±0.05^{d}	1.17±0.06^{f}	0.08±0.01^{a}	8.55±0.36$^{d.e.f}$	0.12±0.00^{a}
Tounsi (B)	32.62±3.77$^{a.b}$	0.75±0.04$^{a.b}$	0.25±0.02^{a}	0.17±0.01$^{b.c.d}$	6.53±0.35^{a}	0.23±0.01^{c}
Nabli (B)	42.47±4.28^{b}	0.82±0.07^{b}	0.38±0.01^{b}	0.2±0.03$^{b.c}$	6.95±0.34$^{a.b}$	0.25±0.01^{d}
Gabsi (B)	23.64±4.11^{a}	0.66±0.04^{a}	0.19±0.02^{a}	0.15±0.04$^{b.c.d}$	7.91±0.77$^{c.d.e}$	0.32±0.01^{e}
Chelfi (B)	29.89±1.61$^{a.b}$	0.86±0.05^{b}	0.42±0.02^{b}	0.29±0.03^{e}	7.8±0.33$^{b.c.d}$	0.31±0.01^{e}

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level of $p < 0.05$.

important amounts of flavonoids were recorded in leaf and flower extracts. However, bark extracts were characterized by a poor fraction of flavonoids (from 0.19 in GB to 0.42 mg CEQ/g DW in CHB). The GL extracts exhibited the highest flavonoids content. This result had been already confirmed by Zhang et al. (2010) who showed that pomegranate leaves include very high amounts of flavonoids, even higher than the pomegranate seed levels. Furthermore, our results mimic data reported by Elfalleh et al. (2011) who also stated that both leaf and flower extracts of Gabsi cultivar exhibited very considerable amounts of flavonoids. Meanwhile, they found more abundant flavonoids in flowers than leaves for the named cultivar. This difference can be due to the environmental and the genetic variability (Jbir et al., 2008; Hasnaoui et al., 2010).

Total anthocyanins content

Total anthocyanins content showed significant differences ($p < 0.01$) among the different plant organs and cultivars (Table 1). The concentration of phenolics was dependent on the plant organs. According to our results, flower extracts monopolized the highest amounts of anthocyanins (0.61 and 0.66 mg/g DW in Gabsi flowers (GFl) and Chelfi flowers (CHFl), respectively), followed by bark and leaf extracts which showed lower levels ranging from 0.08 in Chelfi leaves (CHL) to 0.29 mg/g in Chelfi Barks (CHB). As pigments, anthocyanins are almost exclusively responsible for the red, blue and purple colors in fruits and flowers (Zaouay et al., 2012; Zhang et al., 2011). Miguel et al. (2009) had previously identified one anthocyanin pelargonidin-3,5-diglucoside in pomegranate flowers using high-performance liquid chromatography (HPLC). However, glucosides of delphinidin, cyanidin and pelargonidin have been separated from aril and peel of pomegranates (Gil et al., 2000). This result was later confirmed by works of Zaouay et al. (2012) who studied some characteristic pigment of Tunisian pomegranate juices. This suggests that there are differences in the distributions of the same kind of secondary metabolites in different organs of plants (Zhang et al., 2011) which explains the difference of anthocyanin concentrations in pomegranate flower, leaf and bark samples.

Condensed tannins content

Considerable amounts of condensed tannins occur in pomegranate flowers, leaves and barks (Table 1). Nabli flowers (NAFl) and Gabsi flowers (GFl) exhibited the highest amounts with values of 9.12 and 9.04 mg/g, respectively ($p < 0.05$). However, the lowest content was recorded in Tounsi barks (TNB) and Tounsi flowers (TNFl) (6.53 and 6.62 mg/g, respectively). Our findings are consistent with the data reported by previous studies which mentioned that tannins are mainly found in the pericarp, bark, seed, leaves, and flower (Wang et al., 2004; Lansky and Newman, 2007). This result is in accordance with data reported by Elfalleh et al. (2011) who found higher amounts of tannins in pomegranate leaf and flower extracts than in peel and seed of some Tunisian cultivar.

In addition, as we mentioned above, our samples have

Table 2. Radical scavenging activity (%) and reducing power (mg/g DW) of flower (Fl), leaf (L) and bark (B) extracts in four different Tunisian pomegranate cultivars.

Cultivars	Radical scavenging activity	Reducing power
Tounsi (Fl)	92.2±0.97$^{d.e}$	116.25±7.5^{c}
Nabli (Fl)	93.26±0.05$^{d.e}$	112.88±27.375^{c}
Gabsi (Fl)	82.64±0.18^{c}	35.25±11.25$^{a.b}$
Chelfi (Fl)	89.04±0.91^{d}	140.63±9.38^{c}
Tounsi (L)	63.88±5.49^{a}	85.88±18.375$^{b.c}$
Nabli (L)	69.99±4.42^{b}	94.88±9.375$^{b.c}$
Gabsi (L)	79.11±1.49^{c}	254.25±3.75^{c}
Chelfi (L)	67.6±2.35$^{a.b}$	96.25±12.12$^{b.c}$
Tounsi (B)	94.53±2.24$^{e.f}$	50.75±21.03$^{a.b}$
Nabli (B)	94.26±1.33$^{e.f}$	36±7.5$^{a.b}$
Gabsi (B)	98.38±0.79^{f}	17±1.89^{a}
Chelfi (B)	93.5±2.04$^{d.e}$	33.85±6.58^{a}

Values are expressed as means ± standard deviation (n = 3). Means with different letters in the same column were significantly different at the level of $p < 0.05$.

been picked at the flowering period of pomegranate tree when its tissues are younger and the concentration of simple phenolic compounds are generally higher; once the maturity period is reached, different phenolic acids condense to form complex phenolic compounds such as flavonoids and tannins etc (Arumugam and Perumal, 2012). Hence, bark accumulates phenolic compounds with the maturity of the plant possess relatively higher amounts than other plant organs which coincide with our data.

Non phenolic compound content

Total carotenoids content

Carotenoids are natural pigments that provide leaves, flowers, fruits and vegetables with yellow, orange and some reddish colors as well as several aromas in plants (Cazzonelli, 2011). As recorded in Table 1, the concentration of carotenoïds ranged from 0.1 to 0.32 mg/g DW ($p < 0.05$). The highest amounts were provided by the Gabsi cultivar in both flower and bark extracts (0.33 and 0.32 mg/g, respectively, $p < 0.05$). This result was expected, given the yellowish color of bark extracts characteristic of carotenoïds pigment. However, leaf extracts presented the lowest level of carotenoïds where concentrations ranged from 0.1 to 0.12 mg/g. This may be due to the presence of chlorophyll in leaves, which tend to mask the carotenoïds especially at the flowering period when the green color dominates.

Antioxidant activity

DPPH Radical scavenging activity

As illustrated in Table 2, all the extracts were capable of scavenging the DPPH free radicals ($p < 0.05$). Scavenging abilities on DPPH radicals was in descending order: bark extracts > flower extracts > leaf extracts. The barks of Gabsi cultivar showed the highest percentage (98.38%). The Nabli flower extracts exhibited the highest level (93.26 %). Concerning leaf extracts, it was found that Gabsi cultivar possessed the highest percentage (79.11%). This result is not in accordance with those of Elfalleh et al. (2011) who found higher antioxidant activity in pomegranate leaf extracts than flower extracts in Gabsi cultivar. This difference could be attributed to environmental and seasonal variability of the selected cultivar. Indeed, our cultivars including “Gabsi” were all picked in the flowering period (May 2011) from northwest of Tunisia, whereas Elfalleh et al. (2011) harvested their cultivar on October, 2010 from the southern of Tunisia. We also noticed that the antioxidant activity in DPPH was strongly correlated with carotenoïds content ($r = 0.733$; $p < 0.01$) as well as with anthocyanins ($r = 0.397$; $p < 0.05$), which suggests that carotenoïds are mainly contributing to the DPPH scavenging activity followed by anthocyanins. It is suggested that anthocyanins are important antioxidants in pomegranate flowers (Zhang et al., 2011). Moreover, this result corroborate with those reported by Mena et al. (2011) who found that total anthocyanins content is well correlated to antioxidant capacity assays. However, Zaouay et al. (2012) did not find a significant correlation between total anthocyanins and antioxidant activity.

On the other hand, Kong et al. (2012) demonstrated minimal contribution of carotenoïds towards the antioxidant activity in the leaves of *Barringtonia racemosa* (L.) Spreng plant which is in disagreement with our data. Correlation studies between carotenoïds and antioxidant activities are scarce and those that are available have shown conflicting results with some studies showing positive correlations (Egea et al., 2010) and others showing negative correlation (Müller et al., 2011).

Reducing power

The reducing powers of pomegranate leaf, flower and bark extracts are presented in Table 2. The leaf and flower extracts exhibited stronger reducing power than did the bark extracts (112.88 in NAFl to 140.63 mg/ml in CHFl ($p < 0.05$)). Also, reducing power of the leaf extracts varied from 85.88 in Tounsi leaves (TNL) to 254.25 mg/ml in GL ($p < 0.05$). However, for pomegranate bark extracts, the concentrations ranged from 17.00 in GB to

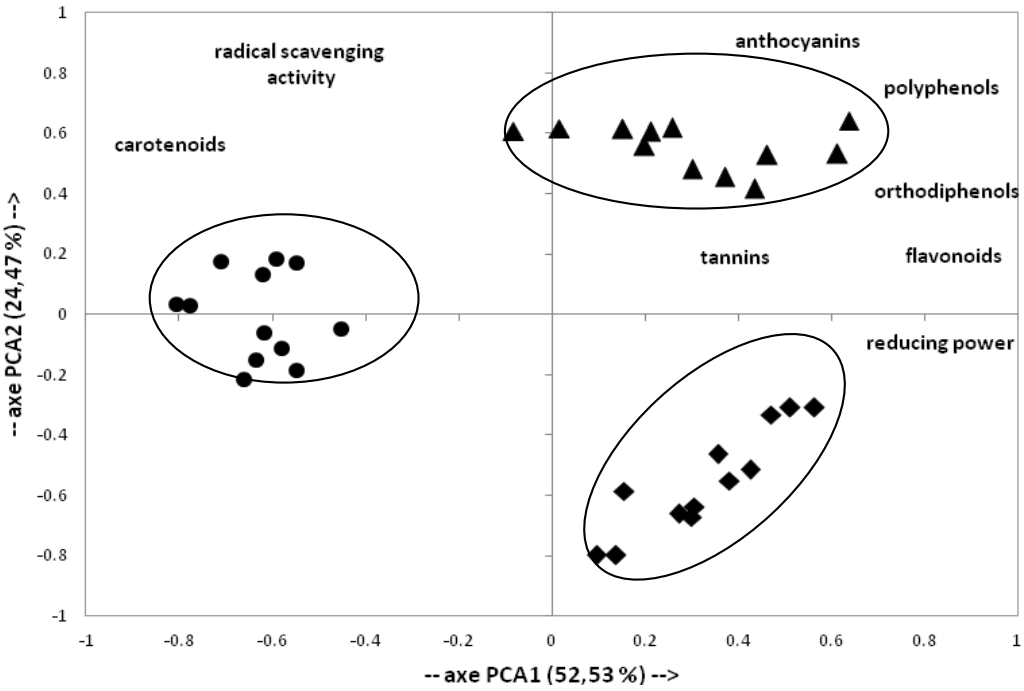

Figure 1. Principal component analysis (scores and loading plots, Biplots) based on phenols, O-diphenols, flavonoids, anthocyanins, tannins, carotenoïds content, DPPH scavenging activity and reduced power in extracts obtained from flower (Fl), leaf (L) and bark (B) extracts of four Tunisian pomegranate cultivars. (⬮): barks; (◆: leaves; flowers (▲

50.75 mg/ml in TNB. This result is similar to those reported by Elfalleh et al. (2011) who found that pomegranate leaf and flower extracts of Tunisian cultivar had higher reducing power than did peel and seed extracts. In addition, we stated that the reducing power was well negatively correlated with the DPPH scavenging activity ($r = -0.333$; $p < 0.05$). Our findings on the reducing power of pomegranate extracts suggest that it is likely to contribute significantly towards DPPH radical scavenging activities. Indeed, many authors reported correlation between reducing power and antioxidant activity of certain plant extracts (Duh, 1998). Furthermore, strong positive correlations were found between the reducing power and phenols, flavonoids and O-diphenols ($r = 0.760$, 0.762 and 0.716; $p < 0.01$, respectively). Adhami and Mukhtar (2006) suggested that the antioxidant activity of pomegranate leaves is positively correlated with the amount of total phenolics and flavonoids. Those results suppose that phenolic antioxidants are mainly contributing to the reducing power of the pomegranate leaf, flower and bark extracts by means of synergic effects. However, carotenoïds which are non-phenolic antioxidants play a major role to enhance the DPPH scavenging activity.

Chemometric analysis

PCA was applied to the dataset of antioxidant compounds, DPPH scavenging activity and reducing power of pomegranate leaf, flower and bark samples. PCA accounted for 52.53% of the total variance (77.00%) on the first component while the second component accounted 24.47%. Figure 1 showed two distinctive groups. The first group is composed of bark samples. The second group is characterized by flower and leaf samples. The first group was negatively associated with PC1 while the last group was positively associated with PC1. PC1 was dominated by the following variables: polyphenols, O-dipenols, flavonoids, anthocyanins, tannins and reduced power. However, PC2 was dominated by the following variables: carotenoïds and DPPH radical scavenging activity. This result clearly indicates that pomegranate by-products have also a strong antioxidant effect. However, the reducing power of our samples is mainly provided by phenolic compounds whereas non phenolic compounds such as carotenoïds are mainly contributing to the DPPH scavenging activity in the flowering period of pomegranate tree. As we previously stated in this study, carotenoïds are the major

compound found in barks and is on its own, the principal generator of antioxidant power of the named by-product. However, the efficacy of pomegranate leaves and flowers antioxidant effect is the result of synergic or antagonist interactions between phenolic compounds. This reveals the difference in the constitution of phytochemicals in various plant parts of the same tree species.

Conclusion

In the present study total phenols as well as carotenoïds were extracted from pomegranate flowers, leaves and barks in four highly consumed Tunisian cultivars (Tounsi, Gabsi, Chelfi and Nabli). Data issued from this work showed that flower and leaf extracts exhibited highest concentrations of phenolics, flavonoïds, anthocyanins and tannins contrary to the barks which showed poor amounts of phenolic compounds but obviously most concentrated on carotenoïds. Among the studied cultivars, no one was considered as phytochemicals dominant despite the statistical differences. This result could be explained since the flowers, leaves and barks of studied cultivars were collected in the same period and from the same orchards. However, obvious variations were found among the different studied parts of pomegranate tree, suggesting the existence of differences in the distributions of the same kind of secondary metabolites in different organs of plants. By measuring the antioxidant activity, we noticed that our samples exhibited very high DPPH scavenging activity reaching over 98% for some bark extracts. Carotenoïds which were very abundant in bark extracts were well correlated with the DPPH scavenging activity.

Furthermore, it was stated that the reducing power of leaf and flower extracts were considerably high and was strongly correlated with phenolic compounds of the same extracts. These results bring attention to the richness of the different part of pomegranate tree with natural antioxidant and can explain the interest of traditional medicine practitioners to pomegranate tree and why this plant is considered as medicinal plant. More studies have to be investigated to better ensure the medicinal proprieties of pomegranate by-products of Tunisian cultivars.

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