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Phenolic nature, occurrence and polymerization degree as marker of environmental adaptation in the edible halophyte *Mesembryanthemum edule*

H. Falleh [a,b,*], R. Ksouri [a], M. Boulaaba [a], S. Guyot [c], C. Abdelly [a], C. Magné [b]

[a] *Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisie*

[b] *Laboratoire d'Ecophysiologie et de Biotechnologies des Halophytes et des Algues Marines, Université de Brest, EA 3877 LEBHAM, IUEM, Rue Dumont d'Urville, 29280 Plouzané, France*

[c] *Unité de Recherches Cidricoles, Biotransformation des Fruits et Légumes, INRA, Domaine de la Motte, BP 35627, 35653 Le Rheu cedex, France*

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Abstract

Mesembryanthemum edule is an edible medicinal halophyte traditionally used to treat several human diseases. In this study, particular importance was attached to the influence of environmental conditions on phenolic composition and antioxidant activities of two *M. edule* provenances from contrasting climatic regions (Djerba and Monastir sampled from arid and superior semi-arid bioclimatic stages, respectively). Shoot phenolic content was evaluated using colorimetric method and its composition was identified by HPLC analysis with or without thiolysis. Antioxidant activities were assessed by five *in vitro* antioxidant systems. Results showed that the two *M. edule* provenances were significantly different according to their antioxidant activity as well as their polyphenol profiles. Indeed, plants from Djerba (lack of rainfall and long light hour periods) exhibited stronger antioxidant activity together with higher phenolic content. For instance, Djerba provenance shoots showed much lower IC_{50} (4.8 μg ml^{-1}) and EC_{50} (80 μg ml^{-1}) values for DPPH and Fe-reducing tests, respectively. In addition, the superiority of this provenance (Djerba) was more marked as compared to positive controls (BHT, BHA, and VitC). HPLC identification revealed also an important difference between the two provenances on major flavonoid components. This difference was confirmed by the mean degrees of tannin polymerization (DP*n*) which was higher in Djerba plants. These data suggest that *M. edule* adaptation to environmental stresses proceeds through induced particular phenol quality and DP*n* for the improvement of their antioxidant capacities to protect plant tissues against oxidative stress.

Keywords: Antioxidant activity; Environmental constraints; DP*n*; *Mesembryanthemum edule*; Phenolic compounds

1. Introduction

The potential of plant antioxidants for their health benefits has raised the interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (Boots et al., 2008). Indeed, food provides not only essential nutrients needed for life, but also other bioactive compounds for health promotion. Accordingly, crude extracts of fruits, herbs, vegetables, cereals, and medicinal plants rich in polyphenols are gaining interest in food industry because they retard oxidative degradation of lipids and thereby improve food quality and nutritional value (Ksouri et al., 2009).

Proanthocyanidins (or condensed tannins), which are potent phenolic compounds, consist of oligomers and polymers of polyhydroxyflavan-3-ol monomer units linked most commonly by acid-labile 4→8 and in some cases by 4→6 bonds (Spranger et al., 2008). Their properties were found to depend strongly on the degree of polymerization (DP*n*) of the tannin

* Corresponding author at: Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisie. Tel.: +216 79 325848; fax: +216 79 325638.

E-mail address: Hanenfalleh@gmail.com (H. Falleh).

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molecule. Actually, Falleh et al. (2011) pointed out that DP*n* and constitutive unit nature s are important structural features that are related to the ability of proanthocyanidins to associate with proteins and polysaccharides. This explains the quite different characteristics between oligomeric and polymeric procyanidins and the availability of several methods to fractionate these types of compounds. Chemical methods are used to determine the structural characterization of these proanthocyanidins. They consist of an acid-catalyzed cleavage of interflavanyl linkages in the presence of a nucleophile reagent such as phloroglucinol or toluene-α-thiol (Guyot et al., 2001). Thiolysis allows distinction between extension and terminal units when coupled to reversed-phased HPLC (Spranger et al., 2008). The method was used to calculate the average DP*n* and to determine the proportions of the constitutive units in proanthocyanidin fraction. Indeed, proanthocyanidins and consequently phenolic synthesis is known to be susceptible to the environmental conditions (Llorach et al., 2008). According to Toor et al. (2006), antioxidant contents are influenced by environmental parameters (solar radiation, temperature, rainfall, and edaphic factors) during physiological plant development, and thus experience seasonal variations. Moreover, environmental constraints may trigger oxidative stress in plants leading to cellular damage and senescence processes (Karray-Bouraoui et al., 2010). Therefore, plants growing in harsh environmental conditions, such as halophytes, are known for their ability to withstand and quench toxic reactive oxygen species (ROS), since they produce polyphenols as a protective mechanism against ROS (Jaleel et al., 2009; Ksouri et al., 2010). In this case, polyphenolic compounds such as phenolic acids, flavonoids, and particularly proanthocyanidins play a key role in plant adaptation to abiotic stresses. Their gradients in concentrations within-species may reflect different requirements for dealing with abiotic stresses. (Hajlaoui et al., 2009; Ksouri et al., 2007).

Mesembryanthemum edule L. (*Carpobrotus edulis*), commonly named sourfig, is well known for its ethnopharmacological utilization in traditional medicine. *M. edule* leaves are used as antiseptic poultice for sores, burns and scalds, and leaf juice are gargled to treat mouth infections (Thring and Weitz, 2006). Besides, sourfig shoots are taken orally for dysentery, tuberculosis, as diuretic, and to ease stomach trouble in African traditional medicine (Martins et al., 2005). Leaves and fleshy fruits are used as food and/or fodder (Springfield et al., 2003). In the present work, we focused on the implication of phenolic compounds in *M. edule* adaptation towards environmental conditions and the possible use of proanthocyanidin polymerization degree as indicator of plant adaptation to stresses. Hence, we examined antioxidant activity and phenolic variation of two *M. edule* from contrasting climates.

2. Material and methods

2.1. Plant sampling and extraction

M. edule is not native to Tunisia, but it was introduced from Europe as seedlings. Thus, Tunisian provenances of this species are not genetically too different. Shoots (leaves and stems) were harvested from two regions: Djerba (South of Tunisia, arid bioclimatic stage) and Monastir (Center of Tunisia, superior semi-arid bioclimatic stage) differing in their climatic conditions (Table 1). Plants were identified by the botanist of the Biotechnology Center of Borj-Cedria (CBBC), and a voucher specimen [AME27] was deposited at the Herbarium of the Laboratory of Extremophile Plants (at CBBC). The collected shoots were rinsed, left at room temperature for 7 days, and then oven-dried before being ground in a Mettler AE 200 1/10 mg (Dangoumeau type). Extracts were obtained by magnetic stirring of 2.5 g dry powder with 25 ml pure methanol for 30 min. Extracts were kept for 24 h at 4 °C, filtered through a Whatman No 4 filter paper, and stored at 4 °C until analyses.

Table 1
Climatic characteristics (temperature, light hours, and precipitations) of the studied localities where *M. edule* shoots were harvested. Jun: June, Jul: July, and Aug: August.

Locality	Average temperature (°C) Jun Jul Aug	Light hours (h) Jun Jul Aug	Precipitations (mm) Jun Jul Aug
Djerba	27.6 27.6 30.1	346.6 391.0 351.0	4.4 0.0 6.0
Monastir	26.0 27.7 29.1	312.0 367.5 312.0	22.8 0.0 16.8

2.2. Determination of antioxidant activity

2.2.1. Total antioxidant capacity

Total antioxidant capacity of methanolic extracts was evaluated through the assay of a green phosphate/Mo^{5+} complex according to the method described by Prieto et al. (1999). Aliquots (0.1 ml) of diluted samples were combined each in a tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a 95 °C water bath for 90 min. Then, the absorbance was measured at 695 nm against blank in UV-Visible spectrophotometer (Anthelie Advanced 2, Secoman). Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE g^{-1} DW). All samples were analyzed in triplicates.

2.2.2. DPPH scavenging activity

Antiradical activity of shoot extracts was measured according to the method described by Hatano et al. (1988). One milliliter from each extract concentration was added to 250 µl methanolic DPPH solution. After 30 min of incubation, absorbance was measured at 517 nm. The potential interference of the solvents used was assessed as negative control and BHT was used as positive control. The scavenging activity of DPPH radical was calculated as follows:

$$\text{DPPH}\cdot\text{scavenging effect}\ (\%) = [(A_0 - A_1)/A_0] * 100 \quad (1)$$

where A_0 and A_1 are the absorbances of the negative control and of the sample at 30 min, respectively. The radical scavenging activity was expressed as IC_{50} (µg ml^{-1}), the extract dose required to cause a 50% inhibition. A low IC_{50} value corresponds to a high antioxidant activity of the plant extract.

2.2.3. $ABTS^+$ scavenging activity

ABTS assay was used as described by Re et al. (1999). $ABTS^+$ solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulfate. The $ABTS^+$ solution (950 µl) was added to 50 µl of the test sample and mixed vigorously. The reaction mixture was allowed to stand for 6 min then absorbance at 734 nm was recorded. BHT prepared in methanol was used as authentic standard and results were expressed as IC_{50} (µg ml^{-1}), the extract dose required to cause a 50% inhibition.

2.2.4. Iron reducing power

The reducing power was determined according to the method of Oyaizu (1986). Sample extracts at different concentrations were mixed with 2.5 ml phosphate buffer (pH 6.6, 0.2 M) and 2.5 ml potassium ferricyanide (1% w/v). The mixture was incubated for 20 min then 2.5 ml of TCA (10%) was added. After 10 min centrifugation (1 000 *g*), supernatant was mixed with distilled water and ferric chloride (0.1% w/v), and the absorbance was read at 700 nm against ascorbic acid as authentic standard. EC_{50} value (µg ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power. It was obtained from linear regression analysis.

2.2.5. β-carotene bleaching inhibition

A slightly modified method of Koleva et al. (2002) was used to estimate shoot capacity to inhibit *β*-carotene bleaching. Briefly, 4 ml of *β*-carotene solution (2 mg/20 ml in chloroform) was added to 40 mg linoleic acid and 400 mg Tween 40. The mixture was then evaporated by means of a rotavapor (model R-200, Büchi) and immediately diluted in 100 ml aired distilled water. An aliquot (150 µl) of the obtained emulsion was distributed in the wells of 96-well microtitre plates and the test samples (10 µl) at different concentrations were added. A standard (BHT dissolved in methanol) was used for comparison. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured at 470 nm using a model EAR 400 microtitre reader (Labsystems Multiskan MS). Three replicates were prepared for each sample as well as the control concentration. The antioxidant activity was evaluated in terms of *β*-carotene bleaching using formula (2).

$$\beta\text{-carotene bleaching inhibition}(\%) = [(S-C_{120})/(C_0-C_{120})] * 100 \quad (2)$$

where C_0 and C_{120} stand for absorbance values of the control at 0 and 120 min, respectively and S stands for sample absorbance at 120 min. Results were expressed as IC_{50} values (µg ml^{-1}).

2.3. Colorimetric quantification of phenolics

2.3.1. Total polyphenol, flavonoid, and condensed tannin contents

2.3.1.1. Total polyphenol content. The amount of total phenolics in shoot extracts was determined using the method described by Dewanto et al. (2002) using a calibration curve of gallic acid. To 125 µl of shoot extracts, 500 µl of Folin-Ciocalteu reagent and 125 µl of distilled water were added. The mixture was shaken before adding 1250 µl Na_2CO_3 (7% w/v) and adjusting with distilled water to 3 ml. After incubation for 90 min in the dark, the absorbance was read at 760 nm. Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW).

2.3.1.2. Total flavonoid content. Quantification of flavonoids was based on the method described by Dewanto et al. (2002). An aliquot of extract or standard ((+)-catechin in methanol) was added to 75 µl $NaNO_2$ (5%) solution and mixed for 6 min before adding 0.15 ml $AlCl_3$ solution (10%). After 5 min at room temperature, 0.5 ml NaOH was added then the mixture was adjusted to 2.5 ml with distilled water. Absorbance was determined at 510 nm and flavonoid concentration was calculated according to the equation obtained from (+)-catechin graph, and were expressed as mg catechin equivalents per gram dry weight (mg CE g^{-1} DW).

2.3.1.3. Total condensed tannin content. Proanthocyanidin contents were determined by the procedure of Sun et al. (1998). Fifty microlitres of shoot extracts were mixed with 3 ml vanillin-methanol solution (4%) and 1.5 ml hydrochloric acid (2 M). The mixture was incubated for 15 min at room temperature then its absorbance was measured at 500 nm. Results were expressed as mg catechin equivalents per gram dry weight (mg CE g^{-1} DW).

2.4. Analysis of individual phenolic compounds by analytical RP-HPLC

RP-HPLC analysis was performed either directly, to assess non condensed phenolics, or after thiolysis, to assess every phenolic compound including the structural units liberated from polymerized tannins. Results from the two analyses allow condensed tannin quantification and identification.

2.4.1. Thiolytic reaction

Thiolysis was carried out on dry powder as described by Guyot et al. (2001) with minor modifications. Toluene-α-thiol (5% in methanol, 800 µl) was added to each sample followed by 400 µl methanol acidified by concentrated HCl (3.3% v/v). Each reaction tube was closed and incubated at 40 °C for 30 min with mixing on a vortex mixer every 10 min. Then, the tubes were put on ice to stop the reaction and the mixture was filtered through a polytetrafluoroethylene (PTFE) membrane (0.45 µm) into a vial closed with a Teflon cap.

2.4.2. HPLC analysis

Ten microlitres from each filtrate was injected into the HPLC system. The HPLC apparatus consisted of a Waters (Milford, MA) system (717 plus autosampler, 600E multisolvent system, 996 photodiode array detector, and the Millennium 2010 Manager system). The column was a 250×4 mm *i.d.*, 60 Å, 5 µm Purospher RP18 (Merck, Germany). The flow rate was maintained at 1 ml min^{-1} and the temperature

kept at 30 °C. The solvent system used was a gradient of solvent A (aqueous acetic acid, 2.5% v/v) and solvent B (acetonitrile). The solvents were filtered through a 0.45 μm PTFE membrane. The following linear gradient was applied: 3% A: 0–5 min, 9% A: 5–15 min, 16% A: 15–45 min, 50% A: 45–51 min, and 90% A, followed by washing and reconditioning the column (Guyot et al., 2001).

When direct (without thiolysis) HPLC analyses were run, 10 mg dry powder from each plant were mixed with 1.2 ml acidified methanol (1% acetic acid). The mixture was vortexed and sonicated for 30 min. All samples were filtered through a 0.45 μm filter (HV, Millipore, Bedford, MA), and 10 μl from each filtrate were injected into the HPLC system as described above.

2.4.3. Polyphenol characterization

Phenolic compounds were characterized by comparison with authentic standards and according to their respective UV-visible spectra. Flavan-3-ols as well as dihydrochalcones were monitored at 280 nm. Phenolic acid derivatives were quantified at 320 nm, and flavonols were characterized by an absorption band with a maximum above 350 nm. Phenolic concentrations are expressed in milligrams per gram dry weight (mg g^{-1} DW). The average degree of polymerization (DP*n*) was measured by calculating the molar ratio of all flavan-3-ol units (thioether adducts + terminal units) to (−)-epicatechin and (+)-catechin corresponding to terminal units.

2.5. Statistical analysis

For all assessed tests, samples were analyzed in triplicates. Results were expressed as means ± standard deviation. Multiple sample comparison was performed using the Statgraphics Plus program version 5.1 for Windows. Analysis of one-way variance (ANOVA) followed by Duncan's multiple range test were used. Whenever ANOVA could not be used, Kruskal–Wallis test was applied after checking for normal distribution of the groups and homogeneity of variances. The level of significance was $P<0.05$.

3. Results

3.1. Antioxidant activity

3.1.1. Total antioxidant capacity

Results showed that *M. edule* shoots exhibit an interesting total antioxidant activity that exceeds 100 mg GAE g^{-1} DW (Table 2) for both provenances. Moreover, this activity varied considerably depending on plant origin. Indeed, Djerba plants exhibited a statistically higher activity (212 mg GAE g^{-1} DW) than Monastir ones (115 mg GAE g^{-1} DW).

Table 2
Total phenolic (TPC, mg GAE g^{-1} DW), flavonoid (mg CE g^{-1}DW), and proanthocyanidin (mg CE g^{-1} DW) contents and total antioxidant activity (TAA, mg GAE g^{-1} DW) of two *M. edule* provenances. Means (±SD) of three replicates followed by the same letter within a column are not significantly different at $P<0.05$.

M. edule provenances	TAA	TPC	Flavonoids	Proanthocyanidins
Djerba	212.2±4.8a	122.3±6.4a	66.0±2.1a	14.2±0.9a
Monastir	114.9±3.7b	48.5±0.4b	35.7±0.7b	8.9±0.0b

3.1.2. Radical scavenging activity against DPPH and $ABTS^{\cdot+}$

Fig. 1A and B shows significant differences closely related to plant origin for both DPPH and $ABTS^{\cdot+}$ tests. Shoot extracts originating from hard climate were more efficient to scavenge DPPH (IC_{50} = 4.8 μg ml^{-1}) and $ABTS^{+}$ (175 μg ml^{-1}) radicals than those collected from the superior semi-arid region (Monastir). Radical scavenging activity of Djerba extracts were even more active than that of the synthetic antioxidant BHT (IC_{50} = 11.5 and 355 μg ml^{-1}, respectively against DPPH and $ABTS^{+}$).

3.1.3. Iron reducing power

As expected, results presented in Fig. 2 shows that Fe^{3+} reducing ability of *M. edule* was also provenance-dependent; it was 2-fold higher (EC_{50} = 80.5 μg ml^{-1}) in Djerba than in Monastir (EC_{50} = 146 μg ml^{-1}). However, the positive control (ascorbic acid) was the most powerful (37 μg ml^{-1}).

3.1.4. β-carotene bleaching inhibition

This method showed important efficiency of plant extracts to inhibit β-carotene bleaching as well as significant differences between the two provenances (Fig. 3). Indeed, Djerba extract exhibited the highest activity (CI_{50} = 22 μg ml^{-1}) as compared to Monastir extract and BHT. Monastir provenance was statistically as efficient as the positive control BHT (74 μg ml^{-1}).

3.2. Phenol contents

As presented in Table 2, total phenolic content measured on *M. edule* shoots varied considerably depending on plant origins from 48.5 to 122 mg GAE g^{-1} DW. The higher amount of total polyphenol was found in Djerba plants. Moreover, this provenance exhibited over 1.5-folder higher total flavonoid (66 mg CE g^{-1} DW) and condensed tannin (14.2 mg CE g^{-1} DW) contents than Monastir.

3.3. Identification of phenolic compounds

3.3.1. Polyphenol characterization

Results presented in Table 3 showed large quantitatively and qualitatively differences between shoot phenolic compounds of the two *M. edule* provenances. Quantitatively, and in an unexpected manner, Monastir exhibited higher shoot phenolic content than Djerba (6 and 5.5 mg g^{-1} DW, respectively). However, these two provenances presented the same major compounds: phloretin and avicularin. Monastir provenance contains also substantial amounts of quercitrin, whereas procyanidin B2 was more abundant in Djerba. On a qualitative basis, shoot phenolic profiles of the two *M. edule* provenances were significantly different. Some compounds were present only in one provenance (*e.g.* unknown n°4 in Monastir and epicatechin in Djerba) and others are nearly absent from a site (*e.g.*

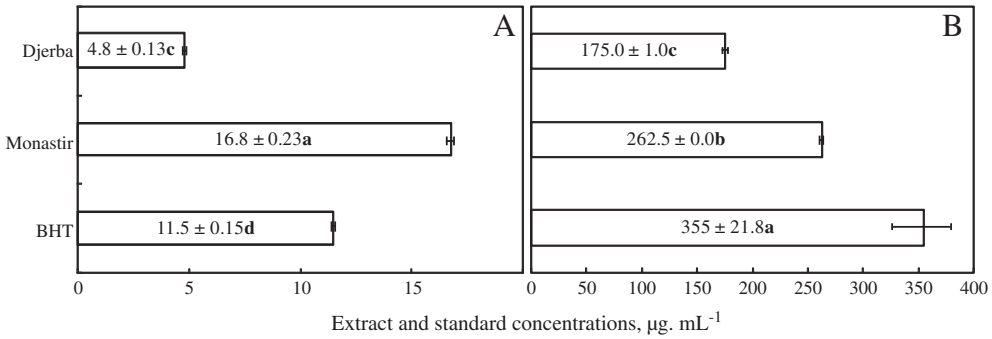

Fig. 1. Neutralization of DPPH (A) and ABTS (B) radicals by *M. edule* shoot extracts and authentic standard (BHT). IC_{50} values (µg ml^{-1}) are means of three replicates. Data followed by the same letter are not significantly different at $P<0.05$.

quercetin and hyperoside from Monastir and isorhamnoside and glucoside from Djerba).

3.3.2. Polymerization degree

Thiolytic degradation coupled with the reversed-phase HPLC was applied to elucidate the average degree of polymerization (DP*n*) of the polymeric proanthocyanidins. The products of thiolytic degradation in Djerba and Monastir shoot extracts showed that the constitutive units was similar in both provenances (Table 4), with (−)-epicatechin representing 96% of constitutive units and epicatechin representing 1% of the terminal unit. However, the two provenances differed by their averages of polymerization degree (DP*n*), being statistically higher in Djerba than in Monastir (32.7 and 26.8, respectively).

4. Discussion

In the present study, the implication of phenolic quality related to antioxidant activity of the edible halophyte *M. edule* in the adaptation to environmental conditions was assessed. Results depicted significant differences between the two studied provenances on the basis of their antioxidant potential depending on phenolic composition, being more important in the southern provenance. Considering antioxidant activity, results revealed that Djerba plants exhibited higher capacity to neutralize ROS than Monastir ones. This relevant difference is attributed to the environmental factors as the harder climatic conditions characterizing Djerba provenance, in terms of low rainfall and high light exposure, are probably related to the increase of antioxidant potential in *M. edule*. Actually, previous studies suggested that abiotic constraints enhance antioxidant activity as a response to the oxidative stress generated in these aggressive environments (Ksouri et al., 2010). Accordingly, Djerba plants (arid bioclimatic stage) living under harsher environmental conditions than Monastir ones (superior semi-arid bioclimatic stage), had an important potential to imbalance the generation of ROS by improving their scavenging system constituents. In this context, our results corroborate positively those of Bahri-Sahloul et al. (2009) studying *Crataegus azarolus* flowers from two provenances, one belonging to the arid bioclimatic stage and the other to the sub-arid bioclimatic stage. These authors found a significant difference in scavenging activity against DPPH and $ABTS^{+}$ radicals between these two provenances with a significantly higher activity in plants from arid climate. For instance, scavenging activity against DPPH radical was 605 and 360 µmol Trolox. 100 g^{-1} DW for the provenances collected from the arid and the sub-arid regions, respectively. Variability depending on climate conditions was also observed by Özgen et al. (2009) when comparing the reducing power of 14 *Moruss nigra* accessions and found that EC_{50} values varied from 7.3 to 16.4 mmol TE g^{-1} FW. An earlier study on two *Cakile maritima* provenances (Ksouri et al., 2007) highlighted also that the stress resistant southern provenance (arid bioclimatic stage) showed stronger

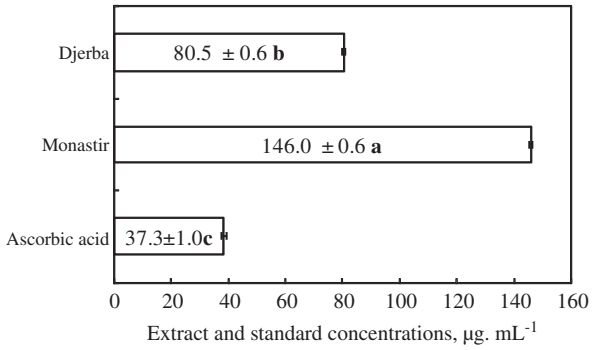

Fig. 2. Ferric ion reducing power in two *M. edule* shoot extracts and authentic standard (Ascorbic acid). EC_{50} values (µg ml^{-1}) are means of three replicates. Data followed by the same letter are not significantly different at $P<0.05$.

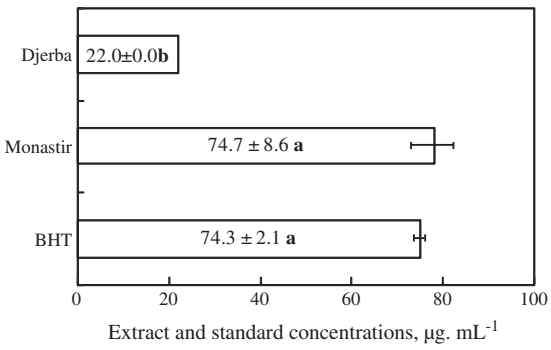

Fig. 3. Inhibition of β-carotene bleaching by *M. edule* shoot extracts and BHT. IC_{50} values (µg ml^{-1}) are means of three replicates. Data followed by the same letter are not significantly different at $P<0.05$.

Table 3
Phenolic compounds of *M. edule* shoots from Djerba and Monastir. Concentrations are given in milligrams per gram dry weight, nd: not detected. Means (three replicates) followed by the same letter within a raw are not significantly different at $P<0.05$.

	Djerba	Monastir
Dihydrochalcons		
Phloretin	0.997	1.263
Flavan-3-ols		
Procyanidin B2	0.653	nd
(−)-Epicatechin	0.149	nd
Flavonols		
Avicularin	0.662	0.966
Quercitrin	0.607	0.958
Isoquercetin	0.162	0.193
Isorhamnoside glucoside	0.059	0.114
Hyperoside	0.154	0.078
Quercetin	0.128	0.013
Kaempferol	0.064	0.079
Phenolic acids		
Para coumaroylquinic acid	0.072	0.045
Chlorogenic acid	0.042	0.055
Phenolic acid 1	0.192	0.123
Phenolic acid 2	0.374	0.424
Phenolic acid 3	0.095	0.057
Phenolic acid 4	0.083	0.040
Unknown compounds		
Unk 1	0.307	0.286
Unk 2	0.231	0.261
Unk 3	nd	0.287
Unk 4	nd	0.268
Total	5.498 b	6.026 a

antiradical activity than the sensitive northern one (humid bioclimatic stage), correlatively with the content of phenolic compounds. As a matter of fact, phenolics were able to reduce damages induced in the photosynthetic systems by absorbing UV-radiation and they were produced to protect plants from stresses such as long exposure to dryness and/or solar radiation (Macheix et al., 2005; Wahid and Ghazanfar, 2006). With this respect, *M. edule* antioxidant activity may be directly linked to their content in phenols, tannins, and flavonoids and consequently to their free radical scavenging activities (Huang et al., 2005). In literature, phenolic compounds are assumed to directly contribute to antioxidative action since their level is strongly correlated with *in vitro*-measured antioxidant activities (Duh and Yen, 1999; Falleh et al., 2008). Accordingly, we found more polyphenols in plants exhibiting higher antiradical activity. Since the two provenances derived from the same mother plants, differences between them are mainly due to environmental conditions. Apart probable edaphic differences, it seems that rainfall scarcity and long light exposure may be involved in the activation of phenol biosynthesis (Naczk and Shahidi, 2006). In this way, a previous work on the halophytic seashore plant (*C. maritima*) showed that polyphenol content varied with plant growing conditions and increased in response to abiotic stresses such as soil salinity (Ksouri et al., 2008). Actually, plant species have inherent physiological differences, as a result of interactions with their environment (Taulavuori et al., 2010). These differences may be reflected by the presence of various chemical compounds that provide information regarding the ecotype conditions (Taulavuori et al., 2010). For example, earlier works showed that plant flavonoid levels can be influenced by environmental factors such as light, temperature, and mineral nutrition (Jaakola et al., 2002). Accordingly, flavonoid contents were higher in plants growing in more stressful climatic conditions (Djerba) than those coming from more appropriate environment (Monastir). In this way, Rodrigues et al. (2011) suggested that the high levels of red onion flavonols are probably related to the high radiation and low rainfall during growing season. Moreover, Germ et al. (2010) showed that the subjection of *Hypericum perforatum* to increasing doses of UV radiation significantly increased its foliar flavonoids (from 6.5 to 9 g 100 g^{-1} DW). Besides, the higher tannin content in Djerba provenance, where minimum daily rainfall values were measured, agrees with earlier studies suggesting that tannins significantly contribute to water stress tolerance of plants (Attia, 2007; Ojeda et al., 2002). In this way, Attia (2007) showed that vine cultivation under water stress significantly increased the levels of condensed tannins as indirect mechanism of drought tolerance.

Additionally, RP-HPLC data depicted significant qualitative and quantitative difference in phenolic compounds when comparing Djerba shoots to Monastir ones. This result confirms that biosynthesis of phenolic compounds is affected by environmental constraints. Thus, quercetin biosynthesis is enhanced in plants subjected to long periods of sunshine because of its ability to inhibit lipid peroxidation in the mitochondria exposed to light (Denisov and Afanas'ev, 2005). In addition, such differences in phenolic composition may explain, at least partially, the difference observed in the antioxidant capacities of the two provenances. For instance, procyanidin B2 and epicatechin, not found in Monastir shoots, are well known for exhibiting high antioxidant activity as recently demonstrated for wine with a positive correlation relating the antioxidant activity to their contents (Gris et al., 2011). Furthermore, Tsai et al. (2008) found that epicatechin and quercetin (significantly more abundant in Djerba provenance in our study) are strongly correlated to the efficiency of antioxidant activity with correlation coefficients higher than 0.6. Therefore, we suggest that these molecules (procyanidin B2, epicatechin and quercetin), present in higher amounts in Djerba provenance, could be the major active compounds in *M. edule* shoots.

For the average polymerization degree, as far as we know, no data concerning environmental effect on DP*n* were available

Table 4
Thiolysis-RP HPLC characterization of procyanidins according to their constitutive units and their average degrees of polymerization (DP*n*) in two *M. edule* provenances. Means (three replicates) followed by the same letter within a raw are not significantly different at $P<0.05$.

	Djerba	Monastir
EC_e (%)	96.23b	96.50b
EC_t (%)	2.76a	2.45a
CT_t (%)	1.02a	1.05a
DP*n*	32.73±0.3a	26.8±0.6b

EC_e: (−)-epicatechin extension units of procyanidins; EC_t: (−)-epicatechin terminal units of procyanidins; CT_t: (+)-catechin terminal units of procyanidins; DP*n*: average degree of polymerization.

neither for *Mesembryanthemum* species nor for other plants. The present results on this parameter suggest that climatic constraints led to the biosynthesis of molecules with higher DP*n*. Nevertheless, it is difficult to explain the DP*n* difference between the two provenances, but we suggest that the orientation in favour of more polymeric molecules is due to the fact that the degree of polymerization is positively correlated with the effectiveness of antioxidant activity and thus to plant protection (Spranger et al., 2008). Actually, proanthocyanidin properties were found to strongly depend on the DP*n* of the tannin molecule. According to Spranger et al. (2008), the antioxidant activity of proanthocyanidins is positively related to their degree of polymerization (*i.e.*, polymer>oligomer>monomer (catechin)), which may explain the increase of this degree in Djerba plants subjected to harder environmental conditions as compared to Monastir ones and may also be considered as a marker for plant adaptation to environmental constraints.

5. Conclusion

In summary, this study demonstrated the implication of phenolic quality and polymerization degree and the antioxidant capacity of *M. edule* in the adaptation towards its environment. Indeed, environmental conditions highly influence the antioxidant potential related to the phenolic composition of *M. edule* shoots. As regard the climatic conditions of the two provenances, it seems that the lack of precipitations and the long period of light hours are probably responsible for improving the level of antioxidant compounds and activity. This study strongly supports the idea that polyphenols play a significant physiological role in *M. edule* adaptation to environmental conditions, particularly against oxidative damage, and suggest that as subjected to environmental constraints, this species should be regarded as an additional health promoting value for use as phytonutrient.

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