Antioxidant capacity and phenolic composition as a function of genetic diversity of wild Tunisian leek (*Allium ampeloprasum* L.)

Accepted 6th August, 2015

ABSTRACT

Wild *Allium* species with an important use in Tunisia, such as *Allium ampeloprasum* L. could provide interesting bioactive compounds to current diet and medical. The bioactive compound content and the antioxidants potentialities of this wild species and the antioxidants potentialities of this wild species and the influence of the environmental condition on theses characteristic have been scarcely known. In order to further assess this assumption, ten accessions originating from different bioclimatic stages of Tunisia, were compared on the basis of the bioactive compounds content and the antioxidant capacity of the edible parts of wild leek (leaves and bulbs). The total polyphenol, flavonoid and tannin contents and antioxidants activities (DPPH and iron chelating power) were strongly affected by above cited factors. Such variability might be of great importance in terms of the valorizing of these species as a source of naturally products, and the methods for phenolic and antioxidant production.

Key words: *Allium ampeloprasum*, wild leek, accessions, bioactive compounds, antioxidant activity, Tunisia.

INTRODUCTION

Plant products, fruits, vegetables, and medicinal herbs have attracted a great interest as functional foods (Chandrashekar et al., 2011; Roberfroid and Delzenne, 1998). Interest in the phytochemical content of these products has increased due to consumer consciousness of their various health and nutraceutical benefits (Goloychenko *et al.*, 2012; Lee *et al.*, 2012; Lorenzi and Matos, 2002). Fruits and vegetables may contain a wide variety of free radical scavenging molecules, particularly phenolic compounds (Adão *et al.*, 2012; Park, 1971). Epidemiological studies have shown that these antioxidants could possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial and/or antiviral activities to a greater or lesser extent (Parente *et al.*, 1985). *Allium* genus is one of the most important field vegetable crops in the world. This genus that include about 700 species, have been recognized as riche sources of secondary metabolites with biological activity (Khanum *et al.*, 2004). *Allium* plants are already well known to be beneficial to human health (Baumgartner *et al.*, 2000; Chen and Tian, 2003; Cérantola *et al.*, 2004; Wack and Blaschek, 2006; Chen *et al.*, 2009). This genus *Allium* exhibits a great diversity as regards widely differing morphological characters, particularly in life form (bulb or rhizome) and ecological habitat. The genus consists mostly of perennial and bulbous plants; and it is widely distributed over holarctic regions from the dry subtropics to the boreal zone (Stearn, 1992). Plants of the genus *Allium* are cultivated worldwide for their nutritional benefits and medicinal properties. Some species have been found to be rich in bioactive polysacharrides, such as *Allium sativum*, *Allium cepa*, and *Allium fistulosum*.

The *Allium ampeloprasum* complex, widely spread in the Mediterranean area, comprises a group of taxa with similar morphology and habitat (Bothmer, 1974; Stearn, 1986). In Tunisia, two varieties have been reported: var. *typicum*...
Considered as a Mediterranean taxon, the *typicum* variety is characterized by a robust port, broad leaves and short ray florets purple flowers in large umbels. The other variety (*duriaeaeum*) distinguished by the port hail, narrow leaves with very prominent ligule and having small flowers in pink. It is a species closely related to leek (*Allium porrum* L.), and has been traditionally considered as its wild progenitor. Although some authors adopt a broad sense of the *A. ampeloprasum* that considers cultivated leeks as a subspecies or variety of *A. ampeloprasum*, others prefer a more restricted taxonomical approach for the species that only includes wild leeks without any subspecies. Recent molecular studies seem to agree that *A. porrum* should be considered a distinct species (Hirschegger et al., 2010). Therefore in this paper we consider *A. ampeloprasum* in this strict sense.

This species has been in used since ancient times by local consumers as a vegetable, spice and herbal remedy. The fresh young leaves and bulbs of *A. ampeloprasum*, or “korath” as called in Tunisia, are consumed in salad and used as spice to prepare traditional recipes. Besides its culinary use, "korath" is also used in folk medicine. Though lesser than other *Allium* species, the wild leek has a very long folk medicinal history of use a wide range of diseases, being mentioned by Dioscorides in the 1st century AD (Osbaldeston, 2000) and also in some modern ethnobotanical works for their perceived anthelmintic, diuretic, anti-hypertensive (Guarrera and Savo, 2013) or digestive properties (Triano et al., 1998). The crushed bulbs are used to treat initial stages of cough, mucous secretion and sore throat. The fresh juice is taken orally as a stomachic and antispasmodic and is also reputed to possess digestive properties (Malafaia et al., 2015). Despite the considerable medicinal potential of this genus, the investigators has tended to focus on the cultivated species *A. cepa* L., *A. fistulosum* L., and *A. sativum* L., as well as on a few wild-growing taxa. Its organosulphur compounds, responsible for the organoliptic parameters, are implicated as contributing in part to its health-promoting properties. In addition, a wealth of classes of compounds, such as polyphenolics including flavonol glycosides are also suggested to contribute to the health promoting properties of these species (Lanzotti, 2006).

It was shown that the amount of polyphenolics in plants, and antioxidant activities, are controlled by biological factors (genotype, organ ontogeny and physiological development stage), edaphic and environmental (temperature, salinity, water stress and light intensity) conditions (Lisiewska et al., 2006). According to Lisiewska et al. (2006), the evolution of phenolic content in higher plants may reflect their physiological status and developmental stages. In fact, Fico et al. (2000) indicate that during the vegetative phase, flavonoids (flavanones, flavones, and flavonols) are only present in the aerial parts and appeared gradually during the plant life. In contrast, these substances are detectable in the roots exclusively during the reproductive phase. On the other hand, Ksouri et al. (2008) indicated that leaf and stem extracts of *Satsola kali* showed a significant decrease of their phenolic contents and consequently their antiradical activities at the reproductive stage, as compared to the vegetative one, while root extract showed the opposite tendency. Other studies reports also that phenolic content varied as a function of plant growth in tomato and *Anethum graveolens* cultivars (Lisiewska et al., 2006; Fico et al., 2000; Ksouri et al., 2008; Toor et al., 2006). The selections of suitable plant material of *A. ampeloprasum* in the nursery constitute the first step to improve natural populations. The present survey has been dedicated to assess the bioactive compounds and the antioxidant capacity of several populations of *A. Ampeloprasum* L. collected from different bioclimatic zones in Tunisia. This paper describes the results of a study on the changes in phenolic content and the antioxidant activities as a function of ten accessions of *A. ampeloprasum* organs and (ii) their variability using several chemical descriptors (phenolic, flavonoids and tannins) and antioxidant assay of the edible parts of this species which could explain the observed ethnopharmacological. This work is, basically, an approach towards the valorization of this species. It also aims to select the effective genotypes which can be cultivated on a wide scale and in order to offer a wide perspective of its potential use in contemporary diets.

**MATERIALS AND METHODS**

**Study sites and harvesting dates**

Ten Tunisian accessions of *A. ampeloprasum* were collected in the wild at several locations with different characteristics (Table 1 and Figure 1) and were transplanted into the *Allium* collection of the Institute of Arid Lands (IRA).

The present study was conducted in the experimental field of Arid Lands Institute (Medenine, Tunisia) located in the lower arid bioclimatic. The plants were propagated in the nursery, the plants of each accession were planted in a separate row. A preliminary study was carried out in January 2013 which corresponds to the vegetative stage of the plant growing cycle. Bulbs were manually harvested plant per plant just before the dissemination (August). Separated stems, leaves and bulbs were cleaned, chopped into ~1 cm pieces and were stored at –80°C prior to freeze drying.

**Plant extracts preparation**

One gram of *A.ampeloprasum* leaves and bulbs dried and ground was extracted with 10 ml of methanol 75% during
Table 1. Main characteristic of ten selected sites of *Allium ampeloprasum*.

<table>
<thead>
<tr>
<th>Name</th>
<th>Geographical coordinates</th>
<th>Bioclimatic zone a</th>
<th>Altitude (m)</th>
<th>Mean annual rainfall (mm)</th>
<th>Mean annual temperature (°C)</th>
<th>Soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Djerba</td>
<td>33°52′3.6″ N, 10°54′28″ E</td>
<td>ASW</td>
<td>13</td>
<td>224</td>
<td>20.2</td>
<td>Salty halomorphe</td>
</tr>
<tr>
<td>Matmata</td>
<td>33°32′32″ N, 09°58′30″ E</td>
<td>ACW</td>
<td>384</td>
<td>226.3</td>
<td>20</td>
<td>Skeleton on hard rock</td>
</tr>
<tr>
<td>Mahdia</td>
<td>35°32′51″ N, 11°01′41″ E</td>
<td>SASW</td>
<td>3</td>
<td>320</td>
<td>18.4</td>
<td>Salty</td>
</tr>
<tr>
<td>Bir Ali</td>
<td>34°46′16″ N, 10°05′41″ E</td>
<td>ASW</td>
<td>129</td>
<td>306</td>
<td>19.7</td>
<td>Brown steppe</td>
</tr>
<tr>
<td>Elouara</td>
<td>32°26′51″ N, 10°27′26″ E</td>
<td>ACW</td>
<td>219</td>
<td>90</td>
<td>20.6</td>
<td>Skeletal non gypsum</td>
</tr>
<tr>
<td>Menzel Habib</td>
<td>34°17′3″ N, 09°35′57″ E</td>
<td>ACW</td>
<td>192</td>
<td>193.5</td>
<td>19.2</td>
<td>Steppe gypsum</td>
</tr>
<tr>
<td>Sousse</td>
<td>35°50′29″ N, 10°33′58″ E</td>
<td>SASW</td>
<td>13</td>
<td>327</td>
<td>18.5</td>
<td>Limestone</td>
</tr>
<tr>
<td>Samaaliette</td>
<td>33°17′3″ N, 10°53′49″ E</td>
<td>ASW</td>
<td>15</td>
<td>170</td>
<td>20</td>
<td>Steppe</td>
</tr>
<tr>
<td>Kef</td>
<td>36°10′32″ N, 09°39′20″ E</td>
<td>SACW</td>
<td>670</td>
<td>509</td>
<td>16.2</td>
<td>Limestone</td>
</tr>
<tr>
<td>Kneiss</td>
<td>34°16′16″ N, 10°12′44″ E</td>
<td>ASW</td>
<td>0</td>
<td>196</td>
<td>18.6</td>
<td>Salty (sebkha)</td>
</tr>
</tbody>
</table>

aBioclimatic zones are defined according to Emberger’s coefficient: ASW: arid at soft winter, SASW: semi-arid at soft winter, ACW: arid at cool winter and SACW: semi-arid at cool winter.

one hour at room temperature. After centrifugation at 8,000 xg, supernatants were recuperated and filtered through a 0.22 µm filter. To prevent denaturation, extraction was achieved rapidly and extracts were immediately used or stored at -20°C until further use.

**Colorimetric quantification of phenolics**

**Determination of total polyphenol content**

Phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton’s method slightly modified (Dewanto et al., 2002). An aliquot (0.125 ml) of appropriately diluted sample extract was added to 0.5 ml of distilled water and 0.125 ml of the Folin-Ciocalteu reagent. After 3 min, 1.25 ml of Na₂CO₃ (7%) solution were added and the final volume was brought to 3 ml with distilled water. The absorbance was measured at 760 nm, after incubation for 90 min at room temperature in dark. Total phenolic content of plant extracts was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g⁻¹ DW) through the calibration curve with gallic acid. The calibration curve range was 0-600 µg ml⁻¹. Triplicate measurements were taken for each sample.

**Estimation of flavonoid content**

The amount of flavonoid content was measured using the method described by Dewanto et al. (2002). An aliquot of suitable diluted sample or standard solution of quercetin was added to a NaNO₂ solution and mixed for 6 min before adding 0.150 ml of a freshly prepared AlCl₃ solution (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml with distilled water and
thoroughly mixed. Absorbance of the mixture was determined at 510 nm. Total flavonoids were expressed as mg quercetin equivalent per gram DW (mg CE g\(^{-1}\) DW), through the calibration curve of quercetin. The calibration curve range was 0-400 µg ml\(^{-1}\). All samples were analyzed in triplicate.

**Total condensed tannins assay**

Contents of condensed tannins were carried out according to Sun et al. (1998). Fifty microliters of properly diluted sample were mixed with 3 ml of vanillin-methanol solution (4%) and 1.5 ml of hydrochloric acid. The mixture was left for 15 min, and the absorption was measured at 500 nm. The concentration of condensed tannins was expressed as mg (+)-equivalent catechin/g DW (mg CE g\(^{-1}\) DW). The calibration curve range of catechin was established between 0 and 250 µg/ml. All samples were analysed in triplicate.

**Determination of antioxidant capacity**

**Scavenging ability on DPPH radical**

The scavenging activity of bulbs and leaves of *Allium ampeloprasum* methanolic extracts was measured in terms of hydrogen donating or radical scavenging ability using the DPPH method (Fattouch et al., 2007). Thirty milliliters of properly diluted sample of each methanolic extracts or the Trolox solution were mixed with 1 ml of a 0.2 mM DPPH (1,1-Diphenyl-2-picylyhydrazyl) methanolic solution. The mixture was vigorously shaken and placed in the dark at room temperature for 30 min. The absorbance of the resulting solution was then read at 517 nm. The antiradical activity was expressed as mg (+)-equivalent Trolox/g DW (mg TE g\(^{-1}\) DW). The calibration curve range of Trolox was

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![Figure 1](image_url). Bioclimatic regions distribution of the studied *Allium ampeloprasum* accessions.
Table 2. Contents of total phenolics, flavonoids and tannins condenses in the bulbs and leaves extracts of 10 wild accessions of A. ampeloprasum.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>Total phenolics (mg GAE/g DM)</th>
<th>Flavonoids (mg CE/g DM)</th>
<th>Tannins (mg CE/g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Bulbs</td>
<td>Leaves</td>
</tr>
<tr>
<td>Djerba</td>
<td>26.22±0.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19.66±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.70±0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kneiss</td>
<td>16.94±0.19&lt;sup&gt;i&lt;/sup&gt;</td>
<td>16.78±0.24&lt;sup&gt;†&lt;/sup&gt;</td>
<td>4.40±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Samaaliette</td>
<td>29.27±0.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.27±0.01&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>5.84±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elouara</td>
<td>30.11±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.37±0.24&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>5.30±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Matmata</td>
<td>48.22±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.56±0.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.70±0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menzel Habib</td>
<td>24.77±0.70&lt;sup&gt;f&lt;/sup&gt;</td>
<td>19.40±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.91±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bir Ali</td>
<td>35.50±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.69±0.13&lt;sup&gt;de&lt;/sup&gt;</td>
<td>5.47±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mehdia</td>
<td>18.15±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.08±0.35&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.66±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sousse</td>
<td>16.64±0.19&lt;sup&gt;i&lt;/sup&gt;</td>
<td>19.77±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.01±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kef</td>
<td>19.39±0.15&lt;sup&gt;s&lt;/sup&gt;</td>
<td>21.51±0.26&lt;sup&gt;s&lt;/sup&gt;</td>
<td>2.03±0.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values as means ± SD of three measurements. Means in column without superscript letters in common differ significantly (P<0.05)

established between 0 and 1000 µg/ml. The ability to scavenge the DPPH radical was calculated using Equation (1):

\[
\text{DPPH scavenging effect (\%) = } \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100
\]

(1)

Where \(A_0\) is the absorbance of the control at 30 min, and \(A_1\) is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

Chelating effect on ferrous ion

According to Zhao et al. (2006), 0.1 ml of each organ extract at different known concentration was added to 0.05 ml of FeCl\(_2\) x 4H\(_2\)O (2 mmol l\(^{-1}\)). Then, 0.1 ml of ferrozine (5 mM) were added, and the mixture was adjusted to 3 ml with deionised water, shaken vigorously, and left standing at room temperature for 10 min. Absorbance of the solutions was then measured spectrophotometrically at 562 nm. The inhibition’s percentage of ferrozine-Fe\(^{2+}\) complex formation was calculated using Equation (2):

\[
\text{Metal chelating effect (\%) = } \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100
\]

(2)

Where \(A_0\) and \(A_1\) have the same meaning as in Equation 1. Results were expressed as EC\(_{50}\): efficient concentration corresponding to 50% ferrous iron chelating.

Statistical analysis

For all plant parameters, three replicates were used. Statistical comparisons between investigated parameters were performed with Duncan’s test (SPSS) (11.5) in order to compare variation between the studied populations. On each parameter a correlation analysis was then used to estimate the relationship between the studied variables. The 10 populations were clustered based on chemical and antioxidant characterization, the scales portray a dissimilarity index calculated using the Euclidean distance coefficient, and the dendrogram was developed using UPGMA (unweighted pair group method using arithmetic averages) clustering procedures; a discriminant analysis was also used.

RESULTS AND DISCUSSION

Total phenolic, flavonoids and tannins content of A. ampeloprasum

Although most antioxidant activities from plant sources are derived from phenolic-type compounds (Cai et al., 2004), these effects do not always correlate with presence of large quantities of phenolics. Therefore, both sets of data need to be examined together, with respect to this, the investigated leek extracts were analysed for total phenolic, flavonoid and tannin contents.

All of the wild leek accessions tested contained significant levels of total phenolics (TP) flavonoids and tannins (Table 2). A. ampeloprasum considered a potential source of TP and compare favourably against those signaled by Bernaet et al. (2012) for 30 leek (Allium ampeloprasum var. porrum) cultivars (14 mg GAE/g DW) and for onion (2-30 mg GAE/g DW) and garlic (20 mg GAE/g DW) (Gorinstein et al., 2009; Kakhonen et al., 1999).

The total phenolic content in the bulbs and leaves of 10 leek accessions varied widely from 16 to 21 mg GAE/g DW and from 16 to 48 mg GAE/g DW, respectively. The
comparison of leaves and bulbs TP content showed that phenolic content was organ-dependant. Leaves were characterized by higher level of polyphenol contents, as compared to the bulb extracts. These findings agree with previous ones indicating that secondary metabolites distribution may fluctuate between different plant organs (Lisiewska et al., 2006; Bano et al., 2003; Falleh et al., 2008). These results are consistent with our expectation that the leaves in major cases would contain a significantly higher amount of total phenolics comparison with the bulbs.

The statically analysis showed a high significant variation among descriptors for all populations and revealed 9 groups of means according to descriptors. Then, the influence of harvest site on chemical composition of A. ampeloprasum accessions was notably clear. The comparison between the ten accessions showed that phenolic content was higher in leaves extracts from Matmata, Bir Ali, Elouara and Samaailette accessions and in bulbs extracts from Kef, Mahdia and Sousse accession. The accession Kneiss showed significantly lower total phenolics content in the bulbs and leaves in comparison with the other accessions.

Several reports showed a correlation between enhanced polyphenol production and exposure to UV-B radiation (sunlight) in barley (Kaspar et al., 2010) and Arabidopsis (Jordan et al., 1998). Besides UV light exposure, insect and microorganism pressure, low temperatures and low nutrient conditions correlates with the synthesis of phenolics and can be responsible for a difference in TP content for the 10 accessions (Duval et al., 1999; Michalak, 2006). Environmental factors are not the only possible explanation for these differences; the method employed to analyse the polyphenols can also conduct to a varying results. The Folin-Ciocalteu method may also determine other reducing compounds as reducing sugars (Vinson et al., 2001) and react also with some nitrogen compounds as amino acids and amines (Ikawa et al., 2003).

The main disadvantage of spectrophotometric assays is that they only give an estimation of the total phenolic content. It does not separate nor does it give quantitative measurement of individual compounds. Similarly, the molecular antioxidant response of polyphenolic compounds in Melo varies remarkably, depending on their chemical structure (Satue-Gracia et al., 1997). Thus, the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content.

Among the 10 leek accessions examined, the whole leek plant of the accession Matmata rated highest for mean total phenolics content (33 mg GAE/g DW). The TP contents reported by Garcia-Herrera et al. (2014) in whole leek plant is 5.77 mg GAE/g FW and Proteggente et al. (2002) a content in the whole leek plant of 22 mg GAE/100g FW are much lower than found for the 10 leek Tunisian accession tested. Santas et al. (2008) reported a TP content of 2.58 mg GAE/g in calçot (A. cepa variety). Again, all of this TP content is lower than our results. Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants (Tomas-Barberan et al., 2000; Lapornik et al., 2005).

The flavonoids content in the leaves and bulbs varied from 1.01 to 5.84 mg CE/g DW and from 4.10 to 4.40 mg CE/g DW, respectively (Table 2). The same tendency of polyphenol was observed for flavonoids content being more important in the leaves than in the bulbs, except for accessions Mahdia, Sousse and Kef. Flavonoids content was stimulated in the plant growing in the arid zone (Samaailette Leaf extract (5.84 mg CE/g DW) and Matmata bulbs extract (4.40 mg CE/g DM)) as compared to those originating from the humid zone. The highest flavonoid levels were recorded in accessions of southern Tunisia (Elouara, Samaailette and Djerba) known for its severe weather condition (temperature particularly) and subjected to prolonged UV light exposure. Rodrigues et al. (2011) observed higher levels of flavonols in onion sample grown in years with higher solar radiation and lower rainfall during the growing season. Overall, variations in the chemical composition of wild A. ampeloprasum, as in other plant tissues may be due to the multiple influences of different factors such as temperature, precipitation, sun exposure, soil composition, growing status and the interaction of other plants or animals in the ecosystem Garcia-Herrera et al., 2014).

Unfavorable environmental conditions (salinity, drought, heat/cold, luminosity and other hostile conditions) may trigger oxidative stress in plants, generating the formation of reactive oxygen species (ROS), leading to cellular damage, metabolic disorders, and senescence processes (Menezes-Benavente et al., 2004). Polyphenols synthesis and their accumulation is generally stimulated in response to abiotic-stress conditions (Naczk and Shahidi, 2004), such as drought (Navarro et al., 2006) leading one to think that secondary metabolites may play a role in the adaptation of xerophyte species to this constraint (Ksouri et al., 2006). It is worth mentioning that all studies dealing with high UV stress found an increase in antioxidant, and especially total phenolics concentration in various plants (Wang and Frei, 2006).

Low levels of flavonoids were recorded by Garcia-Herrera et al. (2014) for the Spanish A. ampeloprasum L. (0.86 mg CE/g extract) and by Gorinstein et al. (2009) in garlic (0.41 mg CE/g DW) and onion with its different varieties (0.76, 0.98 and 1.61 mg CE/g DW for the white onion, red and yellow, respectively). Flavonoids, one of the largest classes of plant phenolic, protect plant cells from UV-B radiation because they accumulate in epidermal layers of leaves and stems and absorb light strongly in the UV-B region while letting visible wavelengths throughout uninterrupted (Lake et al., 2009). Flavonoids are especially important antioxidants due to their high redox potential, which allows them to act as reducing agents hydrogen donors, and
singlet oxygen quenchers. In addition, they have a metal chelating potential (Tsao and Yang, 2003). When consumed regularly by humans, flavonoids have been associated with a reduction in the incidence of diseases such as cancer and heart disease (Beecher, 2003; Cook et al., 1996; Liu et al., 2008). There is currently great interest in flavonoid research due to the possibility of improved public health through diet, where preventative health care can be promoted through the consumption of fruit and vegetables. Flavonoids are a class of flavonoids commonly found in many fruits and vegetables, their content varying widely, depending on environmental factors, such as growing conditions, climate, storage and cooking conditions (Caridi et al., 2007).

Unlike of flavonoids, tannins contents are higher in bulbs than in leaves for the majority of the studied accessions. This content varied between 3.47 and 5.60 mg CE/g DW for leaves and 3.71 and 7.62 mg CE/g DW for bulbs. These results are superior to that recorded by Gorinstein et al. [2009] in the garlic (1.40 mg CE/g DW), red onion (3.67 mg CE/g DW), white onion (1.78 mg CE/g DW) and yellow onion (3.19 mg CE/g DW) for methanolic extracts. These variations could be due to differences among cultivars, growing seasons, agricultural practices and variations in applied total phenolics determinations assays (Bernaert et al., 2012).

### Antioxidant activity

The antioxidant potential of different plant extracts can be measured using numerous in vitro assays. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals, or the inhibition of lipid peroxidation. However, a single method is not recommended for the evaluation of the antioxidant activities of different plant products, due to their complex composition (Chu et al., 2000; Nuutila et al., 2003). Therefore, the antioxidant effects of plant products must be evaluated by combining two or more different in vitro assays to get relevant data. With respect to this, the antioxidant properties of the examined leek wild extract were evaluated, both, as free radical-scavenging capacity (RSC) and as protective effect on the activation of transition metal.

The RSC was evaluated by measuring the scavenging activity of examined leek wild extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and by neutralization of hydrogen peroxide. The DPPH radical is one of the most commonly used substrates for fast evaluation of antioxidant activity because of its stability (in radical form) and simplicity of the assay. On the other hand, although hydrogen peroxide is a non-free radical species, it is the source of the very toxic hydroxyl radical, especially in the presence of metal ions such as copper or iron. Also, hydrogen peroxide can cross membranes and may slowly oxidize a number of cell compounds. Thus, the elimination of hydrogen peroxide, as well as hydroxyl radical, is important for both, human health and the protection of pharmaceutical and food systems.

In the DPPH assay, most of the assessed extracts were able to reduce the stable, purple-coloured radical, DPPH, into the yellow coloured DPPH-H (Table 3). The results of RSC showed a highly significant accession effect (P<0.001) whatever plant parts (leaves or bulbs). The RSC values for the leaves varied between 13.77 and 31.03 mg TE/g DW (Table 3). The considerable antiradical ability was found especially in leaves extract of provenance Mahdia, Elouara and Samaaliette than in other studied provenance. For the bulbs extracts, the DPPH scavenging activity was recorded only in the both of Tunisian south accessions Elouara and Matmata (Table 3). Expressed in Trolox equivalent (TE), this difference between DPPH values difference between DPPH values of organ in A. ampeloprasum var. porrum cultivars are in accordance with earlier published data by Bernaert et al. (2012). Gorinstein et al. (2009) investigated the antiradical activity against the DPPH radical for the Allium species garlic, red, white and yellow onion: 7 µM TE/g dw, 22 µM TE/g dw, 21 and 20 µM TE/g DW, respectively. These findings may be related to the higher

### Table 3. Variability of antioxidant activities in leaves and bulbs of ten accessions of A. ampeloprasum.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>DPPH (mg TE/g DM)</th>
<th>Chelating power (EC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Bulbs</td>
</tr>
<tr>
<td>Djerba</td>
<td>24.50±0.71</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kneiss</td>
<td>13.77±0.64</td>
<td>n.d.</td>
</tr>
<tr>
<td>Samaaliette</td>
<td>30.12±0.54</td>
<td>n.d.</td>
</tr>
<tr>
<td>Elouara</td>
<td>31.03±1.02</td>
<td>14.98±0.13</td>
</tr>
<tr>
<td>Matmata</td>
<td>25.30±1.76</td>
<td>15.66±2.10</td>
</tr>
<tr>
<td>Menzel Habib</td>
<td>27.08±2.38</td>
<td>15.66±2.10</td>
</tr>
<tr>
<td>Bir Ali</td>
<td>28.64±2.43</td>
<td>n.d.</td>
</tr>
<tr>
<td>Mehdia</td>
<td>32.60±4.80</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sousse</td>
<td>24.27±3.06</td>
<td>n.d.</td>
</tr>
<tr>
<td>Kef</td>
<td>26.70±5.70</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d.: not detected. Values are means ± SD of three measurements. Means in column without superscript letters in common differ significantly (P<0.05).
polyphenol and flavonoid content in *A. ampeloprasum*.

Considering the fact that polyphenol compounds contribute directly to the antioxidant activities, the relation level between total phenolic content and antioxidant activities organs seems to be an interesting aspect to explore. In fact, previous reports showed a significant correlation between the antioxidant activity and total phenolic content of Algerian and Chinese medicinal plants (Djeridane et al., 2006; Wong et al., 2006).

The ability to chelate transition metals can be considered as an important antioxidant mode of action. In fact, the chelation and deactivation of transition metals prevent these species from participating in metal-catalysed initiation and hydroperoxide decomposition reactions (Dastmalchi et al., 2007). The chelating power activity (EC$_{50}$) for the leaves and bulbs covered significant ranges: 1.67-13.47 and 0.63-2.30 mg/ml, respectively.

As shown in Table 3, expressed as CE$_{50}$, antioxidant activities were significantly lower in leaves extract from Sousse, Menzel Hbib and Mehdia and bulbs extracts from Kneiss, Sousse, Mehdia and Kef, indicating a notably higher efficiency in these provenances. Nencini et al. (2011) reported similar important CP when analyzing the bulb, leaves and flowers of four *Allium* species (*A. neapolitanum, A. roseum, A. subhirsutum* and *A. sativum*). Halvorsen et al. (2002) analysed the total antioxidants in a variety of dietary plants by the reduction of Fe$^{3+}$ → Fe$^{2+}$. From this study, it was clear that leek contained more antioxidants than tomato, cauliflower and cucumber, but less than spinach, broccoli and red cabbage.

To determine the influence of the phytochemical constituents on antioxidant capacity in *A. ampeloprasum* accesses extract, we determined the correlation between the antioxidant capacity and antioxidant substances (total phenolics, flavonoids and tannins).

Results of DPPH and Chelating power (CP) assay were significantly correlated to the flavonoids compound concentration ($r$=0.501 ($p$<0.01) and $r$=0.504 ($p$<0.01), respectively. No statistically significant correlation was detected between the antioxidant capacity (CP and DPPH) assays and total phenolic content regardless studied (leaves or bulbs) part of *A. ampeloprasum*.

A positive correlation was detected between the results of the two antioxidant capacity assays done on the extracts of the bulbs of *A. ampeloprasum* accesses. This is in agreement with the results of Bernaert et al. (2012) detected a high correlation among DPPH, ORAC and FRAP in 30 leek cultivars. Moreover, the responsibility of phenolics for antioxidant activity, estimated by various methods, depends on their chemical structures, too. Also, the contradictory results are most probably due to differences in the experimental conditions (different reaction mechanisms) used in different assays.

The antioxidant properties of many *Allium* species have been widely proved (Bernaert et al., 2012), as well as the activities of bulbs and aerial parts of garlic (*A. sativum* L.), *A. fistulosum* L., and other species of the genus (Mohammadi et al., 2012).

One of the main mechanisms proposed for explaining *Allium* species bioactivity is radical scavenging. When the balance between the production and neutralization of free radicals by antioxidants tends to the overproduction of reactive oxygen species, the cells suffer the consequences of oxidative stress (Carocho et al., 2013). To avoid this, humans depend on antioxidants present in the diet to maintain free radicals at low levels (Pietta, 2013). Some hydrophilic compounds such as ascorbic acid and other organic acids present antioxidant properties, but there is a lack of data regarding their profile in non-cultivated *Allium* species.

Many of the compounds found in wild leek may have a protective role against various diseases due to their antioxidant activity, being able to chelate metals or to delocalize the electronic charge coming from free radical (Seabra et al., 2006).

In conclusion, the antioxidant capacity of wild *A. ampeloprasum* accesses extracts measured by the two methods (CP and DPPH) assays appears to be influenced by the flavonoides levels. Additionally to their role as antioxidant, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-thrombotic, cardio-protective and vasodilatory effects (Balasundram et al., 2006). In the other hand, the number of contributions on isolation and activity-testing of plant antioxidants has significantly increased in recent years (Huang et al., 2005).

**Classification of *A. ampeloprasum* accesses (PCA)**

A principal component analysis (PCA) was performed reducing the multidimensional structure of data, which provided a two dimensional map for explaining the observed variance (Figure 2). The loadings, eigen values and percentage of cumulative variance are shown in Table 4. Two components of PCA performed explain 80.6% of the total variance (50.16% first, 30.45% second). The graphic representation of the scores and loadings is showed in Figure 2a and b, respectively. The absolute value of loadings is an indicator of the participation of the analysed parameters in the PCs (Helena et al., 2000). The first principal component (PC1) is highly correlated to the variables in the increasing order Chelating power < Tannins < DPPH < Flavonoids and negatively correlated with total phenolics. The second principal component (PC2) is correlated to total phenolics. The relationships between the analysed parameters and also similarities or differences between the leaves and bulbs of the 10 accesses can be detected through investigation of this PCA plot. The chelating power, DPPH, and the flavonoids were the features with positive loadings on PC1 and PC2.

By the end of the analyses based on chemical
composition and antioxidant capacity data, four major groups were defined (Figure 3), the first group (A) comprised accessions Menzel habib, Kef, Sousse and Mahdia, belonging to arid and semi arid climates. The second group (B) included Samaaliette, Bir Ali and Djerba accessions belong to arid climate. The C group contains only the Kneiss accession; the final group (D) is made up of two accessions Matmata and Elouara. This division confirmed the presence of more than one bioclimatic zone in the same group. Ghariani et al. (2004) reported that the aggregation of 16 Lolium perenne L. accessions, using morphological data according to their geographical and bioclimatic originality, is not respected. In contrast, many previous studies consider the geographical and bioclimatic originality as determinant criteria. Ben Fadhel et al. (2000) and Arafh et al. (2002) in their study on two pastoral legumes (Hedysarum carnosum and Argyrolobium uniflorum) using 6 morphological descriptors and Iris haynei and I. atrofusca using floral and vegetative descriptors, respectively confirmed this hypothesis.

Nevertheless, the Menzel habib accession from the arid bioclimatic gathered with the semi arid accessions. These results reveal that chemical composition and antioxidant capacity traits of accessions being variable among the accessions of the same bioclimatic zone. This variability of antioxidant content of leaves and bulbs of A. ampeloprasum could be due to genetic background of the studied accessions, but also abiotic factors (temperature, water, radiation), biotic factors (pathogens), to which the plants are subjected (Mazid et al., 2011; Bernaert et al., 2012).

These factors may partly explain the different accumulation patterns of compounds between accessions and between the part (leaves and bulbs) of the same accession.

The cluster analysis was shown as a dendrogram indicating the estimated relations between A. ampeloprasum populations (Figure 3). It showed four distinct groups which include populations from different bioclimatic areas, such that the regrouped populations could have similar values for each studied descriptors. The first group could be subdivided into two subclusters including different populations from different areas (Figure 3). It was possible to project the populations, according to their bioclimatic area. So it was possible to find two or more populations from the semi arid zone at cool winter climate (group) grouped with populations from the arid climate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols</td>
<td>-0.653</td>
<td>0.712</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.752</td>
<td>0.446</td>
</tr>
<tr>
<td>Tannins</td>
<td>0.723</td>
<td>-0.655</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.752</td>
<td>0.357</td>
</tr>
<tr>
<td>Chelating power</td>
<td>0.654</td>
<td>0.357</td>
</tr>
<tr>
<td>Eigen values</td>
<td>2.508</td>
<td>1.523</td>
</tr>
<tr>
<td>Percentage cumulative variance</td>
<td>50.163</td>
<td>80.618</td>
</tr>
</tbody>
</table>

Table 4. Loadings, eigenvalues and percentage of cumulative variance for the first 2 principal components of the data from the leaves and bulbs from 10 accessions of Allium ampeloprasum.
zone at cool winter (group).

The discriminate analysis showed that *A. ampeloprasum*’s populations were very different from their bioclimatic originality. The populations of the same bioclimatic stage (e.g. semi-arid at cool winter) did not constitute a homogeneous set, and short distances seemed to isolate certain accessions, such as Bir Ali and Menzel habib and Smaalaite and Elouara which perhaps comprised related individuals, progenies of a few foundation plants. These two populations, which are some kilometers apart, were significantly separated. In fact, despite the short distance, they were not in communication. The geographic origin was not a determinant criterion for aggregation of studied populations; the most likely explanation is the coexistence of several varieties of *A. ampeloprasum* L. in Tunisia. However, Cuénod (1954) confirmed that this species comprised 2 varieties.

**Conclusion**

Nowadays, there is a growing interest in substances exhibiting antioxidant properties, which are supplied to human organisms as food components or specific preventive pharmaceuticals.

We investigate the antioxidant capacity in these local, wild leeks well known for their ethnopharmacological utilisations in traditional medicine. We address especially the biological, environmental effects on the phenolic content and antioxidant activities. Both chemical composition (total polyphenol, flavonoid and tannin) and antioxidant activities of *A. ampeloprasum* were influenced by harvest site. These data appeared tightly dependent on a number of biotic (organ and physiological stage) and abiotic (environmental, handling, harvest site) factors. The extreme climatic conditions in terms of drought, low rainfall, and high radiation, characterizing Southern Tunisian, are likely related to the increase of polyphenol and flavonoid content and antioxidant potentialities. The arid zone may enhance phenolic compound synthesis as a response to the oxidative stress generated by the formation of reactive oxygen species in these hostile environments.

The wild leek can be considered a good source of antioxidants to its cultivated relatives and other conventional vegetables. Additionally, the natural yield of this species, although lower than other cultivated *Allium* species, was found to be stable and well-adapted to human disturbed environments for these reasons, this non-conventional wild bulb should be revalorized as a good alternative to increase the diversity of vegetables consumed and enhance the quality of current occidental diets. Further works need to be done in the future to correlate the specific compound with its biological property.

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