

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

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**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 influence of the environmental condition on these characteristic have been scarcely known. In order to further asses 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 (16.64-48.22 mg GEA/g DM), flavonoid (1.01-5.84 mg CE/g DM) and tannin (3.47-7.62 mg CE/g DM) 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.

**Keywords:** accessions, bioactive compounds, DPPH and iron chelating power, Tunisia

### **1. Introduction**

Plant products, fruits, vegetables, and medicinal herbs have attracted a great interest as functional foods (Chandrashekar et al., 2011). Interest in the phytochemical content of these products has increased due to consumer consciousness of their various health and nutraceutical benefits (Lee et al., 2012). *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). 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 Mediteranean area, comprises a group of taxa with similar morphology and habitat (Stearn, 1986). In Tunisia, two varieties have been reported: var. *typicum* (Regel) and var. *duriaeanum* (Batt and Trab.) (Cuénod, 1954). This species was 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 antihelmintic, diuretic, antihypertensive (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 it's 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) 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 *Salsola 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. 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, flavonoïdes and tannins) and antioxidant assay of the edible parts of this specis 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.

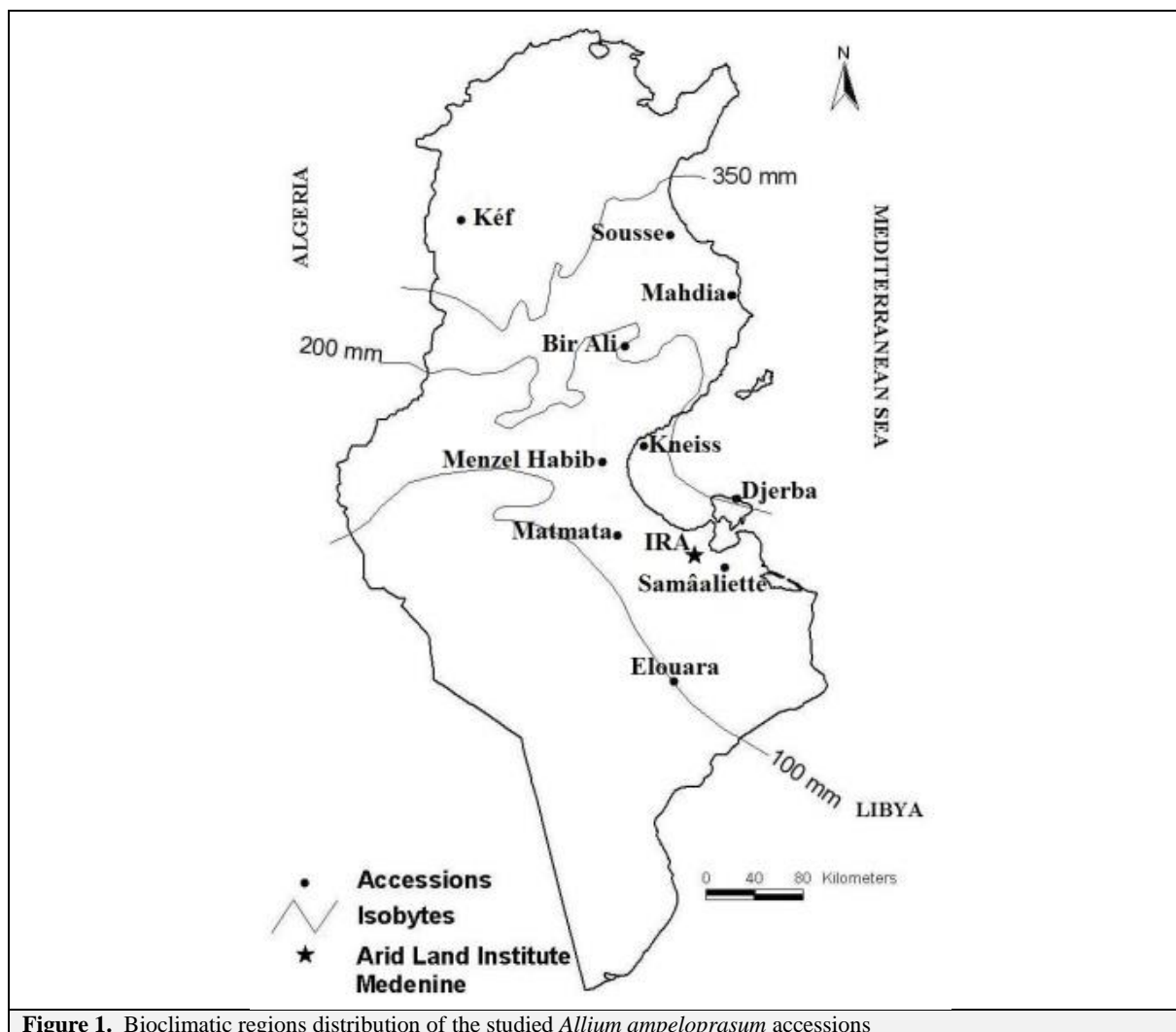
## 2. Material and Methods

### 2.1. Study sites and harvesting dates

Ten Tunisian accessions of *Allium ampeloprasum* (*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).

Name	Geographical coordinates	Bioclimatic zone <sup>a</sup>	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)	Soil type
Djerba	33°52'3.6" N 10°54'28" E	ASW	13	224	20.2	Salty halomorphe
Matmata	33°32'32" N 09°58'30" E	ACW	384	226.3	20	Skeleton on hard rock
Mahdia	35°32'51" N 11°01'41" E	SASW	3	320	18.4	Salty
Bir Ali	34°46'16" N 10°05'41" E	ASW	129	306	19.7	Brown steppe
Elouara	32°26'51" N 10°27'26" E	ACW	219	90	20.6	Skeletal non gypsum
Menzel Habib	34°17'3" N 09°35'57" E	ACW	192	193.5	19.2	Steppe gypsum
Sousse	35°50'29" N 10°33'58" E	SASW	13	327	18.5	Limestone
Samaaliette	33°17'3" N 10°53'49" E	ASW	15	170	20	Steppe
Kef	36°10'32" N 08°39'20" E	SACW	670	509	16.2	Limestone
Kneiss	34°16'16" N 10°12'44" E	ASW	0	196	18.6	Salty (sebkha)

<sup>a</sup> Bioclimatic 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.



**Figure 1.** Bioclimatic regions distribution of the studied *Allium ampeloprasum* accessions

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  $-80^{\circ}\text{C}$  prior to freeze drying.

## 2.2. Plant extracts preparation

One g of *A.ampeloprasum* leaves and bulbs dried and ground was extracted with 10 ml of methanol 75% during one hour at room temperature. After centrifugation at 8.000 xg, supernatants were recuperated and filtered through a 0.22  $\mu\text{m}$  filter. To prevent denaturation, extraction was achieved rapidly and extracts were immediately used or stored at  $-20^{\circ}\text{C}$  until further use.

## 2.3. Colorimetric quantification of phenolics

### 2.3.1. Determination of total polyphenol content

Phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified (Dewanto et al., 2002). Total phenolic content of plant extracts was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE  $\text{g}^{-1}$  DW) through the calibration curve with gallic acid. The calibration curve range was 0-600  $\mu\text{g ml}^{-1}$ . Triplicate measurements were taken for each sample.

### 2.3.2. Estimation of flavonoid content

The amount of flavonoid content was measured using the method described by Dewanto et al. (2002). Total flavonoids were expressed as mg quercetin equivalent per gram DW (mg CE g<sup>-1</sup> DW), through the calibration curve of quercetin. The calibration curve range was 0-400 µg ml<sup>-1</sup>. All samples were analyzed in triplicate.

### 2.3.3. Total condensed tannins assay

Contents of condensed tannins were carried out according to Sun et al. (1998). The concentration of condensed tannins was expressed as mg (+)-equivalent catechin/g DW (mg CE g<sup>-1</sup> DW). The calibration curve range of catechin was established between 0 and 250 µg/ml. All samples were analysed in triplicate.

## 2.4. Determination of antioxidant capacity

### 2.4.1. Scavenging ability on DPPH radical

The scavenging activity of bulbs and leaves of *A. ampeloprasum* methanolic extracts was measured in term of hydrogen donating or radical scavenging ability using the DPPH method (Fattouch et al., 2007). The antiradical activity was expressed as mg (+)-equivalent Trolox/g DW (mg TE g<sup>-1</sup> DW). The calibration curve range of Trolox was established between 0 and 1000 µg/ml. The ability to scavenge the DPPH radical was calculated using the following equation (1):

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

Where A<sub>0</sub> is the absorbance of the control at 30 min, and A<sub>1</sub> is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

### 2.4.2. Chelating effect on ferrous ion

According to Zhao et al. (2006), absorbance of the solutions was then measured spectrophotometrically at 562 nm. The inhibition's percentage of ferrozine-Fe<sup>2+</sup> complex formation was calculated using the following formula (2):

$$\text{Metal chelating effect (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (2)$$

Where A<sub>0</sub> and A<sub>1</sub> have the same meaning as in equation 1. Results were expressed as EC<sub>50</sub>: efficient concentration corresponding to 50% ferrous iron chelating.

## 2.5. Statistical Analysis

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.

## 3. Results and discussion

### 3.1. Total phenolic, flavonoides 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; Kahkonen 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). These resultants are consistent with

our expectation that the leaves in major cases would contain a significantly higher amount of total phenolics comparison with the bulbs.

**Table 2.** Contents of total phenolics, flavonoïdes and tannins condenses in the bulbs and leaves extracts of 10 wild accessions of *A. ampeloprasum*

Accession name	Total phenolics		Flavonoïdes		Tannins	
	(mg GEA/g DM)		(mg CE/g DM)		(mg CE/g DM)	
	Leaves	Bulbs	Leaves	Bulbs	Leaves	Bulbs
Djerba	26.22±0.33e	19.66±0.74b	4.70±0.05cd	4.37±0.07a	5.47±0.11a	4.00±0.15d
Kneiss	16.94±0.19i	16.78±0.24f	4.40±0.05d	4.15±0.15a	5.43±0.18a	7.62±0.21a
Samaaliette	29.27±0.21d	17.27±0.01ef	5.84±0.18a	4.20±0.01a	5.51±0.12a	5.71±0.11c
Elouara	30.11±0.24c	18.37±0.24cd	5.30±0.07b	4.37±0.09a	5.59±0.05a	6.04±0.05b
Matmata	48.22±0.19a	18.56±0.65c	4.70±0.05cd	4.40±0.05a	5.51±0.18a	5.77±0.05c
Menzel Habib	24.77±0.70f	19.40±0.58b	4.91±0.05c	4.18±0.10a	5.60±0.15a	3.87±0.10de
Bir Ali	35.50±0.28b	17.69±0.13de	5.47±0.06b	4.37±0.02a	5.45±0.12a	5.85±0.18bc
Mehdia	18.15±0.20h	20.08±0.35b	1.66±0.15e	4.30±0.10a	3.74±0.18b	4.00±0.10d
Sousse	16.64±0.19i	19.77±0.60b	1.01±0.02f	4.10±0.06a	3.47±0.02b	3.71±0.05e
Kef	19.39±0.15g	21.51±0.26a	2.03±0.63e	4.16±0.03a	3.65±0.65b	3.73±0.02e

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

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 Samaaliette 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). 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 phenolic 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. Santos *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. The flavonoides 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 flavonoides content being more important in the leaves than in the bulbs, except for accessions Mahdia, Sousse and Kef. Flavonoides content was

stimulated in the plant growing in the arid zone (Samaaliette 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, Samaaliette 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 biotic-abiotic stresses (Ksouri *et al.*, 2008), such as drought 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. 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 (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. Flavonols 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 (Ksouri *et al.*, 2008). 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.* 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).

### 3.2. 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 (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  $\mu\text{M}$  TE/g dw, 22  $\mu\text{M}$  TE/g dw, 21 and 20  $\mu\text{M}$  TE/g DW, respectively. These findings may be related to the higher polyphenol and flavonoid content in *A. ampeloprasum*.

**Table 3.** Variability of antioxydant activities in leaves and bulbs of ten accessions of *A. ampeloprasum*

Accession name	DPPH (mg TE/g DM)		Chelating power (EC <sub>50</sub> )	
	Leaves	Bulbs	Leaves	Bulbs
Djerba	24.50±0.71d	n.d.	13.47±0.50a	1.20±0.10f
Kneiss	13.77±0.64e	n.d.	12.80±0.20ab	0.63±0.06h
Samaaliette	30.12±0.54abc	n.d.	6.03±0.95c	1.93±0.11d
Elouara	31.03±1.02ab	14.98±0.13a	11.07±1.00b	2.30±0.10ab
Matmata	25.30±1.76cd	15.66±2.10a	5.50±0.50c	2.23±0.06bc
Menzel Habib	27.08±2.38bcd	n.d.	1.80±0.02d	2.12±0.08c
Bir Ali	28.64±2.43abcd	n.d.	3.10±0.10d	2.40±0.10a
Mehdia	32.60±4.80a	n.d.	2.72±2.00d	1.06±0.05g
Sousse	24.27±3.06d	n.d.	1.67±0.60d	0.68±0.03h
Kef	26.70±5.70bcd	n.d.	3.23±2.17d	1.73±0.06e

n.d.: not detected

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

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). 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<sub>50</sub>) 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<sub>50</sub>, 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. neopolitanum*, *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<sup>3+</sup>- Fe<sup>2+</sup>. From this study, it was clear that leek contained more antioxidants than tomato, cauliflower and cucumber, but less than spanich, broccoli and red cabbage. To determine the influence of the phytochemical constituents on antioxidant capacity in *A. ampeloprasum* accessions extract, we determined the correlation between the antioxidant capacity and antioxidant substances (total phenolics, flavonoides and tannins). Results of DPPH and Chelating power (CP) assay were significantly correlated to the flavonoides 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* accessions. 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 (*Allium sativum* L.), *Allium 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* accessions 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).

### 3.3. Classification of *A. ampeloprasum* accessions (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 < Flavonoides 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 accessions can be detected through investigation of this PCA plot. The chelating power, DPPH, and the flavonoides were the features with positive loadings on PC1 and PC2.

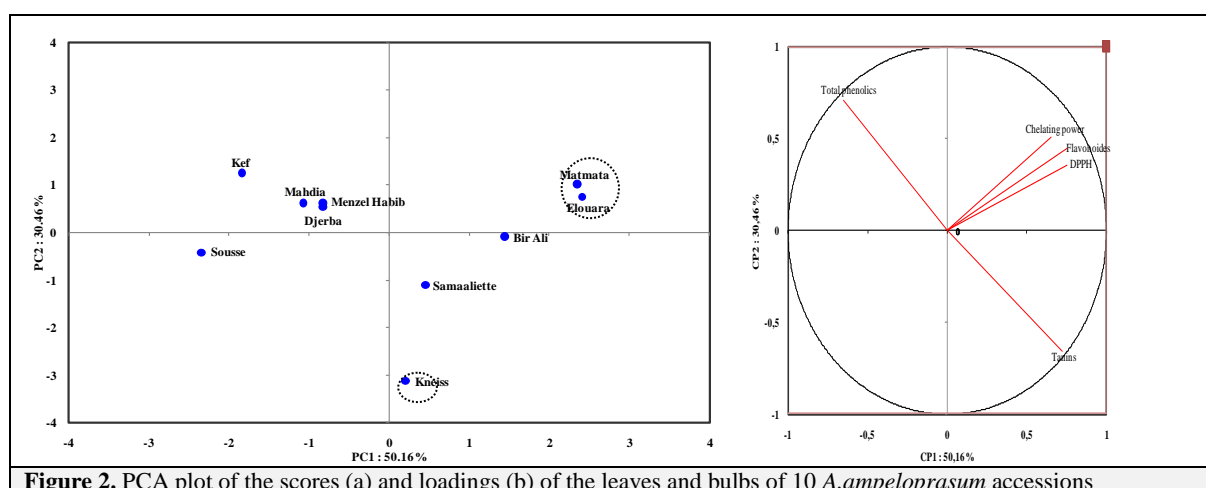


Figure 2. PCA plot of the scores (a) and loadings (b) of the leaves and bulbs of 10 *A. ampeloprasum* accessions



**Table 4.** Loadings, eigenvalues and pourcentage of cumulative variance for the first 2 principal components of the data from the leaves and bulbs from 10 accessions of *Allium ampeloprasum*

Variable	PC1	PC2
Total polyphenols	-0.653	0.712
Flavonoides	0.752	0.446
Tanins	0.723	-0.655
DPPH	0.752	0.357
Chelating power	0.654	0.357
Eigenvalues	2.508	1.523
% cumulative variance	50.163	80.618

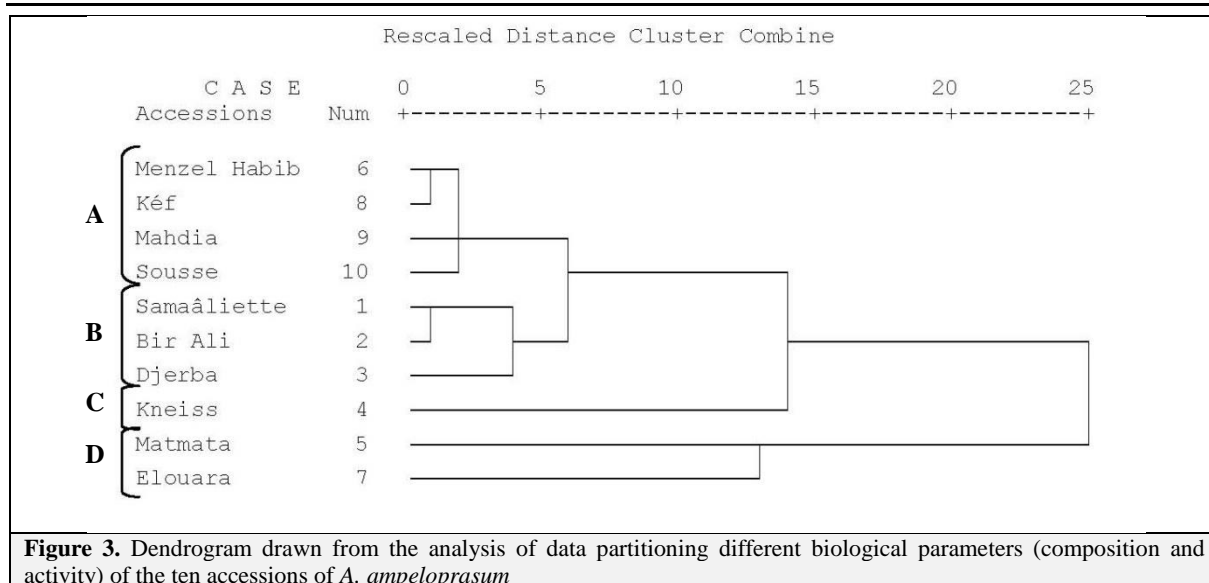
By the end of the analyses based on chemical composition and antioxidant capacity data, four major groups were defined (Figure 3). The first group (A) comprised accessions Menzel habib, Kef, Sousse and Mahdia, belonging to arid and semi arid climates. The second group (B) included Samaaliette, Bir Ali and Djerba accessions belong to arid climat. The C group contains only the Kneiss accession; the final group (D) is made up of tow 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 Arafeh 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 (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 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 Smaalaitte and Eloura 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 likeliest explanation is the coexistence of several varieties of *A. ampeloprasum* L. in Tunisia. However Cuénod (1954) confirmed that this species comprised 2 varieties.



#### 4. 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 here the antioxidant capacity in these local, wild leeks well known for their ethnopharmacological utilizations 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 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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