

# Polyphenolic Composition in Different Organs of Tunisia Populations of *Cynara Cardunculus.L* and Their Antioxidant Activity

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**Abstract** Polyphenols are biologically active molecules that contribute to development and cell growth. This study compared phenolic contents and antioxidant activity of stems and seeds in six Tunisian wild cardoon populations and one population of cultivated cardoon and artichoke located in the north of Tunisia. The analyzed organs exhibited different total polyphenol contents (1.49– 23.25 mg gallic acid equivalents, GAE g<sup>-1</sup> DW). The seeds phenolic and tannins contents were higher than those in stems. The globe artichoke stem phenolic and flavonoid contents were higher than those in wild and cultivated cardoons. Seed extracts displayed the highest DPPH scavenging ability with the lowest IC<sub>50</sub> value (3 µg ml<sup>-1</sup>), followed by globe artichoke stems (12 µg ml<sup>-1</sup>). In contrast, stems showed the highest capacity to quench superoxide (IC<sub>50</sub>: 2.1 µg ml<sup>-1</sup>) as compared to seeds (5.6 µg ml<sup>-1</sup>). These results confirm the beneficial effect of *Cynara cardunculus.L* organs on human health due to their high antioxidant activity.

**Keywords:** antioxidant, *Cynara cardunculus*, flavonoids; polyphenols

## 1. Introduction

Polyphenols are probably the most investigated molecules of nutritional interest. Numerous experimental papers in the literature have tried to evaluate through which mechanisms they exert their health benefits [1].

In plants they contribute to defence, development, cell growth, reproduction, differentiation, flowering and lignification [2]. The phenolic compound content in plants is variable depending on many parameters: genetic, physiological and environmental factors. The most important polyphenols classes are phenolic acids, which include polymeric structures, such as hydrolyzable tannins, lignans, stilbenes, and flavonoids. Flavonoids include flavonols, flavones, isoflavones, flavanones, anthocyanidins (pigments responsible for the colour of most fruits), flavanols (catechins and proanthocyanidins, known as condensed tannins) [3]. There are four main classes of polyphenols namely: phenolic acids, flavonoids, stilbenes and condensed tannins. These compounds exhibit large physiological properties, such as anti-allergic, anti-inflammatory, anti-atherogenic, antioxidant, antimicrobial, cardioprotective and vasodilatory effects [2].

Antioxidants are divided into two main types according to their action. Primary antioxidants can inhibit or delay oxidation by scavenging reactive oxygen species. Secondary antioxidants function by binding metal ions,

converting hydroperoxides to non-radical species, absorbing UV radiations [4]. In fact, the adaptation of many plant species to hostile environmental conditions suggests the presence of antioxidative and antimicrobial constituents in their tissues [5,6].

There is an increasing interest for naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants [7]. Besides, natural compounds had stronger antioxidant activity than that of synthetic ones [6].

The wild cardoon (*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori) is a robust perennial plant non-domesticated, characterized by its rosette of large thorny leaves branched and a flowering stems of blue-purple colour, it belongs to the Asteraceae family, tribe of Cynarace and native to the Mediterranean Basin, where it colonizes dry and quiet lands. Molecular studies [8,9] and cytogenetic and isozyme investigations [10] indicate that this species is the ancestor of both the globe artichoke (*C. cardunculus* var. *scolymus* L.) and the leafy or cultivated cardoon (*C. cardunculus* var. *altilis* DC).

In fact, this plant is used in several dishes, as soups and/or salads [11], their seeds can be used to extract food quality oil [12], and their flowers are widely employed in the making of cheeses [13,14].

Globe artichoke (*Cynara cardunculus.L.var.scolymus* (L.) Fiori) is a herbaceous perennial crop, widely cultivated in the Mediterranean area [15]. The heads, i.e., the large immature inflorescences with edible fleshy

leaves and receptacle, are used worldwide and represent a fundamental ingredient of the Mediterranean diet.

Furthermore, *C. cardunculus* is well-known to contain a high content of bioactive compounds, such as flavonoids and phenolic acids. These are widely studied due to their multiple biological activities [16]. Yet, data related to the antioxidant activity as well as phenolic composition of the stems and seeds of *Cynara cardunculus* L are scarce [17].

The aim of the present study was to determine total polyphenol, flavonoid, and condensed tannin contents at organ level (stems and seeds) and their antioxidant activities in six Tunisian wild cardoon populations from arid and wet regions of Tunisia and one population of cultivated cardoon and artichoke. The antioxidant capacity was evaluated by DPPH radical and  $O_2^-$  superoxide anion scavenging assays.

## 2. Materials and Methods

### 2.1. Plant Material and Preparation for Extract

*C. cardunculus* var. *sylvestris* samples were collected from six different regions of Tunisia: Bahra and Tiwirif (governorate of El Kef, north west of Tunisia with semi arid climate), Wad mliz (governorate of Jendouba, north west of Tunisia with wet climate), Zriba (governorate of Zaghaoun, northeast of Tunisia with semi arid climate) and Bouficha and Enfidha regions (governorate of Sousse, east of Tunisia with semi arid climate).

The stems of artichoke, wild and cultivated cardoons were collected in April 2010, while the mature seeds of wild and cultivated cardoons were harvested in August 2010. The harvested plants were identified at the Biotechnology Centre of the Technopark of Borj-Cedria, and a voucher specimen [ACC27] was deposited at the Herbarium of the Laboratory of Plant Adaptation to Abiotic Stresses at the Biotechnology Centre.

Stems of *Cynara cardunculus* were dried at room temperature for two weeks. Organ extracts were obtained by magnetic stirring for 30min of 2.5g of dry organ powder with 25ml of pure methanol which yielded the highest extracting power (%) as compared to other solvents [19]. Then, extracts were kept at 4 °C for 24h, filtered through a Whatman No ° 4 filter paper, and evaporated to dryness under vacuum and they were stored at 4 °C until analysis.

### 2.2. Phenolic Compounds Analysis

#### 2.2.1. Total Phenolics Content

Total phenolics were assayed using the Folin–Ciocalteu reagent, following Singleton and Rossi's [19] method, based on the reduction of a phosphowolframate–phosphomolybdate complex by phenolics to blue reaction products and slightly modified by [20].

An aliquot of diluted sample extract was added to 0.5 ml of distilled water and 0.125ml of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6min, before addition of 1.25ml of 7%  $Na_2CO_3$ .

The solution was then adjusted with distilled water to a final volume of 3ml and mixed thoroughly. After incubation in dark, the absorbance at 760nm was read

versus the prepared blank. Total phenolic content of stems and seeds of *Cynara cardunculus* was expressed as milligrams of equivalent gallic acid per gram of dry weight ( $mg\ GAE\ g^{-1}\ DW$ ) through the calibration curve with gallic acid. All samples were analyzed in three replications.

#### 2.2.2. Total Flavonoid Content

The total flavonoids were measured using a colorimetric assay developed by [20]. An aliquot of diluted sample or standard solution of (+) - catechin was added to 75 $\mu$ l of  $NaNO_2$  solution (7%), and mixed for 6min, before adding 0.15ml  $AlCl_3$  (10%). After 5min, 0.5ml of 1M NaOH solution was added. The final volume was adjusted to 2.5ml, thoroughly mixed, and the absorbance of the mixture was determined at 510nm. Total flavonoids were expressed as mg of equivalent (+) - catechin  $g^{-1}\ DW$  ( $mg\ CE\ g^{-1}\ DW$ ), through the calibration curve of (+) - catechin (0 – 400 $\mu$ g  $ml^{-1}$  range). All samples were analyzed in three replications.

#### 2.2.3. Total Condensed Tannins

The total condensed tannins were measured using the modified vanillin assay described by [21]. Three millilitres of 4% methanol vanillin solution and 1.5ml of concentrated  $H_2SO_4$  were added to 50 $\mu$ l of suitably diluted sample. The mixture was allowed to stand for 15min, and the absorbance was measured at 500nm against methanol as a blank. The amount of total condensed tannins was expressed as mg of equivalent (+) - catechin  $g^{-1}\ DW$ . All samples were analyzed in three replications.

### 2.3. Antioxidant Activity

#### 2.3.1. DPPH Radical Scavenging Activity

The diphenylpicrylhydrazyl radical (DPPH) scavenging activity was estimated according to [22]. The dried plant extract was diluted in pure methanol at different concentrations ranging from 1 to 50 $\mu$ g  $ml^{-1}$ , and then 2ml were added to 0.5ml of a 0.2 $mmol\ l^{-1}$  DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30min in the dark, and then the absorbance was measured at 517nm. For each dilution of the extract, the DPPH scavenging activity was calculated as  $100 (A_0 - A_1) / A_0$ , where  $A_0$  is the absorbance of the control at 30min, and  $A_1$  is the absorbance of the sample at 30min. The antiradical activity was finally expressed as  $IC_{50}$  ( $\mu$ g  $ml^{-1}$ ), the extract concentration required to cause a 50% inhibition. A lower  $IC_{50}$  value corresponds to a higher antioxidant activity of the plant extract. All samples were analyzed in three replications.

#### 2.3.2. Superoxide Anion Radical-Scavenging Activity

The superoxide quenching activity was assessed using the method described by [23]. The reaction mixture contained 0.2ml of extract diluted in pure methanol at different concentrations (1–50 $\mu$ g  $ml^{-1}$ ), and 0.2ml of 60 $\mu$ M PMS (phenazine methosulfate) solution, 0.2ml of 677 $\mu$ M NADH (Nicotinamide adenine dinucleotide), and 0.2ml of 144 $\mu$ M NBT (nitroblue tetrazolium), all in phosphate buffer (0.1M, pH 7.4). After incubation at ambient temperature for 5min, the absorbance was read at

560nm against a blank. The inhibition percentage of superoxide anion generation was calculated as for DPPH scavenging, and the antioxidant activity in each organ extract was expressed as IC<sub>50</sub>. All samples were analyzed in three replications.

## 2.4. Analysis

A one-way analysis of variance (ANOVA) using the XLSTAT version 2010 statistical program, with the organ as factor, was achieved for the following parameters: polyphenol, flavonoid, and tannin contents, as well as IC<sub>50</sub> on superoxide and DPPH scavenging activities. Means of these parameters were compared using the Newman-Keuls test at the  $P \leq 0.05$  level, when significant differences were found.

## 3. Results

### 3.1. Analysis of Phenolic Compounds

#### 3.1.1. Methanolic Extract Yield and Total Phenolic Content in the Studied Organs

The yield of the methanolic extract in the studied organs depended on their type (Table 1). The highest yield was registered in the stems of globe artichoke (ca. 30.12 %), while that of seeds of wild cardoons was 3 times lower. All organs exhibited high polyphenol content, comprised between 1.49 and 23.25 mg GAE g<sup>-1</sup> DW (Figure 1 and Figure 2). The seeds methanolic extracts of wild and cultivated cardoons displayed higher polyphenol content (23.25 and 15.04 mg GAE g<sup>-1</sup> DW) (Figure 1) than of stems of wild cardoon, cultivated cardoon and globe artichoke (5.79, 4.35 and 8.5 mg GAE g<sup>-1</sup> DW), respectively (Figure 2).

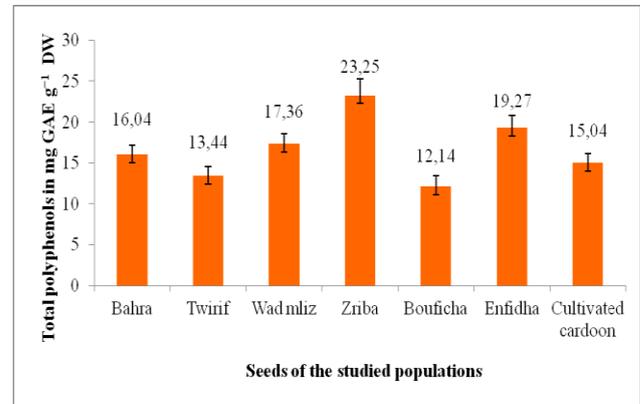
**Table 1. Phenolic compounds from cardoon seeds and aerial organs and globe artichoke aerial organs. Yield of methanolic extracts, total polyphenol, flavonoid and condensed tannin contents in stems and seeds of *Cynara cardunculus***

Organs	Yield (%)	Polyphenol content (mg GAE g <sup>-1</sup> DW)	Flavonoid content (mg CE g <sup>-1</sup> DW)	Tannin content (mg CE g <sup>-1</sup> DW)
Seeds of wild cardoon (Zriba)	9.95	23.25 a	8.93 a	4.62 a
Seeds of wild cardoon (Enfidha)	10.02	19.27 b	10.98 b	3.29 b
Stems of wild cardoon (Twirif)	28.45	5.79 c	7.46 c	0.77 c
Stems of globe artichoke	30.12	8.5 d	12.75 d	0.66 c

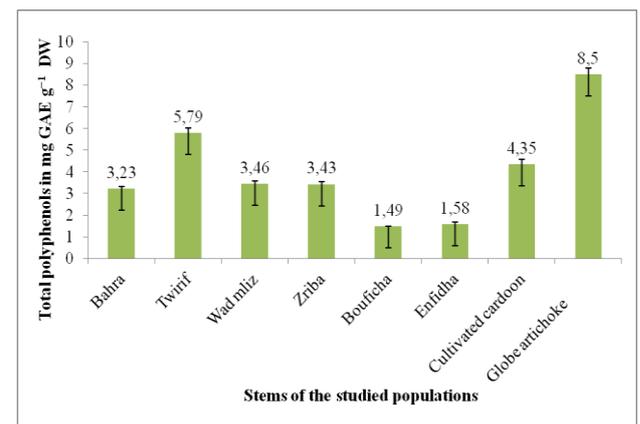
Means (three replicates) followed by at least one same letter are not significantly different at  $P \leq 0.05$ ; mg GAE g<sup>-1</sup> DW: milligrams gallic acid equivalent per gram dry weight; mg CE g<sup>-1</sup> DW: milligrams catechin equivalent per gram dry weight.

From these results, we selected the seeds and stems provenances showing the highest values of total polyphenols which are: seeds of wild cardoon (Zriba): 23.25 mg GAE g<sup>-1</sup> DW, seeds of wild cardoon (Enfidha):

19.27 mg GAE g<sup>-1</sup> DW, artichoke stems: 8.50 mg GAE g<sup>-1</sup> DW and stems of wild cardoon (Twirif): 5.79 mg GAE g<sup>-1</sup> DW. On these samples, we have analyzed the content of flavonoids and tannins in mg catechin equivalent per gram of dry matter (mg CE g<sup>-1</sup> DW), the free radical-scavenging activity of extracts using DPPH and superoxide anion-scavenging activity and we have found the following results:



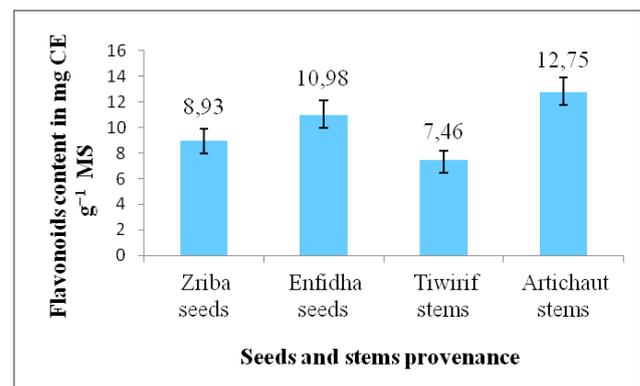
**Figure 1.** Total polyphenol content in milligrams of equivalent gallic acid per gram of dry matter of *Cynara cardunculus* L seeds



**Figure 2.** Total polyphenol content in milligrams of equivalent gallic acid per gram of dry matter of *Cynara cardunculus* L stems

#### 3.1.2. Total Flavonoid Content

Flavonoid content in the studied organs ranged from 7.46 to 12.75 mg CE g<sup>-1</sup> DW (Figure 3). It was lower in seeds and stems of wild cardoons than in stems of globe artichoke.



**Figure 3.** Flavonoid content in milligrams of equivalent catechine per gram of dry matter of *Cynara cardunculus* L organs

### 3.1.3. Condensed Tannins in Methanolic Extract

Condensed tannins were present in all studied plant organs, although in lower abundance than flavonoids, particularly in stems of wild cardoon and globe artichoke (Figure 4). The seeds of wild cardoons showed the highest tannin content (ca. 3.29 to 4.62 mg CE g<sup>-1</sup> DW).

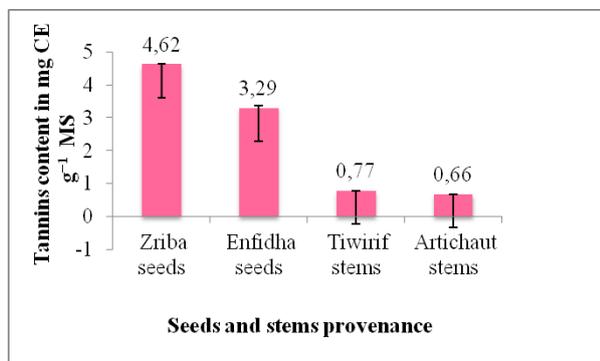


Figure 4. Tannins content in milligrams of equivalent catechine per gram of dry matter of *Cynara cardunculus*.L organs

## 3.2. Antioxidant Activity

### 3.2.1. Free Radical-Scavenging Activity of Extracts Using DPPH

The magnitude of DPPH radical quenching activity depended on the plant organs (Table 2), with IC<sub>50</sub> values extending over a 4-fold range. Seed extracts of wild cardoons displayed the highest DPPH quenching activity as compared to stems of wild cardoons and globe artichoke, which showed, respectively, 1.73 and 4-times higher IC<sub>50</sub> values (Figure 5).

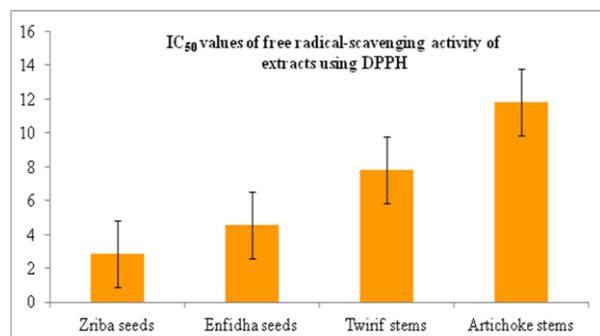


Figure 5. IC<sub>50</sub> values of free radical-scavenging activity of *Cynara cardunculus*.L extracts using DPPH

### 3.2.2. Superoxide Anion-Scavenging Activity

High O<sub>2</sub><sup>-</sup> quenching activity was found for the different plant organs (Table 2). The IC<sub>50</sub> values varied significantly between organs, stems extracts of wild cardoons and globe artichoke displayed the lower IC<sub>50</sub> value (2.1 and 3.5 µg ml<sup>-1</sup>), being one and two times more efficient in scavenging activity than the extract from seeds of wild cardoons, respectively (Figure 6).

### 3.3. Relationship between Content in Total Phenolic Compounds and Antioxidant Activities

The relationships between polyphenol, flavonoid and tannin contents and DPPH or superoxide scavenging

activity (using IC<sub>50</sub> values) were studied using linear regression analysis (Table 3). Significant negative and positive correlation appeared between IC<sub>50</sub> for DPPH scavenging and total polyphenol ( $r = -0.83$ ), flavonoid ( $r = 0.49$ ), and tannin ( $r = -0.91$ ) contents. The O<sub>2</sub><sup>-</sup> quenching activity was more tightly correlated with the concentrations of the three types of compounds ( $r = 0.94$  for total polyphenols;  $r = 0.17$  for flavonoids;  $r = 0.87$  for tannins).

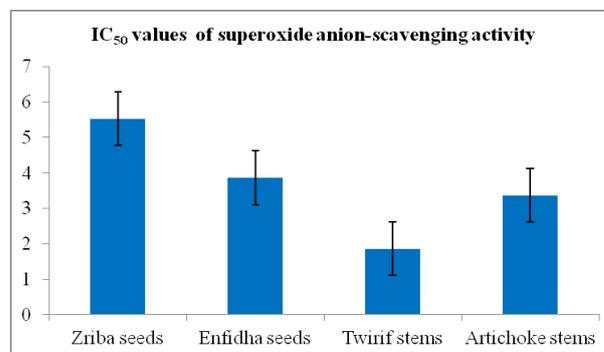


Figure 6. IC<sub>50</sub> values of superoxide anion-scavenging activity of *Cynara cardunculus*.L extracts

Table 2. Antioxidant activity of two methanolic extract concentrations in cardoon seeds and aerial organs and globe artichoke aerial parts (1 and 50 µg ml<sup>-1</sup>). Antioxidant activity expressed as IC<sub>50</sub> values for DPPH radicals and superoxide anion (O<sub>2</sub><sup>-</sup>) scavenging activity

Organs	DPPH (1 µg ml <sup>-1</sup> )	O <sub>2</sub> <sup>-</sup> (1 µg ml <sup>-1</sup> )	DPPH (50 µg ml <sup>-1</sup> )	O <sub>2</sub> <sup>-</sup> (50 µg ml <sup>-1</sup> )	IC <sub>50</sub> of DPPH	IC <sub>50</sub> of O <sub>2</sub> <sup>-</sup>
Seeds of Zriba	42.83 a	26.75 c	79.57 c	79.73 b	3 d	5.6 a
Seeds of Enfidha	14.25 d	24.45 d	79.64 b	85.44 a	4.6 c	4.2 b
Stems of Twirif	33.61 b	30.18 b	74.60 d	57.32 d	8 b	2.1 d
Stems of globe artichoke	27.37 c	32.65 a	96.38 a	65.19 c	12 a	3.5 c

Means (three replicates) followed by at least one same letter are not significantly different at  $P \leq 0.05$

Table 3. Relationship between phenolic compound concentrations in *Cynara cardunculus* extracts and their antioxidant activity. Correlation coefficients ( $r$  values) of total polyphenol, flavonoid and condensed tannin concentration, with DPPH or O<sub>2</sub><sup>-</sup> quenching activity expressed as IC<sub>50</sub> values

Polyphenols		flavonoids		condensed tannins	
DPPH	O <sub>2</sub> <sup>-</sup>	DPPH	O <sub>2</sub> <sup>-</sup>	DPPH	O <sub>2</sub> <sup>-</sup>
-0,832	0,941	0,494	0,171	-0,915	0,879

## 4. Discussion

In the present study, we determined the phenolic composition of customarily consumed organs of six Tunisian wild cardoon (*C. cardunculus*.var. *sylvestris*) populations from arid and wet regions of Tunisia and one population of cultivated cardoon (*C. cardunculus*.var. *altilis*) and globe artichoke (*C. cardunculus*.var. *scolymus*) and we measured the antioxidant activities present in these organs.

Our results show that the seeds of cardoon contain more total polyphenols and tannins than the stems. Similarly, [6] found the highest content of total phenolics and flavonoids

in the extract of seeds (about three times higher than in the flowers). [18] found that leaves and seeds of Tunisian *Cynara cardunculus*.L provenance from El Jem (central arid region of Tunisia) showed the same phenolic contents, twice those of flowers.

Irrespective of the organs, and despite being variable (1.49 to 23.25mg GAE g<sup>-1</sup> DW), the polyphenol content in cardoon was higher than in several species cited in the literature which is characteristic of the Asteraceae family [24]. This may be related to the hard climate conditions of Asteraceae which stimulate the biosynthesis of secondary metabolites such as polyphenols [18].

In this study, the DPPH and regenerated O<sub>2</sub><sup>-</sup> superoxide methods were used to assess the antiradical activities of plant extracts. These activities were organ-dependent, seeds of wild cardoons showing higher DPPH quenching activity than stems of wild cardoons and globe artichoke. Seeds are known to display higher antiradical ability than the other organs of herbs and vegetables [6,25].

The O<sub>2</sub><sup>-</sup> quenching capacity of methanolic extracts was also important in all organs. Stems of wild cardoons and globe artichoke showed the lowest IC<sub>50</sub> value, followed by seeds of wild cardoons. These findings may be due to the higher content in phenolic compounds of seeds of wild and cultivated cardoons as compared to stems of artichoke, wild and cultivated cardoons. However, using the same superoxide inhibition test, [18] found a much lower activity (IC<sub>50</sub> = 6) in seeds of a Tunisian *Cynara cardunculus*.L provenance from El Jem. This difference may be related to the more severe climatic conditions prevailing at Bahra, Twirif, Wad mliz, Zriba, Bouficha and Enfidha (arid and wet regions of Tunisia) than at El Jem (central arid region of Tunisia).

The level of correlation between phenolic content and antioxidant activity among the plant organs, which is an interesting aspect, supports the hypothesis that phenolic compounds contribute directly to antioxidant activity [23].

In our study, the correlation coefficient between phenolic compounds (total polyphenols and condensed tannins) and IC<sub>50</sub> values of DPPH activity (Table 3) was highly significant (-0.832 and -0.915 respectively), and this result indicates that phenolic compounds can play an important role in free radical scavenging [26]. The same type of linear correlation between antioxidant activities and phenolic content was found in whole-plant extracts, fruits, vegetables, and beverages [27], and in extracts of leaves, flowers and seeds of a Tunisian *Cynara cardunculus* from El Jem [18].

Our results show that the artichoke stems are richer in total polyphenols (8.5mg GAE g<sup>-1</sup> DW) than those of wild cardoons. This can be explained by the fact that the artichoke which is a cultivated variety is more sensitive to salinity and heat, therefore it's rich in polyphenols which is a stress factor, and this was confirmed by [28] that have shown that intense synthesis of secondary metabolites (polyphenols) in response to stress conditions is supposed to protect cellular structures from oxidation. Furthermore, [5] and [6] have shown that the adaptation of many species of plants to hostile environmental conditions suggest the presence of antioxidant constituents in their tissues.

The stems of globe artichoke have also shown the highest levels in total flavonoids (12.75mg EC g<sup>-1</sup> DW) which present a beneficial effect on human health;

previous results indicated that the heads of Violetto di Toscana have a higher phenolic content than the leaves [29]. These results indicate that the heads of globe artichoke could represent an important source of polyphenols which may have a therapeutic activity [30,31].

Similar conclusions were drawn by [29] who found that the heads of the variety of Violetto di Toscana are very rich in phenolic compounds and can therefore be regarded as an essential nutrient.

The stems of artichoke, wild and cultivated cardoons and the seeds of wild and cultivated cardoons showed different levels of total polyphenols, condensed tannins, flavonoids and total antioxidant activity. In addition, there was a strong linear relationship between total phenolics and DPPH antiradical capacity. The high content of phenolic compounds is an important factor in determining the antioxidant activity of this plant. In fact, *Cynara cardunculus*.L is interesting specie due to its antioxidant activity, but also a good source of health promotion due to the presence of polyphenols.

## 5. Conclusion

The stems of artichoke, wild and cultivated cardoons and the seeds of wild and cultivated cardoons showed different levels of total polyphenols, condensed tannins, flavonoids and total antioxidant activity. In addition, there was a strong linear relationship between total phenolics and DPPH antiradical capacity. The high content of phenolic compounds is an important factor in determining the antioxidant activity of this plant, *Cynara cardunculus*.L is an interesting food species because of its antioxidant activity, but also a good source of health promotion due to the presence of polyphenols.

## Abbreviations:

CE: (+) - catechin equivalent; DPPH: 2,2 - diphenyl-1-picrylhydrazyl; GAE: gallic acid equivalent, NBT, nitroblue tetrazolium, PMS, phenazine methosulfate.

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