

## A Comparative Evaluation of Total Polyphenolic Content and Antioxidant Potential of Thirty Medicinal Halophytes from the Mediterranean Region

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### ABSTRACT

In the last decades, an increasing interest has been granted to halophytes due to their high phenolic content, which have therapeutic potential in the treatment and/or management of human health. Therefore, it is important to measure the halophyte total polyphenolic content correctly and to valorize their antioxidant capacity. Ethanol extracts from thirty halophytes were analyzed to evaluate the Total Phenol Content (TPC). We employed three testing methods to prove their antioxidant potentialities, including DPPH<sup>•</sup> (1-DiPhenyl-2-PicrylHydrazyl), ABTS<sup>•+</sup> (2,20-Azino-Bis-3-ethylbenzoThiazoline-6-Sulfonic acid) and IRP (Iron Reducing Power) assays. Results showed that plants exhibited different TPC, which varied significantly from 411.5 mg GAE g<sup>-1</sup> DW in *Cynomorium coccineum* to 6.02 mg GAE g<sup>-1</sup> DW in *Ammophila arenaria*. Concerning antioxidant activities, data revealed that *Cynomorium coccineum* (IC<sub>50</sub>= 3.82 µg ml<sup>-1</sup> versus ABTS<sup>•+</sup>) and *Euphorbia paralias* had the highest antiradical capacity (IC<sub>50</sub>= 0.12 µg ml<sup>-1</sup> against DPPH<sup>•</sup>) and exhibited the best efficient concentration with an EC<sub>50</sub> value= 9.57 µg mL<sup>-1</sup> for the IRP. Considering correlation between phenols and antioxidant tests, three groups were distinguished with a higher correlation coefficient between 0.78 and 0.98 for the first group. These data suggest the promising potentialities of the Mediterranean medicinal halophytes as valuable source of powerful antioxidants of industries, especially for *Cynomorium coccineum*, *Carpobrotus edulis*, *Reaumuria vermiculata*, *Tamarix gallica*, and *Euphorbia paralias* regarding their strong phenol content.

**Keywords:** ABTS, Bioactive phytochemicals, DPPH, Therapeutic potential.

### INTRODUCTION

Numerous plants from the North African in Mediterranean regions are of great importance to the health of individuals and communities. Their use is against the oldest and the most assorted of all therapeutic systems (Mahomoodally, 2013). Among them, there are species that have recently been evaluated. These recent investigations have shown biological and phenolic

fingerprints of many plants from Mediterranean regions such *Capparis spinosa* collected from Italy, Turkey, and Morocco (Stefanucci *et al.*, 2018), *Salvia sclarea* L. (Zengin *et al.*, 2018), and *Allium scorodoprasum* L. from Turkey (Mollica *et al.*, 2018).

Halophyte species are among interesting medicinal plants, which have famous folkloric therapeutic applications (Custódio *et al.*, 2012). These plants represent versatile

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group which exhibit powerful antioxidant system mainly polyphenols, to overcome severe conditions (Ksouri *et al.*, 2012).

Nowadays, halophyte polyphenols have drawn an increasing attention thanks to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress- associated to emergent diseases (Jin *et al.*, 2010). Indeed, these molecules have potential uses as health products (functional foods, nutraceuticals, active principle) in various economic fields such as pharmaceutical, agro-food and cosmetic industries. Numerous research teams around the world develop many of their potential applications and recently some products are already on sale in the market (Buhmann and Papenbrock, 2013). In fact, phenols intake can prevent abnormal oxidative stress in the human body and the overproduction of Reactive Oxygen Species (ROS) related to diseases (cancers, atherosclerosis, diabetes, arthritis). They act by inhibiting the initiation or propagation of oxidative chain reaction and by scavenging ROS (Zengin *et al.*, 2017). In this context, it has been proved that halophyte plants are well known for their ethno-pharmacological uses in traditional medicine and culinary uses (Ksouri *et al.*, 2012). For this reason, there is a big interest in measuring and identifying among these species those containing high antioxidant content, especially for their use in the dietary industry and/or medicinal and even cosmetic applications (Meot-Duros *et al.*, 2008).

This study aimed to evaluate the Total Phenol Content (TPC) and to estimate the antioxidant activities of thirty halophyte species by measuring the Iron Reducing Power (IRP) and the radical scavenging assay (DPPH<sup>•</sup> and ABTS<sup>•+</sup>). Moreover, phenolic compounds of the most active shoot extracts were to be identified. The usefulness of the findings is to select the most promising species as source of valuable phenolic compounds, which may be potentially applicable as healthy products for industry.

## MATERIELS AND METHODS

### Plant Material

The thirty halophytes, which were selected thanks to their high biomass in coastal and sebkha areas and for their potential uses in folk medicine, were distributed among 14 botanical families: 7 Amaranthaceae, 6 Asteraceae, 3 Aizoaceae, 3 Zygophyllaceae, 1 Fabaceae, 2 Tamaricaceae, 1 Apiaceae, 1 Brassicaceae, 1 Cynomoriaceae, 1 Euphorbiaceae, 1 Nitrariaceae, 1 Plombaginaceae, 1 Poaceae, and 1 Solanaceae (Table 1). Only shoots of halophytes were collected, in the Northeast (Cap Bon), the East Center (Monastir), and the Southeast (Djerba), in 2016. The harvested plants were identified at the Center of Biotechnology of Borj-Cedria. Shoot plants were dried at room temperature in shadow. Ethanol extracts were obtained by magnetic stirring for 30 minutes of 2.5 g of dry powder with 25 mL ethanol 70%. The extracts were filtered through a Whatman filter paper. All extracts were then stored in the darkness at 4°C until analysis.

### Determination of TPC

The amount of total phenolic extracts was determined with the Folin-Ciocalteu reagent (Dewanto *et al.*, 2002). An aliquot of 125 mL of diluted extract was added to 500 mL of distilled water and 125 mL of the Folin-Ciocalteu reagent. The mixture was shaken, before adding 1,250 mL of Na<sub>2</sub>CO<sub>3</sub> (7%) and adjusting with distilled water to a final volume of 3 mL. After incubation for 90 minutes at 23°C in the dark, the absorbance versus the prepared blank was read at 760 nm. Total phenolic content was expressed as mg GAE (Gallic Acid Equivalent) g<sup>-1</sup> DW (Dry Weight) using a calibration curve with Gallic acid, ranged from 0 to 400 mg mL<sup>-1</sup>. The sample was analyzed in triplicate.

### DPPH<sup>•</sup> Radical Scavenging Assay

DPPH quenching ability of plant extracts was measured according to Hanato *et al.*

Table 1. List of the thirty Tunisian halophyte plants with respect to their family, local name, habit, life form, GPS coordinates location and plant type.<sup>a</sup>

| Family               | Scientific name of species                 | Voucher specimen | English name                             | Local name          | Growth habit        | Life form | Plant types           | GPS coordinates<br>Latitude (N), Longitude (E)                |
|----------------------|--|------------------|--|---------------------|---------------------|-----------|-----------------------|---|
| <b>Alzooaceae</b>    | <i>Aizoon canariense</i> L.                | PHM56            | Purslane-Leaved<br>atzoon                | Zarbiat<br>Iarbiab  | H                   | A         | X-hal                 | Djerba<br>33°51'57.26"N<br>10°47'21.93"E                      |
|                      | <i>Carpobrotus edulis</i> L.               | PHM57            | Sourfig, Hottentot<br>Fig                | Charhabou<br>Red    | H                   | P         | X                     | Monastir (sundy<br>coastal)<br>35°46'43.18"N<br>10°47'37.99"E |
| <b>Amaranthaceae</b> | <i>Mesembryanthemum crystallinum</i> L.    | PHM58            | Hottentot-Fig<br>Crystalline<br>iceplant | Ghassoul            | H                   | A         | F-hal                 | Monastir<br>35°46'58.32"N<br>10°49'59.38"E                    |
|                      | <i>Arthrocnemum macrostachyum</i> (Moric.) | PHM59            | Glaucous<br>glasswort                    | H'madha             | Sh                  | P         | Eu-hal                | Monastir<br>(Sahline)<br>10°42'44.26"E                        |
|                      | <i>Atriplex halimus</i> L.                 | PHM60            | Saltbush                                 | Graf                | Sh                  | P         | X-hal                 | Monastir<br>(Sahline)<br>35°46'8.30"N<br>10°42'44.26"E        |
|                      | <i>Atriplex lindleyi</i> Moq.              | PHM61            | Grey orache                              | Graf                | H                   | P         | Eu-hal                | Monastir<br>35°45'38.24"N<br>10°49'53.07"E                    |
|                      | <i>Salsola kali</i> L.                     | PHM62            | Russian thistle                          | Tasnouma            | H                   | A         | F-hal                 | Monastir<br>35°45'14.19"N<br>10°49'45.79"E                    |
|                      | <i>Salsola soda</i> L.                     | PHM63            | Opposite-<br>Leaved saltwort             | Souida              | H                   | A         | Eu-hal                | Monastir<br>35°46'42.62"N<br>10°47'38.43"E                    |
| <b>Apiaceae</b>      | <i>Suaeda maritima</i> L.                  | PHM64            | Annual scabrite                          | Essouida            | H                   | P         | Eu-hal                | Monastir<br>35°46'42.48"N<br>10°47'37.63"E                    |
|                      | <i>Suaeda pruinosa</i> Lange               | PHM65            | Shrubby                                  | Habet<br>essouida   | Sh                  | P         | Eu-hal                | Cap Bon Soliman<br>36°43'28.40"N<br>10°28'15.92"E             |
|                      | <i>Crithmum maritimum</i> L.               | PHM66            | Sea fennel<br>Rock samphire              | Besbes<br>Iebhar    | H                   | P         | F-hal                 | Monastir<br>35°47'7.93"N<br>10°50'3.50"E                      |
|                      | <i>Eryngium maritimum</i> L.               | PHM67            | Seaside eryngo                           |                     | H                   | P         | P-hal                 | Cap Bon Tazerka<br>36°32'21.64"N<br>10°50'55.51"E             |
|                      | <i>Achillea maritima</i> L.                | PHM68            | Cotton weed plant                        |                     | H                   | P         | P-hal                 | Cap Bon Tazerka<br>36°32'21.89"N<br>10°50'55.38"E             |
|                      | <i>Lananaa resedifolia</i> (Asso) Pau      | PHM69            |  | Ghedida             | H                   | P         | F-Hal                 | Djerba<br>33°49'41.27"N<br>10°53'0.85"E                       |
| <b>Asteraceae</b>    | <i>Limbarda crithmoides</i> L.             | PHM70            | Golden samphire                          |                     | H                   | P         | P-hal                 | Monastir<br>10°49'58.84"E                                     |
|                      | <i>Pallenis maritima</i> L.                | PHM71            | Yellow sea aster                         | Zarbiya             | H                   | P         | Hal                   | Cap Bon<br>36°50'2.92"N<br>11° 7'1.05"E                       |
|                      | <i>Reichardia tingitana</i> (L.) Roth      | PHM72            |  | Lobina or<br>Merr   | H                   | A         | F-hal                 | Monastir<br>35°46'59.10"N<br>10°49'59.25"E                    |
|                      | <i>Cakile maritima</i> Scop.               | PHM73            | European<br>searocket                    | Tarthouth<br>Iahmar | Fleshy red<br>stems | P         | Hal                   | Monastir<br>35°46'45.01"N<br>10°47'16.61"E                    |
|                      | <i>Cynomorium coccineum</i> L.             | PHM74            | Mushroom                                 |                     |                     |           | (parasitic-<br>plant) | Monastir<br>10°49'57.25"E                                     |
|                      | <i>Euphorbia paralias</i> L.               | PHM75            | Sea spurge                               | Loubina             | H                   | P         | P-hal                 | Cap Bon<br>(Menzel Temim)<br>11° 0'19.15"E<br>35°46'56.80"N   |
| <b>Fabaceae</b>      | <i>Lotus cytroides</i> L.                  | PHM76            |  |                     | H                   | P         | F-Hal                 | Monastir<br>10°49'59.56"E                                     |
|                      | <i>Retama raetam</i> (Forssk.)             | PHM77            | Lygos                                    | R'tem               | Sh                  | P         | X-hal                 | Cap Bon Soliman<br>36°43'49.30"N                              |

<sup>a</sup> A: Annual; BA: Biennial; P: Perennial; H: Herbal; Sh: Shrub; T: Tree; Eu-hal: Euhalophyte; F-hal: Facultative halophyte; G-hal: Gypso-halophyte; Hal: halophyte, P-hal: Psammo-halophyte; Ph: Phreatophyte; X-hal: Xerohalophyte; X: Xerophyte.

Table 1 continued...

**Continued of Table 1.** List of the thirty Tunisian halophyte plants with respect to their family, local name, habit, life form, GPS coordinates location and plant type.<sup>a</sup>

| Family         | Scientific name of species         | Voucher specimen | English name                        | Local name          | Growth habit | Life form | Plant types | GPS coordinates<br>Latitude (N), Longitude (E) |
|----------------|------------------------------------|------------------|-------------------------------------|---------------------|--------------|-----------|-------------|--|
| Nitriaceae     | <i>Nitraria retusa</i> (Forssk.)   | PIHM84           | Nabkhas                             | Ghardaq             | Sh           | P         | X-hal       | 33°51'42.43"N<br>10°47'21.71"E                 |
| Plombaginaceae | <i>Limoniastrum monopetalum</i> L. | PIHM78           |                                     | Zita                | Sh           | P         | Eu-hal      | 35°47'2.68"N<br>10°49'57.70"E                  |
| Poaceae        | <i>Ammophila arenaria</i> L.       | PIHM79           | Marram grass                        | Semra lebhar        | H            | P         | P-hal       | 36°46'1.68"N<br>11° 0'18.96"E                  |
| Solanaceae     | <i>Solanum elaeagnifolium</i> Cav. | PIHM80           | Silverleaf Nightshade, Tomato-weed. | Bitter apple,       | H            | P         | F-Hal       | 35°46'2.33"N<br>10°48'57.08"E                  |
| Tamaricaceae   | <i>Tamarix gallica</i> L.          | PIHM81           | Salteedar                           | Tarfia or Athl      | T            | P         | Ph          | 35°46'43.50"N<br>10°47'46.02"E                 |
|                | <i>Recurvata verticillata</i> L.   | PIHM82           |                                     | Oum neda            | Sh           | P         | G-hal       | 35°47'0.09"N<br>10°49'59.38"E                  |
| Zygophyllaceae | <i>Fagonia cretica</i> L.          | PIHM83           |                                     | Oum dhina or Chegaa | H            | P         | X           | 35°47'6.91"N<br>10°50'2.99"E                   |
|                | <i>Zygophyllum album</i> L.        | PIHM84           |                                     | Bougriba            | Sh           | P         | X-hal       | 35°47'6.91"N<br>10°50'2.99"E                   |

<sup>a</sup> A: Annual; BA: Biennial; P: Perennial; H: Herbal; Sh: Shrub; T: Tree; Eu-hal: Euhalophyte; F-hal: Facultative halophyte; G-hal: Gypso-halophyte; Hal: halophyte, P-hal: Psammo-halophyte; Ph: Phreatophyte; X-hal: Xerohalophyte; X: Xerophyte.

(1988). One mL of the samples was added to 250 mL of 0.2 mM solution of DPPH. After 30 minutes of incubation at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of DPPH<sup>•</sup> free radical in percent (I%) was calculated as follows:

$$I (\%) = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

Where,  $A_0$  is the absorbance of the control at 30 minutes, and  $A_1$  is the absorbance of the sample at 30 minutes. All samples were analyzed in three replications. The results are expressed as  $IC_{50}$  ( $\mu\text{g mL}^{-1}$ ), which is the Inhibiting Concentration of 50% of the synthetic radical.

### ABTS<sup>•+</sup> Assay

The ABTS<sup>•+</sup> was produced by the reaction between 5 mL of 14 mM ABTS solution and 5 mL of 4.9 mM potassium persulfate solution, stored in the dark at room temperature for 16 hours. Before usage, this solution was diluted with ethanol to get an absorbance of  $0.700 \pm 0.020$  at 734 nm. In a final volume of 1 mL, the reaction mixture comprised 950 mL of ABTS<sup>•+</sup> solution and 50 mL of each extracts at various concentrations. These mixtures were homogenized and its absorbance was recorded at 734 nm. All measurements were done after at least 6 minutes. Similarly, the reaction mixture of standard group was made with 950 mL of ABTS<sup>•+</sup> solution and 50 mL of BHT (Re *et al.*, 1999). As for the antiradical activity, ABTS scavenging ability was expressed as  $IC_{50}$  ( $\mu\text{g mL}^{-1}$ ). The inhibition percentage of ABTS<sup>•+</sup> radical was calculated using Formula (1).

### Iron Reducing Antioxidant Power (IRP)

The sample extract was mixed with 2.5 mL of sodium phosphate buffer (0.2M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 minutes. After that, 2.5 mL of 10% trichloroacetic acid was added, and the

mixture was centrifuged at  $650\times g$  for 10 minutes. The upper layer fraction (2.5 mL) was mixed with deionized water and 0.5 mL of ferric chloride. The absorbance was measured at 700 nm in a spectro-photometer and ascorbic acid was used as positive control. A higher absorbance indicates a higher reducing power (Oyaizu *et al.*, 1986).  $EC_{50}$  value ( $\text{mg mL}^{-1}$ ) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from linear regression analysis.

### Statistical Analysis

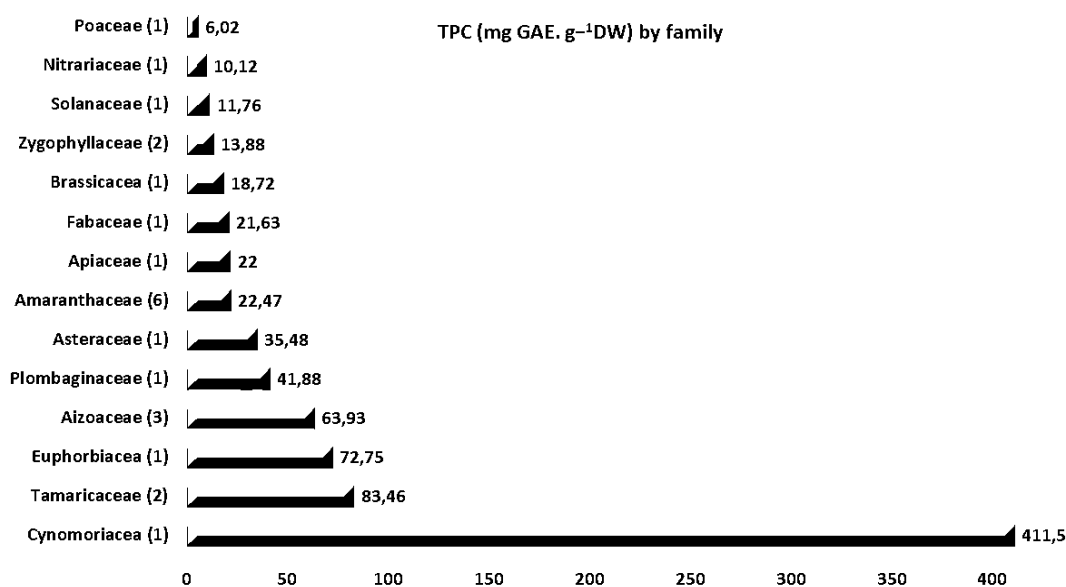
For all parameters, all samples were analyzed in three replications. Data are shown as mean $\pm$ SD. A one-way Analysis Of Variance (ANOVA) using the post hoc analysis with Duncan's test was carried out to test any significant differences at  $P < 0.05$ . The correlation coefficients " $r$ " and the coefficient of determination  $R^2$  between total phenolic content and the methods of antioxidant activity were demonstrated using Excel package 2010.

## RESULTS AND DISCUSSION

### Estimation of TPC by Family

The results (Figure 1) show that TPC of the fourteen-halophyte families revealed a very large inter-familial quantitative variability. The Cynomoriaceae family presented with single species in Mediterranean regions had the best TPC, which was 5.18 to 69 fold higher than Tamaricaceae and Amaranthaceae families, respectively. Indeed, Poaceae family displayed the lowest content of phenols. The present study did not use sufficient number of species for a real inter-familial comparison. However, it can give us just an approximate comparison. Future study would be necessary for the benefit.

One research has shown that endogenic factors (genetic and physiological stage), storage time factor, and environmental factors (biotic and abiotic) have a strong influence on the phenol content, which explains this significant variability (Chaouch *et al.*, 2014).



**Figure 1.** TPC of the fourteen-halophyte sample taxonomic families. TPC shown as Gallic Acid Equivalents per Dry Weight (GAE/DW). Parentheses indicate number of samples included in each halophyte family related to this study. TPC values are the average for the families represented by more than one species.



### Total Phenolic Content (TPC) and Antioxidant Activity

Results in Figure 2 show the different values (2-a: TPC mg GAE g<sup>-1</sup> DW; 2-b: IRP µg ml<sup>-1</sup>; 2-c: DPPH µg mL<sup>-1</sup>; 2-d: ABTS µg mL<sup>-1</sup>) by decreasing order. Our results demonstrate that the polyphenol extracts from plants exhibit a large significant variability in TPC levels. The TPC values are between 411.5 mg GAE g<sup>-1</sup> DW in *C. coccineum* and 6.02 mg GAE g<sup>-1</sup> DW found in *A. arenaria*. The *C. coccineum* TPC value is 2.5 fold higher compared to *C. edulis*, which comes in the second rank and is 7 fold higher than *S. maritima*, 18 fold more than *A. macrostachyum*, and 69 fold higher than the least value in *A. arenaria*.

There are several methods widely used to evaluate antioxidant activities. Therefore, Schlesier *et al.* (2002) recommended that at least two methods should be used. In this study, DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging assays and IRP activity were examined in order to have an indication of antioxidant capacity of the test samples. Indeed, the antioxidant activity is the most important biological property of phenolic substances (Dolek *et al.*, 2018). These substances have been studied intensively in functional foods. Many epidemiological studies have been published that show individual phenolic compounds reduce cancer risk (Elmastas *et al.*, 2015).

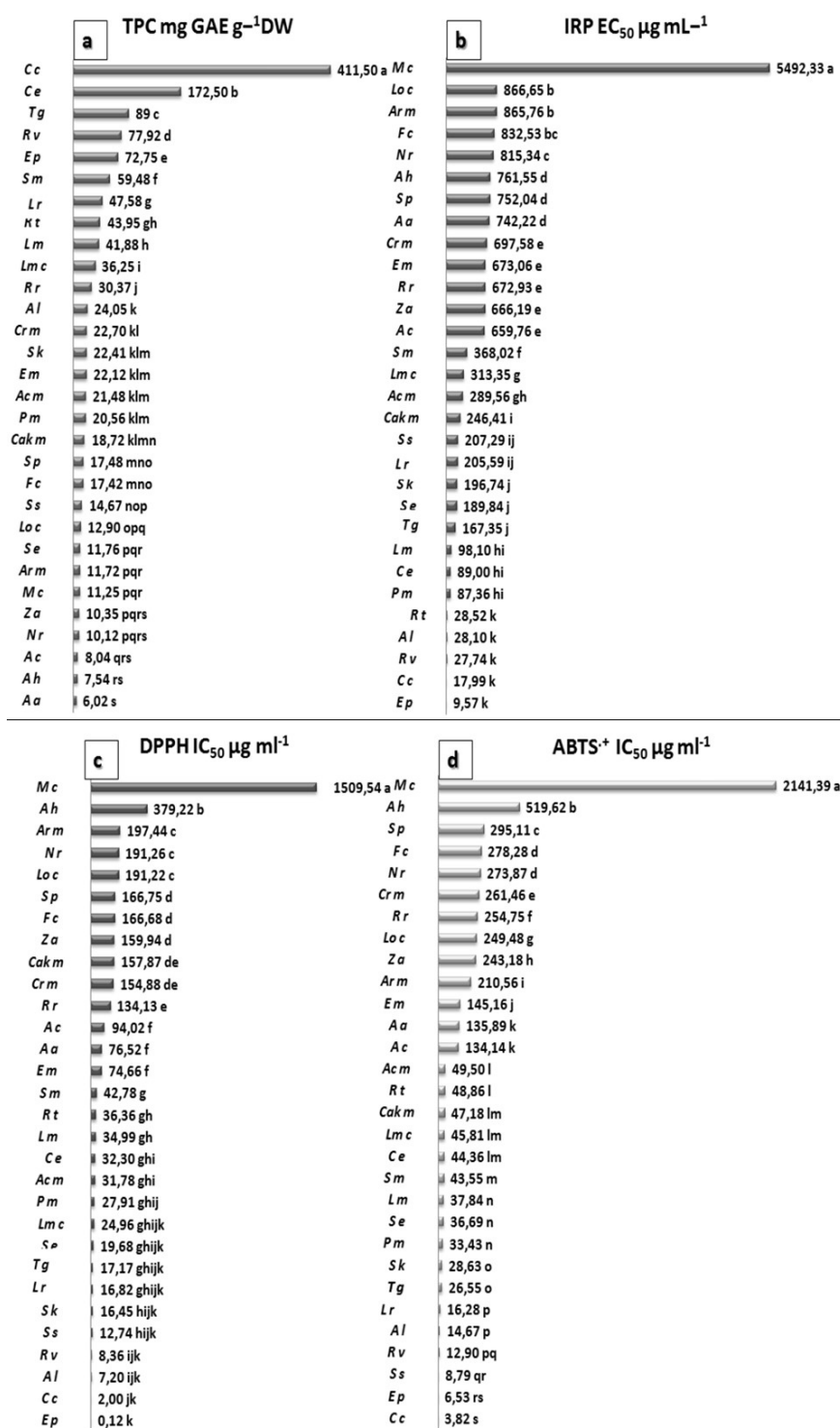
Therefore, the amount of phenols (Figure 2-a) and the antioxidant activities (Figures 2-b, -c and -d) detected by means of the three *in vitro* assays of the 30 aqueous ethanolic extracts are represented in order to determine the relationship between all variables.

On the one hand, several works have proved that the high TPC extracts and synergistic interactions might explain the strong antioxidant properties of halophyte plants (Ksouri *et al.*, 2008). Indeed, knowing that the Inhibition Concentration (IC<sub>50</sub>) (Figures 2-c: DPPH and 2-d: ABTS) and the Efficient Concentration (EC<sub>50</sub>) (Figure 2-b)

are inversely proportional to the antioxidant activity (Figure 2-a). Figure 2 shows that the studied species have in general an antioxidant activity correlated positively with the TPC value.

On the other hand, we have calculated the correlation coefficient (r) and the coefficient of determination (R<sup>2</sup>) by linear regression between TPC and antioxidant capacity by ABTS, DPPH, and by IRP tests to check the relationship between each test and TPC of all these species (Table 2). The correlation coefficient (r) and the (R<sup>2</sup>) values show that there is positive correlation between TPC and antioxidant for all species only against ABTS radical (r= 0.78), while DPPH (r= 0.12) and IRP (r= 0.48) are not correlated with phenol content. This led us to choose the best-correlated species and subdivide them into three groups. For the first group, the relationship between TPC and antioxidant capacity extracts of *C. coccineum*, *R. vermiculata*, and *T. gallica* (Table 2) shows a significant positive relationship with r value equal to 0.98 and R<sup>2</sup>= 0.97. Our findings exhibited that the highest TPC in Tunisian *C. coccineum* was similar to Algerian *C. coccineum* TPC [406.38±1.99 mg GAE g<sup>-1</sup> DW (Rached *et al.*, 2010)]. Therefore, the high TPC of *C. coccineum* might explain its strong antioxidant properties with IC<sub>50</sub> (DPPH)= 2±0.02 µg mL<sup>-1</sup> and the best IC<sub>50</sub> (ABTS)= 3.82±0.10 µg mL<sup>-1</sup> more than the BHA (synthetic agro-alimentary industry antioxidant IC<sub>50</sub>= 4.15 µg mL<sup>-1</sup>) (Rached *et al.*, 2010).

Indeed, Al-humaidi (2016) unveiled that *C. coccineum* extracts contains condensed tannins, flavonoids, glycosides, anthraquinones, and other non-phenolic compounds such as terpenoids and alkaloids. This species has been used in many countries since the middle age, in Qatar as a medicinal tea (Lebling, 2003) and in North Africa for hemorrhoids and diarrhea treated by shoot decoction (IUCN, 2005). Therefore, this non-photosynthetic parasitic plant can be considered as a potential resource of oil, with nutraceutical properties



**Figure 2.** Results of TPC of the 30 halophytes and antioxidant activity (DPPH, ABTS and IRP) ranking by decreasing order. Values not sharing a common letter (a-s) differ significantly at  $P < 0.05$  by Duncan test. *Ac m*: *Achillea maritima*, *A c*: *Aizoon canariense*, *A a*: *Ammophila arenaria*, *Ar m*: *Arthrocnemum macrostachyum*, *A h*: *Atriplex halimus*, *A l*: *Atriplex lindleyi*, *Cak m*: *Cakile maritima*, *C e*: *Carpobrotus edulis*, *Cr m*: *Crithmum maritimum*, *C c*: *Cynomorium coccineum*, *E m*: *Eryngium maritimum*, *E p*: *Euphorbia paralias*, *F c*: *Fagonia cretica*, *L r*: *Launaea resedifolia*, *Lm c*: *Limbarida crithmoides*, *L m*: *Limoniastrum monopetalum*, *Lo c*: *Lotus cytisoides*, *M c*:

**Table 2.** Correlations ( $r$  and  $R^2$ ) between antioxidant capacity of plant extracts by (ABTS, DPPH and IRP assays) and TPC.

| TPC of species <sup>a</sup>   | $r - R^{2b}$        |                     |                     |        |                    |    |
|---|---------------------|---------------------|---------------------|--------|--------------------|----|
|   | DPPH                |                     | ABTS                |        | IRP                |    |
| All species   | 0.12 <sup>ns</sup>  | - 0.06              | 0.78                | **     | 0.48 <sup>ns</sup> | -  |
| Group 1: <i>C. c.</i> , <i>R. v.</i> , <i>T. g.</i>   | 0.98 <sup>***</sup> | -                   | 0.61 <sup>*</sup>   | -      | 0.23 <sup>ns</sup> | ** |
| Group 2: <i>Z. a.</i> , <i>Cak. m.</i> , <i>Cr. m.</i>  | 0.97 <sup>***</sup> | -                   | 0.96 <sup>***</sup> | -      | 0.61 <sup>*</sup>  | -  |
| Group 3: <i>Ar. m.</i> , <i>N. r.</i> , <i>S. k.</i> , <i>A. l.</i> , <i>S. s.</i> , <i>S. e.</i> | 0.94 <sup>***</sup> | -                   | 0.18 <sup>ns</sup>  | - 0.03 | 0.19 <sup>ns</sup> | -  |
|   | 0.90 <sup>***</sup> | -                   | 0.43 <sup>ns</sup>  | - 0.16 | 0.73 <sup>**</sup> | -  |
| <i>e</i>  | 0.80 <sup>**</sup>  | - 0.64 <sup>*</sup> | ns                  | -      | 0.53 <sup>ns</sup> | -  |

<sup>a</sup> *C. c.*: *C. coccineum*; *R. v.*: *R. vermiculata*; *T. g.*: *T. gallica*; *Z. a.*: *Zygophyllum album*; *Cak. m.*: *Cakile maritima*; *Cr. m.*: *Crithmum maritimum*; *Ar. m.*: *Arthrocnemum macrostachyum*; *N. r.*: *Nitraria retusa*; *S. k.*: *Salsola kali*; *A. l.*: *Atriplex lindleyi*; *S. s.*: *Salsola soda*; *S. e.*: *Solanum elaeagnifolium*. <sup>b</sup> Data represents Pearson's correlation coefficient  $r$ . ns: Indicates non-significant; \*: Refers to  $P < 0.05$ ; \*\* and \*\*\*: Indicate significant at  $P < 0.01$  and  $P < 0.001$ , respectively.

and potential benefits in cancer prevention, which has a significant growth inhibitory effect on melanoma and colon cancer cells (Antonella et al., 2015). Another study exhibits that this medicinal plant may have potential as a diet-based solution for combating, preventing, and managing the early stage of type 2 diabetes when coupled with an overall healthy life (Phoboo, 2015).

*Tamarix* spp., especially *T. gallica* species, have a high antioxidant activity (Ksouri et al., 2009; Boulaaba et al., 2015) due to the presence of many polyphenols, which possess both anti-inflammatory and analgesic effects (Chaturvedi et al., 2012).

The *R. vermiculata* species, unknown in folk medicine, is considered as an interesting Tamaricaceae thanks to its important TPC (Figure 2-a). Therefore, Karker et al. (2016) proposed it as a valuable source for bioactive and natural compounds that related to their phenolic compounds, which exhibited interesting biological activity. Interestingly, previous research confirms the anticancer activity against liver, colorectal, breast, and prostate tumor cell lines (Nawwar et al., 2012).

TPC of the second group (Table 2) (*Z. album*, *C. maritima* and *C. maritimum*) has only good correlation with DPPH test ( $r=0.94$ ). Comparing the obtained results with the previously published data, we can

explain the results by the nature of polyphenols. In fact, phenolic compounds structurally differ from simple molecules to highly polymerized compounds (tannins). The different antioxidant results may be due to their richness in flavonoids, which are one of the most numerous and widespread groups of phenolic compounds in higher plants (Tepe, 2005). Recent studies have shown that a big number of flavonoids contribute significantly to the total antioxidant activity of many fruits and medicinal plants (Chaouch et al., 2014).

According to Oszmianski et al. (2007), the antioxidant activities against ABTS<sup>•+</sup> or DPPH<sup>•</sup> were correlated with chemical structures, polymerization, and the concentration degrees of organ antioxidants. Therefore, our study revealed two species that had extreme antioxidant activity values: *E. paralias* with  $IC_{50}=0.12 \mu\text{g mL}^{-1}/6.53\pm 0.09$  and *M. cristallynum* with  $IC_{50}=1509.54 \mu\text{g mL}^{-1}/2141.39\pm 7.92$ , respectively, for DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals (Figure 2). *E. paralias* phenols has very powerful antioxidant compounds that can easily quench free radicals DPPH<sup>•</sup>, ABTS<sup>•+</sup> and has high iron reducing power, while *M. cristallynum* phenols has the lowest antioxidant activity. The antioxidant activity of *E. paralias* against DPPH is 16 fold higher than its level in *C. coccineum*,



however, it has the highest value of TPC (Figure 2-a). According to Besbes Hlila *et al.* (2016), the presence of powerful phenolic compounds seems to be a good reason for the antioxidant activities and  $\alpha$ -glucosidase inhibition. However, *E. paralias* TPC is not the highest and this may be explained by the fact that their polyphenols are not the only antioxidant sources. Indeed, *Euphorbia* genus is known for containing latex (rich in alkaloids) and a wide variety of terpenoids (Özbilgin *et al.*, 2012). Many of these compounds have been used in medicine since ancient times. They have diverse structures and therapeutically importance, and they perform many different biological activities (Tang *et al.*, 2012) such as tumor promoting, antiproliferative, cytotoxic, antimicrobial, antiinflammatory, anti-HIV and anticancer (Özbilgin *et al.*, 2012). However, the Aizoaceae, *M. crystallinum*, with its TPC equal to  $11.25 \pm 0.50$  mg GAE  $g^{-1}$  DW, has exhibited the lowest antioxidant activities (Figures 2-b, -c and -d). Previous research has shown that its antioxidant activity is not due to the polyphenols but to its enzymatic antioxidant activity (Bouftira *et al.*, 2008), which makes it engaged in detoxification of free radicals or ROS (Slesak and Miszalski, 2003). In addition, this ice plant possesses a rapid cell-specific accumulation of secondary metabolites. Flavonol conjugates and non-phenolic compound the betalain pigments (betacyanin include the reddish to violet pigments) have antioxidant and anti-inflammatory activities, making this species a good candidate for pharmaceutical and cosmetic applications, which also act as anticancer agents against colon carcinogenesis (Bouftira *et al.*, 2012).

The current study supports the findings of Falleh *et al.* (2011) for the other Aizoaceae edible plant, *C. edulis*, whose TPC ( $172.50 \pm 3.04$  mg GAE  $g^{-1}$  DW, Table 2) compared with this recent research ( $86.50 \pm 1.86$  mg GAE  $g^{-1}$  DW), is so important. Previous research has proved that *C. edulis* contain phenols with great interest (Liu *et al.*, 2005). Accordingly, a close

relationship may be suggested between phenolic amounts and antioxidant capacities in this halophyte considered as an appreciated source of natural antioxidants for food, medical, cosmetic and pharmaceutical industries (Falleh *et al.*, 2009). Falleh *et al.*, (2011) have also exhibited the cardio-protective effects of the stem and its ability to inhibit lipid peroxidation, to chelate redox-active metals, and to attenuate other processes involving ROS.

Some other species have similar TPC value as (*F. cretica* and *S. pruinosa*) and show the same antioxidant capacity (Figure 2). In fact, *F. cretica* is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well-documented. It has a strong anti-cancerous potential (Hussain *et al.*, 2007). Its ethanolic extract has different effects on the hemoglobin level (Jahala *et al.*, 2014) and it possesses a significant antihyperlipidemic activity (Nagaraj, 2013). In view of their numerous traditional and scientific uses, *F. cretica* seems to be very promising plant.

For the third group (Table 2), *S. elaeagnifolium* and *A. macrostachyum*, although having similar TPC, their antioxidant activity is weakly correlated.

In general, these results suggest that other phytochemical compounds might contribute to the beneficial effects of such species. For some species, the good linear correlations obtained between phenolic concentration and antioxidant capacity determined by the antiradical assays and IRP suggest that phenolic content could be used as an indicator of antioxidant properties of the examined plant species. However, for the other species whose antioxidant capacity and phenolic concentration are not in linear correlations, other compounds might be interfere with the antioxidant effects.

It is worth to note that *A. arenaria* and *L. cytisoides* had never been studied to contain phenolic compounds (respectively, TPC=  $6.02 \pm 0.66$ ;  $12.90 \pm 1.78$  mg GAE  $g^{-1}$  DW), while their antioxidant activities may be interesting. Indeed, this investigation can be



evaluated as the first report on their antioxidant properties in respect to polyphenol content. Further tests are required to improve different ability uses of these coastal widespread species.

For the rest of the thirty halophytes, eleven species (*A. canariense*, *A. halimus*, *A. maritima*, *P. maritima*, *L. crithmoides*, *L. monopetallum*, *E. maritimum*, *R. tingitana*, *R. reatam*, *S. soda* and *S. maritima*) were considered rich in polyphenols but no positive correlations were found between their phenols and antioxidant activities. Some of them (*A. canariense*, *A. maritima*, *S. soda*, *P. maritima*) need further study to unveil more their virtue and especially to confirm their multitude folk medicinal uses.

In fact, our results are a contribution to the valorization of some medicinal halophytes from coastal regions. Generally, richness in polyphenols contributes significantly to the efficient antioxidant activity. Nevertheless, the constituents of bioactive phytochemicals and the antioxidant activity are influenced largely by several variables such as altitude, sunlight, soils, season and region of cultivation (Mobin *et al.*, 2015). No uniform or completely satisfactory procedure is suitable for extraction of all phenols or a specific class of phenolic substances in plant materials (Bruneton, 2006). It has been demonstrated that the recovery of phenolic compounds in different samples is influenced by the solvent extractability and the solubility of these compounds in the used solvent (Sulaiman *et al.*, 2011).

For these reasons, it was necessary to perform several *in vitro* and *in vivo* studies using other solvents to ensure the real antioxidant and biological polyphenol activities contained in each species, especially those studied for the first time.

Finally, according to TPC values, we have found that polyphenols have not usually the most antioxidant activity. Other compounds may play this role such *C. coccineum* oil with its inhibitory cancer effect (Antonella *et al.*, 2015), and alkaloids and different terpenoids in *E. paralias* with their therapeutically importance (Özbilgin *et al.*,

2012). The betalin pigments and enzymatic activity in *M. crystallinum* with their antioxidant and anti-inflammatory activities and their pharmaceutical and cosmetic applications (Bouftira *et al.*, 2012) are other promising antioxidant factors that play a significant bioactive role.

## CONCLUSIONS

The results indicate the large interspecies variability of antioxidant activities and suggest that most of the studied halophytes could be considered as a potential source of bioactive compounds with beneficial proprieties. The results also show that these Mediterranean medicinal halophyte species, especially *C. coccineum*, *C. edulis*, *R. vermiculata*, *T. gallica*, and *E. paralias*, are very promising plants considering their strong phenol content and their high antioxidant capacity, in addition to *F. cretica* for their numerous medicinal uses. Further studies are required regarding the isolation and identification of bioactive constituents responsible for strong antioxidant activity, especially for those that are studied for their phenol content for the first time (*A. arenaria* and *L. cytisoides*). Halophytes need immediate consideration for carrying out detailed chemical and pharmacological evaluations. Such investigations may lead to the discovery of new bioactive compounds that will help to assess the efficacy of herbal remedies. Additional biological testing will be necessary to prove the highly beneficial properties of these plants. *In vivo* assays are essential and they should be carried out to further confirm their uses. Indeed, halophytes can be used as a large source of therapeutic phytochemicals that may lead to the development of newly manufactured biological drugs and functional food.

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## ارزیابی مقایسه ای محتوای پلی فنولیک کل و پتانسیل آنتی اکسیدانی سی گیاه شورپسند دارویی از منطقه مدیترانه

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### چکیده

در دهه های اخیر، توجه روزافزونی به گیاهان شورپسند می شود که دلیل آن محتوای زیاد مواد فنولی آنهاست. این مواد پتانسیل درمانی در درمان و یا مدیریت سلامت انسان دارند. بنا براین، اندازه گیری درست محتوای پلی فنول کل و ارزش گذاری ظرفیت آنتی اکسیدانی این مواد از اهمیت برخوردار است. در این پژوهش، عصاره های اتانول از ۳۰ گیاه شورپسند تجزیه شد تا محتوای فنول کل (TPC) آنها ارزیابی شود. ما سه روش آزمایشی برای اثبات پتانسیل آنتی اکسیدانی، شامل DPPH<sup>•</sup> (1-ABTS<sup>•+</sup> (2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, diphenyl-2-picrylhydrazyl) و قدرت احیا کنندگی آهن (IRP) را به کار بستیم. نتایج نشان داد که گیاهان TPC متفاوتی نشان دادند که بین ۴۱۱/۵mg GAE.g<sup>-1</sup> DW در گیاه *Cynomorium coccineum* و ۱mg GAE.g<sup>-1</sup> DW در گیاه *Ammophila arenaria* به طور معناداری تغییر میکرد. در مورد فعالیت آنتی اکسیدانی، داده ها آشکار ساخت که *Cynomorium coccineum* (با IC<sub>50</sub>= 3.82µg.ml<sup>-1</sup> در برابر ABTS<sup>•+</sup>) و *Euphorbia paralias* (با IC<sub>50</sub>= 0.12 µg.ml<sup>-1</sup>) بیشترین ظرفیت آنتی رادیکالی (IC<sub>50</sub>) بر



علیه<sup>\*</sup> DPPH را داشتند و بهترین غلظت موثر را با  $EC_{50} = 9.57 \mu\text{g. mL}^{-1}$  برای IRP نشان دادند. با در نظر گرفتن همبستگی بین فنول ها و آزمون آنتی اکسیدان، سه گروه شناسایی شد که دارای ضریب همبستگی بالا بین ۰/۷۸ و ۰/۹۸ برای گروه اول بودند. داده ها چنین اشاره دارد که گیاهان مدیترانه ای شورپسند و دارویی، برای صنایع مربوط، به عنوان منابع ارزشمند آنتی اکسیدانها پتانسیل های امیدبخشی دارند، به ویژه در مورد *Reaumuria*، *Carpobrotus edulis*، *Cynomorium coccineum* و *Tamarix gallica vermiculata* و *Euphorbia paralias* به خاطر محتوای فنولی غنی.