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Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes

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Abstract

Halophyte ability to withstand salt-triggered oxidative stress is governed by multiple biochemical mechanisms that facilitate retention and/or acquisition of water, protect chloroplast functioning, and maintain ion homeostasis. Most essential traits include the synthesis of osmolytes, specific proteins, and antioxidant molecules. This might explain the utilization of some halophytes as traditional medicinal and dietary plants. The present study aimed at assessing the phenolic content and antioxidant activities of some Tunisian halophytes (*Cakile maritima, Limoniastrum monopetalum, Mesembryanthemum crystallinum, M. edule, Salsola kali*, and *Tamarix gallica*), depending on biological (species, organ and developmental stage), environmental, and technical (extraction solvent) factors. The total polyphenol contents and antioxidant activities (DPPH and superoxide radicals scavenging activities, and iron chelating and reducing powers) were strongly affected by the above-cited factors. Such variability might be of great importance in terms of valorising these halophytes as a source of naturally secondary metabolites, and the methods for phenolic and antioxidant production. ***To cite this article: R. Ksouri et al., C. R. Biologies 331 (2008).***

Résumé

Influence des facteurs biologiques, environnementaux et techniques sur les teneurs en polyphénols et les activités antioxydantes des halophytes tunisiennes. La capacité des halophytes à surmonter le stress oxydatif déclenché par la salinité est régi par de multiples mécanismes biochimiques qui facilitent le maintien et/ou l'acquisition de l'eau, la protection des chloroplastes et le maintien de l'homéostasie ionique. Ces traits comprennent essentiellement la biosynthèse d'osmolytes, de protéines spécifiques et de molécules antioxydantes. D'où, l'utilisation traditionnelle de ces halophytes comme plantes à intérêts médicinales et alimentaires. On se propose, dans ce travail d'évaluer les teneurs en polyphénols et les activités antioxydantes de quelques halophytes tunisiennes (*Cakile maritima, Limoniastrum monopetalum, Mesembryanthemum crystallinum, M. edule, Salsola kali* et *Tamarix gallica*) en fonction des facteurs biologiques, environnementaux et techniques. L'analyse des résultats a montré que les teneurs en polyphénols, les activités antiradicalaires et les pouvoirs chélateur et réducteur sont significativement affectés par ces différents facteurs. Une telle variabilité pourrait être d'une grande importance dans la valorisation de ces halophytes comme source naturelle de biosynthèse d'antioxydants. ***Pour citer cet article : R. Ksouri et al., C. R. Biologies 331 (2008).***

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Mots-clés : Activités antioxydantes ; Facteurs biologiques ; Environnement ; Halophytes ; Polyphénols ; Solvant

1. Introduction

Halophytes grow in a wide variety of saline habitats, from coastal sand dunes, salt marshes and mudflats to inland deserts, salt flats and steppes [1]. These plants are characterized by a high physiological plasticity not only for their salt tolerance limits, but also for the climatic zone from which they originate. A geographical classification differentiates between hydrohalophytes, typical from brackish wetlands, and xerohalophytes, that are particularly well-adapted to deserts and low-moisture environments [2]. Environmental stresses (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 [3]. Indeed, ROS can react with biological molecules, such as DNA, proteins, or lipids, generating mutations and damaging membranes, leading to cell and tissue injuries [4]. Halophytes are known for their ability to withstand and quench these toxic ROS, since they are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components. Enhanced synthesis of determined secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative effects [5]. Natural antioxidants occur in all plant parts, and the typical compounds that exhibit antioxidant activities include phenolics, carotenoids and vitamins [6]. Among the various kinds of natural antioxidants, polyphenols constitute the main powerful compound, owing to their multiple applications in food industry, cosmetic, pharmaceutical and medicinal materials [7]. Structurally, phenolics comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds [8]. In addition to their role as antioxidant, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-arthero-genic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects [9]. In plants, polyphenol synthesis and accumulation is generally stimulated in response to biotic/abiotic stresses [10], such as salinity [11], leading one to think that secondary metabolites may play a role in the adaptation of halophytic species to this constraint [12]. Previous studies have shown that the amount of polyphenolics in plants, and antioxidant activities, depend on biological factors (genotype, organ and ontogeny), as well as edaphic, and environmental (temperature, salinity, water stress and light intensity) conditions [13]. Besides, the solubility of phenolic compounds is governed by the type of solvent (polarity) used, the degree of polymerization of phenolics, and their interaction [10,14]. A large flora of halophytic species with multiple interests (food, fodder, fuel, oil, wood, pulp therapeutic, and fibre production) was identified in Tunisia [15,16]. For instance, the facultative halophyte *Mesembryanthemum edule* is a succulent plant distributed along coastal areas, known as traditional remedy against fungal and bacterial infections and as treatment of sinusitis, diarrhoea, infantile eczema and tuberculosis [17]. *M. edule* leaf juice is also used as an antiseptic poultice for sores, burns, scalds, and as gargled to treat infections of the mouth and throat [18]. *M. crystallinum*, a prostrate succulent herb covered by large bladder cells that are salt accumulators giving the plant a distinctive glistening aspect, is known for its antiseptic proprieties [15]. *Salsola kali*, a facultative halophytic widespread in the coastal, salt marsh, and desert regions, is a Cd hyper-accumulator, thus potentially useful for phytoremediation [19]. This species is traditionally used for their hypotensive proprieties too [15]. *Cakile maritima* (sea rocket) is an annual succulent and facultative halophyte widely distributed along Tunisian seashore [20]. It shows a potential as oilseed cash crop halophyte [21] and for the production of chemotherapeutic drugs against scorbutic, since rich in vitamin C [20]. The obligate halophyte *Limoniastrum monopetalum* is a shrub from sebkhas and coastal saline depressions which exhibits antidysenteric properties against infectious diseases [15]. *Tamarix gallica* is a tree halophyte from coastal regions and desert, known as astringent, detergent, diuretic, expectorant, and laxative [22] and in cosmetic for hair tinting and skin tanning. This species contains flavonoid sulphates, coniferyl alcohol derivatives, and proanthocyanidin sulphates in the stem bark and other aerial plant tissues [23].

We investigate here the antioxidant capacity in these local halophytic species, well known for their ethnopharmacological utilizations in traditional medicine. We address especially the biological (species, organ, devel-

Table 1
Botanical (scientific and common names, family) data and harvest site characteristics (location, soil type, and climate) of the Tunisian halophyte species investigated

Scientific name (Family)	Common name	Plant organ	Harvest site	Bioclimatic stage
Mesembryanthemum crystallinum (Aizoaceae)	Ice plant	Shoots	Jerba Sandy coastal	arid (MAR > 50 mm)
Mesembryanthemum edule (Aizoaceae)	Sourfig	Shoots	Jerba Sandy coastal	arid (MAR > 50 mm)
Salsola kali (Chenopodiaceae)	Saltwort	Leaves Stems Roots	Soliman Sandy coastal	superior semi arid (MAR > 400 mm)
Limoniastrum monopetalum (Plumbaginaceae)	Faux limonium	Leaves	Enfidha Sebkha	inferior semi arid (MAR > 200 mm)
Tamarix gallica (Tamaricaceae)	Manna plant	Leaves Flowers	Enfidha Sebkha (salinity > 20 g/L)	inferior semi arid (MAR > 200 mm)
			Takelsa Wood land (salinity < 1 g/L)	superior semi arid (MAR > 400 mm)
Cakile maritima (Brassicaceae)	Sea rocket	Leaves	Tabarka	humid (MAR > 600 mm)
			Jerba Seashore	arid (MAR > 50 mm)

MAR: mean annual rainfall.

opmental stage), environmental (biotope and salinity) and extraction (solvent nature) effects on the phenolic content and antioxidant activities.

2. Materials and methods

2.1. Plant sampling

Six species were selected based on their traditional curative traits, their abundance in nature, and their sustainable utilization. For each plant, scientific and common name, family, used organs, original habitat location and climatic characteristics, and the sampling date are given in Table 1. Shoots of *M. crystallinum* and *M. edule* were sampled from the sandy coasts of Jerba, in March 2006. Leaves, stems and roots of *S. kali* L. were collected from Soliman seashore, successively at the vegetative (May 2006) and reproductive (July 2006) stage. *L. monopetalum* leaves were sampled from Enfidha saline land in May 2006. *T. gallica* leaves and flowers were harvested from Enfidha and Takelsa localities in May 2006. Finally, leaves of *C. maritima* were sampled (June 2006) in two Tunisian littoral sites: Tabarka and Jerba.

2.2. Preparation of plant extracts

Plant parts of all species were air dried at room temperature and in the dark for two weeks. Sample extracts were obtained by magnetic stirring of 2.5 g of dry matter powder with 25 mL of pure methanol for 30 min [24]. In the case of *L. monopetalum* leaves, five solvent extracts with increased polarity were used: hexane, ethanol, acetone, methanol and deionizer water. All extracts were kept for 24 h at 4 °C, filtered through a Whatman N°4 filter paper, and evaporated under vacuum to dryness. They were stored at 4 °C until analysis began.

2.3. Determination of total polyphenol content

Colorimetric quantification of total phenolics was determined, as described by [25]. Briefly, 125 µL of suitable diluted sample extract was dissolved in 500 µL of distilled water and 125 µL of the Folin–Ciocalteu reagent. The mixture was shaken, before adding 1250 µL Na_2CO_3 (70 g/L), adjusting with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation for 90 min at 23 °C in darkness, the absorbance versus a prepared blank was read at 760 nm. A standard curve of gallic acid was used. Total

phenolic content of plant parts was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. The calibration curve range was 0–400 μg/mL ($R^2 = 0.99$). All samples were analyzed in three replications.

2.4. Estimation of total flavonoid content

Total flavonoids were measured by a colorimetric assay according to Dewanto et al. [25]. An aliquot of diluted sample or standard solution of (+)-catechin was added to a 75 μL of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg catechin/g dry weight (mg CE/g DW), through the calibration curve of (+)-catechin. The calibration curve range was 0–400 μg/mL ($R^2 = 0.99$). All samples were analyzed in three replications.

2.5. Quantification of total condensed tannins

Proanthocyanidins were measured using the modified vanillin assay described by Sun et al. [26]. To 50 μL of properly diluted sample, 3 ml of methanol vanillin solution and 2.5 mL of H_2SO_4 were added. The absorption was measured at 500 nm against extract solvent as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW. The calibration curve range was 0–400 μg/mL ($R^2 = 0.99$). All samples were analyzed in three replications.

2.6. DPPH radical-scavenging activity

The DPPH· quenching ability of plant extracts was measured according to Hanato et al. [27]. One ml of the extract at different concentrations was added to 0.5 mL of a DPPH· methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical activity was expressed as IC_{50} (μg/mL), the antiradical dose required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

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

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

2.7. Superoxide anion radical-scavenging activity

Superoxide scavenging capacity was assessed using the method of Duh et al. [28]. The reaction mixture contained phosphate buffer, 200 μL of halophyte extracts, 200 μL of PMS solution, 200 μL of NADH, and 200 μL of NBT. After incubation at ambient temperature, the absorbance was read at 560 nm against blank. Evaluating the antioxidant activity in organ extract was based on IC_{50}. The IC_{50} values were expressed as μg/ml. As for DPPH·, lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The inhibition percentage of superoxide anion generation was calculated using the following formula:

$$\text{Superoxide quenching }(\%) = \left[(A_0 - A_1)/A_0\right] \times 100 \quad (2)$$

where A_0 and A_1 have the same meaning as in Eq. (1).

2.8. Metal chelating activity

The chelating of ferrous ions by plant extracts was estimated as described by Dinis et al. [29], moderately modified by Zhao et al. [14]. Briefly, different concentrations of plant part extracts were added to a 0.05 mL $FeCl_2$, $4H_2O$ solution (2 mmol/L) and left for incubation at room temperature for 5 min. After the reaction was initiated by adding 0.1 mL of ferrozine (5 mmol/L), the mixture was adjusted to 3 mL with deionised water, shaken vigorously, and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm (Anthelie Advanced 2, SECOMAN). Analyses were run in triplicates. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the formula given bellow:

$$\text{Metal chelating effect }(\%) = \left[(A_0 - A_1)/A_0\right] \times 100 \quad (3)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample extracts or standard. Results were expressed as EC_{50}: efficient concentration corresponding to 50% ferrous iron chelating.

2.9. Iron reducing power

The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Oyaizu [30]. Each extract was mixed with 2.5 mL of sodium phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (10 g/L), and the mixture was incubated at 50 °C for 20 min. 2.5 mL of trichloroacetic acid (100 g/L) were then added, and the mixture was centrifuged at 650 *g* for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of ferric chloride (0.01 g/L) and thoroughly mixed. The absorbance was measured at 700 nm against a blank in a spectrophotometer. A higher absorbance indicates a higher reducing power. EC_{50} value (mg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained from linear regression analysis. Ascorbic acid was used as control.

2.10. Statistical analysis

Means were statistically compared using the STATICF program with Student's *t*-test at the $P < 0.05$ significance level. A one-way analysis of variance (ANOVA) and Newman–Keuls multiple range test were carried out to test any significant differences between solvents used at $P < 0.05$.

3. Results and discussion

3.1. Inter-specific effect on antioxidant capacity in the genus Mesembryanthemum

The antioxidant capacity within the genus *Mesembryanthemum* was found to be significantly variable, despite the both investigated species (*M. edule* and *M. crystallinum*) were harvested from the same region. Shoot phenolic content was significantly higher in *M. edule* (70.07 mg GAE/g DW) as compared to *M. crystallinum* (1.43 mg GAE/g DW) (Table 2). Similarly, total flavonoïd and condensed tannin contents were considerably higher in the former species (respectively 200-fold and 119-fold of *M. crystallinum* values). Both the antioxidant activity against DPPH radical and the iron reducing power were significantly lower in shoot methanolic extracts of *M. edule*, IC_{50} and EC_{50}, being respectively 5 and 8.5-fold lower than *M. crystallinum*, hence indicating a notably higher efficiency in *M. edule* shoots. These findings may be related to the higher polyphenol contents in *M. edule*, as compared to *M. crystallinum*. Indeed, several authors have reported a positive and significant relationship between the antioxidant components including phenols, polyphenols and tannins, respectively with the reducing power and DPPH radical scavenging capacity [31,32]. Comparing three *Artemisia* species, Djeridane et al. [33] found a significant difference in their antioxidant capacities. For example, total phenolic content varied from 3.42 (*Artemisia arboresens*) to 20.38 mg GAE/g DW (*Artemisia campestris*), while the antioxidant activity ranged from 11.6 to 25 mmol TEAC/g DW. These data were corroborated by Oszmianski et al. [34], who found large inter-species variations of antioxidant capacities between plants from Rosaceae family. A small difference was however observed in the antioxidant capacity of four varieties of *Chrysanthemum morifolium* Ramat [28]. Overall, the literature describes that antioxidant capacities are more variable in plants of different species (inter-specific) than within the same species (intra-specific).

Table 2
Phenolic contents (total polyphenols, flavonoid and condensed tannin) and antioxidant activities (DPPH· scavenging ability and reducing power) of *M. crystallinum* and *M. edule* shoot extracts

Shoot (Jerba)	*M. crystallinum*	*M. edule*
Total phenolic contents (mg GAE/g DW)	1.43b	70.07a
Total flavonoid contents (mg CE/g DW)	0.31b	62.16a
Condensed tannin contents (mg CE/g DW)	0.06b	7.16a
DPPH· scavenging activity (IC_{50} µg/ml)	160a	29.8b
Reducing power (EC_{50} µg/ml)	1070a	126b

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

3.2. Phenolic content and antioxidant activities of T. gallica and S. kali organs

In *T. gallica*, the comparison between leaves and flowers showed that both phenolic content and antioxidant activities were organ-dependent (Table 3). Flower methanolic extracts were characterized by higher polyphenol contents (70.56 mg of GAE/g DW), as compared to the leaf extracts (20.69 mg of GAE/g DW). These findings agree with previous ones indicating that secondary metabolites distribution may fluctuate between different plant organs [13,35,36]. As found for total phenolic content, antioxidant activities of flower were 2 to 8-fold higher than those of leaf extracts. Concerning DPPH scavenging activity, a considerable antiradical ability was found especially in flower methano-

Table 3
Phenolic content, DPPH scavenging activity, reducing and chelating powers in leaf and flower methanolic extracts of *T. gallica* and *S. Kali*

Species organ	*T. gallica* (Monastir)		*S. Kali* (Soliman)	
	Leaves	Flowers	Leaves	Flowers
Phenolic content (mg GAE/g DW)	20.69b	70.56a	17.23a	2.92b
DPPH$^{\cdot}$ scavenging activity (IC_{50} µg/ml)	7.92a	0.97b	10.33b	18a
Reducing power (EC_{50} µg/ml)	205a	84.3b	165b	457.66a
Chelating power (EC_{50} mg/ml)	10.81a	5.3b	–	–

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

lic extracts (IC_{50} value > 1 µg/ml). Similarly, the highest activities with respect to chelating and reducing powers were registered in flower extracts (EC_{50}: 5.3 mg/ml and 84.3 µg/ml, respectively). Such a result may be likely ascribed to the higher polyphenol content in *T. gallica* flowers as compared to the leaves, as found for *M. edule* when compared to *M. crystallinum*.

In contrast to *T. gallica*, polyphenol content and antioxidant capacities were lower in *S. kali* flower than in leaf extracts. The higher phenolic content in leaves (*ca.* 5-time higher than that of flowers) reflected the better antiradical activity and reducing power with the lowest IC_{50} and EC_{50} (respectively, 10.33 and 165 µg/ml). Considering the fact that polyphenol compounds contribute directly to the antioxidant activities [7], the correlation 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 [33,37].

3.3. Evolution of S. kali antioxidant capacities with plant ontogeny

Leaf and stem extracts 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 (Table 4). For instance, the total polyphenol contents of both leaves and stems were 3 times lower at the reproductive stage. Similarly, a 5-fold reduction was observed for total flavonoid and tannin contents, with a more pronounced effect in stem extracts. Our results corroborate previous reports on tomato and *Anethum graveolens* cultivars [13,38], concluding that phenolic content varied as a function of plant growth. With respect to DPPH scavenging activity, data showed that this antiradical activity was significantly different in the same organ at the two developmental stages. As for phenolic contents, this capacity to quench free radical seemed to be related to the physiological stage too, as IC_{50} values largely differed between the two periods. For instance, IC_{50} values of leaves and stems ranged from 11 and 13.5 to 14 and 46 µg/ml, respectively at the vegetative and reproductive stage. On the other hand, phenolic content and antiradical activities seemed also to be related, since they varied in the same way in all studied organs as function of the developmental stage. These results are partially in agreement with those of Zainol et al. [39] who showed a significant correlation between antioxidant activity and phenolic compounds in *Centella asiatica*.

3.4. Environmental conditions effect on antioxidant capacities of C. maritima and T. gallica

Both phenolic contents and antioxidant activities of *C. maritima* were influenced by the harvest site (Table 5). The comparison between the two provenances showed that phenolic content was 1.4 fold higher in Jerba leaves as compared to Tabarka. The same trend was observed for antioxidant activities against DPPH radical and superoxide anion: their IC_{50} values (respectively 610 and 1.7 µg/ml) were significantly lower, indicating a better activity in Jerba provenance than in Tabarka. Thus, these two parameters were stimulated in the plants growing in the arid zone (Jerba) as compared to those originating from the humid zone (Tabarka).

Table 4
Total polyphenol, flavonoid and condensed tannin contents and DPPH quenching activity in *S. Kali* organs (leaves, stems and roots) harvested either at the vegetative (V.S.) or the reproductive (R.S.) stage

Plant part	Leaves		Stems		Roots	
Developmental stage	V.S.	R.S.	V.S.	R.S.	V.S.	R.S.
Phenolic contents (mg GAE/g DW)	17.22a	5.1b	10.59a	3.18b	1.18b	3.8a
Flavonoid contents (mg CE/g DW)	15.27a	4.07b	9.22a	1.76b	0.83a	1.2a
Tanin contents (mg CE/g DW)	1.9a	0.9b	1.4a	0.4b	0.3b	0.9a
DPPH$^{\cdot}$ scavenging activity (IC_{50} µg/ml)	11b	14a	13.5b	46.5a	102a	40b

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

Table 5
Variability of total phenolic content and antioxidant activities against DPPH and superoxide radicals (IC_{50} values) in leaves of *C. maritima* and *T. gallica*

Species	*C. maritima*		*T. gallica*	
Provenance	**Jerba**	**Tabarka**	**Takelsa**	**Enfidha**
Phenolic content (mg GAE/g DW)	7a	5b	34.44b	79.24a
DPPH$^{\cdot}$ scavenging activity (IC_{50} µg/ml)	610b	940a	9.07a	3.88b
Superoxide quenching activity (IC_{50} µg/ml)	1.7b	5.1a	3a	1.85b

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

The extreme climatic conditions in terms of salinity, low rainfall, and high radiation, characterising Jerba, are likely related to the increase of *C. maritima* antioxidant potentialities. Previous studies suggested that abiotic stresses (salinity, luminosity, water deficit, etc.) widely present in 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 [11,40,41].

In order to further assess this assumption, two closer provenances of *T. gallica* originating from two arid regions (superior and inferior bioclimatic stages), differing by edaphic factors especially soil salinity, were compared (Table 1). As expected, total polyphenols content and antioxidant activities against DPPH and superoxide anion in the two provenances were significantly different (Table 5), with values *ca*. twice higher in *T. gallica* harvested from Enfidha salty soil than that originating from Takelsa (woodland). For instance, phenolic contents were 79.24 and 34.44 mg GAE/g DW, respectively in Enfidha and Takelsa plants. Considering that soil salinity is the major different parameter between Enfidha and Takelsa provenances, one may attribute to this factor a major influence on phenolic biosynthesis, and consequently a better antioxidant activity. In agreement with our findings, Parida et al. [42] showed that polyphenol content increased significantly in *Aegiceras corniculatum* plants challenged with 250 mM NaCl. Other authors confirmed this relationship too [11,12].

3.5. Technical factors impact on antioxidant potentialities: Solvent effect on antioxidant capacities of L. monopetalum

Among the several parameters that influence antioxidant capacities in plant analysis, solvent nature is the most controversial one [10,43]. In our study, five solvent kinds with different polarity were used to evaluate the antioxidant potential of *L. monopetalum* leaves and revealed a wide range of leaf polyphenols contents as function of the used solvent, closely dependent on the solvent polarity (Table 6). The extraction with pure methanol showed the highest leaf polyphenol content (15.85 mg GAE/g DW), followed by acetone extract (9.47 mg GAE/g DW). The last group included water, ethanol and hexane extracts which exhibited the lowest amount (1 to 2.6 mg GAE/g DW). As for total phenolics, flavonoid and condensed tannin contents also varied depending on the solvent extraction with maximal values of 4.2 and 3.9 mg EC/g DW, respectively (Table 6). The effect of solvent in flavonoid solubility showed the same classification as phenolics, while differing for tannins. Leaf extract had better tannin content (3.91 mg EC/g DW) in pure acetone, followed by pure methanol (1.47 mg EC/g DW). In the same way, *L. monopetalum* extracts exhibited a variable activity to quench DPPH radical as a function of the solvent type. The IC_{50} values of these extracts ranged from 45 (methanol) to 175 µg/ml (water). Leaf extracts with pure methanol showed the highest ability to reduce DPPH, with an IC_{50} value about 45 µg/ml, followed by acetone (76 µg/ml), ethanol, water and hexane (IC_{50} values over 100 µg/ml for the three last solvents). As discussed above, the significant differences in antioxidant potential between the five solvents used in this experiment was essentially due to the difference in polarity, and thus different extractability, of the antioxidative compounds [7]. Thus, the difference in DPPH scavenging activity of plant extracts might be due to

Table 6
Phenolic contents (total polyphenol, flavonoid and condensed tannin) and DPPH$^{\cdot}$ scavenging activity (IC_{50} values) of *L. monopetalum* (Enfidha provenance) leaf extract using different solvents

Parameter	Hexane	Ethanol	Acetone	Methanol	H_2O
Phenolic contents (mg GAE/g DW)	1.00c	1.64c	9.47b	15.85a	2.6c
Flavonoid contents (mg CE/g DW)	0.02d	0.17d	2.93b	4.2a	1.07c
Tanin contents (mg CE/g DW)	0.36 b	0.65b	3.91a	1.47b	0.46b
DPPH$^{\cdot}$ scavenging activity (IC_{50} µg/ml)	161a	107b	75c	45d	170a

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

the difference in solvent selectivity for extracting certain phenolic groups [33]. Several studies showed that solvent natures, notably polarity, have significantly different extraction capacities for phenolic compounds in plants [42,44]. Therefore, there is no uniform or completely satisfactory procedure that is suitable for extraction of all phenolics or a specific class of phenolic substances in plant materials. Methanol and acetone, and to a lesser extent water and ethanol, and their mixture are frequently used for phenolic extraction [45]. In recent studies, numerous others factors like chemical treatment and agronomical crop management practices have been demonstrated to have a great influence on plants antioxidant pool under abiotic stresses. For instance, exogenous application of triazole derivatives can ameliorates the tolerance to these environmental constraints by enhancing the activities of several enzymes, especially those related to detoxification of active oxygen species and antioxidant metabolism in medicinal plants such as *Catharanthus roseus* [46,47] and *Withania somnifera* [48,49].

4. Conclusion

Halophyte species investigated showed an important and a wide range of polyphenol contents and antioxidant capacities. Phenolic concentrations, especially in *T. gallica* and *M. edule* were significantly higher than those of other halophyte plants. These data appeared tightly dependent on a number of biotic (specie, organ and physiological stage) and abiotic (environmental, handling, solvent extraction) factors. Taken together; these information may confirm the interesting potential of halophytes as a valuable source for natural antioxidant molecules.

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