Salinity impact on seed yield, polyphenols composition and antioxidant activity of Fennel (*Foeniculum vulgarae* Mill) extracts

I. BETTAIEB REBEY, S. BOURGOU, B. MARZOUK, ML. FAUCONNIER, R. KSOURI

[1] Laboratory of Medicinal and Aromatic Plants Biotechnology Center of Borj Cedria, Hammam-Lif 2050, Tunisia.
[2] General and Organic Chemistry Volatomics, Gembloux AgroBio Tech, University of Liege, Passage des déportés, 2-5030 Gembloux Belgium

*Corresponding author: rosainess@yahoo.fr

Abstract - Recently, medicinal and aromatic plants have received much attention in several fields such agroalimentary, perfumes, pharmaceutical industries and natural cosmetic products. The consumption of herbal medicines is widespread and is continuously increasing worldwide. Although, secondary metabolites in the medicinal and aromatic plants were fundamentally produced by genetic processing but, their biosynthesis is strongly influenced by environmental factors. Salinity is one of the major factors that affect essential oil biosynthesis and secretion (Heuer et al. 2002). In Tunisia, salt-affected soils cover about 10% of the total area of the country. Salinity led to biochemical disorders and can change plant behaviour regarding the biosynthesis of primary and secondary metabolites. Among all the secondary metabolites synthesized by plants, phenolic compounds are some of the most widespread. In this context, this research evaluated the effect of salinity on total and individual polyphenols contents as well as the antioxidant activities of fennel (*Foeniculum vulgarae* Mill.) seeds of two geographic origins, Tunisia (TFS) and Egypt (EFS). This plant is one of the most common aromatics in the Mediterranean kitchen. Plants were treated with different concentrations of NaCl treatment: 0, 50, and 75 mmoL. Plant growth was significantly reduced with the severity of saline treatment. This also caused important reductions in the seed yield and yield components. Besides, total polyphenol content was higher in treated seeds, and salinity improved the amount of individual phenolic compounds. Moreover, antioxidant activities of the extracts were determined by four different test systems, namely 2,2-diphenyl-1-picrylhydrazyl, β-carotene/linoleic acid chelating, and reducing power assays. The highest antioxidant activities were reveled in severe stressed plants for both TFS and EFS. In this case, fennel seeds produced under saline conditions may function as a potential source of antioxidant compounds, which could support the utilization of this plant in a large field of applications such as food industry.

Keywords: *Foeniculum vulgarae* Mill., seeds, salinity, polyphenols, antioxidant.

1. Introduction

Foeniculum vulgare (Apiaceae) commonly known as fennel is a well known and important medicinal and aromatic plant widely used as carminative, digestive, lactogogue and diuretic and in treating respiratory and gastrointestinal disorders. Its seeds are used as flavourings in baked goods, meat and fish dishes, ice cream, alcoholic beverages and herb mixtures (Rather et al. 2012). Phenols, phenolic glycosides and volatile aroma compounds such as trans-anethole, estragole and fenchone have been reported as the major phytoconstituents of this species. Different pharmacological experiments in a number of in vitro and in vivo models have convincingly demonstrated the ability of F. vulgare to exhibit antifungal, antibacterial, antioxidant, antithrombotic and hepatoprotective activities, lending support to the rationale behind several of its therapeutic uses (El-Awadi and Hassan, 2010; Bahmani et al. 2012). Phenolic compounds isolated from F. vulgare are considered to be responsible for its antioxidant activity while the volatile aroma compounds make it an excellent flavouring agent (Rather et al. 2012).

Salinity is one of the most important environmental stresses that reduce growth, development and production of plants. The major inhibitory effect of salinity on plant growth and yield has been attributed to osmotic effect, ion toxicity and nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders (Mahajan and Tutija, 2005; Zhu, 2007). If global food production is to be maintained, it seems reasonable to predict that enhancement of salt tolerance of crops will be increasingly important to many plant breeding programs (Arzani, 2008). In view of the wide extending soil salinity, the study of the chemical responses of the medicinal and aromatic plants to salinity needs some focus in Tunisia. Salinity impact on essential oil and fatty acid composition has been recently reported in different aromatic and medicinal plants, such as coriander (Neffati et al. 2010), black fennel (Bourgou et al. 2010), sweet majorana (Baatour et al. 2012).

In plants, polyphenol biosynthesis and accumulation are generally stimulated in response to biotic/abiotic stresses (Rice Evans et al. 1996) such as salinity (Naczk and Shahidi, 2004). Indeed, phenolic compounds participate in the defense against reactive oxygen species (ROS), which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental constraints (Navarro et al. 2006). Thus, salt-stressed plants might represent potential sources of polyphenols for economical use. However, environmental constraints have two opposite effects on polyphenol yield, because they both augment polyphenol concentration in the tissues and restrict biomass production (Sreenivasulu et al. 2000). Thus, It may be hypothesized that optimal polyphenol yield would be obtained using stress-tolerant species. In our previous work, we examined for the first time the effect of salinity on growth, essential oil yield and composition of Foeniculum vulgarae Mill (Bettaieb Rebey et al. 2016).

To the best of our knowledge, no study has been conducted concerning the phenolic composition as well as the antioxidant response in fennel seeds, under salt treatment. After all the above, in order to strengthen the valorisation of the Tunisian variety as a new source of antioxidant compounds, we compared it with the Egyptian variety by investigating their polyphenol compositions and their antioxidant activities under salt stress. The results will be important to indicate the effect of salinity and geographic origin on the phenolic compounds biosynthesis and thus to improve their economic and health utilisation as a source of natural bioactive compounds.

2. Material et methods

2.1. Plant material and growth conditions

Two accessions of mature fennel seeds (*Foeniculum vulgarae* Mill) were used in this work. The first called (TFS) were harvested in June 2015 from the region of Korba in the northeast of Tunisia; latitude 36340 38. 22''(N); longitude 10510 29.63''(E) and the altitude is 637 m. The precipitation average was 400-500 mm/year and the monthly average temperature was 17.7 C. The other seeds were reported to be imported from Egypt (EFS). Botanical identification of this species was carried out by Prof. A. Smaoui (Biotechnologic Center in Borj-Cedria Technopark, Tunisia), and a voucher specimen has been kept in our laboratory for future reference. The two provenances were cultivated under the same environmental conditions. Thus, seeds were germinated on an inert substrate at 25 °C. After 10 days, seedlings were transferred to 6-l plastic pots (6 plants per pot) and were hydroponically cultivated, using aerated quarter strength Hoagland's solution, containing macronutrients (mM): $MgSO_4$ (0.5), KH_2PO_4 (0.25), KNO_3 (1.25), $Ca (NO_3)_2$ (1.25). The medium also contained iron as complex EDTA–K–Fe and micronutrients as a mixture of salts: $MnCl_2$, $CuSO_4\_5H_2O$, $ZnSO_4\_7H_2O$, $Mo_7O_{24} (NH_4)_6\_4H_2O$ and H_3BO_3 (Hoagland and Arnon, 1950). Two leaf seedlings were separated in four groups irrigated with a nutrient solution supplemented with different NaCl concentrations (0, 50 and 75 mM). To avoid osmotic shock, salt concentrations increased stepwise daily with 25 mM NaCl. The nutrient solutions were replaced each 4 days. The experiment was performed under controlled conditions (18–25 °C temperature, 16/8 h light/darkness, 60–80% relative humidity and under artificial light of 141 µmol/m/s (6000 lx)). Seeds were harvested at the fruiting stage, 15 weeks after treatment and weighted.

2.2. Preparation of extracts

Seed extracts were obtained by stirring 1 g of dry material powder with 10 ml of 80 % acetone for 30 min. Extraction was carried out using maceration at room temperature for 24 h followed by filtration through Whatman No. 4 filter paper and after evaporation to dryness. The yield (%) of evaporated dried extracts was calculated as 100 x DWextr/DWsamp, where DWextr is the weight of extract after

evaporation of solvent, and DW samp is the dry weight of original sample. Samples were stored at 4 °C until analysis.

2.3. Total phenolic amounts

The total phenolic amount of the acetone extracts was determined by using Folin–Ciocalteu reagent (Merck), according to the procedure described by Dewanto et al. (2002). Briefly, 125 µl of sample extract were dissolved in 500 µl of distilled water and 125 µl of Folin–Ciocalteu reagent. The mixture was shaken, before addition of 1.25 ml of 7% Na_2CO_3, adjusting with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation in the dark for 90 min, the absorbance at 760 nm was measured versus the prepared blank. Total phenolic amounts were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW), through a calibration curve with gallic acid.

2.4. RP-HPLC evaluation of phenolic compounds

Dried samples from fennel seeds were hydrolysed according to the method of Proestos et al. (2006) which was slightly modified. 20 ml of acetone 80% containing BHT (1g/l) were added to 0.5g of a dried sample. Then 10 ml of 1 M HCl were added. The mixture was stirred carefully and sonicated for 15 min and refluxed in a water bath at 90°C for 2 h. The obtained mixture was injected to HPLC. The phenolic compound analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP–HPLC) coupled with an UV-Vis multiwavelength detector. The separation was carried out on a 250×4.6-mm, 4-µm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml/min. The gradient programme was as follows: 15% A/ 85% B 0-12 min, 40% A/ 60% B 12-14 min, 60% A/ 40% B 14–18 min, 80% A/ 20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 µl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45 µm membrane filter before injection. Peaks were identified by congruent retention times compared with standards.

2.5. DPPH radical scavenging assay

Radical-scavenging activity was determined according to Hanato et al. (1998). Two millilitres of the extract at different concentrations were added to 0.5 ml of a 0.2 mM DPPH methanolic solution. After shaking, the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm. BHA was used as positive reference while methanol was used as negative reference. DPPH radical-scavenging activity was expressed as the inhibition percentage (I %) and was calculated using the following formula:

$$I\% = 100 \times (Ablank - Asample)/Ablank$$

where A blank is the absorbance of the control at 30 min reaction (containing all reagents except the test compound), and Asample is the absorbance of the sample at 30 min. Antiradical activity was expressed as IC50, defined as the concentration of the extract generating 50% inhibition.

2.6. β-Carotene/linoleic acid bleaching assay

Antioxidant activity was evaluated according to the *β*-carotene bleaching method described by Tepe et al. (2004). A stock solution of *β*-carotene/linoleic acid mixture was prepared by dissolving 0.5 mg of *β*-carotene in 1 ml of chloroform and adding 40 mg of linoleic acid together with 400 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water were added to the residue; 3 ml of this mixture were dispensed to test tubes and 200 µl of each extract were added. The emulsion system was incubated for 2 h at 50 °C, together with two controls, one containing BHT as a positive control and another with the same volume of distilled water instead of the extracts. In the test tube with BHT, the yellow colour is maintained during the incubation period, and the absorbance was measured at 470 nm.

2.7. Chelating effect on ferrous ions

The ferrous ion chelating activity of different organ extracts and essential oils was assessed as described by Zhao et al. (2006). Different concentrations of the sample were added to 0.05 ml of $FeCl_2 \cdot 4H_2O$ solution (2 mM) and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.1 ml of ferrozine (5 mM), and the mixture was adjusted to 3 ml with deionized water, shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given below:

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

where A_0 is the absorbance of the ferrozine-Fe^{2+} complex and A_1 is the absorbance of the test compound. Results were expressed as IC_{50}, efficient concentration corresponding to 50% ferrous iron chelating. EDTA was used as a positive control.

2.8. Reducing power

The method of Oyaizu (1986) was used to assess the reducing power of different seed extracts and essential oils. 1 ml of different concentrations of seed extracts in acetone 80 % were mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide (K3Fe (CN)6), and incubated in a water bath at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650 g for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue-green colour was measured at 700 nm. The EC50 value (mg/ml) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control.

2.9. Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA. Percentage of each parameter was the mean of six replicates ± S.D and the differences between individual means were deemed to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Salinity effect on total phenolic content

The total phenolic content (TPC) of control plants as estimated by the Folin–Ciocalteu method was of 7.06 and 6.25 mg of GAE/g DW, respectively, for TFS and EFS. TPC extracted from TFS were 1.12 fold higher compared to EFS (Fig.1).
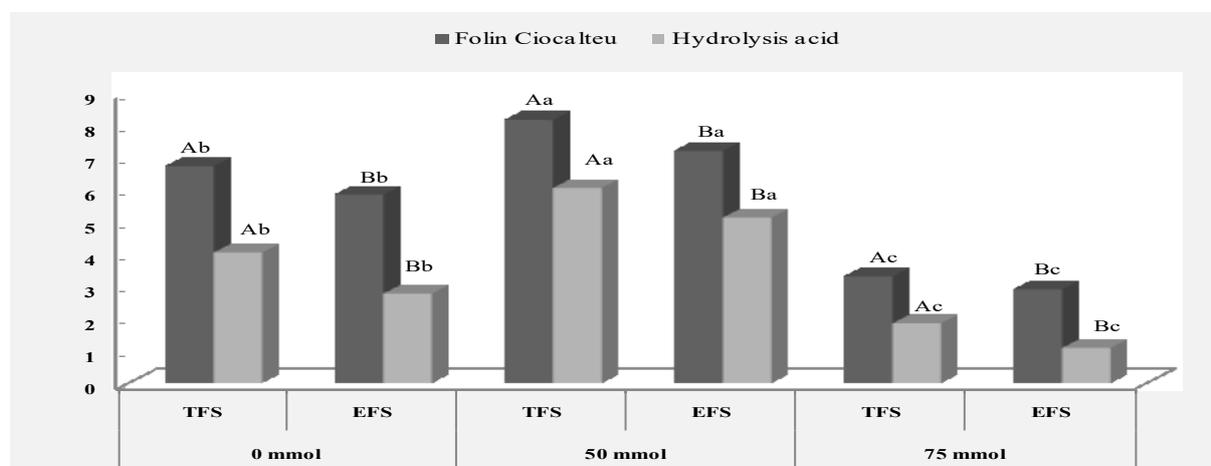
Figure 1. Salinity impact on total phenolic contents of fennel seed extracts. Values are means of three replications (N=3±SD). The data marked with the different capital letter, for the provenance, and small letter, for the treatment, in the table, value share significant differences at P<0.05 (Duncan test).

TPC was significantly increased when plants subjected to moderate salt treatment. Thus, under 50 Mm NaCl, TPC were about 35.21 % and 70.44% higher than the control one, correspondingly, for TFS and EFS. Besides, TPC were decreased significantly by 1.89 and 2.11 folds under 75 mM NaCl for TFS and EFS, respectively. On the other hand, the assessment of phenolic compounds as determined by RP-HPLC was 4.60 and 3.18 mg/g DW in the control samples. An increase in polyphenol content has been found under the moderate salt treatment as determined by this method. Thus, total polyphenol content was about 1.48 and 1.74 times higher than that observed in control plants, respectively, for TFS and EFS under 50 mM NaCl.

Besides, it increased significantly by 1.93 and 2.07 folds under severe salt treatment. According to these results, the contents of phenolic compounds as assessed by RP-HPLC are too inferior to those obtained by the Folin-Ciocalteu method. These differences could be explained by the weak selectivity of the Folin-Ciocalteu reagent, as it reacts positively with different antioxidant compounds (Bettaieb et al. 2010). However, these two methods confirmed that the total phenolic content of fennel seeds was improved under moderate salt treatment and this improvement suggested the salt tolerance of fennel under 50mM NaCl.

Furthermore, depending on the salt treatment, the amount of TPC of fennel seeds was highly ($p < 0.05$) affected by the origin of varieties. Tunisian variety was the rich one in total phenolic content. Similarly, previous studies suggested that abiotic constraint may increase phenolic compound biosynthesis, as a response to the oxidative stress generated by the formation of reactive oxygen species in these hostile environments (Navarro et al. 2006). Likely, the total phenolic content of sprouts treated with 100 mM of NaCl was significantly increased, which is similar to that of *Cakile maritima* and red pepper reported by Ksouri et al (2007) and Navarro et al. (2006). Conversely, in the case of coriander (*Coriandrum sativum* L), Naffeti et al. (2010) found a significant decrease in polyphenol accumulation under different levels of salinity.

3.2. Salinity effect on phenolic composition

Polyphenol qualitative and quantitative determination in fennel seeds were performed by RP-HPLC coupled with an UV-visible multi wavelength detector. Phenolics occur in plants mainly as aglycones, glycosides, esters or are bounded to the cell wall. Therefore, acidic hydrolysis was used to release aglycones. Qualitative and quantitative differences were found between the two accessions (Table 1).

Table 1. Quantitative (mg/g DW) changes of phenolic compounds in Tunisian and Egyptian fennel (*Foeniculum vulgarae* Mill.) seed extracts as influenced by salinity

		0 mmol	50 mmol	75 mmol
Phenolic acids	*TFS*	**2.58**	**4.28**	**0.98**
	EFS	**1.79**	**3.40**	**0.74**
Gallic acid	*TFS*	0.29 ± 0.02^{aA}	0.30 ± 0.01^{aA}	0.17 ± 0.02^{bA}
	EFS	0.09 ± 0.01^{aAB}	0.15 ± 0.01^{aAB}	0.05 ± 0.01^{abB}
Chlorogenic acid	*TFS*	1.22 ± 0.01^{bA}	2.18 ± 0.04^{aA}	0.71 ± 0.01^{cA}
	EFS	0.96 ± 0.01^{bB}	2.13 ± 0.01^{aA}	0.66 ± 0.02^{cA}
Ferrulic acid	*TFS*	1.07 ± 0.03^{aA}	1.80 ± 0.05^{aA}	0.10 ± 0.05^{bA}
	EFS	0.74 ± 0.04^{bAB}	1.12 ± 0.01^{abAB}	0.03 ± 0.01^{cA}
Flavonoids	*TFS*	**1.46**	**1.79**	**0.72**
	EFS	**1.04**	**1.82**	**0.29**
Luteolin-7-O glucoside	*TFS*	0.29 ± 0.24^{aA}	0.56 ± 0.03^{aA}	0.08 ± 0.11^{abA}
	EFS	0.09 ± 0.11^{abAB}	0.12 ± 0.01^{aB}	0.02 ± 0.02^{aA}
Quercetin-3-O rutinoside	*TFS*	1.04 ± 0.01^{aA}	1.50 ± 0.03^{aB}	0.42 ± 0.01^{bA}
	EFS	0.93 ± 0.01^{bA}	1.69 ± 0.01^{aA}	0.25 ± 0.01^{aB}
Apigenin	*TFS*	0.13 ± 0.00^{bA}	0.03 ± 0.01^{cA}	0.22 ± 0.02^{aA}
	EFS	0.02 ± 0.01^{aAB}	0.01 ± 0.01^{aA}	0.02 ± 0.01^{aAB}
Total	*TFS*	4.04	6.07	1.88
	EFS	2.83	5.22	1.03

Values are means of three replications (N=3±SD). The data marked with the different capital letter, for the provenance, and small letter, for the treatment, in the table, value share significant differences at *p*<0.05 (Duncan test).

TFS contained more phenolic acids than EFS (2.58 mg/g DW and 1.79 mg/g DW, respectively). Additionally, TFS contained more flavonoids than EFS (1.46 mg/g DW and 1.04 mg/g DW,

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respectively). A total of six phenolic compounds were successfully identified in TFS and EFS. Independently of the applied treatments, chlorogenic acid was detected as the major phenolic acid for the two ascensions and was more present in TFS (1.22 mg/g DW) compared to EFS (0.96 mg/g DW). Seeds were also characterized by the presence of other phenolic compounds in interesting proportions. TFS showed higher proportions of ferulic acid (1.07 mg/g DW) and even quercetin-3-Ɵ- rutinoside (1.04 mg/g DW), compared to EFS. Polyphenol composition and content in plant foods can vary greatly according to many factors such as plant genetics, soil composition and growing conditions, stage of maturity and post-harvest conditions (Faller and Fialho, 2009).

Results indicated that, moderate salt treatment resulted in a significant increase of the biosynthesis of the different phenolic acids which resulted in the enhancement of their contents by about 1.65 and 1.89 folds, respectively, for TFS and EFS. Hence, the level of chlorogenic acid increased in TFS and EFS by about 1.78 and 2.21 folds, consecutively, as compared to the control. Besides, a significant increase was observed in the level of ferulic acid for both varieties under moderate treatment. On the other hand, results demonstrated that severe salt treatment (75 mM) affected significantly the biosynthesis of phenolic acids. Analysis of the effect of salinity on flavonoids contents of fennel seeds indicated that 50 mM NaCl improved significantly the accumulation of these metabolites by about 1.22 and 1.75 folds, respectively, for TFS and EFS. Moderate stress improved appreciably the biosynthesis of all the flavonoids as compared to the control. These results suggest the stimulation of isoprenoids pathway by salinity, while shikimate and phenypropanoid pathways were enhanced. Unlike, severe salt treatment affected significantly the content of flavonoids by 49.31 and 27.88%. This decrease was expressed mainly by the reduction of the contents of lutelin-7-Ɵ-glucoside and quercetin-3-Ɵ-rutinoside for the two varieties as compared to control. These variations were related to the relative contents of the constituents and not to the presence of new ones or the absence of particular components. In general, phenolic compounds in plants are produced through the phenylpropanoid pathway, and they can be induced by environmental stresses and elicitor (Giorgi et al. 2009; Kim et al. 2006). The phenolic compounds in plants can be changed by salinity, but this is critically dependent on the salt sensitivity of plants (Kim et al. 2008).

3.3. Salt effect on antioxidant activity of fennel seed extracts

The antioxidant activity, determined by four different methods namely DPPH, and ß-carotene-linoleic acid, reducing power and chelating ability was presented in Table 2. Lower IC50 value indicates higher antioxidant activity. This activity was evaluated by the capacity to neutralize DPPH radical.

Table 2. Effect of salinity on antioxidant activities of Tunisian and Egyptian fennel seed extracts

	DPPH (IC50, μg/ml)		β-carotene bleaching (IC$_{50}$, μg/ml)		Chelating ability (IC$_{50}$, mg/ml)		Reducing power (EC$_{50}$, μg/ml)	
	TFS	EFS	TFS	EFS	TFS	EFS	TFS	EFS
0 mmol	76.24±0.64[a][bA]	95.14±0.04[cB]	125.86±0.23[bA]	137.05±0.05[aAB]	1.65±0.87[aAB]	3.73±0.83[aA]	190.34±3.74[cA]	213.87±2.1[bA]
50 mmol	54.03±0.06[a][A]	67.25±0.01[aAB]	82.12±0.97[aA]	92.80±0.04[b][AB]	1.12±0.09[abA]	2.52±0.22[bB]	52.11±1.34[bA]	88.14±1.19[aA]
75 mmol	155.83±0.02[abA]	212.66±0.01[bB]	259.23±0.54[bA]	354.77±0.01[abB]	8.75±1.43[bA]	12.13±0.14[cB]	110.23±0.77[aA]	157.22±1.2[aA]
EDTA			-		0.03±0.01		-	
Ascorbic acid			-		-		43±0.84	
BHT	0.18±0.01		41±0.56		-		-	

GAE: gallic acid equivalent; IC$_{50}$: the concentration of the extract generating 50% inhibition; EC$_{50}$: the effective concentration at which the absorbance was 0.5; Each value in the table was obtained by calculating the average of three experiments; The data marked with the different capital letter, for the provenance, and small letter, for the treatment, in the table of each IC$_{50}$ or EC$_{50}$ value share significant differences at $p < 0.05$ (Duncan test)

The DPPH assay provides basic information on the antiradical activity of the extracts. The actual reaction that is taking place between the DPPH stable radical and the antioxidant (AH) is DPPH$^{.}$ + (AH)$_0$ /DPPH-H+ (A$^{.}$)$_0$. The radical that is formed (A$^{.}$) in general is less reactive, depending on the structure of the molecule, or it can follow a radical radical interaction to create a stable molecule (Huang et al.

2005). The DPPH radical scavenging activities of the seed extracts obtained from the TFS extracts showed the highest capacity to neutralize this radical. Moreover, the IC50 values obtained were 76.24 µg/ml and 95.14 µg/ml Tunisian and Egyptian varieties, respectively. The IC50 of BHT was equal to 0.18 µg/mL. In this study, DPPH radical scavenging activity of test samples was in the order BHT > Tunisian variety > Egyptian variety.

By analyzing the antioxidant activity of the different extracts under different levels of salinity, it was demonstrated that they all had the capacity to scavenge DPPH free radicals (Table 2). Indeed, DPPH scavenging activity increased significantly by 29.13 and 29.31 % under 50 mM NaCl, respectively, for TFS and EFS, as compared to the control. Conversely, the IC50 values of TFS and EFS affected by about 2.04 and 2.23 folds, correspondingly, under 75Mm NaCl. Moreover, the DPPH radical scavenging activity was strongly ($p<0.05$) affected by the fennel varieties. Benavente-Garcia et al. (2000) reported that the radical scavenging activity of plant extracts depends on the amount of polyphenolic compounds in the extracts. The antioxidant activity of fennel seed extracts was also evaluated by the *β- carotene-linoleate bleaching method* (Table 2) because *β-carotene* shows strong biological activity and constitutes physiologically important compound (Sarkar et al. 1995; Sakanaka et al. 2005). This method is based on the loss of the yellow colour of *β-carotene* due to its reaction with radicals formed after linoleic acid oxidation in emulsion. The rate of *β-carotene* bleaching can be slowed down in the presence of antioxidants. TFS had the strongest ability to prevent the bleaching of *β- carotene* than EFS (IC50 = 125.86 µg/ml and IC50 = 137.05 µg/ml, respectively). On the other hand, TFS and EFS extracts had lower antioxidant activities than BHT with IC50 of 41 µg/ml (Table 2). Salinity improved significantly the TFS activity by about 1.53 folds under 50 mM, in comparison to the control. Conversely, the aptitude to prevent the bleaching of *β- carotene* of EFS extracts was found to be altered significantly under 75 mM NaCl by about 2.58 folds, as compared to control. Thus, the *β-carotene-linoleate* bleaching values were highly ($P < 0.05$) affected by the provenance of varieties. The effect of salinity and provenance on the antioxidant ability of fennel seeds was also assessed by the estimation of chelating and reducing powers. Independently of salt treatment applied, both provenances presented high chelating power with IC50 of 1.65 and 3.73 mg/ml as well as a strong reducing capacity with IC50 of 190.34 and 213.87 mg/ml, respectively, for TFS and EFS. Nevertheless, it was found to be damaged significantly by 75 mM NaCl. Finally, we should point out that TFS and EFS extracts were able to reduce Fe^{3+} ions in the reaction medium. Hence, the reducing power evaluation showed that both 50 and 75 mM NaCl increased considerably the reducing ability by 3.42 and 2.11 folds for TFS and 2.04 and 1.78 folds for EFS, respectively. The reducing property is generally associated with the presence of reductones (Duh, 1998), such as ascorbic acid, which have been shown to exert antioxidant action by breaking the free radical chain (Gordon, 1990). Reductones are also reported to react directly with peroxides and also with certain precursors of peroxides, thus preventing peroxide formation (Shimada et al. 1992; Xing et al. 2005). On the other hand, statistical analysis revealed no effect ($p< 0.05$) of varieties on reducing power. Total phenolic content has been reported to be responsible for the antioxidant activities of botanical extracts. DPPH, *β-carotene-linoleate* bleaching assay, chelating ability and reducing power have been used to measure antioxidant activity and these results should correlate with those of total phenolic content. Do et al. (2004) demonstrated that some bioactive compounds present in medicinal plant possessed high total antioxidant activity, which was due to the presence of phenolic, carotenoids and flavonoids. In this way, salinity can lead to increased production of free radicals and other oxidative species in plants, which respond by increasing their capacity to scavenge reactive oxygen species (ROS), phenolics being very significant in this field (Mittler 2002). To protect themselves against these toxic oxygen intermediates, plant cells and their organelles like chloroplasts, mitochondria and peroxisomes employ antioxidant defence systems. Phenolics are well-known antioxidants acting as powerful radical scavengers and ions chelators, and their content increment under water deficit is thought to be involved in the prevention of stress-induced oxidative damage or in maintenance of osmotic balance (Balasundram et al. 2006). Besides, plant resistance to various stresses is associated with antioxidant capacity and increased levels of antioxidants may prevent constraints damage (Bor et al. 2003). These activities may be directly linked to the seed phenol contents and consequently to their free radical scavenging properties (Huang et al. 2005), since phenolic compounds contribute directly to antioxidant activity. In comparison with our result, previous reports showed a significant correlation between the antioxidant activity and total phenolic contents in *Coriandrum sativum* (Neffati et al. 2010) and in *Cakile maritime* (Ksouri et al. 2007). It is also proposed that under stress conditions phenylpropanoid

biosynthesis may represent an alternative pathway for photochemical energy dissipation, which has the added benefit of enhancing the antioxidant capacity of the cell (Grace and Logan 2000).

4. Conclusion

This study showed that moderate salt treatment induced biochemical changes in fennel seeds which could reflect an adaptation response to stress. Moreover, our results demonstrated that cultivation of medicinal plants like *F. vulagarae* under salt conditions could increase its secondary metabolism as shown by the enhancement of total polyphenols. On the other hand, under salt treatment, fennel seed extracts were characterized by the prevalent of phenolic content for both accessions. Furthermore, TFS grown at 50 mmol showed higher antioxidant ability as compared to EFS. The highest response of TFS under moderate salt treatment was correlated with the antioxidant abilities. These activities could be directly linked to the content of phenols. TFS is promising and therefore further investigations should be targeted on such important issues as activity in real food systems relative to commercially used antioxidant extracts and economic feasibility of practical applications due to higher phenolic content and antioxidant activities than in EFS.

5. References

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