Leaf mineral element content and soil characteristics on *in vitro* antioxidant and enzymatic inhibitory activities of aqueous fennel extracts

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ABSTRACT

This study was conducted to evaluate the biochemical characterization of three harvested *Foeniculum vulgare* plants collected from two bioclimatic zones in order to investigate the soil growing conditions effect. The results showed a great variability of the phenolic amounts and biological properties of samples rely on localities. Fv$_{SEN}$ contained the highest amounts of phenolic compounds. These amounts were accompanied by the greatest antioxidant ability through almost studied assays. Fv$_{SEN}$ and Fv$_{ZO}$ were significantly different. In addition, the samples exhibited a significant and variable enzymatic inhibition activity with values ranging from 30 to 50 µg/mL for lipoxygenase assay. But these extracts did not revealed significant differences on their tyrosinase abilities. On the other hand, the levels of mineral elements were also estimated. These contents varied depending on sample and locality. The growing soil conditions of samples in terms of different parameters is likely related to their antioxidant and enzyme inhibition potentialities added to their mineral composition which settled by Spearman's correlation. These data may confirm the interesting potential of *F. vulgare* as a valuable source for natural antioxidant molecules but the growing soil conditions can affect all the potentialities of these plants set for human consumption and other uses.

Keywords: *Foeniculum vulgare*, enzymatic inhibition, antioxidant ability, soil

INTRODUCTION

Foeniculum vulgare Mill. (Apiaceae), with the common name fennel, is a perennial umbelliferous herb native from the Circum-Mediterranean area and widespread in central Europe and Mediterranean region (Muckensturm et al., 1997; Diao et al., 2014). In Tunisia, F. vulgare has a large geographical distribution in Aïn Draham, El Haounria, Sfax, Mahdia, Kerkenna, Djerba,Oasis, Moularès, generally in limestone soils and essentially widespread in the edge of the watercourses (Pottier-Alapetite, 1979; Rejeb et al., 2006).

Fennel is used since antiquity due to its characteristic aniseed flavour (Muckensturm et al., 1997). Moreover, in folk medicine this species has been used for more than forty types of illnesses, particularly gastrointestinal and neurological disorders, kidney stones, vomiting, diarrhoea, antispasmodic, antiseptic, carminative, galactagogue for lactating mothers, and antiulcer properties (Badgujar et al., 2014; Sayed-Ahmad et al., 2017). The review undertaken by Badgujar et al. (2014) allowed to compile the diverse pharmacological properties that have been attributed to fennel (antimicrobial, antiviral, anti-inflammatory, antioxidant, antimutagenic, antinociceptive, antipyretic, antispasmodic, antithrombotic, apoptotic, cardiovascular, chemomodulatory, antitumor, hepatoprotective, hypoglycemic, hypolipidemic, and memory enhancing property). These activities can be attributed to several compounds such as phenylpropanoids in volatile essential oils, phenolic acids, flavonoids, fatty acids and amino acids (Badgujar et al., 2014). However, differences in the chemical profiles of samples may occur, which afterwards will be responsible for unlike biological properties. Several factors may contribute to this variability. For example, and in what

concerns the essential oils of fennel, several studies have proved that the relative chemovariability found depends on the geographical origin, extraction methods, maturation stages, and plant parts (Díaz-Maroto et al., 2005, Díaz-Maroto et al., 2006; Telci et al., 2009).

The physicochemical or biological properties of soil affect the quality of soil fertility. For instance, high soil acidity can reduce the plant absorption of macroelements, such as nitrogen, phosphorus, potassium, calcium, and magnesium but it improves their absorption of microelements, such as iron, manganese, copper, and zinc (Zhu et al., 2020), though the effect of pH on the plant depends on the species (da Silva et al., 2019). Microelements play a key role on plant growth (Majdoub et al., 2017). In the same trend, these authors also demonstrated that Zn excess influence not only the growth of fennel, but also the antioxidant activity of their aqueous extracts.

As far as we know there are no previous reports on the biological activities of Tunisian *F. vulgare* related with the soil characteristics and macro- and micro-elements in the aerial parts of fennel growing spontaneously in two bioclimatic zones. In particular, the objectives of the present study were (a) to relate the soil qualitative characteristics and the macro- and micro-element contents of the aerial parts of fennel on the biological properties (e.g. antioxidant activity and inhibitory activity towards tyrosinase, acetylcholinesterase, α-amylase and lipoxygenase).

MATERIAL AND METHODS

Plant material
The fresh aerial part samples of *F. vulgare* were collected just before flowering from the region of Bizerte and Siliena, in May 2015. In Bizerte, the wild *F. vulgare* (Fv) was harvested in two places of Alia (Latitude: 37°10′08″ N; Longitude: 10°02′00″ E; Elevation above sea level: 1022 m). In Siliena, Fv was collected in Makthar (35° 51′ 0″ N, 9° 12′ 0″ E). Taxonomic identification of the plant material was confirmed by Doctor Mouhiba Ben Nasri-Ayachi, member of Botanical Laboratory, Faculty of Science of Tunis, according to "Flore de la Tunisie" (Alapetite, 1981). Fresh material was separated and stored in -80 °C and the rest of material was dried at the room temperature. Samples in the text were defined by Fv $_{ZO}$ and Fv $_{SEN}$ which were collected from sites named respectively Zoghba and Senia in the region of Bizerte and Fv $_{SEL}$ collected from Makthar in the region of Seliana

Mineral elements of the aerial parts of Fv
Fennel aerial part samples were digested by applying the optimized procedure using nitric and perchloric acids.

Briefly, 0.05 g of well-powdered fennel samples were added to 5 mL of HNO_3 and $HClO_4$ (3:1)(V/V) and the mixtures were digested firstly in ambient temperature for 24 h and then by increasing subsequently the temperature from 60 to 90 °C and finally to 105 °C until total dissolution. After cooling of the digest, Milli Q water was added up to an equal volume to dilute left over acid. Blank solutions were prepared by digesting the mixture of reagents following the same procedure. Duplicate digestions were carried out for each sample of fennel. The level of all elements in the sample solutions were determined by MP-AES (Microwave Plasma Atomic Emission Spectrometry) (Agilent 4200 MP-AES, Santa Clara, CA)

Soil characteristics
Soils were collected from the surface of two sampling sites (Bizerte "Zo" and Seliana "Sel"). Chemical and physical parameters were determined after mixing and air drying for two days. Both soils are calcareous according to the pedological chart of Tunisia – a rendzines (and calcareous brown soils) associated with lithosols and Mediterranean red soils (Bizerte) and a rendzines (and calcareous brown soils) associated with lithosols or regosols on geological rock (generally humic soils) (Seliena) (ESDAC – European Commission, 2018). From the soil samples, 'available' P was extracted with 0.5 N of $NaHCO_3$ and determined colorimetrically as previously reported by Olsen and al. (1954) with slight modification. Organic matter was determined by dichromate oxidation (Walkley and Black 1934) and total N by titration of Kjeldahl digests (Bremner, 1965). Electrical conductivity (EC) of soil solutions was measured to quantify the soluble salts concentration as suggested by Dahnke and Whitney (1988) and soil pH was usually measured potentiometrically in a slurry system using an electronic pH meter (McLean, 1982). Soil analysis was performed by "Núcleo de Apoio à Produção Agrícola do Algarve-lab Químico Agrícola Rebelo da Silva". Exchangeable cations, including titratable acidity, and cation exchange capacity (CEC) were determined by (Mehlich, 1953). Total limestone was estimated by using volumetric closed circuit (Sheibler calcimeter – Richard 1954). Active lime was estimated by the method of Drouineau (1942) - briefly, soil was agitated with ammonium oxalate solution and the amount of calcium carbonate reacted on ammonium oxalate which was determined by titration with permanganate solution.

Major element and heavy metal concentrations were determined by MP-AES (Microwave Plasma Atomic Emission Spectrometry) (Agilent 4200 MP-AES, Santa Clara, CA) after digestion in a mixture of acetic acid and ammonium acetate and EDTA according to the method proposed by Lakanen and Ervio (1971).

Preparation of the aqueous extracts of *F. vulgare*
It was weighed 5 g of the aerial parts of each sample, air dried and then macerated with distilled water (100 mL) at room temperature for 24 h, filtered, and finally, the filtrate was frozen and freeze-dried. All extracts were stored at +4 ºC until further analysis.

Total phenolic and flavonoid contents
Total phenolic contents (TPC) of the extracts were determined by using Folin-Ciocalteu reagent according to the slightly modified method of (El-Guendouz et al., 2016). An aliquot of extracts was mixed with Foline Ciocalteu and sodium carbonate solution. After 2 hours of incubation in the dark, the absorbance of the mixture was measured at 760 nm. TPC in the extracts were expressed in terms of mg gallic acid equivalents/mL extract using equation of a regression curve of gallic acid standard.

A standard colorimetric assay with slight modifications was used to quantify total flavonoid contents (TFC) of the extracts. An aliquot of sample extracts was mixed with sodium nitrate solution and aluminum chloride solution which allowed standing for 5 minutes. Then sodium hydroxide solution was added, and the mixture was incubated for 30 min. The absorbance was measured at 510 nm. TFC of the extracts were expressed as mg quercetin equivalents/mL extract using a regression curve obtained from standard quercetin solutions (El-Guendouz et al., 2016).

The total quantification of flavanone and dihydroflavonol compounds was estimated according to El-Guendouz et al. (2016). The values are expressed as naringin equivalents (mg NE/mL extract).

Antioxidant activity
The antioxidant activity was measured through diverse methods, as reported below.

ABTS (2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid) radical scavenging activity
The scavenging activity against ABTS cation radical was measured by employing the method previously reported by Aaza et al. (2011). The ABTS radical cation scavenging activity was expressed as DPPH radical scavenging activity.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity
The DPPH radical scavenging activities of the extracts were estimated by modifying the method of Albano and Miguel (2010). The percentage of DPPH scavenging activity was calculated according to the following formula: $\% = [(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample.

Superoxide anion radical scavenging activity
The superoxide anion radical scavenging activities of the extracts were measured as previously reported (Albano and Miguel, 2010) using nitroblue tetrazolium (NBT) reduction method. The percentage of superoxide anion radical scavenging activity was calculated according to the following formula: $\% = [(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample.

Nitric oxide radical scavenging activity
Nitric oxide scavenging activity was measured spectrophotometrically. Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction as described by Miguel et al. (2013).

Total antioxidant activity (Phosphomolybdenum assay)
The total antioxidant activity of the samples was estimated based on phosphomolybdenum method described by Majdoub et al . (2017). The total antioxidant capacity was expressed as Ascorbic Acid equivalent (mg AA/mL extract).

Metal chelating activity on ferrous ions
The metal chelating activity on ferrous ions was determined as previously described by (Bulanouar et al., 2013). The metal chelating activity was expressed as $\% = [(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample.

Inhibition of lipid peroxidation by thiobarbituric acid reactive species (TBARS) method using egg yolk as fat source
The TBARS assay is the most commonly used method for measuring lipid peroxidation. The assay was determined according to method of Majdoub et al. (2017). The percentage of inhibition was calculated as follows: $[(A_0 - A_1/A_0) \times 100]$, in which A_0 is the absorbance of the control reaction (without extract), and A_1 is the absorbance of the extracts. Analyses were run in triplicate. The inhibition percentage was plotted against extract concentration (w/v) and IC_{50} values were determined (concentration of extract able to prevent 50% of lipid peroxidation).

Inhibition of lipid peroxidation by thiobarbituric acid reactive species (TBARS) method using liposomes as fat source
The liposome assay was determined by following the method reported by Majdoub et al. (2017). Tests were carried out in triplicate. The assay was performed as reported above for thiobarbituric acid reactive species (TBARS) method.

Tyrosinase inhibition assay

Tyrosinase inhibitory activity was measured using the modified dopachrome method with L-DOPA as substrate (El-Guendouz et al., 2016). The percentage of tyrosinase inhibition was calculated as follows: $\% = [(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample and IC_{50} was expressed.

Acetylcholinesterase (AChE) inhibition assay

The inhibitory activity of the water extract on AChE was determined following the method of El-Guendouz et al. (2016). The absorbance was measured at 295 nm. The percentage of (AChE) inhibition was calculated as follows:

$\% = [(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample and IC_{50} was calculated.

Lipoxygenase (LOX) inhibition assay

Lipoxygenase inhibition assay was performed according to the slightly modified procedure of El-Guendouz et al (2016). The % inhibition for different concentrations of the extracts was determined. $\% = [(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample and IC_{50} was calculated.

α-Amylase inhibition assay

A modification of the method of El-Guendouz et al. (2016) was used to assay α-amylase inhibition activity. Fifty μL of extract was mixed with sodium phosphate buffer and α-amylase, the resulting solution was incubated at 37°C for 10 min. Then, soluble starch was added to the mixture. The homogenate was reincubated for 30 min at 37°C. The enzymatic reaction was stopped by the addition of HCl (10%), followed by iodine reagent. After vigorous vortexing, distilled water was added. The absorbance was read at 620 nm. Each experiment was carried out in triplicate. The inhibition percentage of the enzyme was calculated using the following formula:

$$\% \text{ of α-amylase inhibition: } [1- [(A^-_{control})-(A^+_{control})]- (A_{sample})/ (A^-_{control})-(A^+_{control})] \times 100$$

where $A^-_{control}$ denotes the absorbance of 100% enzyme activity (ethanol 70% with enzyme), $A^+_{control}$ denotes absorbance of 0% enzyme activity (ethanol 70% without enzyme), and A_{sample} denotes the absorbance of sample.

Statistical analysis

The experimental design was a randomized block design with three replication (divided as plots). Nine plants from each plot were harvested. Soil samples were collected from each plot surface of two sampling sites of Bizerte in the order of (0–20) cm from the bottom to the top with a row spacing (1.5-2 m). The experiments were conducted in triplicate, and the data were expressed as mean ± standard deviation (SD) by using the Statistical Package for the Social Sciences (SPSS) 23.0 software (SPSS Inc., Chicago, IL, USA) as given in Tables. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons were used for comparing means at the significance level $p < 0.05$. Correlations were performed by Spearman's correlation coefficient (r) at a significance level of 95% or 99%.

RESULTS AND DISCUSSION

Soil characteristics

Soil texture and structure regulate water retention and infiltration, susceptibility to erosion, gaseous exchanges, nutrient dynamics, and root penetration, and consequently, the plant physiological responses (Shah et al., 2017; Rabot et al., 2018). If *F. vulgare* is widely distributed throughout the world (Mishra et al. 2016), it can mean that this species will adapt to diverse types of soil, nevertheless the metabolic behaviour shall be different and expectedly the biological properties.

Tables 1-3 depict the general characteristics of the soils from where fennel plants were collected. Clay and sand content, soil pH, soil electrical conductivity, total and active limestone contents are shown in Table 1; Table 2 shows soil organic matter, nitrogen, available phosphorous contents, cation exchangeable capacity and % of saturation of basic cations. Soil Mn, Fe, Zn, Cu and Pb contents are shown in Table 3.

In both soils the levels of soil exchangeable calcium (Ca) were relatively high, ranging from 10.9 cmol(+)/kg to 13.1 cmol(+)/kg of soil, in Fv_{SEN} and Fv_{ZO}, respectively. Those values represent, respectively, 80.7 and 84.8 % of the exchangeable soil capacity. The level of soil exchangeable potassium (K) was considered high for Fv_{SEN} and was higher [0.98 cmol(+)/kg of soil − 7.3 % of soil exchangeable capacity] when compared to the level found in Fv_{ZO} [0.36 cmol(+)/kg of soil − 2.3 %

Table 1: Physical and chemical properties of soil samples

Samples	Soil texture	Clay (%)	Sand (%)	pH (H₂O)	pH (KCl)	Electrical Conductivity (μS/cm)	Total limestone (%)	Active limestone (%)
Soil Fv_{ZO}	loam	9.63±0.65*	80.6±0.4*	7.61±0.02*	7.16±0.01*	415.66±1.52*	25.1	11.88
Soil Fv_{SEN}	loam	12.1±1.34*	75.5±0.45*	7.7±0.01*	7.05±0.03*	343.33±1.52*	1.6	-

Each value expressed as the mean of three replicates ± S.E. *Values are significantly different at p < 0.05

of soil exchangeable capacity] (Table 2). The levels of exchangeable Mg and Na were almost similar in both cases (Table 2). The high percentage of soil exchangeable calcium reflects that both soils are well structured. The relation Ca/Mg of both soils are high (9.1 and 8.7, respectively for Fv_{SEN} and Fv_{ZO}), meaning that Mg plant nutrition should be conditioned (ILACO, 1981).

Table 3 shows significant differences (p < 0.05) between Fv_{ZO} and Fv_{SEN} soil's samples in the microelements' amounts, although growing in the same bioclimatic zone (sub-humid). Fv_{ZO} is a cultivated soil, whereas Fv_{SEN} is not subjected to any type of agronomic practice. Fvzo soil has a higher content of active limestone which may affect the soil availability of P, Fe, Mn, Zn and Cu, however soil Fe content is slightly higher in Fv_{ZO} soil. Fv_{SEN} had higher concentrations of Mn (114.05 µg/g), Zn (11.31 µg/g), and Cu (0.58 µg/g), whereas Fv_{ZO} had only higher amount of iron (0.14 mg/g). In this case, iron was the most important mineral element of Fv_{ZO} sample and not Mn, as found for Fv_{SEN} (Table 3). Pb content in both soil samples were not statistically different (Table 3). The results show that even in the same climatic conditions, the difference of the soil type may influence the phytochemical and biological properties of plants. The soil from the Makthar region was not assessed.

Macro- and micro-elements in the aerial parts of F. vulgare collected at different places

Most of micro-elements (Cu, Mn, Fe and Zn) play a crucial role in the plant metabolism, being also essential elements in the human or animal organisms (Chizzola et al., 2003). The results of mineral elements are depicted in Table 4. This Table pinpoints significant variations between Fv_{ZO} and Fv_{SEN} samples within the same bioclimatic zone (sub-humid) as well as between both and that of semi-arid zone (Fv_{SEI}), since the samples were found to show variability in their accumulation of mostly elements (Table 4). The greatest differences between Fv_{ZO} and Fv_{SEN} were clearly observed in Zn, Cu and Na content. The highest Cu level was recorded in Fv_{ZO} whereas Fv_{SEN} was found to be Zn rich. The highest amount of Zn in the aerial parts of Fv_{SEN} is coincident with the highest amount of this element in the soil where this plant has grown; nevertheless, the highest level of Cu detected in the aerial parts of Fv_{ZO} did not correspond to the highest amount of this element in the same place. The high active limestone content of the soil may affect the availability of mineral elements for the plants. Zengin and his co-authors (2008) revealed that the uptake of micro-elements depends on their high amounts in the soil but in another recent study, it has been demonstrated that mineral elements accumulation was influenced by soil pH (Bravo et al., 2017). The soil sample from SEN had the highest amount of Cu. It is also noteworthy the significant lower levels of all elements in F_{SEL} and, particularly, Fe (Table 4).

The levels of Zn found in the samples are much higher than those values found by Zengin et al. (2008) for air dried aerial parts of fennel (6 µg/g). The values found for Cu and Na in Fv_{SEL} were within the range (4.44 and 4266.7 µg/g, respectively) found by Zengin et al. (2008), nevertheless in

Table 2: Soil organic matter (OM), nitrogen (N), available phosphorous (P), cation exchangeable capacity (CEC) and exchangeable cations (Ca, Mg, K, Na) contents

Samples	OM (%)	N (%)	P (ppm)	CEC	Exch Ca	Exch Mg	Exch K	Exch Na	Degree of saturation
					(cmol(+)/kg soil)				(%)
Soil Fv_{ZO}	2.72±0.69*	0.23±0.00*	0.13±0.02*	13.5	100	1.2	0.98	0.42	100
Soil Fv_{SEN}	6.14±0.61*	0.15±0.10*	0.48±0.02*	15.4	100	1.5	0.36	0.48	100

Each value expressed as the mean of three replicates ± S.E. *Values are significantly different at p < 0.05

Table 3: Soil available Fe, Mn, Zn, Cu, Cd and Pb contents

Samples	Fe (mg/g DW)	Mn (µg/g DW)	Zn (µg/g DW)	Cu (µg/g DW)	Cd (µg/g DW)#	Pb (µg/g DW)
S_{ZO}	0.14±0.00*	44.11±0.87*	5.68±0.03*	0.33±0.00*	<6.61	60.21±15.41
S_{SEN}	0.07±0.00*	114.08±1.60*	11.31±0.24*	0.58±0.00*	<6.61	51.99±3.40

Each value expressed as the mean of three replicates ± S.E. *Values are significantly different at p < 0.05 using t-student Test
NA means not analyzed.
Estimated based on LOD for Cd, which was 0.022 mg/L

Table 4: Macro-, micro-elements and heavy metal contents in the aerial parts of F .vulgare samples

SAMPLES	K (mg/g DW)	Mg (mg/g DW)	Fe (mg/g DW)	Na (mg/g DW)	Zn (µg/g DW)	Cu (µg/g DW)	Mn (µg/g DW)	Cd (µg/g DW)*	Pb (µg/g DW)#
Fv_{ZO}	34.89±1.31[a]	1.94±0.2[b]	0.13±0.01[a]	20.09±0.89[a]	49.735±5.28[b]	56.89±3.76[a]	38.87±9.37[a]	<6.61	<39.04
Fv_{SEL}	33.18±1.31[ab]	1.28±0.2[b]	0.03±0.01[b]	3.81±0.89[c]	19.445±5.28[c]	2.59±3.76[b]	34.75±9.37[a]	<6.61	<39.04
Fv_{SEN}	28.04±1.31[b]	2.82±0.2[a]	0.17±0.01[a]	8.25±0.89[b]	104.765±5.28[a]	11.79±3.76[b]	35.15±9.37[a]	<6.61	<39.04

Each value expressed as the mean of three replicates ± S.E. Values with different letters in the same column are significantly different at p < 0.05using
ANNOVA One Way Test / * Estimated based on LOD for Cd, which was 0.022 mg/L / # Estimated based on LOD for Pb, which was 0.13 mg/L

the remaining samples, the values were much higher. Mn and K in all samples were within the range (42.29 µg/g and 28.8 mg/g, respectively) of those reported by Zengin et al. (2008) for the same species. Fv_{SEL} samples had lower amounts of Mg and Fe than the remaining samples, being like those reported by Zengin et al. (2008), which means that Fv_{ZO} and Fv_{SEN} had much higher amounts of the same elements. Cd and Pb, even at low doses, are toxic for human beings. They were detected in samples, although not quantified because the concentrations in both soils were below the method detection limit, and therefore impossible to compare the results among samples.

Shortly, Fv_{SEL} had lower concentration of almost all mineral elements (Zn, Cu, Fe, Mg and Na) (Table 4). Plants growing in the semi-arid climate accumulated much less mineral elements when compared to the two samples collected in the sub-humid place, although between these two samples there were also differences. Although analysis of the soil from the semi-arid clime (SEL) had not been performed, it is known that these places are less crowded and industrialized, therefore, the levels of minerals in the soils will not as remarkable as observed in the locations of the sub-humid zone (ZO and SEN), which can partly explain the differences detected among samples. Moreover, these differences and in particular the highest levels of some mineral elements in Fv_{ZO} and Fv_{SEN} can be a double-edged sword: on the one hand the richness in some elements can be useful for a healthy life due to the supplementation in some minerals needed for a normal metabolism, but on the other hand, it may constitute a dangerous problem and even compromise the safety of this kind of products since an accumulation may occur after a continuous take of these plants, either through food, in herbal teas or in food supplements, with the consequent negative effects for the health.

Polyphenols' contents in the aqueous extracts of fennel

Medicinal plants are known by their richness with polyphenolic compounds including flavonoids which exhibited effective antioxidant activities (Zengin et al., 2015). Table 5 depicts total phenolic contents (TPC) and total flavonoid content (TFC), which measures the content of flavones and flavonols, including dihydrofavonols and flavanones in the aqueous extracts of *F. vulgare*. TPC ranged from 44.55, in Fv_{SEL}, to 186.79 meq GA/mL, in Fv_{SEN}. Concerning TFC, the minimal and maximal values were also found in the same samples and the values were 10.59 and 158.88 meq Q/mL, respectively. Fv_{ZO} had the highest concentration of dihydrofavonols (5.45 meq NA/mL) whereas Fv_{SEL} had the highest amounts of these secondary metabolites (9.00 meq NA/mL). The values of TPC are much higher when compared to the aqueous extracts of two cultivars of sweet fennel plants:

Table 5: Polyphenols' content and antioxidant activities (IC$_{50}$, µg/mL) of aqueous extracts of *F. vulgare*, measured by different methods

Samples	Phenolic (mg GAE/mL Extract)	Flavonoids (mg QE/ml Extract)	Dihydro-flavone (mg NE/mL Extract)	Phosphomolybdenum (AAE/mL)	DPPH	ABTS	Ferric chelating	Liposome	NO	Superoxide anion radicals	Egg yolk
F_{ZO}	76.91±0.10[b]	36.96±0.34[b]	5.45±0.79[c]	43.95±0.77[b]	19.79±0.37[b]	10.33±0.33[b]	13.95±0.24[b]	64.39±0.36[a]	393.77±17[b]	2.47±0.00[ab]	26.57±0.62[b]
F_{SEL}	44.55±0.35[c]	10.59±0.77[c]	9±0.34[a]	41.63±0.69[c]	75.85±0.76[a]	22.23±1.10[a]	32.07±0.57[a]	26.74±0.40[b]	1246.02±4.54[b]	4.04±0.28[ab]	5.38005±0.20[c]
F_{SEN}	186.79±0.99[a]	158.88±0.26[a]	6.71±0.49[b]	131.97±0.48[a]	3.48±0.20[c]	5.23±0.26[c]	5.07±0.13[c]	19.78±0.57[c]	359.80±2.83[b]	1.26±0.10[b]	41.43±0.65[a]

Each value expressed as the mean of three replicates ± S.E. Values with different letters in the same column are significantly different at p < 0.05

"Latina" and "Doux de Florence" previously studied by this team (Majdoub et al., 2017). TFC found in Fv_{ZO} and Fv_{SEN} were also higher to those found in cultivars of sweet fennel samples. The levels found in FV_{SEL} were within the range of those cultivars. The dihydrofavonols and flavanones amounts of all samples were within the range of sweet fennel plants (Majdoub et al., 2017).

The flavonoid content in Fv_{SEN} represented around 80% of the total phenolic content as reported for medicinal fennel from different Mediterranean countries (Faudale et al., 2008). However, the same authors also found lower ratio flavonoid/total phenols (< 70%) in some samples, particularly in fruits of the Italian populations Bologna, Giara, and Messina.

A positive correlation was found between the levels of Zn and K and the amount of phenols and flavonoids, nevertheless a negative correlation between the levels of Cu, Mg and Na on the accumulation of the same type of metabolites (Table 6). The presence of some metals is sometimes associated with increased activities of enzymes of secondary pathway such as shikimate dehydrogenase, phenylalanine ammonialyase (PAL), and polyphenols oxidase (PPO) (Ali et al., 2006; Castáneda and Pérez, 1996; Van de Mortel et al., 2006; Wang et al., 2011), nevertheless some authors also reported that that phenolic accumulation and type of phenol are also dependent on the type of metals (Kovácik et al., 2009). In addition, the present work also showed that Fe only positively act on the induction of production of flavanones and dihydroflavonols. The remaining mineral elements correlated neither TPC nor flavones/flavonols (Table 6).

Antioxidant activity

Water extracts of fennel aerial part were tested for their antioxidant capacity through diverse *in vitro* assays (Table 5).

Table 6: Spearman's correlation coefficients between total phenol, flavonoid, dihydroflavonol contents and antioxidant activities

	Phenol	Flavone/ flavonol	Dihydroflavonol/ flavanone
Phenol	1	0.999**	-0.355
Flavone/flavonols	0.999**	1	-0.312
Dihydroflavonol/ flavanone	-0.355	-0.312	1
Phosphomolybdenum	0.981**	0.989**	-0.179
DPPH	-0.818**	-0.788*	-0.800**
Ferric chelating	-0.877**	-0.852**	0.737
NO	-0.700*	-0.662	0.877**
Superoxide	-0.921**	-0.902**	0.668
ABTS	-0.869**	-0.842**	0.738*
Liposome	-0.435	-0.481	-0.640
Egg yolk	0.918**(?)	0.897**??	-0.676*

*Correlation is significant at the P < 0.05 level. **Correlation is significant at the P < 0.01 level

Ability for scavenging DPPH and ABTS free radicals of the aqueous fennel extracts

The free radical-scavenging activity of the samples was assessed against radical species generated in the reaction system, such as DPPH and ABTS radicals. As shown in Table 5, Fv_{SEN} was found to exhibit the highest radical-scavenging activity in both assays DPPH and ABTS, since this extract presented the lowest IC_{50} values. The capacity for scavenging DPPH free radicals were better than those reported by Majdoub et al. (2017) for the cultivars of sweet fennel plants, nevertheless the capacity for scavenging ABTS free radicals were within the same range. The scavenging effect of extracts on DPPH radicals have also been reported by other authors and using different plant parts (shoots, steams, inflorescences and leaves) of *F. vulgare*, collected in Portugal. The results obtained showed IC_{50} values ranging from 1.34 to 12.16 mg/mL (Barros et al., 2009), that is much higher than the results of the present work, which means weaker activity. Similarly, more previously investigation of Mata et al. (2007) suggested that aqueous extract of *F. vulgare* leaves exhibited lower DPPH radical scavenging activity as well as lower accumulation of total phenolic compounds. In this case, it can be observed that our tested samples were quantitatively richer in polyphenolic compounds, which can explain the activity found. In fact, a linear negative correlation between the total phenols and flavonoids and the IC_{50} values for both methods was found (Table 6). Zengin et al. (2015) suggested that the accumulation of polyphenolic substances can contribute to effective free radical scavenging capacity and subsequently observed a positive correlation between phenolic content and the antioxidant activity against DPPH free radicals.

Parejo et al. (2004) reported a strong antiradical scavenging activity of several phenolic acids (3-caffeoylquinic acid, 4-caffeoylquinic acid, 1,5-*O*-dicaffeoylquinic acid, rosmarinic acid) and flavonoid glucosides (eriodictyol-7-*O*-rutinoside, quercetin-3-*O*-galactoside, kaempferol-3-*O*-rutinoside, and kaempferol-3-*O*-glucoside) isolated from an aqueous extract of fennel waste.

Ability for scavenging superoxide anion radicals by the aqueous fennel extracts

Fv_{SEN} extract was found to be the most effective as superoxide scavenger (Table 5). As reported for the capacity for scavenging DPPH and ABTS free radicals, a negative correlation between the IC_{50} values and the phenol and flavonoids' content were also found (Table 6). This correlation was also reported by Majdoub et al. (2017) for two cultivars of sweet fennel plants. The capacity for scavenging superoxide anion radicals of some phenolic acids and flavonoids isolared from an aqueous extract of fennel waste were also reported by Parejo et al. (2004).

However, in a study undertaken by Albano and Miguel (2010), aqueous extract of aerial parts of *F. vulgare* was shown to be unable to scavenge superoxide anion radicals. Such results may confirm that the amount but also the nature of polyphenols, both contribute to the scavenging effect on the superoxide anion radicals. In other investigation, Schinella et al. (2002) reported the possible role of flavonoids on the ability of plant extracts, such as *Inula viscosa*, for scavenging superoxide radicals.

Ability for scavenging nitric oxide by the aqueous fennel extracts

As a bio-regulatory molecule, nitric oxide (NO) contributes to several physiological processes such as neural signal transmission, immune response and control vasodilatation (Shukla et al., 2016). Nevertheless, excessive NO generation may cause many pathologies, but some plants or plant-derived products may have the property to scavenge NO radicals and thus preventing the damage produced by an overproduction of NO (Jagetia et al., 2004). In the present study, all tested samples displayed NO radical scavenging activities. As shown in Table 5, F_{ZO} and F_{SEN} exhibited the highest NO scavenging activity with no significant differences ($p < 0.05$), whereas F_{SEL} had the least activity. Based on Spearman's analysis; a negative correlation between TPC and IC_{50} values, nevertheless a positive correlation was found between these values and the concentration of total flavanones and dihydroflavonols (Table 6). No similar relationship was observed between flavonoids content and NO scavenging activity, meaning that the type of polyphenols is, at least, as important as their amounts. It has been previously reported by Shukla et al. (2016) that the effective NO radical scavenging activity of starter culture Doenjang water extract samples was owing to their high amount of phenolic compounds but this capability depended on the type of polyphenolic compound. The results of the present work may indicate that flavanone sand dihydroflavonols possess a negative role on the capacity for scavenging NO radicals, although further studies need to be performed to confirm or not this statement. Acker et al. (1995) showed that among the flavonoids studied by the authors, including anthocyanins, these ones were the most effective as scavengers of NO radicals. On the other hand, Haernen and Bast (1999) reported no general relationship between the structure of diverse subtypes of flavonoids and activity.

Total antioxidant activity (phosphomolybdenum assay)

The antioxidant activity was studied in terms of phosphomolybdenum assay and the results were shown in Table 5. The molybdenum ion-reducing capacity measured spectrophotometrically the green phosphate/Mo(V) complex formed by the reduction of Mo(VI) to Mo(V) in the presence of antioxidants. The greatest activity occurred with Fv_{SEN} extract (131.97 mg AAE/g extract) followed by Fv_{ZO} (43.95mg AAE/ mL extract) and then Fv_{SEL} (41.63mg AAE/ mL extract) extracts with statistically differences. In the present investigation, a highly positive relationship was found between phosphomolybdenum-reduction activity and phenolic as well as flavonoid contents (Table 6). This correlation was already reported by Majdoub et al. (2017) for two cultivars of sweet fennel plants, nevertheless the activity of Fv_{SEN} was remarkably higher when compared to those two cultivars, which may be related to the highest amount of TPC found in Fv_{SEN}.

Metal chelating activity

Transition metals are co-factors required to ensure enzyme activity in the human body. Nevertheless, they can also trigger the formation of alkoxyl radicals after reacting with peroxides. These reactions occur due to the presence of unpaired electrons in these transition metals. Hence, chelating compounds may be considered important agents in the prevention of oxidation processes (Zengin et al., 2015). The metal-chelating activity of *F. vulgare* extracts was tested in our investigation by measuring the formation of ferrous ion-ferrozine complexes. The values of IC_{50} values are depicted in Table 5. In the present study, all samples were able to chelate ferrous ions, nevertheless with diverse strength. As aforementioned for antioxidant activity, in this assay Fv_{SEN} also exhibited the best chelating ability, followed by Fv_{ZO}. Fv_{SEL} showed a low activity. In line with the DPPH, ABTS and superoxide, an inverse correlation between the IC_{50} values and total phenol and flavonoides was observed (Table 6). These findings are in accordance with the results of Zengin et al. (2015), whom studied the chelating effects of *Ornithogalum narbonense*. The authors concluded that the relative high occurrence of chelating effect of tested samples could be attributed to their polyphenolic content. This hypothesis can be corroborated with the results reported by Majdoub et al. (2017) for two cultivars of fennel. The authors described lower total phenols as well as flavonoids than those reported in the present work. Moreover, the authors also stated an inverse correlation between IC_{50} values and the amounts of phenols and flavones and flavonols. In the present work, the capacity for chelating metal ions was higher than those reported by Majdoub et al. (2017) and an inverse correlation between the same factors was found (Tables 5 and 6). In contrast, no correlation was found between the ability for chelating metal ions and the amount of flavanones and dihydroflavovols, as also found by Majdoub et al. (2017).

Inhibition of lipid peroxidation using thiobarbituric acid-reactive substances (TBARS)

Several techniques are available for measuring the oxidation rate of lipids. The capacity of extracts to inhibit lipid

peroxidation may use these techniques. The ability of the fennel extracts to prevent lipid oxidation was assayed by one method (TBARS) but using two lipid substrates (egg yolk and liposomes). This procedure estimates the malondialdehyde (MDA) formed as the split product of an endoperoxide of unsaturated fatty acids due to the oxidation of a lipid substrate. In the present study, all samples were tested for their ability to inhibit lipid peroxidation (Table 5). With egg yolk as lipid substrate, Fv_{SEL} exhibited the highest capacity for inhibiting lipid peroxidation followed by Fv_{ZO} and then Fv_{SEN}. However, when liposomes constitute the lipid substrate, the trend was different since Fv_{SEN} was the best extract for preventing liposome peroxidation and Fv_{ZO} the worst one. The effect antioxidant depends on the composition and physical properties of the lipid substrate and type of inducer as well (Schnitzer et al., 2007).

When egg yolk was used as lipid system, a positive correlation between IC_{50} values and phenols/flavonoid content was obtained, nevertheless a negative correlation was found between IC_{50} values and dihydroflavonol content (Table 6). These results suggest that phenols, flavones and flavonols have a negative role on the lipid peroxidation, that is, they induce the oxidation (pro-oxidants). Only flavanones and dihydroflavonols prevent lipid oxidation. No correlation was found when liposomes were used as lipid substrate. The prevention of liposomal oxidation found in the work cannot be attributed to this kind of compounds. These results were in disagreement with those findings of Barros et al. (2009) who showing significant negative linear correlation with IC_{50} values of inhibition and phenolic compounds. However, the absence of correlation between phenols/flavonoid content and liposomal oxidation was also reported by Majdoub et al. (2017) for fennel, dill and anise extract samples. The pro-oxidant activity of fennel phenols can be partly explained by the presence of relative high amounts of some metal ions. For example, Rufián-Henares et al. (2006) reported an increased linoleic acid oxidation promoted by phenolic acids with high reducing power in the presence of high concentration of Cu^+, measured through the TBARS method.

Inhibitory activity of enzymes
5-Lipoxygenase inhibition activity
The biosynthesis of leukotrienes catalyzed by lipoxygenase caused several inflammatory diseases including asthma, cancer and allergic disorders (Rackova et al., 2007). The inhibition of this enzyme can be considered not only an indicator of anti-inflammatory activity but also a possible indicator of antioxidant because this enzyme promotes the oxidation of arachidonic acid or linoleic acid through the insertion of a diatomic oxygen generating a hydroperoxy derivative of the fat acid (Smith and Murphy, 2002).

Table 7 depicts the ability of fennel extracts for inhibiting some enzymes (lipoxygenase, acetylcholinesterase and tyrosinase). All samples presented capacity for inhibiting lipoxygenase activity. Fv_{SEN} extract was the most effective inhibitor of 5-lipoxygenase and F_{SEL} extract exhibited relatively lower ability to inhibit this enzyme. The IC_{50} values found in the present work are within the range already found by Majdoub et al. (2017) for cultivated fennel samples. The inhibitory activities (IC_{50} values) correlated well with the phenols, including flavones and flavonols' contents (Table 8), such already reported by those authors, revealing the role of this sort of compounds on the lipoxygenase inhibition, in contrast to dihydroflavonols or flavanones in which no relationship between their amounts in samples and activity was found. Previous reports proved the ability of some phenol acids, such as carnosic acid (Kamatou et al. 2010) or some flavonoids for inhibiting 5-lipoxygenase (Redrejo-Rodriguez et al., 2004). These authors demonstrated that the inhibitory potency of flavonoids cannot be only attributed to their structures but also to the delocalization of LUMO (lowest unoccupied molecular orbital) orbital.

Acetylcholinesterase (AChE) inhibitory activity
All tested samples exhibited inhibitory activity toward AChE. As shown in table 7, the pronounced inhibitory activity was attributed to Fv_{ZO} and the worst to Fv_{SEN}, even so more effective than water extract of Portuguese fennel ($IC_{50} = 1490$ µg/ml), as previously registered by Mata et al. (2007). Majdoub et al. (2017) found diverse inhibitory activity of samples on AChE according to the cultivars of fennel and presence of absence of zinc supplementation. "Doux de Florence" was not able to inhibit the activity of this enzyme, whereas the cultivar "Latina" possessed such activity but dependent on the presence or absence of Zinc.

A positive correlation between the IC_{50} values and the amounts of phenols/flavonoids was obtained, which

Table 7: Enzyme inhibitory activities (IC50 = µg/ mL) of aqueous extracts of F.vulgare

	Lipoxygenase	Acetylcholinesterase	Tyrosinase
F_{ZO}	40.33±0.33[b]	59.68±48.88[b]	180.88±0.93[a]
F_{SEL}	50.81±0.38[a]	117.59±10.77[b]	155.54±5.92[a]
F_{SEN}	30.12±2.21[c]	678.54±30.85[a]	155.84±10.61[a]

Each value expressed as the mean of three replicates ± S.E. Values with different letters in the same column are significantly different at p < 0.05

Table 8. Spearman's correlation coefficients between total phenol, flavonoid contents and enzyme inhibitory activities

	Phenol	Flavone/ flavonol	Dihydrofavonol/ flavanone
Acetylcholinsterase	0.950**	0.963**	-0.076
Tyrosinase	-0. 259	-0.306	-0.616
Lipoxygenase	-0.931**	-0.931**	0.646

*Correlation is significant at the P < 0.05 level. **Correlation is significant at the P < 0.01 level

means that these compounds induce the AChE activity. In a previous work, Majdoub et al. (2017) did not find any correlation between phenolic content of fennel extracts and inhibitory activity on AChE. Quercetin and caffeic acid were good inhibitors of this enzyme, that rapidly lost activity if under the glycosilated form (Nugrobo et al., 2017). In addition, phenolic acids (4-hydroxyphenylpyruvic acid > nordihydroguaiaretic acid > rosmarinic acid > caffeic acid > gallic acid = chlorogenic acid) and flavonoids (cyanidin, delphinidin, kaempferol, myricetin, phloridzin, pelargonidin or quercetin) were also considered by Szwajgier (2015) as being able to inhibit AChE, nevertheless phenolic acids in pairs with flavonoids presented, in most cases, lost inhibitory activity.

Tyrosinase inhibition activity

Tyrosinase converts both L-tyrosine and L-dihydroxyphenylalanine (L-dopa) into *o*-dopaquinone, a precursor of the polymeric pigment's eumelanin (black-brown) and pheomelanin (yellow-reddish-brown). Higher activity of tyrosinase may lead to disfunctions of skin pigmentation, including melasma associated with age, age spots, and post-inflammatory hyperpigmentation (Burlando et al., 2017). Inhibitors of tyrosinase can be used as lightening skin agents, but also in the prevention of post-harvest food browning, due to the inhibition of hydroxylation of monophenols and the oxidation of *o*-diphenols, into *o*-quinones (Burlando et al., 2017).

Fennel water extracts were tested to evaluate their ability in tyrosinase inhibition. As shown (Table 7), all examined extracts exhibited tyrosinase-inhibition capacities without statistical significance. The IC_{50} values found were lower than those previously reported (Majdoub et al., 2017) for fennel extracts, being, therefore, better inhibitors of tyrosinase. No correlation was found between IC_{50} values and the amount of phenols/flavonoids (Table 8), although such correlation had been widely reported (Lee et al., 2016 and references therein). Polyphenols have been reported as inhibitors of tyrosinase with different strength and acting by diverse mechanisms: direct inhibition of enzyme (quercetin, kmapferol), acting as substrate for the enzyme (competitive inhibition) (catechin), acting as a free radical scavenger (rhamnetin) or both (gallic acid) (Lee et al., 2016). In fact, in our previous work with fennel, anise and dill aqueous extracts, a negative correlation between IC_{50} values and phenol/flavonoid concentrations was detected (Majdoub et al., 2017). The absence of correlation in the present work is unexpected and difficult to find an explanation with the sole data that we have in the moment.

α-Amylase inhibitory activity

Pancreatic α-amylase is an enzyme of dietary carbohydrate digestion in humans. Inhibitors of α -amylase may be effective in retarding carbohydrate digestion and glucose absorption and, therefore, in suppressing postprandial hyperglycaemia (El-Guendouz et al., 2016). The tested samples were evaluated for their α-amylase inhibitory activity which was expressed as percentage inhibition at final concentration of 100 mg/mL. In fact, all samples exhibited weak α-amylase ability and the respective percentage values were estimated as 19.59%; 18.55% and 14.09% for F_{ZO}, F_{SEL} and F_{SEN} (Fig. 1). The antidiabetic activity of fennel was only reported for its essential oils (Badgujar et al., 2014). The absence of activity was reported by Gholamhoseinian et al. (2008) for methanolic and aqueous extracts of fennel.

Mineral content on phenol/flavonoid content and biological properties

In the present investigation, the relationship between some mineral content and phenol/flavonoid content as well as antioxidant activity and inhibitory activity on diverse enzymes was evaluated. To the best of our knowledge, there are no previous report highlights this relationship in fennel aqueous extracts.

The results showed that Zn and K have a positive role on the phenol/flavonoid content, nevertheless Cu, Mg and Na had a negative role on the same secondary metabolites.
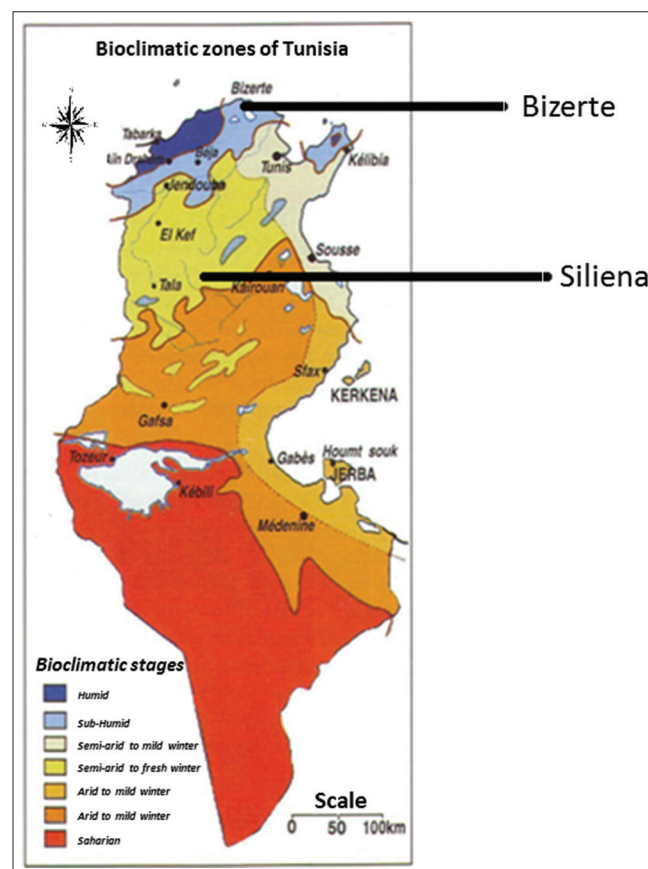

Fig 1. Map of bioclimates (Adapted from: Atlas Jeune Afrique)

Higher levels of Zn promoted higher accumulation of phenols/flavonoids and consequently also enhanced the antioxidant activity (scavenging ability of DPPH, ABTS, NO, and superoxide free radicals). The capacity for chelating ferric ions and for inhibiting lipoxygenase activity was also promoted by higher concentrations of Zn, nevertheless had an inverse action on the acetylcholimesterase activity and lipid peroxidation, that is, induced the activity of acetylcholinesterase and has a prooxidant activity in what concerns the lipid peroxidation of egg yolk (Table 9). Fe had a negative role on the antioxidant activity, since higher concentrations of this element was responsible for the decrease of antioxidant activity (positive correlation between IC_{50} values of DPPH, ABTS, NO, superoxide and Fe concentration). Higher amounts of Fe induce the lipoxygenase activity and decrease the chelating metal ions' capacity. In what concerns the antioxidant activity, including the action on lipoxygenase, and the capacity for preventing egg yolk peroxidation, Fe and Zn act in an opposite manner, nevertheless higher amounts of Zn promoted the accumulation of phenol/flavonoids whereas no correlation could be found between the amounts of these secondary

metabolites and the concentration of Fe. The action of Zn on the antioxidant activity can be due not only to a possible direct action on the process of free radicals' scavenging but also, indirectly, through the induction of phenol biosynthesis, which are known to possess antioxidant activity. Fe did not influence the biosynthesis of phenols, since there is no correlation between the concentration of this element and the production of phenol/flavonoids, therefore the negative action on the antioxidant activity can only be attributed to a direct effect on the scavenging ability.

A negative correlation between Na amount and phenol/flavonoid content was observed, therefore, as Na concentration increases as the phenol amounts decreases. The correlation between the concentration of Na and IC_{50} values found for the assays that evaluated the capacity for scavenging DPPH, ABTS, NO, superoxide free radicals, or for chelating iron ions, or inhibiting the lipoxygenase activity were positive, which means lower biological activities for higher concentrations of Na. This correlation can be partly attributed to the lowest concentrations of phenol/flavonoids in the extracts possessing higher levels of Na. However, a negative correlation between Na amounts and the IC_{50} values for preventing egg yolk peroxidation or inhibiting the activity of acetylcholinesterase was observed (Table 9).

Several elements seem exert an effect on the acetylcholinesterase activity: higher levels of Cu, Mn, Mg and Na promoted the inhibition of acetylcholinesterase since a negative correlation between IC_{50} values and the concentrations of these elements were found, whereas Zn and K had an opposite effect (Table 9). In the case of lipoxygenase activity, only Zn, Fe and Na correlated with the enzyme activity: higher levels of Zn increased the inhibition of lipoxygenase negative correlation between IC_{50} values and Zn concentration), whereas Fe and Na had
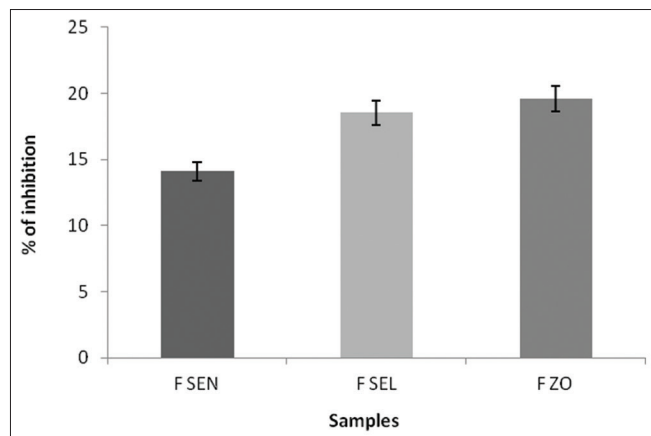

Fig 2. α Amylase inhibitory activity of *F. vulgare* samples

Table 9. Spearman's correlation coefficients between elements content and antioxidant and enzyme inhibitory activities

	Zn	Cu	Fe	K	Mn	Mg	Na
Phenol	0.990**	-0.845**	-0.561	0.748*	-0.518	-0.760*	-1.000**
Flavone/flavonol	0.982**	-0.872**	-0.518	0.781*	-0.561	-0.792*	-1.000**
Dihydroflavonol/flavanone	-0.475	-0.170	0.931**	0.320	-0.571	-0.303	0.338
DPPH	-0.890**	0.385	0.935**	-0.231	-0.067	0.249	0.806**
ABTS	-0.928**	0.473	0.892**	-0.325	0.032	0.343	0.858**
NO	-0.792*	0.211	0.984**	-0.050	-0.248	0.068	0.685*
Superoxide	-0.964**	0.583	0.821**	-0.446	0.164	0.462	0.914**
Liposome	-0.306	0.849**	-0.501	-0.923**	0.995**	0.916**	0.454
Egg yolk	0.964**	-0.566	-0.842**	0.425	-0.138	-0.441	-0.910**
Phosphomolybdenum	0.944**	-0.933**	-0.389	0.863**	-0.675*	-0.872**	-0.985**
Ferric chelating	-0.935**	0.486	0.889**	-0.338	0.045	0.355	0.867**
Acetylcholinesterase	0.899**	-0.962**	-0.287	0.908**	-0.746*	-0.915**	-0.956**
Lipoxygenase	-0.971**	0.598	0.814**	-0.462	0.180	0.478	0.923**
Tyrosinase	-0.140	0.681*	-0.566	-0.767*	0.872**	0.758*	0.279

*Correlation is significant at the P < 0.05 level. **Correlation is significant at the P < 0.01 level

an opposite effect (Table 9). For tyrosinase, the positive correlation between IC$_{50}$ values and concentration means lower inhibitory activity for higher concentrations of these elements, in contrast to K in which higher concentrations lead to better inhibitory activity of tyrosinase (Table 9).

CONCLUSION

In conclusion, the aqueous extracts of fennel exhibited antioxidant activity and inhibitory effect on acetylcholinestrase; tyrosinase and lipoxygenase but these abilities as well as the amounts of polyphenols and mineral elements varied considerably depending on their growing soil conditions. The physicochemical properties of soil showed differences from a site to another thoroughly in the same bioclimatic zone. Further investigation will be needed for carefully choose the best growth conditions of medicinal plants in order to promote the highest possible production of the targeted plant-based products.

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Authors′ contributions
Maria Graça Miguel, Clara Costa and Carlos Guerrero helped with experimental design, writing and editing the manuscript. Nessrine Majdoub, Soukaïna El-Guendouz, João Duarte and Jorge Carlier helped with sample analyses. Nessrine Majdoub, Soukaïna El-Guendouz carried out the experiments, performed the statistical analysis, and helped with the manuscript draft.

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84

Emir. J. Food Agric ● Vol 33 ● Issue 1 ● 2021

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Emir. J. Food Agric ● Vol 33 ● Issue 1 ● 2021

85

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86

Emir. J. Food Agric ● Vol 33 ● Issue 1 ● 2021