Systematic evaluation of the antioxidant potential of different parts of *Foeniculum vulgare* Mill. from Portugal

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Abstract

Fennel (*Foeniculum vulgare* Mill.) is a widespread perennial umbeliferous (Apiaceae) herb, traditionally used for medicinal purposes and human consumption. It is highly recommended for diabetes, bronchitis and chronic coughs, and for the treatment of kidney stones; some of those chronic diseases are related to the production of radical species involved in the oxidative stress. Therefore, the antioxidant potential of this herb might explain some of their empirical uses in folk medicine. This is the first time that a systematic study on different parts of fennel is performed, in order to understand differences in the antioxidant potential of shoots, leaves, steams, and inflorescences, particularly related to their composition in antioxidant compounds such as vitamins (ascorbic acid and tocopherols) and phenolics. The shoots seems to have the highest radical scavenging activity and lipid peroxidation inhibition capacity (EC_{50} values < 1.4 mg/ml), which is in agreement with the highest content in phenolics (65.85 ± 0.74 mg/g) and ascorbic acid (570.89 ± 0.01 μg/g) found in this part. The shoots also revealed high concentration of tocopherols (34.54 ± 1.28 μg/g) and were the only part with flavonoids.

Keywords: Fennel; Portuguese folk medicine; Vitamins; HPLC; Phenolics; Antioxidant potential

1. Introduction

Our research group has been interested in functional foods and nutraceuticals targeting oxidative stress (Ferreira et al. 2009). Public health authorities consider prevention and treatment with nutraceuticals a powerful instrument in maintaining and promoting health, longevity and life quality. The beneficial effects of nutraceuticals will undoubtedly have an impact on nutritional therapy; they also represent a growing segment of today's food industry. Folk medicine in Portugal is still very important in several regions where many wild plants are related to many different traditional medicinal uses. Ethnobotanical data currently available on wild useful plants in Portugal highlight the importance of fennel (*Foeniculum vulgare* Mill.), which is traditionally used for medicinal purposes and human consumption (Camejo-Rodrigues, 2003; Novais et al., 2004; Salgueiro, 2004; Carvalho, 2005; Santayana et al., 2007). Fennel is a widespread perennial umbeliferous (Apiaceae) herb, with a characteristic aniseed flavour, native to the Circum-Mediterranean area but naturalized elsewhere. In Portugal, as well as in the Iberian Peninsula, fennel has a long history of herbal use and is generally associated with gypsy communities' medicinal practices and diet. Roots, young shoots, leaves, flowering steams, mature inflorescences and fully ripened and dried seeds are commonly used for homemade remedies, being useful in the treatment of a variety of complaints (**Table 1**), especially those of the digestive system. Fennel is also highly recommended for diabetes, bronchitis and chronic coughs, and for the treatment of kidney stones. The species is also considered to have diuretic, stomachic and galactagogue properties. Infusions of leaves, steams or seeds, root or seeds decoctions, liqueurs prepared with steams and inflorescences, baths, ointments and poultices are some of the therapeutic applications reported in Portuguese folk medicine.

74 Therefore, it was decided to explore fennel as source of crucial compounds in the
75 neutralization of radical species involved in the oxidative stress, and responsible for several
76 chronic diseases such as cancer, cardiovascular diseases and diabetes (Ramarathnam et al.,
77 1995; Fang et al., 2002; Valko et al., 2007). This species might be used directly in diet and
78 promote health, taking advantage on the additive and synergistic effects of all the bioactive
79 compounds present. Our main question is what is the contribution of each one of the
80 different parts of the plant to the overall antioxidant activity?
81 It was reported the antioxidant activity of *Foeniculum vulgare* seeds (Oktay et al., 2003;
82 Surveswaran et al., 2007), leaves (Heinrich et al., 2005) and fruits (Marino et al., 2007), but
83 not their contents in vitamins, well-known as powerful antioxidants. Fennel volatile oil
84 mainly composed by linoleic, palmitic and oleic acids revealed strong antioxidant
85 properties, even higher than the standards butylated hydroxyanisole (BHA) and butylated
86 hydroxytoluene (BHT) (Singh et al., 2006). The essential oils extract (mainly composed by
87 (*E*)-anethole, (*Z*)-anethole and α-thujone) also revealed some antioxidant potential and
88 mostly antiacetylcholinesterase activity (Mata et al., 2007). Nevertheless, nothing is
89 reported on fennel shoots or steams, and it is the first time that a systematic study on
90 different parts of *Foeniculum vulgare* is performed, in order to understand differences in
91 the antioxidant potential of shoots, leaves, steams and inflorescences, particularly related to
92 their composition in antioxidant compounds such as vitamins and phenolics.
93
94 **2. Materials and methods**
95 *2.1. Samples*
96 Samples of shoots, leaves, steams and inflorescences were gathered in Bragança, Trás-os-
97 Montes, north-eastern Portugal. The selected sites and gathering practices took into account

local consumers gathering criteria for the medicinal use of fennel and the optimal growth stage. The plant material was collected in half shade sites at the edges of woods, in early spring (shoots), in June (leaves) and during and after the flowering period in July (steams and inflorescences). Shoots are the young steams that sprouted from the caudexes (**Figure 1a**); leaves, fully expanded, were collected in the median nodes of annual flowering steams; (**Figure 1c**); steams correspond to the herbaceous portion of the annual caulis; (**Figure 1d**); inflorescences are the fully developed compound umbels, with fertile flowers and immature seeds (**Figure 1e**).

Morphological key characters from the Flora Iberica (Castroviejo coord., 2003) were used for plant identification. Voucher specimens are deposited in the Herbarium of the ESAB. The material was lyophilized (Ly-8-FM-ULE, Snijders, HOLLAND) and kept in the best conditions for subsequent use.

2.2. Standards and Reagents

Acetonitrile 99.9% pure, of HPLC grade was purchased from Lab-Scan (Lisbon, Portugal). All the other reagents were of analytical grade purity: methanol and diethyl ether were supplied by Lab-Scan (Lisbon, Portugal); toluene from Riedel-de-Haën; sulphuric acid from Fluka (St. Gallen, Switzerland). The fatty acids methyl ester (FAME) reference standard mixture 37 (fatty acids C4 to C24; standard 47885-U) was from Supelco (Bellefonte, PA, USA) and purchased from Sigma (St. Louis, MO, USA), as well as other individual fatty acid isomers and the sugar standards. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

122 *2.3. Determination of vitamins*

123 *2.3.1. Tocopherols*

124 Tocopherols content was determined following a procedure previously optimized and
125 described by Barros et al. (2008a). BHT solution in hexane (10 mg/ml; 100 µl) and IS
126 solution in hexane (tocol; 2.0 µg/ml; 250 µl) were added to the sample prior to the
127 extraction procedure. The samples (~500 mg) were homogenized with methanol (4 ml) by
128 vortex mixing (1 min). Subsequently, hexane (4 ml) was added and again vortex mixed for
129 1 min. After that, saturated NaCl aqueous solution (2 ml) was added, the mixture was
130 homogenized (1 min), centrifuged (5 min, 4000*g*) and the clear upper layer was carefully
131 transferred to a vial. The sample was re-extracted twice with hexane. The combined
132 extracts were taken to dryness under a nitrogen stream, redissolved in 1 ml of n-hexane,
133 dehydrated with anhydrous sodium sulphate, filtered through a 0.22 µm disposable LC
134 filter disk, transferred into a dark injection vial and analysed by HPLC.

135 The HPLC equipment consisted of an integrated system with a Smartline pump 1000
136 (Knauer, Germany), a degasser system Smartline manager 5000, an AS-2057 auto-sampler
137 and a 2500 UV detector at 295 nm (Knauer, Germany) connected in series with a FP-2020
138 fluorescence detector (Jasco, Japan) programmed for excitation at 290 nm and emission at
139 330 nm. Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic
140 separation was achieved with a Polyamide II (250 x 4.6 mm) normal-phase column from
141 YMC Waters (Japan) operating at 35ºC (7971 R Grace oven). The mobile phase used was a
142 mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min, and the
143 injection volume was 20 µl. The compounds were identified by chromatographic
144 comparisons with authentic standards. Quantification was based on the fluorescence signal

145 response, using the internal standard method. Tocopherol contents in the samples are
146 expressed in µg per g of dry matter.
147
148 *2.3.2. Ascorbic acid*
149 Ascorbic acid was determined according to the method of Klein and Perry (1982). A fine
150 powder (20 mesh) of sample (150 mg) was extracted with metaphosphoric acid (1%, 10 ml)
151 for 45 min at room temperature and filtered through Whatman Nº 4 filter paper. The filtrate
152 (1 ml) was mixed with 2,6-dichloroindophenol (9 ml) and the absorbance was measured
153 within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the
154 basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/ml; $y = 3.0062x +$
155 0.007; $R^2 = 0.9999$), and the results were expressed as µg of ascorbic acid per g of dry
156 weight.
157
158 *2.4. Determination of phenolics and flavonoids*
159 A fine dried powder (20 mesh) of the leaves, steams, inflorescences and shoots (~1g) was
160 extracted by stirring with 50 ml of methanol at 25 ºC at 150 rpm for 12 h and filtered
161 through Whatman No. 4 paper. The residue was then extracted with one additional 50 ml
162 portion of methanol. The combined methanolic extracts were evaporated at 35ºC under
163 reduced pressure (rotary evaporator Büchi R-210), re-dissolved in methanol at a
164 concentration of 50 mg/ml, and stored at 4 ºC for further use.
165 Total phenolics were estimated by a colorimetric assay, based on procedures described by
166 (Wolfe et al., 2003) with some modifications. An aliquot of the extract solution was mixed
167 with *Folin-Ciocalteu* reagent (5 ml, previously diluted with water 1:10 v/v) and sodium
168 carbonate (75 g/l, 4 ml). The tubes were vortexed for 15 s and allowed to stand for 30 min

169 at 40 °C for colour development. Absorbance was then measured at 765 nm (Analytikijena
170 200-2004 spectrophotometer). Gallic acid was used to calculate the standard curve (0.05-
171 0.8 mM; $y = 1.9799x + 0.0299$; $R^2 = 0.9997$), and the results were expressed as mg of gallic
172 acid equivalents (GAEs) per g of extract.
173 Total flavonoid content was determined spectrophotometrically using the method of Jia et
174 al. (1999) based on the formation of a complex flavonoid-aluminum, with some
175 modifications. An aliquot (0.5 ml) of the extract solution was mixed with distilled water (2
176 ml) and subsequently with $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution
177 (10%, 0.15 ml) was added and allowed to stand further 6 min, thereafter, NaOH solution
178 (4%, 2 ml) was added to the mixture. Immediately, distilled water was added to bring the
179 final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15
180 min. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to
181 calculate the standard curve (0.0156-1.0 mM; $y = 0.9186x - 0.0003$; $R^2 = 0.9999$) and the
182 results were expressed as mg of (+)-catechin equivalents (CEs) per g of extract.
183
184 *2.5. Antioxidant activity.*
185 *DPPH radical-scavenging activity.* This methodology was performed using an ELX800
186 Microplate Reader (Bio-Tek Instruments, Inc). The reaction mixture in each one of the 96-
187 wells consisted of extract solution (30 µl) and aqueous methanolic solution (80:20 v/v, 270
188 µl) containing DPPH radicals ($6x10^{-5}$ mol/l). The mixture was left to stand for 60 min in the
189 dark. The reduction of the DPPH radical was determined by measuring the absorption at
190 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH
191 discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the

192 absorbance of the solution when the sample extract has been added at a particular level and
193 A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of
194 radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage
195 against extract concentration. Trolox was used as standard.
196 *Reducing power.* This methodology was performed using the Microplate Reader described
197 above. The extract solutions (0.5 ml) were mixed with sodium phosphate buffer (200
198 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixture was
199 incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The
200 mixture (0.8 ml) was poured in the 48-wells, as also deionised water (0.8 ml) and ferric
201 chloride (0.1% w/v, 0.16 ml), and the absorbance was measured at 690 nm. The extract
202 concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of
203 absorbance at 690 nm against extract concentration. Trolox was used as standard.
204 *Inhibition of β-carotene bleaching.* The antioxidant activity of the extracts was evaluated
205 by the β-carotene linoleate model system, as described previously by us (Barros et al.,
206 2008b). A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in
207 chloroform (10 ml). Two millilitres of this solution were pipetted into a round-bottom flask.
208 After the chloroform was removed at 40ºC under vacuum, linoleic acid (40 mg), Tween 80
209 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous
210 shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes
211 containing different concentrations of the extracts (0.2 ml). The tubes were shaken and
212 incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero
213 time absorbance was measured at 470 nm using a spectrophotometer. A blank, devoid of β-
214 carotene, was prepared for background subtraction. β-Carotene bleaching inhibition was

calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used as standard.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 ml) of the supernatant was incubated with the extracts solutions (0.2 ml) in the presence of $FeSO_4$ (10 μM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against extract concentration (Barros et al., 2008b). Trolox was used as standard.

2.6. Statistical analysis

For each one of the fennel components three samples were analysed and also all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD) or standard errors (SE). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program. The regression analysis between phenolics and EC_{50} values for antioxidant activity were performed using the same statistical package.

3. Results and discussion

The present study reports the antioxidant compounds (vitamins and phenolics), the free radical scavenging activity and lipid peroxidation inhibition of different parts of *Foeniculum vulgare* (fennel) (**Figure 1**; see section 2.1.). As it was described, young shoots, leaves and flowering steams are commonly used for homemade remedies, being useful in the treatment of a variety of complaints (**Table 1**), including chronic diseases; some of them are related to the production of radical species involved in the oxidative stress. Therefore, the antioxidant potential of this herb might explain some of the empirical uses in folk medicine, namely as infusions of leaves or seeds, root or seeds decoctions, liqueurs prepared with steams and inflorescences, baths, ointments and poultices.

Vitamin E (tocopherols) and vitamin C (ascorbic acid) are naturally-occurring antioxidant nutrients that play important roles in health by inactivating harmful free radicals produced through normal cellular activity and from various stressors (Chew, 1995). The water-soluble chain radicals, such as vitamin C, function as a primary defence against aqueous radicals, while vitamin E acts as lipophilic chain-breaking antioxidant and is responsible for scavenging lipid peroxyl radicals (Liu et al., 2008). The different parts of fennel revealed high contents of both vitamins, particularly ascorbic acid (**Table 2**). In generally, shoots

and leaves showed the highest levels of both vitamins, while steams revealed the lowest vitamins content. The four tocopherols (α, β, γ, δ-tocopherols) were quantified in inflorescences and shoots (**Figure 2**); δ-tocopherol was not detected in leaves and steams. α-Tocopherol was the major vitamin E in all the samples. The presence of these vitamins in the different parts of fennel is very important since it has been described in literature the existence of cooperative interactions among vitamin C and vitamin E. They interact synergistically at the membrane-cytosol interface to regenerate membrane-bound oxidized vitamin E (Li and Schellhorn, 2007). The interactions among these antioxidant nutrients are likely very important in protecting cells because the concentration of each antioxidant alone may not be adequate to effectively protect these cells against lipid peroxidation (Chew, 1995).

The antioxidant properties of phenolic compounds are well-known, playing a vital role in the stability of food products, as well as in the antioxidative defense mechanisms of biological systems. They might provide health benefits associated with reduced risk of chronic diseases that may be due to their ability to reduce agents by donating hydrogen and quenching singlet oxygen (Nijveldt et al., 2001). The extraction yields and total phenolics including flavonoids of the different parts of fennel are presented in **Table 3**. Shoots and leaves revealed the highest extraction yields (measured as ration between the extract weight and the dry weight of each sample) and also phenolics contents. The yields obtained in this study, using methanol, were higher than the values obtained for water (26.5%) and ethanolic (6.9%) extracts from another Portuguese fennel sample (Mata et al., 2007). Oktay et al. (2003) obtained 16.20% for water extracts and 10.95% for ethanolic extracts from Turkish seeds.

Phenolics were the main antioxidant compounds found in all the parts and flavonoids, in

285 particular, were only detected in the shoots. The shoots revealed the highest content in
286 phenolics (65.85 ± 0.74 mg/g); this content is higher than the phenolics found in methanolic
287 extracts of fennel leaves from India (11.1 mg/g; Surveswaran et al., 2007), but lower than
288 the amounts found in ethanolic extracts from Greece samples (178.87 mg/g; Heinrich et al.,
289 2005). The phenolics content found in our methanolic inflorescences extracts (34.68 ± 0.74
290 mg/g; **Table 3**) was lower than the content found in buthanolic extracts from Italian fruits
291 (103.13 mg/g), but higher than aqueous extracts from the same sample (21.74 mg/g;
292 Marino et al., 2007). As expected, Mata et al. (2007) reported higher levels of phenolics
293 using the all plant, either in aqueous extracts (63.8 mg/g) or ethanolic extracts (63.1 mg/g).
294 This is the first report on bioactive compounds present in shoots and steams of *Foeniculum*
295 *vulgare*.
296 The studied parts of *Foeniculum vulgare* proved to be free radical scavenging activity and
297 lipid peroxidation inhibition, but in different extensions. The free radical scavenging
298 activity of the samples was measured against radical species generated in the reaction
299 system, such as DPPH radicals (scavenging effects on DPPH assay), linoleate-free radical
300 (β-carotene bleaching inhibition assay) or Fe^{3+}/ferricyanide complex (reducing power
301 assay). The first two are hydrogen atom transfer reaction based assays ($X^{\bullet} + AH \rightarrow XH +$
302 $A^{\bullet}$), while the third is an electron transfer reaction based assay ($M^{3+} + AH \rightarrow AH+ + M^{2+}$)
303 (Prior, 2005). From the analysis of **Figure 3**, we can conclude that the scavenging effects of
304 the fennel extracts on DPPH radicals (measured by the decrease in DPPH radical
305 absorption after exposure to radical scavengers), increased with the concentration increase
306 and were good for shoots, leaves and inflorescence extracts (> 50% at 10 mg/ml). The RSA
307 values at 10 mg/ml were moderate for steams extracts (~40%). The reducing power was

evaluated measuring the conversion of a Fe^{3+}/ferricyanide complex to the ferrous form. It can be observed that reducing power increased with concentration, and the values obtained for shoots, inflorescence and leaves extracts were excellent (**Fig. 3**); at 5 mg/ml were higher than 1.5. The extract obtained with steams showed lower reducing power values (~1 at 5 mg/ml). The β-carotene bleaching inhibition, evaluated by the capacity to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models, is also presented in **Figure 3**. Again, shoots and leaves extracts were the most effective for antioxidant activity (>90% at 20 mg/ml).

Lipid peroxidation is a complex process and occurs in multiple stages. It is well accepted that antioxidants retard lipid peroxidation in foods and biological samples. Hence, many techniques are available for measuring the oxidation rate of membranes, food lipids, lipoproteins, and fatty acids, which are particularly useful for antioxidant evaluation. In the present study, it was used TBARS assay, the most commonly used method to detect lipid oxidation (Kishida et al., 1993). This procedure measures the malondialdehyde (MDA) formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate. The results for lipid peroxidation inhibition in brain tissue (measured by the colour intensity of MDA-TBA complex), were excellent and in the order of leaves ≈ shoots > inflorescences > steams (**Figure 3**). The vitamins present in the samples can play a vital role in this process: vitamin E (tocopherol) can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA (polyunsaturated fatty acid), thereby breaking the radical chain reaction and preventing the peroxidation of PUFA in cellular and subcellular membrane phospholipids (Lampi et al., 1999).

$$LH + \text{oxidant initiator} \rightarrow L^{\bullet}$$

331 $$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

332 $$LOO^{\bullet} + \text{tocopherol} \rightarrow LOOH + \text{tocopherol}^{\bullet}$$

333 As a reducing agent, vitamin C (ascorbic acid) reacts with a vitamin E radical to yield a
334 vitamin C radical while regenerating vitamin E (Chew, 1995; Nagaoka et al., 2007).

335 $$\text{vitamin C} + \text{tocopherol}^{\bullet} \rightarrow \text{ascorbate}^{\bullet} + \text{tocopherol}$$

336 $$\text{ascorbate}^{\bullet} + \text{NADH} \rightarrow \text{ascorbate} + \text{NAD}^{\bullet}$$

337

338 Overall, the shoots gave the best results in all the antioxidant activity assays (EC_{50} values <
339 1.4 mg/ml; **table 4**), which is in agreement with the highest content in phenolics (65.85 ±
340 0.74 mg/g) and ascorbic acid (570.89 ± 0.01 μg/g) found in this part. The shoots also
341 revealed high concentration of tocopherols (**Table 2**) and were the only part with
342 flavonoids (**Table 3**). The steams were the fennel part with the lowest antioxidant activity
343 (highest EC_{50} values; **table 4**) which is also in agreement with its lowest content in all the
344 antioxidant compounds (ascorbic acid, tocopherols and phenolics). Particularly for
345 phenolics, significantly negative linear correlations with antioxidant activity EC_{50} values
346 were observed: DPPH scavenging activity (y = -0.1705x + 13.626; R^2 0.9179; $p < 0.001$),
347 reducing power (y = -0.0585x + 3.2856; R^2 0.9488; $p < 0.001$), β-carotene bleaching
348 inhibition (y = -0.0404x + 2.7182; R^2 0.9828, $p < 0.001$) and TBARS inhibition (y = -
349 0.0013x + 0.2845; R^2 0.6260, $p < 0.001$).

350 Despite being widely adopted and generally considered to be a very safe herb, a few
351 ethnobotanical inventories conducted in Portugal (Carvalho, 2005) have also reported some
352 adverse effects from the use of fennel. Particularly older users/informants (more than 60
353 years old) have mentioned a certain degree of toxicity present in seeds and roots decoctions

354 (Carvalho, 2005). Based on their empirical knowledge, transmitted between successive
355 generations, these people stated that the continued use of fennel decoctions should be
356 avoided because it can produce abdominal pains (*sensu latu*). Instead they advised the
357 infusion of leaves or the liqueur of steams or inflorescences, which they considered less
358 aggressive for stomach. It was also reported that the plant should be avoid during
359 pregnancy.
360
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365
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444

445 **Table 1**. Medicinal and edible uses of fennel (*Foeniculum vulgare* Mill.) reported in

446 Portuguese ethnobotanical studies.

Portuguese Region	Local name	Medicinal uses and properties	Edible uses
Trás-os-Montes (northeast)	Fiolho, fionho, erva-doce	Respiratory, gastrointestinal and genitourinary systems, diabetes. Depurative, diuretic, antiseptic, digestive, carminative, galactagogue. Panacea	Condiment/spices flavouring and seasoning soup and stews Raw in salads Spirits, cakes and pastries To cook chestnuts
Arrábida and Açor (center)	Funcho, erva-doce	Indigestion, cold, cough, throat pain, cystitis, skin diseases	Condiment/spices flavouring cakes and pastries To cook chestnuts
Alentejo and Algarve (south)	Funcho, fiolho, funcho-doce, funcho-amargo	Diarrhea could, cough, kidney regulator, diabetes. Stomachic, digestive, carminative, expectorant, galactogogue.	Cooked with different kinds of beans and chickpeas. Fried with eggs. Omelettes. Stewed or roasted fish. Fish dishes in general. To prepare and preserve olives and dried figs.

447

448 **Table 2**. Vitamins (ascorbic acid and tocopherols) composition (μg/g dry weight) of
449 different parts of *Foeniculum vulgare*. The results are expressed as mean ± SD (n=3). In
450 each column different letters mean significant differences (p<0.05).

Parts of the plant	Ascorbic acid	α-tocopherol	β-tocopherol	γ-tocopherol	δ- tocopherol	Total
Shoots	570.89 ± 0.01 a	28.37 ± 1.25 b	0.10 ± 0.02 c	2.54 ± 0.06 b	3.53 ± 0.56 a	34.54 ± 1.28 b
Leaves	360.41 ± 0.23 b	50.22 ± 1.44 a	0.76 ± 0.07 b	4.70 ± 0.27 a	*n.d*	55.68 ± 1.77 a
Steams	181.77 ± 0.53 d	1.10 ± 0.18 c	1.52 ± 0.18 a	0.27 ± 0.08 c	*n.d*	2.89 ± 0.44 c
Inflorescences	311.40 ± 0.13 c	4.72 ± 0.44 c	1.71 ± 0.21 a	2.71 ± 0.06 b	0.18 ± 0.03 b	9.32 ± 0.35 c

451

452 **Table 3**. Extraction yields, total phenolics and flavonoids of different parts of *Foeniculum*
453 *vulgare*. The results are expressed as mean ± SD (n=3). In each column different letters
454 mean significant differences ($p<0.05$).

455

Parts of the plant	η (%)	Phenolics (mg/g extract)	Flavonoids (mg/g extract)
Shoots	34.77 ± 8.43 b	65.85 ± 0.74 a	18.64 ± 0.90
Leaves	40.64 ± 9.85 a	39.49 ± 0.62 b	*n.d*
Steams	29.68 ± 5.25 c	8.61 ± 0.09 d	*n.d*
Inflorescences	20.39 ± 6.93 d	34.68 ± 0.74 c	*n.d*

Table 4. Antioxidant activity EC_{50} values (mg/ml) of different parts of *Foeniculum vulgare*. The results are expressed as mean ± SD (n=3). In each column different letters mean significant differences ($p<0.05$).

Parts of the plant	DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	Lipid peroxidation inhibition
Shoots	1.34 ± 0.07 d	0.48 ± 0.02 d	0.49 ± 0.03 d	0.13 ± 0.03 d
Leaves	6.88 ± 0.70 c	1.17 ± 0.07 b	1.14 ± 0.03 c	0.22 ± 0.02 c
Steams	12.16 ± 0.94 a	2.82 ± 0.04 a	2.38 ± 0.12 a	0.27 ± 0.01 a
Inflorescences	7.72 ± 0.87 b	1.02 ± 0.02 c	1.29 ± 0.03 b	0.25 ± 0.01 b

Figure 1. Fennel, *Foeniculum vulgare* Mill (Apiaceae), in Trás-os-Montes, Portugal: a – shoots, b – woody steams from the last year, c – leaves, d – steams, e – inflorescences, f – Informant showing dried chopped steams prepared for infusions.

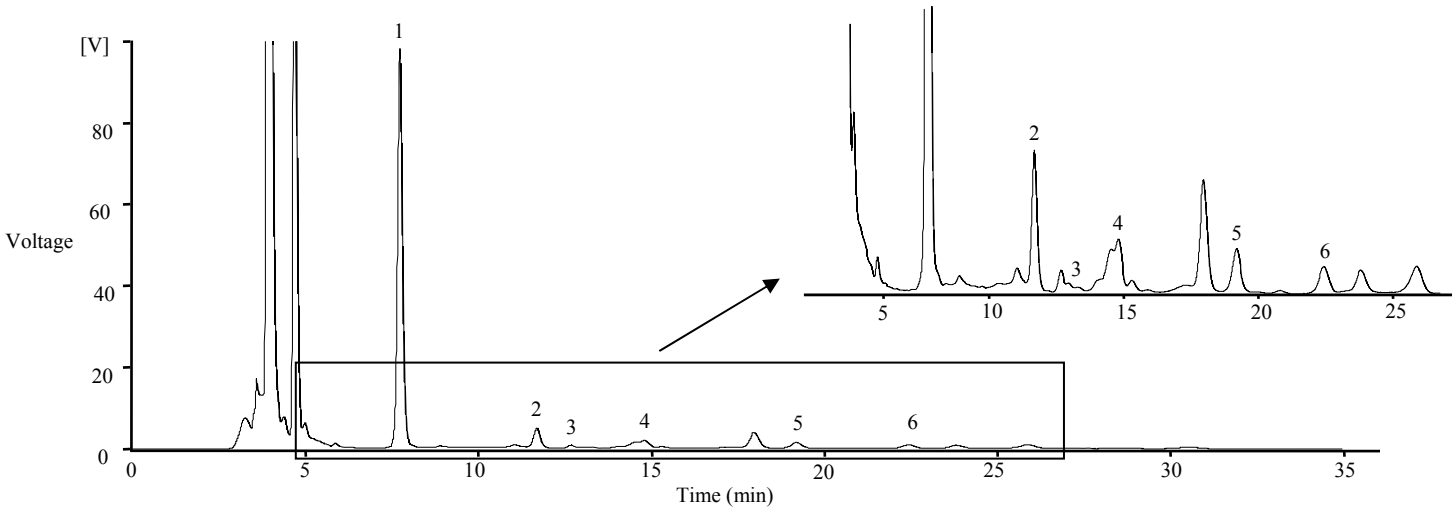

Figure 2. HPLC fluorescence chromatogram of fennel shoots. Peaks: 1- α-tocopherol; 2-BHT (butylated hydroxytoluene); 3-β-tocopherol; 4- γ-tocopherol; 5- δ-tocopherol; 6- I.S.- internal standard (tocol).

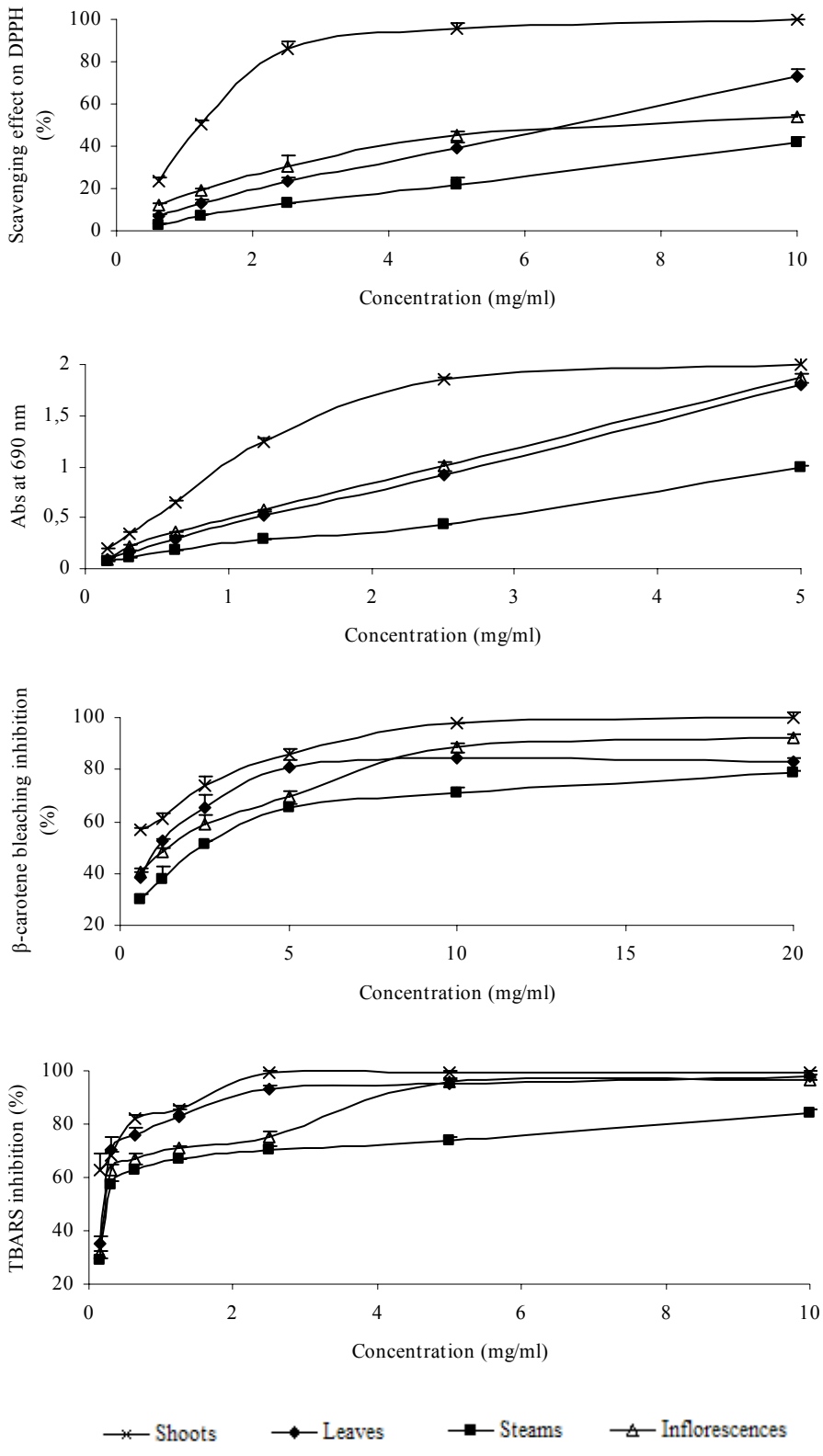

Figure 3. Antioxidant activity of the different parts extracts of *Foeniculum vulgare*: Scavenging activity on DPPH radicals, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition. Each value is expressed as mean ± standard error (n=3).