View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Sapientia

Elsevier Editorial System(tm) for Industrial Crops and Products
Manuscript Draft

Manuscript Number: INDCRO-D-17-01815R3

Title: Growth, photosynthetic pigments, phenolic content and biological activities of Foeniculum vulgare Mill., Anethum graveolens L. and Pimpinella anisum L. (Apiaceae) in response to zinc

Article Type: Research Paper

Section/Category: Non-food bioactive products

Keywords: Anethum graveolens L., Foeniculum vulgare Mill., Pimpenilla anisum L., Zinc, antioxidant activity, enzyme inhibition

Corresponding Author: Professor Maria Miguel,

Corresponding Author's Institution: Universidade do Algarve

First Author: Nessrine Majdoub

Order of Authors: Nessrine Majdoub; Soukaina el-Guendouz; Marwa Rezgui; Jorge Carlier; Clara Costa; Leila Kaab; Maria da Graca Miguel

Abstract: The effect of zinc (Zn) on phenols, antioxidant activities (free radicals' scavenging activities, inhibition of lipid peroxidation, chelating activity and reducing power), and enzyme inhibition activities of acetylcholinesterase, lipoxygenase and tyrosinase of anise, dill and two cultivars of fennel [Latina" (FL) and "Doux de Florence" (FDF)] aqueous extracts was evaluated for the first time. At the same time, growth and photosynthetic pigment contents were also estimated.
A significant decrease in all growth parameters, such as plant height, fresh and dry weights of aerial parts and roots was statistically proved in the presence of 2 mM Zn. An enhancement of Zn accumulation was observed, particularly in aerial part. Exposure to 2 mM Zn induced quantitative changes in the phenols of all tested extracts. In fennel extracts, a decrease of phenol content was observed when treated with Zn, whereas an increase was found in dill and anise extracts submitted to Zn treatment. All samples, either in the presence or absence of Zn, showed antioxidant activity, independent on the tested assay, with the exception of the cultivar FDF (treated and non-treated), which was unable to scavenge NO radicals. Zn-treated dill and anise presented usually higher antioxidant activity. Concerning inhibitory activities against acetylcholinesterase, lipoxygenase and tyrosinase enzymes, both treated and non-treated FDF did not inhibit acetylcholinesterase activity. Zinc-treated FDF was also unable to inhibit lipoxygenase activity. The remaining species presented capacity for inhibiting those enzymes and tyrosinase as well, but their abilities varied in response to Zn excess.

Date: 04/08/2017

Subject: Manuscript Ref. No.: INDCRO-D-17-01815R2

Title: Growth, photosynthetic pigments, phenols and in vitro biological activities of aqueous anise, dill and fennel extracts in response to 2 mM Zinc

Dear Prof. Maria Pascual-Villalobos,

We thank you for your e-mail of 24 July 2017 with the comments on our manuscript (MS) INDCRO-D-17-01815R2 on “Growth, photosynthetic pigments, phenols and in vitro biological activities of aqueous anise, dill and fennel extracts in response to 2 mM Zinc”.

We have read it carefully and we do understand most of the referee’s comments. Please find bellow our reply and comments following each point raised by the reviewers. All corrections and additions performed in the MS text have been marked in blue.

Reviewers' comments:

Reviewer #1: The revised version of the manuscript INDCRO-D-17-01815R2 meets all the criteria of scientific work.

Paper may be published after several technical corrections:

- Suggestion for the title (please accept the proposal, the current version is incorrect):

Growth, photosynthetic pigments, phenolic content and biological activities of Foeniculum vulgare Mill., Anethum graveolens L. and Pimpinella anisum L. (Apiaceae) in response to zinc

RESPONSE:This proposal made by reviewer 1 was taken into account.

- Technical errors in paper need to be corrected, for example line 76 and 78 chronology, 110 (ZnSO4x7H2O is ok, not with point ZnSO4·7H2O), 184 (50% is ok, not with space 50 %) 190, ...803 (pat or part)....etc..

RESPONSE: The group of references cited in the text was listed alphabetically, according to the guide for authors. ZnSO4·7H2O was used, and not ZnSO4x7H2O, according to the generally found in chemical manuscripts.

All cases in which % was separated of the number was corrected.

pat was corrected and replaced by part.

- The Latin names of the species should be in italics as well as in vitro, in vivo etc. (lines 577, 597, 634, 650, 658, 666, 675, 689, 700, 713, 716, 739, 765, 784, 792, 798 etc...)

RESPONSE: All cases were corrected.

- Grammatical errors were noticed in the text of paper and not respected the rules in writing the English language.

41 RESPONSE: Tentatively done. The revision of the text by an English native was not carried out.
42
43 - There are errors in the text when typing, spacing, double spacing, etc.. all the errors should be
44 corrected.
45 RESPONSE: All cases were corrected.
46
47 - References should be technical checked in accordance with the instructions for authors. Also, a
48 number of typographical errors have been noted in the references.
49 RESPONSE: All cases were corrected.
50
51 - Conclusion must be accurately, clearly and precisely written, based on the results.
52 RESPONSE: Conclusion was re-written.
53
54
55 Reviewer #2: The present manuscript studied the effect of Zn in the antioxidant activity of fennel, dill
56 and anise. Zn is a heavy metal and is also the responsible for the weakness of several soils that cannot
57 be used for agriculture so that is very important to control the amount of this metal.
58 The introduction is very complete as also the other sections, especially the results and discussion that
59 are clear and compared with the available literature, and the authors concluded that the Zn
60 concentration directly affects the antioxidant activity and also the amount of phenolic compounds.
61 This is very interesting given the importance of the safety in the agriculture practices.
62 This manuscript was already revised and after revision the authors already made the requested
63 corrections according to the journals rules.
64 The manuscript can be accepted in the present form.
65
66
67 Reviewer #3: I have evaluated the manuscript (INDCRO-D-17-01815R2) titled "Growth,
68 photosynthetic pigments, phenols and in vitro biological activities of aqueous anise, dill and fennel
69 extracts in response to 2 mM Zinc" submitted to Industrial Crops and Products. The study is original
70 and content of the study was studied properly. My comments on the paper were given below.
71 1- The title of the manuscript is not appropriate.
72 RESPONSE: The title was re-written according to the proposal of Reviewer 1.
73
74 2- Give more details about the statistical analysis. Please specify the type of statistical analysis for
75 each experiment. Also, indicate if data showed normality and homocedasticity, or if you assumed
76 them directly before analysis.
77 RESPONSE: A chapter of statistical treatment was introduced in the present form. By lapse it was
78 not introduced in the previous version of the manuscript.
79
80 3- The results of the work do not show much novelty. The effects of heavy metals (including Zn
81 excess) have already been studied on many plants, although it does not seem that they have been tested
82 on fennel, dill and anise.
83 RESPONSE: I agree with the reviewer, nevertheless this subject is new for these species.
84
85 4- Deep explanation of the mechanisms of action of the aqueous extracts is required in "Results and
86 discussion" section. Authors must establish comparisons between them and possible conclusions of the
87 work itself.
88 RESPONSE: The discussion was tentatively ameliorated, at least in some cases.
89
90 5- It would be useful to determine the chemical composition of the aqueous extracts of anise, dill and
91 fennel.
92 RESPONSE: The chemical composition was not introduced, because it is not done.
93
94 6- All information necessary to understand the table should be included in the title or subtitle.
95 RESPONSE: Information introduced.

96
97 7- I suggest to delete Table 8. It is too long and not necessary. The explain of the major points in the
98 table is enough.
99 RESPONSE: Table 8 was deleted from the main document, but at this moment it is a
100 supplementary information. We consider that the information compiled in previous Table 8,
101 now Table S1, is important and must remain.
102
103 We hope that we have addressed correctly all the reviewers and Editor remarks and questions.
104 Hoping that everything is in the correct form and looking forward to hearing from you.
105
106 Yours sincerely,
107
108 Graça Miguel

Highlights

Zn excess reduced growth and photosynthetic pigments level in fennel, anise and dill

Zn excess affected phenol amounts and antioxidant potency of extracts

Zn treatment affected the enzymatic inhibitory ability of extracts

*Manuscript
Click here to view linked References

1 # Growth, photosynthetic pigments, phenolic content and biological activities
2 # of *Foeniculum vulgare* Mill., *Anethum graveolens* L. and *Pimpinella anisum*
3 # L. (Apiaceae) in response to zinc

4 Nesrine Majdoub[a,b] , Soukaina el-Guendouz[b], Marwa Rezgui[a], Jorge Carlier[c], Clara Costa[bc], Leila
5 Bettaieb Ben Kaab[a], Maria Graça Miguel[b,*]

6 [a]Unité de Recherche: Nutrition et Métabolisme Azotés et Protéines de Stress (99 UR /09-20), Campus
7 Universitaire, Faculté des Sciences de Tunis, Département de Biologie, Université Tunis EL Manar,
8 2082 Tunis, Tunisia

9 [b]Faculdade de Ciências e Tecnologia, MeditBio, Universidade do Algarve, Campus de Gambelas,
10 8005-139 Faro, Portugal

11 [c]Centro de Ciências do Mar (CCMAR), Universidade do Algarve, 8005-139 Faro, Portugal

12

13 *Corresponding author

14 M. G. Miguel

15 Phone: +351289800100

16 E-mail: mgmiguel@ualg.pt

17

18

19

20

21

22

23

24 **Abstract**

25 The effect of zinc (Zn) on phenols, antioxidant activities (free radicals' scavenging activities,
26 inhibition of lipid peroxidation, chelating activity and reducing power), and enzyme inhibition
27 activities of acetylcholinesterase, lipoxygenase and tyrosinase of anise, dill and two cultivars
28 of fennel [Latina" (F_L) and "Doux de Florence" (F_{DF})] aqueous extracts was evaluated for the
29 first time. At the same time, growth and photosynthetic pigment contents were also estimated.
30 A significant decrease in all growth parameters, such as plant height, fresh and dry weights of
31 aerial parts and roots was statistically proved in the presence of 2 mM Zn. An enhancement of
32 Zn accumulation was observed, particularly in aerial part. Exposure to 2 mM Zn induced
33 quantitative changes in the phenols of all tested extracts. In fennel extracts, a decrease of
34 phenol content was observed when treated with Zn, whereas an increase was found in dill and
35 anise extracts submitted to Zn treatment. All samples, either in the presence or absence of Zn,
36 showed antioxidant activity, independent on the tested assay, with the exception of the
37 cultivar F_{DF} (treated and non-treated), which was unable to scavenge NO radicals. Zn-treated
38 dill and anise presented usually higher antioxidant activity. Concerning inhibitory activities
39 against acetylcholinesterase, lipoxygenase and tyrosinase enzymes, both treated and non-
40 treated F_{DF} did not inhibit acetylcholinesterase activity. Zinc-treated F_{DF} was also unable to
41 inhibit lipoxygenase activity. The remaining species presented capacity for inhibiting those
42 enzymes and tyrosinase as well, but their abilities varied in response to Zn excess.

43

44 **Keywords**: *Anethum graveolens* L., *Foeniculum vulgare* Mill., *Pimpenilla anisum* L., Zinc,
45 antioxidant activity, enzyme inhibition

46

47

48 **1. Introduction**

49 Zinc (Zn), presented in available form, is an essential micronutrient for plants, animals,
50 and microorganisms (Dhankhar et al., 2012). It plays crucial roles as a functional, structural,
51 and regulatory cofactor in many enzymes and regulatory proteins (Fujiwara et al., 2015).
52 Regarding plant physiology and metabolism, Zn is known to be required at optimal
53 concentration for growth and is involved in the protective effect of plasma membrane integrity
54 and permeability (Paradisone et al., 2015). However, various soils are detrimental for plant
55 growth owing to high concentration of metal ion, either due to natural processes or man's
56 activities (Levitt, 1980). As a heavy metal, Zn toxicity is one of the major problems of
57 worldwide agricultural production causing a potential health risks (Marichali et al., 2014). In
58 Tunisia, soils around open-cast mining exhibit a very high content of Pb, Zn, and Cd (Sebei et
59 al., 2005). Owing to this fact, plants on these mining areas may accumulate some metals such
60 as Pb, Zn, and Cd with an average of 0.7%; 0.9% and 0.003% respectively (Sebei et al., 2006).
61 High amounts of zinc accumulated in plants leads to the induction of oxidative stress and,
62 consequently, to the alteration of their physiological processes, according to the investigation
63 made in model plants. Nevertheless this sort of research, using medicinal and aromatic plants
64 is scarce (Marichali et al., 2016).

65 *Foeniculum vulgare* Mill. (fennel) is a perennial umbelliferous (Apiaceae) herb, used by
66 humans since antiquity, due to its characteristic aniseed flavor. Fennel is native from the
67 Circum-Mediterranean area but is cultivated throughout the world (Muckensturm et al., 1997).
68 This aromatic plant has been traditionally used for the preparation of herbal teas and even for
69 daily consumption, in the raw form as salads, stewed, boiled, grilled, or baked in many dishes
70 (Barros et al., 2010). Several studies have demonstrated the effective *in vitro* activity of fennel
71 as antimicrobial, antiviral, antiprotozoal (Dua et al., 2013), antioxidant, antitumor, anti-

72 inflammatory, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic (Badgujar et
73 al., 2014).

74 *Anethum graveolens* L. (dill) is an annual and sometimes biennial umbelliferous
75 (Apiaceae) herb, native to south-west Asia or south-east Europe, in the Mediterranean region
76 (Zehtab-Salmasi et al., 2006; Oshaghi et al., 2017). This species has been used in traditional
77 medicine for digestive disorders as carminative, stimulating lactation, and antispasmodic
78 (Zehtab-Salmasi et al., 2006; Oshaghi et al., 2017). Other properties have also been attributed
79 to dill: lowering lipids, anticancer, antimicrobial, antidiabetic, antigastric irritation,
80 antioxidant, and anti-inflammatory (Oshaghi et al., 2017). In addition, dill has also been used
81 as a vegetable and as an inhibitor of sprouting in stored potatoes (Zehtab-Salmasi et al., 2006).

82 *Pimpinella anisum* L. (anise), a plant belonging to the Umbelliferae family (Apiaceae),
83 which can be found in the Eastern Mediterranean region, is known for its medicinal and
84 culinary properties. This genus exhibits biological and pharmacological properties, such as
85 antimicrobial, galactogogue, antioxidant, antiseptic, digestive, and anti-inflammatory activities
86 (Martins et al., 2016).

87 There are growing interests for *F. vulgare*, *A. graveolens* and *P. anisum* therapeutic and
88 medicinal activities but little attention has been paid to the zinc monitoring (toxic heavy metal)
89 in these medicinal plants. To the best of our knowledge, there are no reports in the literature
90 investigating the biological activities, particularly *in vitro* antioxidant, acetylcholinesterase,
91 lipoxygenase and tyrosinase inhibitory activities of *F. vulgare, A. graveolens* and *P. anisum* in
92 response to Zn excess. Thus, this study highlights the capacity of accumulation of Zn by
93 fennel, dill and anise as well as its effect on some morphophysiological parameters, along with
94 the effect on some biological properties in terms of antioxidant, anti-inflammatory activities,
95 anti-acetylcholinesterase and anti-tyrosinase activities effectiveness.

96

97 **2. Material and Methods**

98 *2.1. Plant cultivation and Zn treatment*

99 Two cultivars of sweet fennel plants: "Latina" (F_L) and "Doux de Florence" (F_{DF}), as well
100 as dill (D) and anise (A) plants, were used in the present study. The plants were cultivated
101 according to the recommended technology reported by Senatori et al. (2013). Surface-
102 sterilized seeds were germinated and grown in the spring of 2014 at the experimental
103 greenhouse, in a plane area of North of Tunisia (EL Alia, Bizerte, Latitude: 37°10′ 08″ N;
104 Longitude: 10°02′00″ E; Elevation above sea level: 102 m), filled with a loamy sand soil,
105 naturally lit with sunlight, with a temperature range of 20 - 30 °C and a relative humidity
106 range of 50−80%, and supplied with distilled water every 3 days. The 30-d-old seedlings were
107 transferred to individual pots (26 cm upper diameter, 18 cm lower diameter, 25 cm in height)
108 of 10 L volume, filled with the same soil. Throughout the 60 days of the experiment, healthy
109 and uniform seedlings were submitted to Zn treatment using 2 mM $ZnSO_4 \cdot 7H_2O$, that is
110 renewed every 5 days. A control treatment (0 mM Zn) was made in the same conditions but
111 without adding Zn. The experimental design was a randomized block with two treatments
112 (control and treated with Zn) of each variety arranged in individual pots, with one plant each,
113 and nine replicates. Samples, obtained by mixing the aerial part collected at the end of the
114 experiment from the nine replications of each treatment, were submitted for analysis.

115 *2.2. Determination of growth parameters*

116 For each treatment, the fresh and dry weights, and high of roots and aerial part were determined.

117 *2.3. Determination of chlorophyll content*

118 The pigments *Chla*, *Chlb*, total chlorophyll and carotenoids were extracted from fresh
119 material in 80% acetone at room temperature. The chlorophyll contents were determined
120 spectrophotometrically as reported by Marichali et al. (2014) and using the followed
121 equations:

122 $Chla = 10.05\ OD_{663} - 1.97\ OD_{645}$

123 $Chlb = 16.36\ OD_{645} - 2.43\ OD_{663}$

124 $Chla + Chlbb = 7.62\ OD_{663} + 14.39\ OD_{645}$

125 Where *Chla* = Chlorophyll a; *Chlb* = Chlorophyll b; *Chla* + *Chlb* = total chlorophyll; OD =
126 optical density (nm).

127 Carotenoid amounts were evaluated in the same extracts and were calculated according to
128 Lichtenthaler (1987) using the formula:

129 $C_{\text{carotenoïds}} = (1000\ OD_{470} - 1.82\ Chl_a - 85.02\ Chl_b) / 198$

130 Where *Chla* = Chlorophyll a; *Chlb* = Chlorophyll b; OD = optical density (nm).

131 2.*4. Determination of zinc (Zn) content*

132 Fennel, dill and anise treated and non-treated samples (roots and aerial part) were digested
133 by applying the optimized procedure using nitric and perchloric acids. In brief, a 0.05 g of
134 well-powdered samples were added to 5 mL of HNO_3 and $HClO_4$ (3:1) (v/v) and the mixtures
135 were digested firstly in ambient temperature for 24 h and then with an increased temperature
136 starting from 60°C to 90°C and finally to 105°C until total dissolution. After cooling of the
137 digest, an equal volume of Milli Q water was added to dilute left over acid. Blank solution was
138 prepared by following the same procedure but without samples, only with reagents. The

139 amount of Zinc in the sample solutions was determined by MP-AES (Microwave Plasma
140 Atomic Emission Spectrometry) (Agilent 4200 MP-AES, Santa Clara, CA).

141 *2.5. Preparation of the extracts*

142 The air dried material of fennel, dill and anise species were powdered by using electric
143 mixer grinder and were weighed (5 g for each) and then macerated with distilled water
144 (100 mL) without agitation, at room temperature, for 24 h, filtered, and finally, the filtrate was
145 freeze dried.

146 *2.6. Determination of total phenols*

147 Phenolic compounds were determined in accordance with Folin–Ciocalteau's method (El-
148 Guendouz et al., 2016). In brief, a serial of dilutions of aqueous extracts (50 µL) were
149 prepared and mixed with 750 µL of Folin–Ciocalteau's reagent and 600 µL of sodium
150 carbonate (75 mg/mL). The reaction mixtures were further incubated at room temperature for
151 two hours. Afterward, absorption was measured at 760 nm versus a blank prepared without
152 extract. Tests were carried out in triplicate. A number of dilutions of gallic acid (standard)
153 were obtained to prepare a calibration curve. Total phenol content of the extracts was
154 expressed as gallic acid equivalent/mL extract (mg GAE/mL extract).

155 *2.7. Determination of total flavonols and flavones*

156 Flavonols and flavones content of the extracts were evaluated by aluminum chloride
157 colorimetric method (El-Guendouz et al., 2016). The extracts (150 µL) were mixed with
158 aluminum chloride (2%) (75 µL) and sodium acetate (1%) (75 µL). After reaction for 5 min,
159 150 µL of sodium hydroxide (40 mg/mL) was added and left at room temperature for 30 min.
160 The absorbance of the reaction mixture was measured at 510 nm. The flavonols and flavones
161 content of the extracts were expressed as quercetin equivalents (mg QE/mL extract)

162 *2.8. Flavanone and dihydroflavonol content*

163 The total quantification of flavanone and dihydroflavonol compounds was determined
164 according to El-Guendouz et al. (2016). Briefly, 75 µL of sample or standard (naringin) and
165 2 mL DNP (2,4-dinitrophenylhydrazine) (1 g DNP in 2 mL 96% sulphuric acid diluted to
166 100 mL with methanol) were heated at 50 ºC, for 50 min. After cooling at room temperature,
167 175 µL of KOH 10% were finally added to the mixture. The resulting solution was diluted to
168 3 mL with methanol. The absorbance was measured at 486 nm. All determinations were
169 performed in triplicate. The values are expressed as naringin equivalents (mg NE/mL extract).

170 *2.9. Antioxidant activity*

171 *2.9.1. Inhibition of lipid peroxidation by Thiobarbituric Acid Reactive Species (TBARS) method*

172 This assay was performed using two distinct lipid substrates: egg yolk and liposomes.

173 The TBARS assay is the most commonly used method for measuring lipid peroxidation.
174 The assay was carried out as described by Boulanouar et al. (2013). Briefly, egg yolk
175 (100 mg/mL) (250 µL) homogenates were used as a lipid-rich medium and mixed with acetic
176 acid (750 µL) and the solution of TBA (8 mg/mL) (750 µL) dissolved in sodium dodecyl
177 sulfate (SDS) (11 mg/mL). The method involved heating the reaction mixture for one hour in
178 water bath at 95 °C. After cooling at room temperature, the final volume was adjusted to 2 mL
179 by adding butanol followed by vigorous vortexing and centrifugation for 10 min at 3,000 *g*.
180 The absorbance of the upper layer was measured at 532 nm against a blank that contained all
181 reagents minus the sample; the percentage of inhibition was calculated as follows:

182 $[(A_0 - A_1 / A_0) \times 100]$, in which A_0 is the absorbance of the control reaction (without
183 extract), and A_1 is the absorbance of the extracts. Analyses were run in triplicate. The

184 inhibition percentage was plotted against extract concentration (w/v) and IC_{50} values were
185 determined (concentration of extract able to prevent 50% of lipid peroxidation).

186 The liposome assay started with the preparation of liposome solution: 0.4 g lecithin in
187 80 mL chloroform (Boulanouar et al., 2013). This solution was dried and submitted to
188 nitrogen flux for 30 s and then to vacuum for at least two hours until complete dryness. In
189 brief, the reaction was initiated by mixing 50 µL of different extract dilution with 100 µL of
190 liposome suspension, Fe Cl_3 (100 µL, 4 mM) and 50 µL of ascorbic acid (0.18 mg/mL diluted
191 to 1/10). After incubation at 37 °C for one hour, 2 mL of TBA solution (0.6%) was added and
192 the resulting mixture was further heated for 10 min at 95 °C. Afterwards, 2 mL butanol was
193 subsequently added and final solution was centrifuged for 5 min at 3,000 *g*. The absorbance of
194 the supernatant was determined at 532 nm. Tests were carried out in triplicate. The assay was
195 performed as reported above for thiobarbituric acid reactive species (TBARS) method.

196 *2.9.2. Ability for scavenging $ABTS^{+\bullet}$ free radicals*

197 The scavenging activity against ABTS cation [2,2-azinobis(3-ethylbenzothiazoline)-6-
198 sulfonic acid)] radical assay was carried out as described by Boulanouar et al. (2013). The
199 $ABTS^{+\bullet}$ solution was diluted with ethanol to obtain an absorbance ranging between 0.700 and
200 0.800, at 734 nm. Then, 25 µL of samples were mixed to 275 µL $ABTS^{+\bullet}$ solution and the
201 absorbance was read at 734 nm and recorded for 6 min. Three independent experiments were
202 performed. The ABTS radical cation scavenging activity was expressed as:

203 $[(A_0 - A_1 / A_0) \times 100]$, in which A_0 is the absorbance of the control reaction (without extract),
204 and A_1 is the absorbance of the extract. The results were expressed as IC_{50} values.

205 *2.9.3. Ability for scavenging DPPH free radicals*

206 The method of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity
207 previously reported by Boulanouar et al. (2013) was followed by mixing a methanolic solution
208 of DPPH 25 µM (275 µL) with extract (25 µL) and then maintained in the dark at room
209 temperature for 1 h. The absorbance of the reaction mixture was measured at 517 nm. The
210 percentage inhibition of the DPPH radical of samples was calculated by the formula:

211 $[(A_0 - A_1 / A_0) \times 100]$ in which A_1 and A_0 are absorbances of solvent with and without
212 sample, respectively. The results were expressed as IC_{50} values.

213 *2.9.4. Total antioxidant activity (Phosphomolybdenum assay)*

214 The total antioxidant activity of the samples was evaluated by phosphomolybdenum
215 method as reported by Zengin et al. (2015) with slight modifications. Fifty microliters of
216 sample were added to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate
217 and 4 mM ammonium molybdate). The mixture was incubated for 90 min at 95 ºC and the
218 absorbance was measured at 695 nm. The total antioxidant capacity was expressed as Ascorbic
219 Acid equivalent (mg AA/mL extract)

220 *2.9.5. Ferric reducing power activity*

221 The reducing power of each extract was measured as previously reported by Boulanouar et
222 al. (2013) with slight modifications. Fifty microliters of each sample was mixed to 0.5 mL of
223 potassium phosphate buffer (200 mM, pH 6) and 0.5 mL of potassium ferricyanide (1%). The
224 reaction mixtures were incubated at 50 °C in a water bath, for 20 min .Subsequently, 0.5 mL
225 of trichloroacetic acid (10%, w/v) was mixed and the solution was centrifuged at 3,000 *g*, for
226 10 min. Finally, distilled water (0.5 mL) and 0.1 mL $FeCl_3$ (0.1%, w/v) were added to the
227 supernatant and the absorbance was measured at 700 nm. The ferric reducing power capacities
228 of the extracts were expressed graphically by plotting absorbance against concentration.

229 *2.9.6. Ferrous chelating metal ions assay*

230 Ferrozine can chelate with Fe^{2+} and form a complex with a red color which can be
231 quantified. The ferrous ion-chelating effect of all extracts was estimated according to Aazza et
232 al. (2013). Briefly, the reaction was initiated by mixing samples with 25 µL of $FeSO_4 \cdot 7H_2O$
233 (0.52 mg/mL), 150 µL of distilled water and 25 µL of ferrozine (2.5 mg/mL). The absorbance
234 of the reaction mixture was measured at 562 nm. The ratio of inhibition of ferrozine–Fe^{2+}
235 complex formation was calculated as follows: $[(A_0–A_1 /A_0) \times 100]$ in which A_0 is the
236 absorbance of the control and A_1 is the absorbance of the test sample. The results were
237 expressed as IC_{50} values.

238 *2.9.7. Nitric oxide scavenging capacity*

239 The nitric oxide (NO) scavenging activity of samples was determinate in accordance with
240 the method previously reported by Aazza et al. (2013) with slight modifications. In brief,
241 75 µL of extracts were added to 75 µL of 10 mM sodium nitroprusside into a 96-well plate
242 and left standing at ambient temperature for 35 min. The reaction was initiated by adding
243 Griess reagent [40 µL of sulfanilamide solution and 40 µL of *N*-1-napthylethylenediamine
244 dihydrochloride (NED) solution] to the mixture and the absorbance was measured at 532 nm
245 without previous incubation. The percentage of inhibition was calculated from the formula:
246 $[(A_0–A_1 /A_0) \times 100]$ where A_0 is the absorbance of the control reaction (without extract), and
247 A_1 is the absorbance of the extracts. The results were expressed as IC_{50} values.

248 *2.9.8. Superoxide anion scavenging activity (non-enzymatic method)*

249 The superoxide anion scavenging activity of samples was determinate according to the
250 method previously described by Albano and Miguel (2010). The method involved mixing the
251 extract (25 µL) with 25 µL nitroblue tetrazolium (0.42 mg/mL) and 25 µL NADH
252 (1.32 mg/mL) and ethanol (125 µL) followed by 25 µL phenazine methosulfate (PMS)

253 (0.25 mg/mL). The absorbance was measured at 560 nm, after 10 minutes of reaction. The
254 decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the
255 reaction mixture. Tests were carried out in triplicate. The percentage inhibition was calculated
256 by the formula given bellow: $[(A_0-A_1 /A_0) \times 100]$ in which A_1 and A_0 are absorbances of
257 solvent with and without sample, respectively. The results were expressed as IC_{50} values.

258 *2.10. Inhibitory activity of enzymes*

259 *2.10.1. Lipoxygenase (LOX) inhibitory activity*

260 The inhibition of lipoxygenase enzyme was performed by following the previous method
261 described by El-Guendouz et al. (2016) with some modifications. The reaction consisted on
262 the mixture of 10 µL of each sample and 5 µL of enzyme solution (0.054 g/mL) and 50 µL of
263 linoleic acid (0.001 M) and borate buffer 937 µL (0.1M, pH 9). The measurement of the
264 absorbance was recorded at 234 nm. The analyses were carried out in triplicate. The
265 percentage of inhibition was calculated from the formula: $[(A_0-A_1 /A_0) \times 100]$, where A_0 is the
266 absorbance of the control reaction (without extract), and A_1 is the absorbance of the sample
267 solution. The results were expressed as IC_{50} values.

268 *2.10.2. Acetylcholinesterase (AChE) inhibitory activity*

269 Acetylcholinesterase inhibitory activity was carried out according to the method reported
270 by El-Guendouz et al. (2016) with minor modification. Briefly, an aliquot of sample (60 µL),
271 425 µL Tris-HCl buffer (0.1 M, pH 8) and 25 µL enzyme (0.28 U/mL) were mixed and
272 incubated for 15 min at room temperature. After pre-incubation, 75 µL of substrate (0.005 g of
273 iodine acetylcholine in 10 mL of buffer) and 475 µL of 5,5'-dithiobis(2-nitrobenzoic acid)
274 (DTNB) (0.059 g in 50 mL of buffer) were added to terminate the reaction. The absorbance of
275 the reaction mixture was read after 30 min of incubation. The control was prepared in the
276 same way, except that the sample solution was replaced by Tris-HCl buffer. Each experiment

277 was conducted in triplicate, and enzyme inhibitory rates of samples were calculated as
278 follows: [(A_0−A_1 /A_0) × 100]. The results were expressed as IC_{50} values.

279 *2.10.3. Tyrosinase inhibitory activity*

280 Tyrosinase inhibitory activity was measured according to the method reported by El-
281 Guendouz et al. (2016). An aliquot of each sample (40 µL) was mixed with (10 µL) enzyme
282 solution (100 U/mL) and 140 µL phosphate buffer (50 mM, pH 6.8) in a 96-well microplate.
283 The mixture reaction was incubated for 40 min at room temperature, then 60 µL of L-3,4-
284 dihydroxyphenylalanine (L-DOPA) (5 mM) was finally added as substrate. The absorbance
285 was measured at 492 nm. The percentage of inhibition of tyrosinase activity was calculated as
286 follows: [(A_0−A_1 /A_0) × 100]. The results were expressed as IC_{50} values.

287 *2.11. Statistical analysis*

288 Statistical analysis was performed with the Statistical Package for the Social Sciences
289 (SPSS) 23.0 software (SPSS Inc., Chicago, IL, USA). Statistical comparisons were made with
290 one-way analysis of variance followed by Tukey's multiple comparisons. The level of
291 significance was set at $p < 0.05$. Correlations were achieved by Spearman's correlation
292 coefficient (r) at a significance level of 95% or 99%.

293

294 **3. Results and discussion**

295 *3.1. Zinc content*

296 Table 1 depicts the Zn amounts in roots and aerial parts of fennel, anise and dill. Zn
297 accumulation varied according to the organ. Zn levels recorded in non-treated and treated
298 roots ranged from 21.89 to 89.02 µg/g DW (dry weight) and from 61.02 to 1312.77 µg/g DW,
299 respectively. Concerning the aerial part, anise and dill exposed to Zn excess exhibited a

300 remarkable increase in their Zn content reaching a maximum values of 16940.27 µg/g, DW,
301 and 23665.70 µg/g, DW, respectively.

302 Zinc toxicity involves perturbation of metabolic activity in terms of competition for uptake
303 of other elements and inhibition of enzymatic action (Marichalli et al., 2016). It has been
304 reported by Cayton et al. (1985) that the absorption and translocation of plant nutrients such as
305 Fe, Mg, K, P and Ca were related to Zn concentration in soil. Metal may enter inside plant
306 cells by simple diffusion, passive transport, through channel proteins and finally by active
307 transport through carrier proteins (the most important). Zinc, through this process, enters in the
308 cytosol via members of the ZIP transporter family (ZRT-IRT like protein: **Z**inc-regulated
309 transporter, **I**ron-regulated transporter **P**rotein). After entering, there are mechanisms that
310 establish plant Zn homeostasis. Such may be achieved through Zn efflux transporters which
311 are involved in Zn and other metals redistribution, translocation, and detoxification (Lin and
312 Aarts, 2012). Plants that are unable to prevent entry or improve efflux of Zn or other metals,
313 for surviving, they have other mechanisms that are based on sequestration of such metals in
314 order to store them in storage tissues or specific organelles (e.g. vacuole). For example, the
315 metal tolerance protein 1 (MTP1), is probably the most important Zn vacuolar sequestration
316 transporter in plants (Lin and Aarts, 2012), or the heavy metal ATPase 3 (HMA3) (Chilian et
317 al., 2015).

318 Our findings show that the accumulation of Zn in the aerial part of fennel (independent on
319 the variety) is mediated by different mechanisms when compared to those of dill, in which the
320 amounts of Zn in the aerial parts are much higher than in roots. According to Chilian et al.
321 (2015) such could be attributed to higher HMA4 activity than that of HMA3, because HMA3
322 protein mediates Zn accumulation in root vacuoles, whereas HMA4 is involved in the metal
323 translocation from root towards the shoot (Lin and Aarts, 2012). Following this reasoning, we
324 could hypothesize that in dill, HMA4 could act more intensely than in fennel or anise, due to

325 the higher amounts of Zn in the aerial part. However, these hypotheses need further
326 confirmation since it was not the aim of the present work.

327 *3.2. Effects of Zn on plant growth and its pigment content*

328 Zinc (Zn), manganese (Mn), iron (Fe), copper (Cu) and nickel (Ni) are essential
329 micronutrients necessary for normal plant growth (Bashir et al., 2016; Sarwar et al., 2017).
330 Several cellular organelles need these microelements for plant growth and development. Fe,
331 Cu and Mn regulate chlorophyll synthesis and photosynthesis; Fe and Cu regulate respiration;
332 and zinc is a co-factor required for the structure and function of diverse proteins (more than
333 300 enzymes and 200 transcription factors essential for maintaining membrane integrity,
334 reproduction and auxin metabolism) (Bashir et al., 2016; Grotz and Guerinot, 2006; Singh et
335 al., 2016). Deficiencies or high amounts of such elements may lead to dysfunctional
336 chloroplasts and mitochondria (Bashir et al., 2016). The toxic effects due to higher amounts of
337 essential elements, such as Zn, include inhibition of growth and photosynthesis, altered water
338 balance and nutrient assimilation, senescence and plant death (Singh et al., 2016). Some of
339 these effects were observed in the species and varieties studied in the present work,
340 nevertheless more noticeable in dill and anise plants (Table 2), which can suggest that these
341 species are more sensitive to high concentration of Zn than fennel.

342 Results depicted in Table 2 show that all tested plants remained alive and have grown until
343 the end of treatment, nevertheless a depressing effect caused by Zn exposure in all growth
344 parameters (plant height, root length, fresh and dry weight of aerial parts and roots) was
345 observed. Indeed a strong negative correlation ($p < 0.01$) between Zn amount and growth
346 parameters was estimated, which confirmed the toxic effect of Zn treatment on samples (Table
347 3). The action of Zn on growth plant was more evident in anise, independent on the plant part.

348 For all samples, the results presented in Table 1 indicate that the chlorophyll *a* content
349 (*Chla*) is lower in treated plants comparing with non-treated samples. The same trend was
350 noted for the chlorophyll *b* content (*Chlb*) after the treatment with zinc. The Zn exposure
351 caused a significant decrease of carotenoid contents in all studied samples. The results
352 confirmed that these cultivars of fennel, dill and anise are sensitive to Zn excess.

353 According to the results depicted in Table 4, the measured parameters such as high length,
354 dry weight and fresh weight of roots and aerial part are positively correlated with total
355 chlorophyll and carotenoid contents.

356 Our results also showed a dramatically reduction in carotenoid contents in response to Zn
357 excess as compared to other photosynthetic pigments. In addition, the significant decrease in
358 chlorophyll contents (total chlorophyll (*Chla* + *Chlb*), *Chla*, and *Chlb*) seems to be more
359 pronounced in *Chlb* than *Chla*, which is similar to the results of Marichali et al. (2016) who
360 reported that the content of photosynthetic pigments of *Nigella sativum* was significantly
361 reduced as well as *Chlb* content, being this pigment more sensitive to Zn excess than *Chla*.
362 The photosynthetic pigments decline, induced by Zn, might be resulted from several causes
363 such an iron deficiency; an inhibition of enzyme activities involved in the chlorophyll
364 biosynthesis; inhibition of other enzymes (δ-aminolevulinic acid dehydratase and
365 protochlorophyllide reductases); a Mg removal from chlorophyll; advanced peroxidation of
366 chloroplast membrane lipids by reactive oxygen species (ROS) which would inhibit the
367 reductive steps in the biosynthesis pathway of chlorophylls; degradation of thylakoids; and
368 reduction in the rate of RubisCO synthesis and/or modification in its activities (Di Baccio et
369 al., 2009; Fernàndez-Martínez et al., 2014; Islam et al., 2014; Marichali et al., 2016; Parlak
370 and Yilmaz, 2012; Subba et al., 2014).

371 *3.3. Zn effects on total phenols and flavonoid contents*

372 As shown in Table 5, the accumulation of phenols and flavonoids depends on the variety.
373 Under stressful conditions, the two varieties of fennel plants showed a decrease in phenol and
374 flavonoid contents, whereas in the other species higher accumulation of phenols was detected
375 in the Zn treated plants (dill followed by anise) than in non-treated samples. Data from Table 5
376 show that treatment had a significant increment ($p < 0.05$) in the amount of dihydroflvonols in
377 the F_L as well as in anise, whereas in dill their accumulation in treated plants decreased by
378 two-fold, when compared to the control.

379 The results of the present work show a variability of total phenol contents for the different
380 tested samples. Treated anise and dill samples showed a significant increase of their total
381 phenol concentrations while a reduction in phenolic production was observed for both F_{DF} and
382 F_L cultivars exposed to elevated Zn concentration. Thus, it can be argued that the response in
383 terms of phenolic accumulation due to Zn excess is species dependent. Such higher amounts
384 of total phenols in dill and anise samples were coincident with the highest levels of Zn
385 detected in the same samples. This phenol accumulation may be a response to the oxidative
386 damage induced by Zn in order to permit plant survival, such as previously reported for
387 different plant species by several authors (Marichali et al., 2014; Morina et al., 2010). In fact,
388 some studies have revealed that metal application, including Zn, are associated with the
389 increased activities of enzymes of secondary pathway namely shikimate dehydrogenase,
390 phenylalanine ammonialyase (PAL), and polyphenols oxidase (PPO) (Ali et al., 2006;
391 Castáneda and Pérez, 1996; Van de Mortel, 2006; Wang et al., 2011). Nevertheless, Basak and
392 co-workers (2001) have been reported that *Camellia sinensis*, submitted to Ni treatment,
393 resulted in reduction of their phenolic amounts despite the stimulation of PAL activity as
394 observed for our samples of fennel submitted to Zn. These dissimilar results are in accordance
395 with those obtained in the present work, whereby the effect of metals on phenol accumulation
396 in plants is dependent on the species. Therefore, no significant correlation between aerial part

397 Zn accumulation and phenol content was observed (Table 6). Hence, these results may support
398 the observation of Kováčik et al. (2009) suggesting the use of modulators of phenolic
399 metabolism in response to Ni treatment. In addition, they reported that phenolic accumulation
400 and type of phenol are also dependent on the type of metals and on the plant organ.

401 Most of reports have studied the effect of Zn excess on the oxidative machinery but little
402 information is available about the relationship between Zn excess and the non-enzymatic
403 antioxidant activity.

404 *3.4. Zn effects on antioxidant activities of samples*

405 Generally, the extracts of anise had higher antioxidant activity than fennel (Table 7). On
406 the other hand, the zinc exposure enhanced the activity in dill and anise extracts, in contrast to
407 the fennel extracts. In this case and in both varieties, zinc had a negative effect on the
408 antioxidant activity (Table 7). Accordingly, our results revealed that treated samples, rich in
409 polyphenols, when compared to the non-treated ones, responded by an enhancement of their
410 capacity for scavenging DPPH, ABTS, nitric oxide and superoxide radicals, as well as
411 through the total antioxidant measured by phosphomolybdenum method. The antioxidant
412 activity correlated well with the phenols' amounts. FRAP assay showed that dill extracts
413 (treated and non-treated), and Zn-treated anise extract possessed the greatest reducing power
414 followed by anise, and fennel (F_L and F_L Zn). However, treated and non-treated F_{DF} was not
415 able to reduce the iron metal ion. Moreover, the activity was dose-dependent (Figure 1).

416 In order to discuss our results, we present a reference Table gathering the previous
417 investigations about fennel, anise and dill (Table S1) which is organized as follows: the type
418 of assay studied in the work followed by the plant part used, extraction type as well the
419 solvent used and, finally, the chemical composition for finding a possible relationship
420 between activity and chemical composition.

421 The results found for fennel samples are within the range reported by other authors for
422 extracts obtained by diverse methods, but particularly for DPPH method, and solvents (Table
423 S1). This Table only compiles antioxidant activities, measured through diverse methods, of
424 the aerial parts of fennel, anise and dill as a whole or as parts (leaves, stems, shoots,
425 inflorescences and flowers). The term "Plant material" was also considered. Fruits and seeds
426 were not taken into account, because we considered that we should compare results obtained
427 from the same plant part. In the evaluation of the antioxidant activity, the authors almost
428 always use, at least two assays, although the capacity for scavenging DPPH radicals
429 predominates in practically all works. Beyond the diversity of unities used by the authors for
430 presenting the results, Table S1 permit to show great differences among the results of
431 antioxidant activity, whereby our ones are within the large range found by other authors. Type
432 of extraction and solvent, part of plant used and plant itself can be altogether factors that
433 determine this variability.

434 In the majority of cases, the chemical composition of the extracts was not performed,
435 nevertheless in those in which such was done (nine), practically did not contribute to correlate
436 the activity with the components identified in the extracts. Only in three cases, the authors
437 described a correlation between the antioxidant activity of extracts with some components
438 detected in the same extracts (Faudale et al., 2008; Hossain et al., 2011; Parejo et al., 2004).
439 In these cases, the authors considered that chlorogenic acid isomers, rosmarinic acid, gallic
440 acid and luteolin-7-*O*-glucoside were determinant in the antioxidant activities found in
441 extracts.

442 Independent on the assay tested, the authors of the works (Table S1) found a correlation
443 between the activities and the concentration of total phenols, such was observed in our
444 investigation (Table 8). In the present work, a correlation was also found between the
445 amounts of flavones/flavonols and antioxidant activity, but not detected between activity and

446 flavanones and di-hydroflavonols (Table 8). However, the capacity for preventing lipid
447 peroxidation, either using egg yolk or liposomes as lipid substrates, as well as the capacity for
448 scavenging NO free radicals did not correlate with total phenol content. Only the
449 concentrations flavone/flavonols correlate with the capacity for preventing lipid oxidation,
450 when egg yolk was used as lipid substrate, although no correlation had been found when
451 liposomes constitute the lipid substrate of the reaction (Table 8).

452 The antioxidant activity of the aerial parts of anise and dill (Table S1) was also reviewed
453 and much lower references were found when compared to fennel. When the activity unities
454 were presented as IC_{50}, it was possible to compare the results of the present work with them.
455 They were within the same range. Correlations between antioxidant activities of extracts and
456 the phenols identified in the same extracts were only reported by Zielinski et al. (2015) and
457 Stankevičius et al. (2011) for anise and dill samples, respectively (Table S1). The former
458 attributed the capacity for scavenging DPPH free radicals to gallic acid, catechin, *epi*catechin
459 and quercetin. Although the importance of the identification of the phenol compounds of the
460 extracts by the authors, they did not give additional information about their role as
461 antioxidants. Nevertheless they also had detected correlation between the concentration of
462 total phenols and antioxidant activity as observed in the present work in the majority of tests.

463 Despite numerous papers describing different antioxidant assays testing the ability of
464 fennel, dill and anise extracts, little investigations has been performed for evaluating the
465 potentiality of these plants under Zn excess conditions, whereby our work permit finding the
466 effect of this metal not only in the production of phenols but also in the antioxidant activity.
467 Such results allowed detecting that fennel, independent on the variety, presented a negative
468 effect by decreasing the activity whereas in anise and dill such was not evident.

469 The accumulation of low molecular phenols by plants in stress or pollution conditions has
470 been reported by diverse authors. According to Mongkhonsin et al. (2016), caffeic acid and
471 rutin were at higher amounts in the leaves of *Gynura pseudochina* treated with zinc and/or
472 cadmium. However, there was a threshold above which a decrease in the contents of those
473 compounds was observed. In the same work, the authors also reported that higher amounts of
474 rutin and caffeic acid correspond stronger capacity for scavenging DPPH free radicals.
475 *Echium vulgare* plants exposed to Zn or lead (Pb) stress increased accumulation of
476 chlorogenic acid and rosmarinic acid (Dresler et al. (2017). The young leaves of *Betula*
477 *pubescens* trees, near to copper-nickel smelter (Finland), had higher amounts of 1-*O*-galloyl-
478 *β*-D-(2-*O*-acetyl)-glucopyranose, neochlorogenic acid (*trans*-5-caffeoylquinic acid), *trans*-5-
479 *p*-coumaroylquinic acid and quercetin-3-*O*-*β*-D-galactopyranoside. Mature leaves had higher
480 concentrations of 1-*O*-galloyl-*β*-D-(2-*O*-acetyl)-glucopyranose and neochlorogenic acid
481 (Loponen et al., 1997). Some of these compounds were reported by some authors (Faudale et
482 al., 2008; Hossain et al., 2011; Parejo et al., 2004; Stankevičius et al., 2011; Zielinsli et al.,
483 2015) (Table 8) as being constituents of fennel, anise and dill that correlated well with the
484 capacity for scavenging free radicals.

485 Recently, the findings of Marichali et al. (2016) highlighted the importance of studying the
486 induced-Zn toxicity in Tunisia toward antioxidant response of *Nigella sativa*. The results
487 revealed that treatment of *N. sativa* with Zn excess was accompanied by an enhanced
488 antioxidant activity of this plant. On the other hand, in comparison with previously tested
489 metals, such increase was observed in FRAP values assay which indicates that excess of boron
490 application stimulates the non-enzymatic antioxidant mechanism in the apple rootstock EM 9
491 explants (Molassiotis et al., 2006). These results supported the conclusion of Elzaawely et al.
492 (2007) suggesting that improvement of antioxidant activity of plants may be caused by their
493 exposure to abiotic stress. Copper-treated plants, with higher amount of phenols, were found

494 to increase their antioxidant activity determined by DPPH radical scavenging and β-carotene
495 bleaching methods, which could be suggested as a protective response of these plants from
496 copper induced damages (Elzaawely et al., 2007).

497 Anise plants under Zn toxicity, with higher amounts of phenolics, revealed a capacity for
498 metal chelating four-fold more efficient than non-treated plants (Table 7). All treated samples,
499 without exception, increase their ability to inhibit lipid peroxidation, particularly when
500 liposomes were used as lipid substrate, in response to Zn stress. These results explain the
501 strong correlation between the effective ability of samples against lipid peroxidation and their
502 Zn accumulation (Table 6). In contrast, the higher accumulation of Zn in all treated samples
503 did not correspond to stronger capacity for scavenging free radicals (DPPH, ABTS,
504 superoxide, NO) in all samples, which may explain the absence of correlation between zinc
505 accumulation and capacity for scavenging some types of free radicals. Zinc is a mineral
506 antioxidant, such as selenium, that does not act on free radicals, but act preventing lipid
507 peroxidation (Prasad et al., 2004).

508 *3.5. Zn effects on inhibitory activity of enzymes*

509 Inhibitory activity of extracts on lipoxygenase (LOX), acetylcholinesterase (AChE) and
510 tyrosinase were performed.

511 Anise extracts exhibited strong LOX inhibitory activity with IC_{50} values of 0.015 and
512 0.035 mg/mL for treated and non-treated samples, respectively. F_L treated samples (F_LZn)
513 showed the lowest ability to inhibit LOX activity (IC_{50} = 0.062 mg/mL), followed by non-
514 treated ones (IC_{50} = 0.052 mg/mL). The extract from non-treated F_{DF} plants revealed an IC_{50}
515 value of 0.049 mg/mL, whereas Zn treated plants ($F_{DF}Zn$) did not present the ability for
516 inhibiting 5-LOX enzyme. The exposure to Zn excess showed insignificant changes in the

517 potency of dill extract as a LOX inhibitor (Table 9). These results implying that Zn application
518 influence the LOX inhibitory capacity of samples but depending on variety and species.

519 The LOX inhibition assay can be considered not only as an indicator of antioxidant but
520 also an indicator of possible anti-inflammatory activity (Albano and Miguel, 2010;
521 Kontogiorgis et al., 2016). These authors studied the antioxidant activity and LOX inhibitor
522 activity of *F. vulgare* extracts. However, there is no study, at least to our knowledge, focused
523 on fennel and anise Zn treated extracts as enzymatic inhibitors. It has been previously ascribed
524 by Molassiotis et al. (2006) that treatment with boron was associated with an enhancement in
525 LOX activity in leaves and stems of explants of the apple rootstock EM 9 (*Malus domestica*
526 Borkh). According to these authors, LOX activity may be considered as indicator of oxidative
527 stress. Indeed, it has been suggested that propagation of lipid peroxidation in plants under
528 stress condition induced by higher lipolytic activity on the membrane was associated with
529 stimulation of LOX activity (Lacan and Baccou, 1998).

530 As shown in Table 10, all samples had a significantly moderate ability for inhibiting AchE
531 except F_{DF} samples (treated and non-treated), which were not able to inhibit AchE. Moreover,
532 different trends were observed in the studied varieties after treatment with Zn. Extracts from
533 Zn stressed F_L and anise plants revealed higher capacity of AchE inhibition than extracts from
534 the respective non-treated plants, while the opposite occurred with the extracts form dill plants
535 treated and non-treated with Zn.

536 Up to date, searching for new AchE inhibitors derived from natural sources with few side
537 effects is required (Hasbal et al., 2014) but no relevant studies have been reported about
538 anticholinesterase activity of fennel, dill and anise exposed to Zn excess which prompted us to
539 concentrate on the efficiency of our samples for AchE inhibition. Among several
540 investigations, Gomathi and Manian (2015) suggested the effectively AchE inhibitory effect

541 stem bark and leaves of *Stevia crenata* might be attributed to their increased amounts of non-
542 enzymatic antioxidants.

543 In this work, although anise and some fennel plants were shown to possess an inhibitory
544 effect against AchE, it was not revealed a significant correlation between AchE inhibitory
545 activity and phenolic compounds including dihydroflavonols, suggesting the contribution of
546 other bioactive constituents against AchE (Table 6). It is noteworthy to refer that there is not a
547 clear trend on the AchE inhibitory activity of tested samples under Zn excess. In fact, no
548 correlation between zinc content and the inhibitory effect of samples against AchE was
549 observed in the present data (Table 6), and to best of our knowledge, scientific information
550 remains absent for better understanding the zinc contributing function in the inhibitory AchE
551 activity.

552 In the present research, all tested extracts exhibited tyrosinase inhibitory activities; however
553 these were higher for dill and anise plants than for both F_{DF} and F_L cultivars, which were in the
554 lower range (Table 9). In addition, the inhibitory activity values of dill and anise Zn treated
555 extracts were 1.3 fold higher than those measured for non-treated extracts, suggesting that Zn
556 treatment increased the ability of samples to inhibit tyrosinase enzyme. The extract form dill
557 plants treated with Zn was found as a potent tyrosinase inhibitor. The inhibitory activity of
558 tyrosinase of dill extracts was better than those reported by Orhan et al. (2013). The tyrosinase
559 inhibitory activity, expressed as IC_{50}, was negatively correlated with the amounts of total
560 phenols and flavonoids (Table 8), which can be explained by the essential role of hydroxyl
561 group of phenolic compound for forming hydrogen bond with a site of tyrosinase enzyme.

562 There was no correlation between zinc accumulation and antityrosinase activity.
563 Tyrosinase inhibitory activity of kojic acid and its synthetic derivatives is related to the ability
564 to coordinate metals (Lachowicz et al. 2015). Synthetic kojic acid derivatives obtained by

565 these authors had affinity for Fe^{3+}, Al^{3+}, Zn^{2+}, and Cu^{2+}. However, the chelating capacity for
566 Zn^{2+}, and Cu^{2+} was weaker. The absence of correlation between zinc accumulation and
567 antityrosinase activity of extracts may be supported by the finding of Lachowicz et al. (2015).
568 The chelating ability of the compounds present in the extracts with antityrosinase activity for
569 zinc is weak and, consequently, without any supplemental inhibitory effect on tyrosinase
570 activity.

571

572 **4. Conclusions**

573 The results of the present work showed that growth parameters and photosynthetic
574 pigment contents were negatively affected by Zn treatment but depending on the plant species
575 and even variety. An accumulation of zinc was observed in both roots and aerial parts of all
576 species studied, though more noticeable in the aerial parts of anise and dill. The plants
577 responded differently to Zn excess exposure, especially in their accumulation of phenols and,
578 therefore, in their abilities for scavenging free radicals and inhibit tyrosinase activity. The
579 accumulation of zinc in plants was only negatively correlated with the IC_{50} values of TBARS
580 assay, independent on the lipid substrate used (egg yolk and liposomes), which may indicate
581 the positive role of zinc on the prevention of lipid peroxidation.

582 The extracts of zinc-treated anise and dill plants had higher antioxidant activity and the
583 extracts of anise-treated plants also had higher ability for inhibiting the activity of
584 acetylcholinesterase, lipoxygenase and tyrosinase. This study gives new insights about the
585 effect of Zn excess exposure on the ability of tested extracts against some enzymes but it is
586 important to pay attention about the high accumulation of Zn in the aerial parts of these plants
587 which make their consumption dangerous for human health, due to the toxicity of this metal.
588 On the other hand, the ability of dill and anise plants to accumulate high amounts of Zn raises
589 the hypothesis of their use for bioremediation of soils contaminated with this metal.

590

591 **Acknowledgements**

592 The authors wish to acknowledge the financial support provided by the Portuguese
593 National Funding Agency for Science, Research and Technology (*Fundação para a Ciência e*
594 *Tecnologia* – FCT; Portugal), under the projects UID/BIA/04325/2013 – MEDTBIO and
595 UID/Multi/04326/2013.

596

597 **References**

598 Aazza, S.S., Lyoussi, B., Antunes, D.D., Miguel, M.G., 2013. Physicochemical
599 caracterization and antioxidant activity of commercial Portuguese honeys. J. Food. Sci. 78,
600 1159–1165.

601 Albano, S.M., Miguel, M.G., 2010. Biological activities of extracts of plants grown in
602 Portugal. Ind. Crops Prod. 33, 338–343.

603 Ali, M.B., Singh, N., Shohael, A.M., Hahn, E.J., Paek, K.Y., 2006. Phenolics metabolism and
604 lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress.
605 Plant Sci.171, 147–154.

606 Badgujar, S.B., Patel, V.V., Bandivdekar, A.H., 2014. *Foeniculum vulgare* Mill: A review of
607 its botany, phytochemistry, pharmacology, contemporary application, and toxicology.
608 BioMed Res. Int. 1- 31. http://dx.doi.org/10.1155/2014/842674.

609 Barros, L., Carvalho, A.M., Ferreira, I.C.F.R., 2010. The nutritional composition of fennel
610 (*Foeniculum vulgare*): shoots, leaves, stems and inflorescences. LWT - Food. Sci. Technol.
611 43, 814–818.

612 Barros, L., Heleno, S.A., Carvalho, A.M., Ferreira, I.C.F.R., 2009. Systematic evaluation of
613 the antioxidant potential of different parts of *Foeniculum vulgare* Mill. from Portugal. Food
614 Chem. Toxicol. 47, 2458–2464.

615 Basak, M., Sharma, M., Chakraborty, U., 2001. Biochemical responses of *Camellia sinensis*
616 (L.) O. Kuntze to heavy metal stress. J. Environ. Biol. 22, 37–41.

617 Bashir, K., Rasheed, S., Kobayashi, T., Seki, M., Nishizawa, N.K., 2016. Regulating
618 subcellular metal homeostasis: the key to crop improvement. Front. Plant Sci. 7, 1192. doi:
619 10.3389/fpls.2016.01192.

620 Boulanouar, B., Abdelaziz, G., Aazza, S., Gago, C., Miguel, M.G., 2013. Antioxidant
621 activities of eight Algerian plant extracts and two essential oils. Ind. Crops Prod. 46, 85–96.

622 Castáneda, P., Pérez, L.M., 1996. Calcium ions promote the response of *Citrus limon* against
623 fungal elicitor sorwounding. Phytochemistry. 42, 595–598.

624 Cayton, M.T.C., Reyes, E.D., Neue, H.U., 1985. Effect of zinc fertilization on the mineral
625 nutrition of rice differing in tolerance to zinc deficiency. Plant Soil 87, 319–327.

626 Chilian, A., Bancuta, R.O., Bancuta, I., Setnescu, R., Ion, R.M., Radulescu, C., Setnescu, T.,
627 Stihi, C., Gheboianu, A.I., Chelarescu, E.D., 2015. Study of the influence of Zn concentration
628 on the absorption and transport of Fe in maize by AAS and EDXRF analysis techniques.
629 Rom. Rep. Phys. 67, 1138–1151.

630 Dhankhar, R., Sainger, P.A., Sainger, M., 2012. Phytoextraction of zinc: physiological and
631 molecular mechanism. Soil Sediment. Contam. 21, 115–133.

632 Di Baccio, D., Tognetti, R., Minnocci, A., Sebastiani, L., 2009. Responses of the *Populus x*
633 *euramericana* clone I-214 to excess zinc: Carbon assimilation, structural modification, metal
634 distribution and cellular localization. Environ. Exp. Bot. 67, 153–163.

635 Dresler, S., Wójciak-Kosior, M., Sowa, I., Stanisłaski, G., Bany, I., Wójcik, M., 2017. Effect
636 of short-term Zn/Pb or long term multi-metal stress on physiological and morphological
637 parameters of mettalicous and nonmetallicolous *Echium vulgare* L. populations. Plant
638 Physiol. Biochem. 115, 380–389.

639 Dua, A., Garg, G., Mahajan, R., 2013. Polyphenols, flavonoids and antimicrobial properties
640 of methanolic extract of fennel (*Foeniculum vulgare Miller*). Eur. J. Exp. Biol. 3, 203–208.

641 El-Guendouz, S., Aazza, S., Lyoussi, B., Antunes, M.D., Faleiro, M.L., Miguel, M.G., 2016.
642 Anti-acetylcholinesterase, antidiabetic, anti-inflammatory, antityrosinase and antixanthine
643 oxidase activities of Moroccan propolis. Int. J. Food Sci. Technol. 51, 1762–1773.

644 Elzaawely, A.A., Xuan, T.D., Tawata, S., 2007. Changes in essential oil, kava pyrones and
645 total phenolics of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. leaves exposed to copper
646 sulphate. Environ. Exp. Bot. 59, 347−353.

647 Faudale, M., Viladomat, F., Bastida, J., Poli, F., Codina, C., 2008. Antioxidant activity and
648 phenolic composition of wild, edible, and medicinal fennel from different Mediterranean
649 countries. J. Agric. Food Chem. 56, 1912–1920.

650 Fernàndez-Martínez, J., Zacchini, M., Fernández-Marín, B., García-Plazaola, J.I., Fleck, I.,
651 2014. Gas-exchange, photo- and antioxidant protection, and metal accumulation in I-214 and
652 Eridano *Populus* sp. clones subjected to elevated zinc concentrations. Environ. Exp. Bot. 107,
653 144–153.

654 Fujiwara, T., Kawachi, M., Sato, Y., Mori, H., Kutsuna, N., Hasezawa, S., Maeshima, M.,
655 2015. A high molecular mass zinc transporter MTP12 forms a functional heteromeric
656 complex with MTP5 in the Golgi in *Arabidopsis thaliana*. FEBS. J. 282, 1965−1979.

657 Gomathi, R., Manian, S., 2015. Analgesic and acetylcholinesterase inhibition potential of
658 polyphenols from *Scolopia crenata* (Flacourtiaceae): An endemic medicinal plant of India.
659 Ind. Crops Prod .73, 134–143.

660 Grotz, N., Guerinot, M.L., 2006. Molecular aspects of Cu, Fe and Zn homeostasis in plants.
661 Biochim. Biophys. Acta 1763, 595–608.

662 Hasbal, G., Yilmaz-Ozden, T., Can, A., 2014. Antioxidant and antiacetylcholinesterase
663 activities of *Sorbus torminalis* (L.) Crantz (wild service tree) fruits. J. Food Drug Anal. 23,
664 57–62.

665 Hossain, M.B., Patras, A., Barry-Ryan, C., Martin-Diana, A.B., Brunton, N.P., 2011.
666 Application of principal component and hierarchical cluster analysis to classify different
667 species based on *in vitro* antioxidant activity and individual polyphenolic antioxidant
668 components. J. Funct. Foods 3, 179–189.

669 Islam, F., Yasmeen, T., Riaz, M., Arif, M.S., Ali, S., Raza, S.H., 2014. *Proteus mirabilis*
670 alleviates zinc toxicity by preventing oxidative stress in maize (*Zea mays*) plants. Ecotoxicol.
671 Environ. Saf. 110, 143–152.

672 Kontogiorgis, C., Deligiannidou, G.E., Hadjipavlou-Litina, D., Lazari, D., Papadopoulos, A.,
673 2016. Antioxidant protection: the contribution of proper preparation of fennel (*Foeniculum*
674 *vulgare* Mill.) beverage. Ind. Crops Prod. 79, 57–62.

675

676 Kovácik, J., Klejdus, B., Backor, M., 2009. Phenolic metabolism of *Matricaria chamomilla*
677 plants exposed to nickel. J. Plant Physiol. 166, 1460–1464.

678 Lacan, D., Baccou, J.C., 1998. High levels of antioxidant enzymes correlate with delayed
679 senescence in nonnetted muskmelon fruits. Planta 204, 377–382.

680 Lachowicz, I.J., Nurchi, V.M., Crisponi, G., Pelaez, J.G.M., Rescigno, A., Stefanowicz, P.,
681 Cal, M., Szewczuk, Z., 2015. Metal coordination and tyrosinase inhibition studies with Kojic-
682 βAla-Kojic. J. Inorg. Biochem. 151, 36–43.

683 Levitt, J., 1980. Responses of Plant to environmental stress chilling, freezing, and high
684 temperature stresses, second ed. Academic Press, New York.

685 Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic
686 biomembranes. Methods Enzymol. 148, 350–382.

687 Lin, Y.F., Aarts, M.G.M., 2012. The molecular mechanism of zinc and cadmium stress
688 response in plants. Cell. Mol. Life Sci. 69, 3187–3206.

689 Loponen, J., Ossipov, V., Koricheva, J., Haukioja, E., Pihlaja, K., 1997. Low molecular mass
690 phenolics in foliage of *Betula pubescens* Ehrh. In relation to aerial pollution. Chemosphere
691 34, 687−697.

692 Marichali, A., Dallali, S., Ouerghemmi, S., Sebei, H., Casabianca, H., Hosni, K., 2016.
693 Responses of *Nigella sativa* L. to zinc excess: Focus on germination, growth, yield and yield
694 components lipid and terpene metabolism and total phenolics and antioxidant activities. J.
695 Agric. Food Chem. 64, 1664−1675.

696 Marichali, A., Dallali, S., Ouerghemmi, S., Sebei, H., Hosni, K., 2014. Germination, morpho-
697 physiological and biochemical responses of coriander (*Coriandrum sativum* L.) to zinc
698 excess. Ind. Crops Prod. 55, 248−257.

699 Martins, N., Barros, L., Santos-Buelga, C., Ferreira, I.C.F.R., 2016. Antioxidant potential of
700 two Apiaceae plant extracts: A comparative study focused on the phenolic composition. Ind.
701 Crops Prod. 79, 188–194.

702 Molassiotis, A., Sotiropoulos,T., Tanou, G., Diamantidis, G., Therios, I., 2006. Boron-
703 induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of
704 the apple rootstock EM 9 (*Malus domestica* Borkh). Environ. Exp. Bot. 56, 54–62.

705 Mongkhonsin, B., Nakbampote, W., Hokura, A., Nuengchamnong, N., Maneechai, S., 2016.
706 Phenolic compounds responding to zinc and/or cadmium treatments in *Gynura pseudochina*
707 (L.) DC. extracts and biomass. Plant Physiol. Biochem. 109, 549–560.

708 Morina, F., Jovanovic, L., Mojovic, M., Vidovic, M., Pankovic, D., Jovanovic, S.V., 2010.
709 Zn-induced oxidative stress in *Verbascum Thapsus* is caused by an accumulation of reactive
710 oxygen species and quinhydrone in the cell wall. Physiol. Plant. 140, 209–224.

711 Muckensturm, B., Foechterlen, D., Reduron, J.P., Danton, P., Hildenbrand, M., 1997.
712 Phytochemical and chemotaxonomic studies of *Foeniculum vulgare*. Biochem. Syst. Ecol. 25,
713 353–358.

714 Orhan, I.E., Senol, F.S, Ozturk, N., Celik, S.A., Pulur, A., Kan, Y., 2013. Phytochemical
715 contents and enzyme inhibitory and antioxidant properties of *Anethum graveolens* L. (dill)
716 samples cultivated under organic and conventional agricultural conditions. Food Chem. 59,
717 96–103.

718 Oshaghi, E.A., Khodadadi, I., Mirzaei, F., Khazaei, M., Tavilani, H., Goodarzi, M.T., 2017.
719 Methanolic extract of dill leaves inhibits AGEs formation and shows potential
720 hepatoprotective effects in CCl_4 induced liver toxicity in rats. J. Pharm.
721 https://doi.org/10.1155/2017/6081374.

722 Paradisone, V., Barrameda-Medina, Y., Montesinos-Pereira, D., Romeiro, L., Esposito, S.,
723 Ruiz, J.M., 2015. Roles of some nitrogenous compounds protectors in the resistance to zinc
724 toxicity in *Lactuca sativa* cv. Phillipus and *Brassica oleracea* cv. Bronco. Acta Physiol.
725 Plant. 37, 137. doi:10.1007/s11738-015-1893-9.

726 Parejo, I., Viladomat, F., Bastida, J., Schmeda-Hirschmann, G., Burillo, J., Codina, C., 2004.
727 Bioguided isolation and identification of the non-volatile antioxidant compounds from fennel
728 (*Foeniculum vulgare* Mill.) waste. J. Agric. Food Chem. 52, 1890–1897.

729 Parlak, K.U., Yilmaz, D.D., 2012. Response of antioxidant defences to Zn stress in three
730 duckweed species. Ecotoxicol. Environ. Saf. 85, 52–58.

731 Prasad, A.S., Bao, B., Beck, F.W.J., Kucuk, O., Sarkar, F.H., 2004. Antioxidant effect of zinc
732 in humans. Free Rad. Bio. Med. 37, 1182–1190.

733 Sarwar, N., Imran, M., Shaheen, M.R., Ishaque, W., Kamran, M.A., Matloob, A., Rehim, A.,
734 Hussain, S., 2017. Phytoremediation strategies for soils contaminated with heavy metals:
735 modifications and future perspectives. Chemosphere 171, 710–721.

736 Sebei, A., Chaabani, F., Ouerfelli, M.K., 2005. Impacts of mining wastes on the soil and
737 plants in the Boujaber area (NW. Tunisia). Chemical fractionation of heavy metals in soils.
738 Geo-Eco-Trop 29, 37–50.

739 Sebei, A., Chaabani, F., Ouerfelli, M.K., Abdeljaoued, S., 2006. Evaluation de la
740 contamination des sols par des métaux lourds dans la région minière de Fedj Lahdoum (NW
741 de la Tunisie). Rev. Méditer. Environ. 1, 1–13.

742 Senatore, F., Oliviero, F., Scandolera, E., Taglialatela-Scafati, O., Roscigno, G., Zaccardelli,
743 M., De Falco, E., 2013. Chemical composition, antimicrobial and antioxidant activities of
744 anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp.
745 *vulgare* var. *azoricum* (Mill.) Thell]. Fitoterapia 90, 214–219.

746 Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy metal tolerance in
747 plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6,
748 1143. doi: 10.3389/fpls.2015.01143.

749 Stankevičius, M., Akuņeca, I., Jākobsone, I., Marušca, A., 2011. Comparative analysis of
750 radical scavenging and antioxidant activity of phenolic compounds present in everyday use
751 spice plants by means of spectrophotometric and chromatographic methods. J. Sep. Sci. 34,
752 1261–1267.

753 Subba, P., Mukhopadhyay, M., Mahato, S. K., Bhutia, K.D., Mondal, T.K., Ghosh, S.K.,
754 2014. Zinc stress induces physiological, ultrastructural and biochemical changes in mandarin
755 orange (*Citrus reticulata* Blanco) seedlings. Physiol. Mol. Biol. Plants. 20, 461−473.

756 Van de Mortel, J.E., Villanueva, L.A., Schat, H., Kwekkeboom, J., Coughlan, S., Moerland,
757 P.D., VanThemaat, E.V.L., Koornneef, M., Aarts, M.G.M., 2006. Large expression
758 differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis
759 distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi*
760 *caerulescens*. Plant Physiol.142, 1127–1147.

761 Wang, C., Lu, J., Zhang, S., Wang, P.F., Hou, J., Qian, J., 2011. Effects of Pb stress on
762 nutrient uptake and secondary metabolism in submerged macrophyte *Vallisneria natans*.
763 Ecotoxicol. Environ. Saf. 74, 1297–1303.

764 Zehtab-Salmasi, S., Chassemi-Golezami, K., Moghbeli, S., 2006. Effect of sowing date and
765 limited irrigation on theseed yield and quality of dill (*Anethum graveolens L.*). Turk. J. Agric.
766 Forest. 30, 281–286.

767 Zengin, G., Uysal, S., Ceylan, R., Aktumsek, A., 2015. Phenolic constituent, antioxidative
768 and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A
769 phytochemical study. Ind. Crops Prod. 70, 1–6.

770 Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M., Granato, D.,
771 2014. A comparative study of the phenolic compounds and the *in vitro* antioxidant activity of
772 different Brazilian teas using multivariate statistical techniques. Food Res Int. 60, 246–254.

773

774 **Table 1**

775 Zn content in roots and aerial parts of non-treated and Zn-treated *F. vulgare*, *A. graveolens* and *P. anisum* plants

Samples	**Zn (µg/g DW)**	
	Roots	**Aerial Part**
F_{DF}	56.84±7.50 ^{cd}	52.38±0.84^{c}
F_{DF} Zn	87.93±11.21^{c}	71.03±0.08^{c}
F_{L}	21.89±0.54^{d}	54.12±0.40^{c}
F_{L} Zn	61.02±12.3 9^{cd}	82.51±4.11^{c}
D	89.02±5.23^{c}	66.62±4. 99^{c}
D Zn	186.27±9.42^{b}	23665.70±3466.64^{a}
A	59.75±6.26^{cd}	30.45±4.73^{c}
A Zn	1312.77±25.64^{a}	16940.27±2466.04^{b}

776 DW: Dry Weight.

777 Each value represents the mean of three replicates ± S.E (standard error). Values with different letters in the same column are significantly different at $p < 0.05$.

778

779

780

781

782

783 **Table 2**

784 Growth parameters of non-treated and Zn treated *F. vulgare*, *A. graveolens* and *P. anisum* plants and their photosynthetic pigment amounts

	Growth (cm)		**Fresh weight (FW) (g)**		**Dry weight (DW) (g)**		**Pigment contents (mg/g FW)**			
Sample	Aerial part	Roots	Aerial part	Roots	Aerial part	Roots	*Chla*	*Chlb*	*Chla+Chlb*	*Carotenoids*
$\mathbf{F_{DF}}$	90.50 ± 3.85^{b}	17.33 ± 1.46^{b}	399.55 ± 63.33^{a}	24.84 ± 2.00^{a}	296.22 ± 41.78^{b}	18.06 ± 1.24^{a}	0.98 ± 0.01^{d}	0.68 ± 0.01^{b}	1.67 ± 0.02^{c}	0.48 ± 0.01^{a}
$\mathbf{F_{DF}}$ **Zn**	73.08 ± 3.88^{d}	12.13 ± 1.68^{d}	273.08 ± 3.88^{bc}	17.26 ± 2.94^{c}	195.86 ± 41.71^{cd}	14.93 ± 1.09^{b}	0.66 ± 0.00^{f}	0.40 ± 0.04^{d}	1.06 ± 0.05^{e}	0.08 ± 0.01^{cde}
$\mathbf{F_{L}}$	79.27 ± 2.81^{cd}	16.40 ± 1.20^{b}	262.94 ± 34.37^{c}	23.48 ± 3.65^{ab}	356.72 ± 44.30^{a}	17.04 ± 1.43^{a}	1.12 ± 0.05^{bc}	0.84 ± 0.08^{a}	1.97 ± 0.03^{a}	0.49 ± 0.06^{a}
$\mathbf{F_{L}}$ **Zn**	62.17 ± 2.74^{e}	11.55 ± 1.56^{d}	214.26 ± 41.34^{d}	13.32 ± 1.10^{d}	156.54 ± 20.34^{ef}	11.37 ± 1.29^{c}	0.80 ± 0.01^{e}	0.59 ± 0.01^{bc}	1.39 ± 0.00^{d}	0.01 ± 0.00^{e}
D	106.63 ± 4.46^{a}	24.30 ± 1.73^{a}	304.02 ± 2.42^{bc}	21.23 ± 1.11^{b}	203.74 ± 2.53^{cd}	14.61 ± 0.95^{b}	1.32 ± 0.06^{a}	0.56 ± 0.04^{c}	1.88 ± 0.02^{b}	0.55 ± 0.01^{a}
D Zn	80.88 ± 6.13^{c}	15.1 ± 1.49^{bc}	166.62 ± 30.66^{e}	11.12 ± 1.60^{d}	130.15 ± 5.42^{f}	9.21 ± 1.39^{d}	0.46 ± 0.04^{i}	0.25 ± 0.03^{ef}	0.72 ± 0.00^{f}	0.12 ± 0.01^{cd}
A	59.73 ± 2.85^{e}	22.28 ± 3.02^{a}	273.65 ± 34.06^{bc}	16.11 ± 0.87^{c}	156.98 ± 15.32^{ef}	12.58 ± 1.37^{c}	1.03 ± 0.01^{cd}	0.91 ± 0.02^{a}	1.94 ± 0.01^{ab}	0.31 ± 0.02^{b}
A Zn	12.90 ± 1.05^{f}	12.90 ± 1.05^{cd}	114.90 ± 2.72^{f}	7.28 ± 1.39^{e}	70.40 ± 4.55^{i}	4.70 ± 1.13^{e}	0.32 ± 0.01^{i}	0.16 ± 0.03^{f}	0.48 ± 0.00^{i}	0.06 ± 0.02^{de}

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

786

787

788

789

790

791 **Table 3**

792 Spearman correlation coefficients between Zn content and growth parameters

	Growth		Fresh weight		Dry weight	
	Roots	Aerial part	Roots	Aerial part	Roots	Aerial part
Zn content	-0.265*	-0.470**	-0.720**	-0.284**	-0.744**	-0.390**

793 * Significant at $p< 0.05$; ** Significant at $p < 0.01$.

794

795 **Table 4**

796 Spearman correlation coefficients between chlorophyll / carotenoid content and growth / fresh weight

	Chlorophyll content (mg/g FW)	Carotenoid content(mg/g FW)
Growth(cm)	0.511**	0.717**
Fresh weight(g)	0.654**	0.766**

797 FW: Fresh Weight

798 * Significant at $p< 0.05$; ** Significant at $p < 0.01$.

799

800

801

802 **Table 5**

803 Phenolic contents of aqueous extracts of non-treated and Zn treated *F. vulgare*, *A. graveolens* and *P. anisum* plants

Samples	Phenols (mg GAE/mL Extract)	Flavonols and flavones (mg QE/mL Extract)	Dihydroflavonols and flavanones (mg NE/mL Extract)
F_{DF}	24.42±0.06[f]	13.86±0.13[d]	9.51±0.31[bcd]
F_{DF} Zn	20.61±0.16[i]	10.48±0.08[e]	9.80±0.07[bc]
F_L	35.76±0.08[d]	14.46±0.60[d]	2.60±0.13[f]
F_L Zn	20.85±0.01[i]	10.65±0.31[e]	10.22±0.07[b]
D	67.22±0.64[b]	27.80±0.88[b]	10.44±0.52[b]
D Zn	74.07±0.3[a]	35.40±0.50[a]	5.06±0.53[e]
A	29.08±0.08[e]	15.01±1.14[d]	8.43±0.04[d]
A Zn	38.25±0.03[c]	22.63±1.21[c]	14.49±0.01[a]

804 GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; NE: Naringin Equivalent.

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

806

807

808

809

810

811 **Table 6**

812 Spearman correlation coefficients between Zn amount ,antioxidant and enzyme inhibitory activities

	Zinc
Total phenol	0.331
Flavones + flavonols	0.294
Dihydroflavonols + flavanones	0.310
DPPH	-0.539
ABTS	-0.166
Phosphomolybdenum	0.307
Ferric chelating	-0.331
TBARS	-0.644**
Liposome	-0.709**
Superoxide	-0.257
NO	0.298
Lipoxygenase	-0.166
Acetylcholinesterase	-0.038
Tyrosinase	-0.271

813 ** Significant at $p < 0.01$.

814

815

816

817

818 **Table 7**

819 Antioxidant activity (IC_{50}, mg/mL) of aqueous extracts of non-treated and Zn-treated F. *vulgare*, *A. graveolens* and *P. anisum* plants, measured by using different assays

Samples	Phospho-molybdenum (mg AAE/mL)	DPPH	ABTS	Ferric chelating	Liposome	NO	TBARS	Superoxide
F_{DF}	31.692±0.793[i]	0.379±0.005[a]	0.020±0.000[bc]	0.116±0.004[b]	0.067±0.000[a]	-	0.021±0.000[d]	0.021±0.002[c]
F_{DF} Zn	27.121±0.465[j]	0.354±0.001[b]	0.025±0.002[a]	0.145±0.001[a]	0.010±0.000[i]	-	0.048±0.001[b]	0.028±0.002[b]
F_L	42.700±0.462[de]	0.073±0.002[d]	0.007±0.000[fi]	0.056±0.001[c]	0.042±0.000[c]	1.735±0.023[c]	0.051±0.002[a]	0.005±0.000[e]
F_L Zn	24.498±0.744[k]	0.310±0.007[c]	0.012±0.000[de]	0.143±0.001[a]	0.017±0.000[e]	3.798±0.117[a]	0.004±0.000[f]	0.040±0.001[a]
D	82.717±1.223[b]	0.032±0.000[e]	0.010±0.001[ef]	0.023±0.000[e]	0.062±0.001[b]	2.007±0.027[b]	0.037±0.000[c]	0.010±0.000[d]
D Zn	95.713±0.457[a]	0.017±0.000[f]	0.014±0.002[d]	0.016±0.000[f]	0.012±0.000[f]	1.821±0.047[b]	0.021±0.000[d]	0.003±0.000[ef]
A	38.008±0.515[f]	0.306±0.010[c]	0.018±0.000[c]	0.040±0.000[d]	0.023±0.000[d]	0.009±0.000[d]	0.050±0.001[ab]	0.005±0.000[e]
A Zn	46.716±1.298[c]	0.071±0.000[d]	0.010±0.000[ef]	0.010±0.000[i]	0.009±0.000[i]	0.007±0.000[d]	0.013±0.000[e]	0.004±0.000[ef]

820 -: Not detected; AAE: Antioxidant Activity Equivalent.

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

822

823

824

825 **Table 8**

826 Spearman coefficients between phenolic compound amounts and antioxidant and enzyme inhibitory activities

	Total phenol	Flavones+flavonols	Dihydroflavonols + flavanones
Total phenol	1	0.953**	-0.118
Flavone+flavonol	0.953**	1	-0.110
dihydroflavonol	-0.118	-0.110	1
DPPH	-0.892**	-0.855**	0.04
ABTS	-0.563**	-0.437*	-0.067
Phosphomolybdenum	0.959**	0.953**	-0.173
Ferric chelating	-0.897**	-0.893**	-0.108
TBARS	-0.040	-0.006	-0.626**
Liposome	0.021	0.001	-0.277
NO	0.381	0.312	0.044
Superoxide	-0.801**	-0.825**	0.320
Lipoxygenase	-0.022	-0.131	-0.159
Acetylcholinesterase	0.382	0.370	-0.188
Tyrosinase	-0.949**	-0.957**	0.098

827 ** Significant at $p < 0.01$

828

829 **Table 9**

830 Enzyme inhibitory activities (IC_{50} = mg/ mL) of aqueous extracts of non-treated and Zn-treated *F. vulgare, A.*

831 *graveolens and P. anisum* plants

Samples	Lipoxygenase	Acetylcholinesterase	Tyrosinase
F_{DF}	0.049 ± 0.000^{b}	-	0.826 ± 0.015^{b}
F_{DF} Zn	-	-	0.941 ± 0.010^{a}
F_{L}	0.052 ± 0.001^{b}	0.331 ± 0.015^{bc}	0.592 ± 0.019^{c}
F_{L} Zn	0.062 ± 0.000^{a}	0.209 ± 0.010^{cd}	0.953 ± 0.035^{a}
D	0.043 ± 0.000^{c}	0.120 ± 0.007^{de}	0.190 ± 0.004^{f}
D Zn	0.041 ± 0.000^{c}	0.227 ± 0.007^{bcd}	0.157 ± 0.001^{f}
A	0.035 ± 0.000^{d}	0.809 ± 0.128^{a}	0.403 ± 0.008^{d}
A Zn	0.015 ± 0.000^{e}	0.351 ± 0.026^{b}	0.308 ± 0.005^{e}

832 Each value represents the mean of three replicates ± S.E (standard error). Values with different letters in the

833 same column are significantly different at $p < 0.05$.

834 -not detected

835

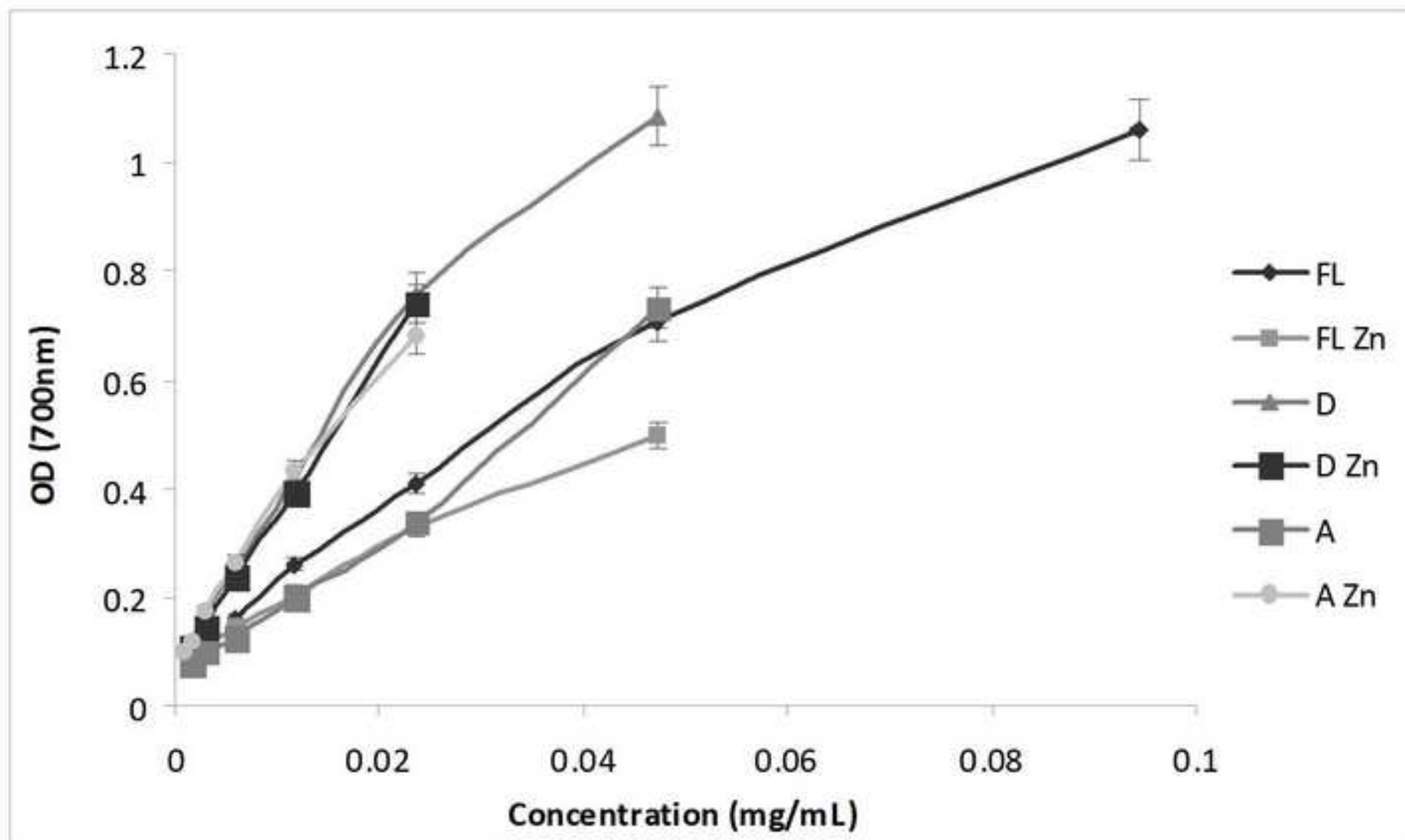
836 **Figure. 1.** Ferric reducing power of aqueous extracts of non-treated and Zn-treated *F.vulgare, A. graveolens and*

837 *P. anisum* plants.

838

839

Figure



Supplementary Interactive Plot Data (CSV)
Click here to download Supplementary Interactive Plot Data (CSV): Supplementary material final.doc