Research Article

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Antioxidant activity, polyphenolic contents and essential oil composition of *Pimpinella anisum* L. as affected by zinc fertilizer

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Abstract

BACKGROUND: The antioxidant activity and essential oil content of plants may vary considerably with respect to environmental conditions, especially nutrient availability. Among micronutrients, zinc (Zn) is needed by plants in only small amounts but is crucial to plant development. This study aimed to evaluate the effects of Zn fertilization on the antioxidant activity, polyphenolic contents and essential oil composition of *Pimpinella anisum* fruit.

RESULTS: Foliar application of Zn fertilizer considerably increased the number of detected essential oil components from 27 to 45. Zinc application at a rate of 0.2% (w/v) significantly enhanced the levels of β-bisabolene, germacrene D, *n*-decane and α-zingiberene, whereas the opposite trend was observed for (*E*)-anethole and geijerene. Application of 0.2% Zn considerably increased the levels of phenolic compounds, with chlorogenic acid showing the highest content among eight phenolic compounds detected in treated plants. The maximum antioxidant activity was achieved through application of 0.2% Zn fertilizer.

CONCLUSION: These findings indicated that the quality and quantity of anise fruit essential oil components were significantly altered by application of low levels of Zn. After foliar application of Zn, polyphenolic contents as well as antioxidant activity of anise fruit increased. Using Zn fertilizer is an efficient method to improve the pharmaceutical and food properties of anise fruit.

Keywords: anise; antioxidant activity; essential oil; polyphenolic content; zinc fertilizer

INTRODUCTION

Herbal therapeutic substances are generally utilized for various purposes. Commonly used by ancient societies on account of their organoleptic, psychotherapeutic, mystic, preservative and essentially medicinal properties, a few recent investigations have clarified the healing potential of various plant extracts and phytochemicals.[1–3]

Pimpinella anisum L. (anise) is a plant species of the Apiaceae family widely utilized for its medicinal and culinary properties as well as in cosmetic and other industrial applications. Fruits/seeds of anise are generally suggested as antioxidant, antimicrobial, digestive, antiseptic, antispasmodic, aperitif (in respiratory and gastrointestinal tracts), galactogogic, expectorant, estrogenic, anti-inflammatory and diuretic agents, these properties being mainly connected with the essential oil.[4,5] Aniseeds contain around 1.5–5.0% (w/w) essential oil fundamentally made up of unstable phenylpropanoids such as *trans*-anethole (~90%).[6] The essential oil of anise fruit also contains other major compounds such as estragol, anisaldehyde, γ-himachalene and *cis*-anethole.[7–9] Several studies have reported the antioxidant activity of anise extracts obtained from the whole plant,[10] seeds,[1,11,12] roots[13] and essential oil.[14–17] Similarly, there are a number of reports on the phenolic content of anise in the seeds,[1,18] fruits, leaves,[19] roots[13] and whole plant.[10]

The antioxidant activity and essential oil content of plants may vary considerably with respect to environmental conditions, nutrient and water accessibility and genetic potential of the crop.[20,21] Among nutrients, zinc (Zn) is one the most important basic micronutrients and has a specific physiological function within plants, being required in membrane integrity,[22] carbohydrate metabolism,[23] indole-3-acetic acid (IAA) biosynthesis and transport,[24,25] control mechanisms of generation and detoxification of oxygen-derived radicals and activation of various antioxidant enzymes,[26–29] protein synthesis and RNA metabolism[30,31] and photosynthesis.[23,32] Zinc is known to have an essential role either as a metal segment of enzymes or as a functional, structural or controlling cofactor of a large number of enzymes. Around 200 enzymes and transcription elements require Zn as a useful part.[33] Inferable from the previously mentioned unsubstituted activities, Zn plays a basic part in the growth and development of plants. It has been demonstrated that Zn application markedly influences the essential oil components, total phenols and antioxidant capacity of *Mentha canadensis*,[34] *Cuminum cyminum*[35,36] and *Chrysanthemum balsamita*.[37] The aim of the present study was to evaluate

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Table 1. Some physical and chemical properties of studied soil

FC (%, DM basis)	PWP (%, DM basis)	pH (paste)	EC (dS m^{-1})	CEC (cmol kg^{-1})	Organic C (g kg^{-1} soil)	N (%)	P (mg kg^{-1} soil)	K (mg kg^{-1} soil)	DTPA-extractable Zn (mg kg^{-1} soil)
22.0	9.0	7.4	1.3	11.0	8.4	0.04	13.0	59.0	1.21

FC, field capacity; DM, dry matter; PWP, permanent wilting point; EC, electrical conductivity; CEC, cation exchange capacity.

the impact of various levels of Zn foliar application on the essential oil composition, phenolic contents and antioxidant activity of aerial parts of the anise plant.

MATERIALS AND METHODS

Plant materials and growth conditions

A greenhouse experiment was performed at the research station of Shiraz Payame Noor University situated in Golestan town (29° 36′ N, 52 ° 32′ E, 1490 m above sea level). The soil used in the study was a fine loam taken from 0–30 cm of a virgin soil. Some physicochemical properties of the soil are given in Table 1. Considering the crucial deficiency level for diethylenetriaminepentaacetic acid (DTPA)-extractable soil Zn (0.5 mg kg^{-1}),[38] the soil was seriously deficient in available Zn. The soil was air dried and crushed to pass through a 2 mm sieve. Nitrogen (N) and phosphorus (P) at the rate of 50 mg kg^{-1} soil and copper (Cu) and manganese (Mn) at the rate of 5 mg kg^{-1} soil were applied uniformly to the soil as NH_4NO_3, KH_2PO_4, $CuSO_4 \cdot 5H_2O$ and $MnSO_4 \cdot H_2O$ respectively. The soil was put in 8 L plastic pots at the rate of 7.5 kg per pot. Thirty seeds of anise (*P. anisum*) were planted in each pot, irrigated with deionized water twice weekly to near field capacity and maintained at this moisture level by adding water to a constant weight. After 15 days, the plants were thinned to 15 uniform stands in each pot. At two stages, after thinning and upon the onset of inflorescence appearance, Zn was sprayed at rates of 0.1 and 0.2% (w/v) in the form of Zn-ethylenediaminetetraacetic acid (EDTA) chelate. Deionized water was also sprayed as control. Plants containing mature fruits were harvested 12 weeks after planting. Seed heads also were collected while they were still green. Fruit samples were dried in the shade and made ready for analysis.

Plant extract preparation

Dried fruit samples (20 g) were soaked in 250 mL of methanol/water (90:10 v/v) for 2 days. The extracts were filtered and concentrated in a rotary evaporator for up to 15 min. The fine powders were weighed and their yields recorded. Before utilization, the powders were maintained at −20 °C. The desired concentration of powder in methanol was prepared before each measurement, then the total phenol content and antioxidant activity were evaluated.[39]

Antioxidant activity measurement

The antioxidant activity of methanolic fruit extracts was measured as their 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH•)-scavenging capacity.[40] Aliquots (20 μL) of 12.5–3200 µg mL^{-1} methanolic extracts or gallic acid were blended with 200 μL of 100 mmol L^{-1} DPPH• solution in methanol. The solutions were kept at room temperature for about 30 min. Using an ELx808 absorbance microplate reader (BioTek Instruments Inc., Winooski, VT, USA), DPPH•-scavenging capacity was assessed at 515 nm. IC_{50} (concentration of extract (mg L^{-1}) needed to reduce DPPH• by 50%) values were computed from the nonlinear regression between log concentration (µg mL^{-1}) of sample extracts and mean % radical-scavenging activity using MATLAB (The MathWorks Inc., USA). Antioxidant activity was calculated as

$$\text{antioxidant activity } (\%) = 100 - \left[\left(A_{\text{sample}} - A_{\text{blank}}\right)/A_{\text{control}}\right] \times 100$$

where A_{blank} and A_{sample} are the absorption values of the blank reaction ($t = 0$ min) and the test solution ($t = 30$ min) respectively. DPPH• (without plant extract) and methanol were utilized as control and blank separately.

Zinc ion concentration

Concentrations of Zn were assessed in anise shoot (stem + leaf) and fruit samples. Each sample (100 mg) was finely ground in 2 mL of concentrated HNO_3. After processing, the sample volume was made up to 100 mL with distilled water. Zinc concentration was evaluated by an atomic absorption spectrophotometer (SpectrAA 220, Varian, Mulgrave, Victoria, Australia).

Headspace volatile extraction

Dried fruit samples (3 g) were pulverized and put in 20 mL headspace vials. The vials were immediately sealed with silicone elastic septa and aluminum tops and transferred to a headspace plate. Using a CombiPAL system (CTC Analytics AG, Zwingen, Basel-Land, Switzerland), headspace extraction was performed. Under agitation, the vials were warmed to 80 °C for 20 min.[41]

Isolation and analysis of essential oils

Anise fruit samples (100 g) were finely ground in an analytical grinder (A 11 basic analytical mill, IKA, Werke Staufen, Germany) and subjected to hydrodistillation for 6 h in a Clevenger apparatus. After decanting and drying of the oil over anhydrous sodium sulfate, the mild yellowish oil was recovered and stored in tightly closed dark vials under refrigeration (4 °C) until analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using an Agilent 7890 system (Agilent Technologies Inc., Shanghai, China) and an HP-5 MS capillary column (Chrom Tech, Apple Valley, MN, USA) (phenyl methyl siloxane, 30 m × 0.25 mm i.d., 25 µm) with helium as carrier gas and a split ratio of 1:50. Retention indices (RIs) were assigned utilizing retention times of *n*-alkanes (as standard) that were injected after the essential oil. Compounds were identified by comparison of their RIs (HP-5) with those reported in the literature and by comparison of their mass spectra with published mass spectral data in the Adams Library, Wiley GC/MS Library and MassFinder 2.1 Library. The most critical components estragol and *trans*-anethole were further confirmed by co-injection of authentic standards (Roth, Karlsruhe, Germany).[42]

Table 2. Effect of Zn-EDTA foliar application on concentrations of chemical compounds (g kg^{-1}) identified in essential oil of *Pimpinella anisum* fruit

No.	Compound	Retention index	Applied Zn (% w/v)		
			0.1	0.2	0 (control)
1	α-Pinene	932	2.00 ± 0.3a	1.70 ± 0.4a	1.00 ± 0.2b
2	Sabinene	970	8.00 ± 0.5b	14.90 ± 0.5a	5.30 ± 0.2c
3	*n*-Decane	998	19.20 ± 0.4b	30.40 ± 0.9a	18.40 ± 0.5b
4	*p*-Cymene	1023	0.18 ± 0.03a	0.13 ± 0.04b	0.21 ± 0.03a
5	Limonene	1025	0.56 ± 0.03b	2.11 ± 0.06a	0.63 ± 0.03b
6	1,8-Cineole	1029	0.27 ± 0.04b	0.98 ± 0.05a	0.33 ± 0.025b
7	γ-Terpinene	1056	0.54 ± 0.04b	0.69 ± 0.03a	0.42 ± 0.03c
8	*n*-Undecane	1098	2.44 ± 0.35b	3.58 ± 0.40a	0.66 ± 0.04c
9	*n*-Nonanal	1104	1.45 ± 0.05b	2.22 ± 0.06a	0.95 ± 0.07c
10	*n*-Amyl isovalerate	1108	0.46 ± 0.12b	0.74 ± 0.06a	0.66 ± 0.05a
11	Geijerene	1139	13.52 ± 0.55a	7.39 ± 0.75b	14.35 ± 0.83a
12	Menthol	1174	0.07 ± 0.004c	0.27 ± 0.03a	0.12 ± 0.05b
13	Methyl chavicol	1197	10.38 ± 0.75a	1.89 ± 0.55c	2.88 ± 0.55b
14	*n*-Decanal	1204	7.04 ± 1.14b	27.36 ± 1.67a	3.44 ± 0.48c
15	*trans*-Carveol	1220	1.71 ± 0.33b	2.87 ± 0.42a	2.44 ± 0.71a
16	*cis*-Carveol	1225	2.21 ± 0.45b	3.55 ± 0.44a	1.11 ± 0.45c
17	(*Z*)-Anethole	1251	0.66 ± 0.04a	0.59 ± 0.03a	0.73 ± 0.06a
18	(2*E*)-Decenal	1260	9.77 ± 0.73b	118.34 ± 3.45a	5.79 ± 0.77c
19	(*E*)-Anethole	1284	435.95 ± 16b	294.12 ± 18c	802.41 ± 11a
20	Thymol	1291	3.34 ± 0.48a	1.22 ± 0.27b	2.09 ± 0.44b
21	Carvacrol	1298	0.16 ± 0.03b	0.19 ± 0.05a	–
22	Undecanal	1305	0.42 ± 0.03b	0.87 ± 0.04a	–
23	δ-Elemene	1335	0.57 ± 0.04b	1.05 ± 0.02a	0.38 ± 0.04b
24	α-Cubebene	1347	0.07 ± 0.005b	0.15 ± 0.03a	–
25	Eugenol	1363	1.83 ± 0.05b	2.71 ± 0.18a	0.64 ± 0.02c
26	α-Copaene	1373	0.23 ± 0.03a	0.08 ± 0.006b	–
27	β-Bourbonene	1382	0.96 ± 0.04a	0.59 ± 0.06b	–
28	β-Elemene	1389	0.32 ± 0.04a	0.29 ± 0.05a	–
29	*n*-Tetradecane	1397	2.13 ± 0.03b	4.21 ± 0.04a	0.61 ± 0.04c
30	Dodecanal	1407	1.35 ± 0.05b	3.40 ± 0.06a	–
31	α-Gurjunene	1416	1.82 ± 0.05a	1.04 ± 0.02a	–
32	β-Cedrene	1426	0.53 ± 0.04a	0.29 ± 0.04b	–
33	*trans*-α-Bergamotene	1433	0.54 ± 0.38a	0.62 ± 0.05a	–
34	α-Himachalene	1445	0.18 ± 0.03a	0.03 ± 0.05b	–
35	α-Humulene	1450	0.48 ± 0.05b	0.96 ± 0.06a	–
36	(*E*)-β-Farnesene	1455	2.07 ± 0.03a	1.86 ± 0.04a	–
37	(2*E*)-Dodecenal	1464	8.08 ± 0.37b	39.33 ± 1.22a	–
38	γ-Himachalene	1478	7.47 ± 0.29a	3.24 ± 0.28b	3.78 ± 0. 53b
39	Germacrene D	1481	65.61 ± 2.22a	51.87 ± 0.93b	22.76 ± 0.77c
40	α-Zingiberene	1493	28.08 ± 1.66b	37.93 ± 2.04a	13.62 ± 1.07c
41	β-Bisabolene	1507	352.13 ± 6.54b	369.30 ± 4.77a	94.00 ± 2.48c
42	γ-Cadinene	1511	1.27 ± 0.11a	1.19 ± 0.05a	–
43	δ-Cadinene	1521	3.66 ± 0.06a	3.87 ± 0.28a	–
44	α-Cadinene	1535	0.05 ± 0.01a	0.03 ± 0.01a	–
45	Caryophyllene oxide	1580	0.18 ± 0.05b	0.49 ± 0.07a	–

Data are mean ± standard deviation of eight replications. Means followed by the same letter within a row are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Extraction and analysis of polyphenols

Polyphenol extraction was carried out according to Justesen *et al.*[43] with minor modifications. Reference standards of 13 polyphenols (catechin, gallic acid, chlorogenic acid, caffeic acid, cinnamic acid, quercetin, *p*-coumaric acid, coumarin, carvacrol, rutin, *trans*-ferulic acid, vanillin and hesperidin) were bought from Merck (Darmstadt, Germany). Gradient elution was selected to achieve maximum separation and sensitivity. Elution was performed by varying the ratio (v/v) of solvent A (formic acid 1% (v/v) in deionized water) to solvent B (methanol) as follows:[44] B/A 10:90 at 0 min, B/A 25:75 at 10 min, B/A 60:40 at 20 min and finally B/A 70:30 at 30 min and B/A 70:30 at 40 min. High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1200 system fitted with a Zorbax Eclipse XDB-C18 column

(10 cm × 5 μm i.d., 150 mm film thickness) and a photodiode array detector. Elution was observed at 230 and 280 nm.

Statistical analysis

Statistical analysis of the data was done using SPSS Version 20 (IBM, Armonk, NY, USA) to check the significance of different treatments, while Duncan's multiple range test at the level of 5% probability was utilized to compare differences between means.

RESULTS AND DISCUSSION

Twenty-seven essential oil compounds were identified in the control, whereas 45 compounds were obtained with application of Zn fertilizer at both concentrations. The main essential oil components of anise fruit in the control were characterized by a high content of (*E*)-anethole (802.41 g kg^{-1}) followed by *β*-bisabolene (94.00 g kg^{-1}), germacrene D (22.76 g kg^{-1}), *n*-decane (18.40 g kg^{-1}), geijerene (14.35 g kg^{-1}) and *α*-zingiberene (13.62 g kg^{-1}) (Table 2). Foliar application of Zn-EDTA at 0.1% (w/v) considerably altered the amounts of these components to (*E*)-anethole (435.95 g kg^{-1}), *β*-bisabolene (352.13 g kg^{-1}), germacrene D (65.61 g kg^{-1}), *n*-decane (19.20 g kg^{-1}), geijerene (13.52 g kg^{-1}) and *α*-zingiberene (28.08 g kg^{-1}). Upon treatment with 0.2% Zn, the levels were (*E*)-anethole (294.12 g kg^{-1}), *β*-bisabolene (369.30 g kg^{-1}), germacrene D (51.87 g kg^{-1}), *n*-decane (30.40 g kg^{-1}), geijerene (7.39 g kg^{-1}) and *α*-zingiberene (37.93 g kg^{-1}).

As shown in Table 2, Zn-EDTA application significantly increased the levels of *β*-bisabolene (+393%), germacrene D (+288%), *n*-decane (+165%) and *α*-zingiberene (+278%) compared with the control, whereas the opposite trend was seen for (*E*)-anethole (−273%) and geijerene (−194%). Meanwhile, 18 compounds, namely carvacrol, undecanal, *α*-cubebene, *α*-copaene, *β*-bourbonene, *β*-elemene, dodecanal, *α*-gurjunene, *β*-cedrene, *trans*-*α*-bergamotene, *α*-himachalene, *α*-humulene, (*E*)-*β*-farnesene, (2*E*)-dodecenal, *γ*-cadinene, *δ*-cadinene, *α*-cadinene and caryophyllene oxide, were identified in the essential oil of treated plants which were not detected in the control.

In agreement with our findings, previous studies have also confirmed the importance of Zn fertilization in improving essential oil components. The maximum proportions of some essential oil components of *C. cyminum* such as *γ*-terpinene, *p*-cymene and *β*-pinene were achieved by Zn application.[36] Said-Al Ahl and Mahmoud[45] likewise demonstrated that Zn treatment led to increased amounts of 1,8-cineol, *β*-pinene, camphor, nerolidole and camphene in *Ocimum basilicum*. As also indicated by Ghorbanpour *et al.*,[46] the content of essential oil components of *O. basilicum* was notably enhanced by Zn application. As Zn is included in photosynthesis and saccharide metabolism and as CO_2 and glucose are the most probable sources of carbon used in terpene biosynthesis, the role of Zn in impacting essential oil composition appears to be especially critical.[36]

trans-Anethole (a derivative of phenylpropene) is the main component of aniseed essential oil. Phenylpropenoids can potentially metabolize and degrade to aldehyde intermediates. It has been demonstrated that *trans*-anethole is metabolized to protocatechuic acid through *trans*-anethole-diol, anisaldehyde, anisic acid, *p*-hydroxybenzoic acid and 2-phenylacetic acid. The last compound is one of the endogenously synthesized auxins in plants. Zinc is an essential micronutrient for the synthesis of auxin in plants.[47,48] In the present study, Zn application decreased the content of *trans*-anethole in the fruit essential oil (Table 2). This result might be due to the metabolization of *trans*-anethole to 2-phenylacetic acid (auxin) which was catalyzed by Zn.

Table 3. Effect of Zn-EDTA complex on uptake of zinc by stem + leaf and seed of anise

Treatment	Zinc content (mg kg^{-1} dry weight)	
	Aerial part (stem + leaf)	Seed
Zn-EDTA 0.1% (w/v)	128.34 ± 1.04d	137.54 ± 1.14c
Zn-EDTA 0.2% (w/v)	136.27 ± 1.09b	145.29 ± 1.11a
Control	60.45 ± 1.23e	74.16 ± 0.95e

Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

Analysis of the aerial plant parts revealed a significant increase in Zn content of shoots (+112 and +125%) and fruits (+85 and +95%) treated with 0.1 and 0.2% Zn-EDTA respectively (Table 3). These results are in concurrence with those of Derakhshani *et al.*,[37] Ghorbanpour *et al.*[46] and Pande *et al.*,[49] who also noted an increase in Zn uptake with increased Zn supply.

Phenolics are aromatic secondary plant metabolites that are widely spread throughout the plant kingdom. Phenolics have been connected with color, sensory qualities, nutritional and antioxidant properties.[50] Four phenolic compounds were identified in the control. Foliar use of Zn raised the number of detected compounds to eight, namely catechin, chlorogenic acid (CGA), *p*-coumaric acid, coumarin, vanillin, *trans*-ferulic acid, hesperidin and eugenol, with CGA being present in the highest amount (Table 4). CGA (Fig. 1) is an ester formed from cinnamic and quinic acids and is also called 5-*O*-caffeoylquinic acid (5-CQA) (IUPAC numbering) or 3-CQA (pre-IUPAC numbering).[51] The most widely recognized type of CGA is 5-CQA (Fig. 2). There is evidence that CGA has numerous biological properties, including antibacterial, antioxidant, anticarcinogenic activities, particularly hypoglycemic and hypolipidemic effects and insulin sensitizer.[52] Zinc application had markedly positive effects on phenolic content. Zinc treatment at 0.2% initiated substantially higher phenolics compared with the control and plants treated with 0.1% Zn (Table 4). It has been reported that Zn application enhanced the phytoestrogen content of pomegranate seeds.[53] Foliar utilization of Zn also amplified isoflavone levels in clover seedlings.[54] Venkatesan *et al.*[55] found a positive and highly significant correlation between Zn level and the polyphenol content of developed leaves of tea (*Camellia sinensis*). Similarly, another review demonstrated that the total phenol content of costmary (*C. balsamita* L.) was increased by Zn supply.[37]

The results of the antioxidant assay of the essential oils are shown in Fig. 3. All extracts showed a considerable DPPH•-inhibitory effect, with IC_{50} ranging from 809.35 mg L^{-1} in the control to 384.22 mg L^{-1} with application of 0.2% Zn-EDTA. The linear regression equation between IC_{50} (Y, mg L^{-1}) and Zn fertilization level (X, % w/v) was determined as $Y = -212.57X + 1216.9$ ($R^2 = 0.98$, $P \leq 0.01$), indicating that IC_{50} decreased in response to applied Zn-EDTA. Our findings revealed high topical antioxidant potential of anise oil. The maximum antioxidant effect was observed with application of 0.2% Zn fertilizer, which led to a 3.6-fold increase in CGA concentration in comparison with the control. The results showed a significant correlation within one species.

Screening of the antioxidant properties of some Umbelliferae fruits from Iran (including *P. anisum*) by the DPPH•-scavenging test demonstrated that all extracts had antioxidant capability, with

Table 4. Phenolic compounds of *Pimpinella anisum* fruit as affected by Zn-EDTA

Phenolic compound (mg g^{-1})	Foliar-applied Zn (% w/v)		
	0.1	0.2	0 (control)
Catechin	0.50 ± 0.02b	0.56 ± 0.01a	ND
Chlorogenic acid	0.28 ± 0.04b	0.90 ± 0.03a	0.25 ± 0.02c
Coumarin	0.14 ± 0.01a	0.19 ± 0.02a	0.09 ± 0.006b
Vanillin	0.04 ± 0.002a	0.07 ± 0.001a	0.02 ± 0.005b
trans-Ferulic acid	0.25 ± 0.03a	0.47 ± 0.03a	0.13 ± 0.02b
p-Coumaric acid	0.01 ± 0.006	0.01 ± 0.005	ND
Hesperidin	0.10 ± 0.01	ND	ND
Eugenol	0.24 ± 0.04	0.25 ± 0.04	ND

Values are calculated mean amount of polyphenol based on weight of ground dry plant in eight replicates ± standard deviation. Means followed by the same letter within a row are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. ND, not detected.

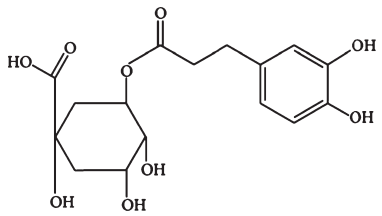

Figure 1. Chemical structure of chlorogenic acid (CGA).

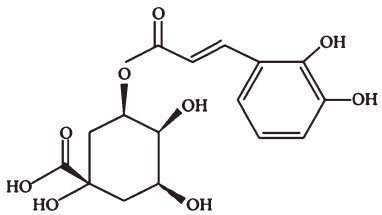

Figure 2. Chemical structure of 5-*O*-caffeoylquinic acid (chlorogenic acid).

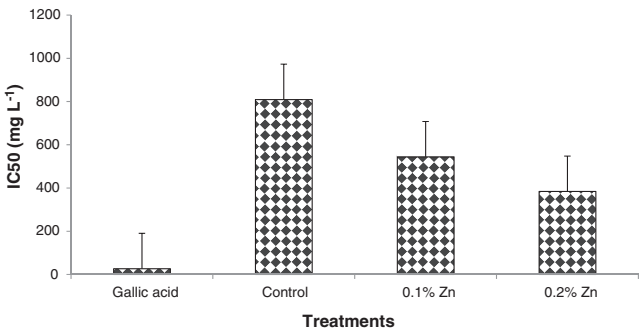

Figure 3. Effect of Zn-EDTA foliar application on antioxidant activity of *Pimpinella anisum* fruit measured by DPPH assay, and comparison with gallic acid (IC_{50} is concentration required to inhibit DPPH• formation by 50%). Vertical bars represent ± standard error of mean.

P. anisum extract showing the strongest activity and its ethyl acetate fraction displaying the highest activity and flavonoid content.[56] In concurrence with our findings, it was also demonstrated that Zn application upgraded antioxidant activity in peanut (*Arachis hypogaea*)[57] and costmary (*C. balsamita*).[37] Zinc diminishes reactive oxygen species (ROS) accumulation by enhancing antioxidant systems in plant compartments.[58] Zinc also acts as a cofactor of most antioxidant enzymes.[59] The antioxidant activity of assumed antioxidants, chiefly polyphenols, has been ascribed to various mechanisms, among which are binding of transition metal ion catalysts, prevention of chain initiation, prevention of continued hydrogen removal, decomposition of peroxides, reductive capacity and radical scavenging.[60] As shown in Table 4, total phenolic content was significantly enhanced by Zn applications. This increase in phenolics is the main cause of improved antioxidant activity under Zn treatments. Phenolics are differing secondary metabolites (flavonoids, tannins, phenolic acids and lignins) abundant in plant tissues.[61] The antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize unpaired electrons (chain-breaking function) and chelate transition metal ions.[62] Accordingly, foliar treatment with a low concentration of Zn might give a helpful method of enhancing the pharmacological properties of *P. anisum* fruit.

CONCLUSION

Our findings demonstrate that the quality and amount of anise (*P. anisum* L.) fruit essential oil components were fundamentally modified by application of low levels of Zn. Also, anise plants accumulated high levels of Zn in their aerial tissues at moderate levels of Zn fertilization, with no diminishment in growth. After foliar application of Zn, the polyphenolic contents as well as antioxidant activity of anise fruit intensified. CGA and catechin were the principal phenolic compounds of anise essential oil positively influenced by Zn treatments. Overall, the results presented here support the hypothesis that Zn possibly alters the production of essential oil; however, for a more extensive comprehension, the exact mechanism of such modifications in plant metabolism needs to be clarified. Nonetheless, Zn treatment of therapeutic plants such as anise can be established as a promising strategy to produce more desired pharmaceutically active compounds, e.g. *β*-bisabolene for drug industries and medical supplies.

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