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Antioxidant Properties of Aqueous Extracts from Selected Lamiaceae Species Grown in Turkey

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Water-soluble extracts from black thyme (*Thymbra spicata* L.), savory (*Satureja cuneifolia* Ten.), Spanish oregano (*Coridothymus capitatus* (L.) Reichb. f.), sweet marjoram (*Majorana hortensis* Mœnch), Syrian oregano (*Origanum syriacum* L.), Toka oregano (*Origanum minutiflorum* O. Schwarz et P. H. Davis), and Turkish oregano (*Origanum onites* L.) were screened for antioxidant properties in a battery of six in vitro assays. Total phenol content and qualitative−quantitative compositional analyses were also carried out. The extracts demonstrated varying degrees of efficacy in each screen. The savory extract was the most effective at reducing iron(III), scavenging 1,1-diphenyl-2-picrylhydrazyl radicals, inhibiting ascorbate-iron(III)-catalyzed hydroxyl radical-mediated brain phospholipid peroxidation, and site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation. The Syrian oregano extract was the most effective chelator of iron(II), while Spanish and Turkish oregano extracts were the most effective inhibitors of nonsite-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation. All the extracts contained Folin−Ciocalteu reagent-reactive substances, which was confirmed by the presence of polar phenolic analytes (i.e., hydroxybenzoates, hydroxycinnamates, and flavonoids).

KEYWORDS: Antioxidants; chelation; composition; DPPH•; high performance liquid chromatography; hydroxyl radical; iron reduction; Lamiaceae; water-soluble extracts

INTRODUCTION

Herbs and spices have been utilized since antiquity for their culinary qualities, and in addition, have also been used for their preservative and medicinal properties. Laboratory-based attempts to characterize their antiseptic qualities date back to the 1900s (*1*); while more recently, in the 1950s, Chipault and co-workers identified the ability of herbs and spices to inhibit deleterious oxidative reactions (*2*). Numerous research groups have since demonstrated that plant extracts, foodstuffs and specific beverages possess a variety of in vitro antioxidant properties. Furthermore, many studies have shown that the consumption of foodstuffs rich in antioxidative phytochemicals can exert positive effects upon human health and the aging process (*3*−*5*). This has resulted in investigations focusing upon the discovery of naturally occurring antioxidants by researchers in the fields of phyto-pharmacognosy and natural product and food chemistry.

The family Lamiaceae (Labiatae) includes between 200 and 250 genera and between 3200 and 6500 species (*6*, *7*) and is characterized by aromatic herbage, quadrangular stems, and verticillate inflorescences. Distribution is throughout the world but is particularly well represented in tropical and temperate areas such as the Mediterranean region and tropical upland savannas (*6*). This family is represented in Turkey by 556 species and 741 taxa in toto (*8*). The aerial material of most aromatic plants belonging to this family, such as members of the genera *Origanum*, *Satureja*, *Thymus*, etc., are added to foods for their organoleptic properties and are often consumed as herbal teas in Turkey (*9*). Members are used in folk remedies to treat various ailments including asthma, cramping, diarrhea, indigestion, infectious diseases, muscle pain, nausea, and rheumatism. A survey of the literature reveals that Lamiaceae species possess a variety of activities, including antiinflammatory, antioxidant, antibacterial, antifungal, and antiviral properties (*10*−*14*), among others.

The aim of this study was to characterize the antioxidant activities of seven members of the family Lamiaceae belonging to the genera *Coridothymus* (*C. capitatus*), *Majorana* (*M. hortensis*), *Origanum* (*O. syriacum*, *O. onites*, *O. minutiflorum*), *Satureja* (*S. cuneifolia*), and *Thymbra* (*T. spicata*), as these species have not been assessed for such properties previously except for *O. onites* (*11*). Furthermore, a review of the literature revealed that, other than for their volatile oils, the chemical composition of these specific species has rarely been reported (*11*, *15*). Therefore, the phenolic content, chemical composition, and in vitro antioxidant (iron reduction and chelation, 1,1-diphenyl-2-picrylhydrazyl radical scavenging, ascorbate-iron(III)-catalyzed hydroxyl radical-mediated phospholipid degradation inhibition and nonsite- and site-specific hydroxyl radical-

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mediated 2-deoxy-D-ribose degradation inhibition) properties of water-soluble extracts were examined.

MATERIALS AND METHODS

Materials. Steam-distilled−deodorized air-dried aerial plant material was obtained from Türer Ltd., Turkey. Pycnogenol was obtained from Biolandes Arômes, France. The chromatography standards were purchased from Extrasynthese (Genay, France). Ultrapure water (18.2 MΩcm) was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Bedford, MA). All reagents and solvents were of either analytical or HPLC grade and were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Freeze-Dried Extracts. The botanical material was sieved, suspended in ultrapure H_2O and was hydrodistilled for 2 h in a European Pharmacopoeian hydrodistillation apparatus. This process was repeated, and the combined aqueous extracts were then filtered, reduced in volume in vacuo (45 °C), freeze-dried, and stored at 4 °C.

Total Phenols. The total phenols were estimated as gallic acid equivalents, according to the Folin−Ciocalteu method (*16*). First, 0.25 mL of sample was transferred to a 25.0-mL volumetric flask containing 6 mL of H_2O, to which was subsequently added 1.25 mL of undiluted Folin−Ciocalteu reagent. After 1 min, 3.75 mL of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 mL with H_2O. The controls contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents and are presented as mean of duplicate analyses.

Chromatographic Fingerprint Analyses. The liquid chromatographic system (Waters 600E) consisted of an in-line degasser and controller coupled to a 991 photodiode array detector equipped with a 717 autosampler (25-μL injection volume) interfaced to a PC running PDA 991 software (Waters Corp., Milford, MA). Separations were performed on a 250 × 4.6-mm i.d., 5 μm reverse-phase Hypersil BDS−C18 analytical column (Agilent Technologies, Milford, MA) operating at room temperature with a flow rate of 0.8 mL/min. Detection was carried out with a sensitivity of 0.1 aufs between the wavelengths of 200 and 450 nm. Elution was by a ternary nonlinear gradient of the solvent mixture MeOH/H_2O/CH_3COOH (10:88:2, v/v/v) (solvent A), MeOH/H_2O/CH_3COOH (90:8:2, v/v/v) (solvent B) and MeOH (solvent C). The composition of the mobile phase was changed from 85:15:0 (A/B/C) to 70:30:0 (A:B:C) in 15 min, changed to 60:40:0 (A/B/C) in 3 min, held for 12 min, changed to 0:100:0 (A/B/C) in 5 min, 0:85:15 (A/B/C) in a further 2 min, and to 0:70:30 (A/B/C) in 11 min, then returned to initial conditions in 2 min. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and by comparison of their UV spectra with an in-house PDA-library. A 10-min equilibrium time was allowed between injections.

Chromatographic Standards and Sample Preparation. Stock solutions of the extracts and standards were prepared in 70% aqueous methanol to final concentrations of 10 and 1 mg/mL, respectively. The calibration concentration ranges used were 0.01−0.10 mg/mL for caffeic acid, naringenin, and apigenin and 0.05−0.50 mg/mL for rosmarinic acid and luteolin-7-*O*-glucoside.

Iron(III) to Iron(II) Reducing Activity. The ability of the extracts to reduce iron(III) was assessed by the method of Oyaizu (*17*). A 1-mL aliquot of each extract dissolved in water was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. After 30 min incubation at 50 °C, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. A 2.5-mL aliquot of the upper layer was mixed with 2.5 mL of water and 0.5 mL of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm. The mean absorbance values were plotted against concentration, and a linear regression analysis was carried out. The data are presented as ascorbic acid equivalents (AscAE) in mmol ascorbic acid per gram of extract. The bigger the AscAE, the greater the reducing power of the sample. Means are the average of at least nine measurements.

Iron(II) Chelation Activity. The chelation of iron(II) ions by the different extracts was carried out as described by Carter (*18*). To 200 μL of each extract was added to 100 μL of 2.0 mM aqueous $FeCl_2 \cdot 4H_2O$ and 900 μL methanol. The controls contained all the reaction reagents except the extract or positive control substance. After a 5 min incubation, the reaction was initiated by 400 μL of 5.0 mM ferrozine. After a 10 min equilibrium period, the absorbance at 562 nm was recorded. The iron(II) chelating activity was calculated using the eq 1 and the IC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2001 version 7.0, SPSS Inc., Chicago, IL). EDTA was used as a positive control. The values are presented as the mean of eight measurements.

$$\text{percentage inhibition (\%)} = \left(\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}}\right) \times 100 \quad (1)$$

1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging. The ability of the extracts to scavenge DPPH• free radicals was determined by the method of Gyamfi et al. (*19*). A 50-μL aliquot of each extract, in Tris-HCl buffer (50 mM, pH 7.4), was mixed with 450 μL of Tris-HCl buffer and 1.0 mL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl in methanol. The controls contained all the reaction reagents except the extract or positive control substance. After a 30-min incubation in darkness and at ambient temperature (23 °C), the resultant absorbance was recorded at 517 nm. The percentage inhibition values were calculated using eq 1, and the IC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2001 version 7.0). Ascorbic acid, BHA, BHT, and Pycnogenol were used as positive controls. The values are presented as the mean of at least nine measurements.

Ascorbate−Iron(III)-Catalyzed Phospholipid Peroxidation. The ability of the extracts to scavenge hydroxyl radicals was determined by the method of Aruoma et al. (*20*). Bovine brain extract (Folch type VII) was mixed with 10 mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath until an opalescent suspension was obtained, containing 5 mg/mL phospholipid liposomes. The liposomes (0.2 mL) were combined with 0.5 mL of PBS buffer, 0.1 mL of 1 mM $FeC1_3$, and 0.1 mL of extract dissolved in PBS. Peroxidation was initiated by adding 0.1 mL of 1 mM ascorbic acid. The mixture was incubated at 37 °C for 60 min, after which, 50 μL of 2.0% butylated hydroxytoluene (BHT) was added to each tube, followed by 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 1.0% 2-thiobarbituric acid (TBA) in 0.05 M NaOH. The samples were vortexed and heated in a water bath at 100 °C for 20 min. The reaction was stopped by a 5 min ice H_2O bath. To each tube was added 2 mL of *n*-butanol, and the mixture was vigorously vortexed. After centrifugation, the extent of oxidation was estimated from the absorbance of the organic layer at 532 nm. The percentage inhibition was calculated using eq 1, where the controls contain all the reaction reagents except the extract or positive control substance, and the IC_{50} values were estimated using a nonlinear regression algorithm (SigmaPlot 2001 version 7.0). The values are presented as the mean values of five measurements, except for Spanish oregano ($n = 3$).

Nonsite-Specific Hydroxyl Radical-Mediated 2-Deoxy-D-ribose Degradation. The ability of the extracts to inhibit nonsite-specific hydroxyl radical-mediated peroxidation was carried out essentially as described by Halliwell et al. (*21*). The reaction mixture contained 500 μL of extract dissolved in KH_2PO_4−KOH buffer (50 mM, pH 7.4), 100 μL of 28 mM 2-deoxy-D-ribose in KH_2PO_4−KOH buffer, 200 μL of a premixed 100 μM $FeCl_3$, and 104 mM EDTA (1:1 v/v) solution, 100 μL of 1.0 mM H_2O_2 and 100 μL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 37 °C for 60 min. Thereafter, 50 μL of 2.0% BHT was added to each tube followed by 1 mL of 2.8% TCA and 1 mL of 1.0% TBA. The samples were vortexed and heated in a water bath at 100 °C for 20 min. The reaction was stopped by a 5 min ice H_2O bath. To each tube was added 2 mL of *n*-butanol, and the mixture was vigorously vortexed. After centrifugation, the extent of oxidation was estimated from the absorbance of the organic layer at 532 nm. The percentage inhibition was calculated using eq 1, where the controls contained all the reaction reagents except the extract or positive control substance, and the IC_{50} values were estimated

Table 1. Extract Yield, Total Phenols, and HPLC Qualitative and Quantitative Data for Water-Soluble Extracts[a]

			identified components					unidentified components			
sample[b]	extract yield[c]	total phenols[d]	caffeic acid	luteolin-gluc[e]	naringenin	rosmarinic acid	apigenin	HBA[f]	HCA[g]	Fl[h]	Σ
(1)	292	87.3	0.13 ± 0.01	1.84 ± 0.14	nd[i]	6.75 ± 0.32	0.15 ± 0.02	4.18 ± 0.20	0.34 ± 0.02	4.99 ± 0.06	18.38 ± 0.41
(2)	318	151.0	0.07 ± 0.00	1.04 ± 0.10	0.30 ± 0.01	5.47 ± 0.22	0.29 ± 0.03	1.12 ± 0.91	1.12 ± 0.01	3.03 ± 0.03	12.44 ± 0.94
(3)	252	84.1	0.12 ± 0.01	1.37 ± 0.00	nd	6.37 ± 0.36	0.01 ± 0.01	0.95 ± 0.05	0.73 ± 0.01	2.46 ± 0.04	12.01 ± 0.37
(4)	242	97.9	0.12 ± 0.00	2.58 ± 0.24	nd	5.09 ± 0.59	0.42 ± 0.04	0.64 ± 0.04	0.87 ± 0.01	4.26 ± 0.02	13.98 ± 0.64
(5)	290	119.1	0.21 ± 0.02	4.03 ± 0.40	0.23 ± 0.00	7.84 ± 0.07	0.19 ± 0.01	1.24 ± 0.01	2.46 ± 0.00	4.32 ± 0.03	20.52 ± 0.41
(6)	290	77.6	0.14 ± 0.00	1.70 ± 0.14	nd	6.36 ± 0.01	0.10 ± 0.01	0.57 ± 0.21	1.03 ± 0.03	6.87 ± 0.05	16.77 ± 0.26
(7)	304	93.9	0.12 ± 0.02	tr[j]	nd	3.96 ± 0.53	0.15 ± 0.01	7.49 ± 0.09	0.41 ± 0.02	3.45 ± 0.04	15.58 ± 0.54

[a] Values (mg/g) are expressed as means ± standard error. [b](1) Black thyme, (2) savory, (3) Spanish oregano, (4) sweet marjoram, (5) Syrian oregano, (6) Toka oregano, and (7) Turkish oregano. [c] Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material. [d] Total phenols expressed as gallic acid equivs, milligrams of gallic acid per gram (dry weight) of extract. [e] glu, glucoside. [f] HBA, hydroxybenzoic acid derivatives; quantified using *p*-hydroxybenzoic acid. [g] HCA, hydroxycinnamic acid derivatives; quantified using caffeic acid. [h] Fl, flavonoids; quantified using apigenin. [i] nd, not detected. [j] tr, trace.

using a nonlinear regression algorithm (SigmaPlot 2001 version 7.0). The values are presented as the mean of five measurements. BHA and BHT were not used as positive controls, due to solubility, as solvents DMSO, ethanol, or methanol cannot be used in this assay (*21*, *22*).

Site-Specific Hydroxyl Radical-Mediated 2-Deoxy-D-ribose Degradation. The ability of the extracts to inhibit site-specific hydroxyl radical-mediated peroxidation was carried out as described in the nonsite-specific hydroxyl radical-mediated peroxidation inhibition procedure, except that EDTA was replaced by buffer.

Statistical Analysis. All statistical analyses were carried out using Minitab Release 10.5 Xtra for Windows (Minitab Inc., State College, PA). Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $p < 0.05$.

RESULTS AND DISCUSSION

Extract Yields and Total Phenols. The extract yields and total phenol content data for the water-soluble extracts are shown in **Table 1**. The extract yields for the samples ranged from 242 mg/g (sweet marjoram) to 318 mg/g (savory), with the order of increasing yield being sweet marjoram < Spanish oregano < Syrian and Toka oregano < black thyme < Turkish oregano (also known as Cretan oregano and pot marjoram) < savory. No significant association between the amount of extractable components (extract yield) and Folin−Ciocalteu ($r^2 = 0.249$, $p = 0.254$) and HPLC ($r^2 = 0.068$, $p = 0.572$) total phenols, AscAE ($r^2 = 0.363$, $p = 0.152$), iron(II) chelation ($r^2 = 0.369$, $p = 0.147$), DPPH• ($r^2 = 0.304$, $p = 0.200$) and hydroxyl ($r^2 = 0.175$, $p = 0.350$) radical scavenging and nonsite- ($r^2 = 0.125$, $p = 0.437$) and site- ($r^2 = 0.034$, $p = 0.691$) specific 2-deoxy-D-ribose degradation indices was identified by linear regression analysis. The absence of a significant correlation between extract yield and quantitative and antioxidant indices has previously been reported for water-soluble *Mentha* extracts prepared in an identical fashion (*23*). This may be due to the extraction efficiacy (i.e., not only polar secondary metabolites such as hydroxylated compounds are released into solution but also polar primary metabolites, which do not possess active moieties (e.g., phenolic groups) within their structures, and therefore, are either poorly active or inactive in the test systems used).

The total phenol content determined by the Folin−Ciocalteu method, expressed as milligrams of gallic acid per gram of extract, ranged from 77.6 mg gallic acid/g for Toka oregano to 151.0 mg gallic acid/g for savory, with the order of increasing phenolic content being Toka oregano < Spanish oregano < black thyme < Turkish oregano < sweet marjoram < Syrian oregano < savory (**Table 1**). No significant association between Folin−Ciocalteu determined total phenols and HPLC total phenols ($r^2 = 0.021$, $p = 0.759$), iron(II) chelation ($r^2 = 0.299$, $p = 0.205$), nonsite- ($r^2 = 0.143$, $p = 0.403$), and site- ($r^2 = 0.291$, $p = 0.211$) specific 2-deoxy-D-ribose degradation indices was identified. There was, however, a strong association between total phenols and AscAE ($r^2 = 0.877$, $p = 0.002$), DPPH• ($r^2 = 0.835$, $p = 0.011$; excluding Toka oregano data), and hydroxyl ($r^2 = 0.720$, $p = 0.016$) radical scavenging data. It is often reported that a strong correlation exists between total phenolic content and iron(III) reduction (*24*, *25*) and free radical scavenging activity (*23*, *26*−*28*); however, the former relationship may not always be linear (*29*). The strong correlation between the Folin−Ciocalteu phenol data and the AscAE, DPPH• and hydroxyl radical scavenging data may be explained by a common underpinning mechanism of reduction and system polarity. Although the Folin−Ciocalteu method is recognized as nonspecific for phenolic compounds (*16*), other interfering substances such as sugars and ascorbic acid would also contribute to these specific antioxidant indices. They would not, however, be expected to affect HPLC determined total phenols, iron(II) chelation activity (*30*), or iron(III) chelation (site specific hydroxyl radical scavenging).

The total phenol/extractable compounds ratio ranged from 26.8% (Toka oregano) to 47.5% (savory). This suggests that there is a large amount (73.2%) of non-Folin−Ciocalteu-reactive substances in the Toka oregano extract, while the extract of savory contained only 52.5% of non-Folin−Ciocalteu-reactive substances.

Compositional Analysis. Lamiaceae species are known to contain a range of secondary metabolites, such as terpenoids, flavonoids, both glycosides and aglycones, and hydroxybenzoic and hydroxycinnamic acids. The qualitative−quantitative analysis of the extracts, made using high performance liquid chromatography coupled with PDA detection, is presented in **Table 1**, and representative chromatograms are presented in **Figure 1**. The components caffeic acid, luteolin-7-*O*-glucoside, naringenin, rosmarinic acid, and apigenin (**Figure 2**) were identified by comparisons to the retention time and UV spectra of authentic standards, while the quantitative data were calculated from their calibration curves. A number of components could not be identified; however, their chemical class was determined from their chromatographic behavior and UV spectra. Accordingly, all the water-soluble extracts contained relatively polar compounds (i.e., hydroxybenzoates, hydroxycinnamates and flavonoids), with calculated levels of caffeic acid, luteolin-7-*O*-glucoside, rosmarinic acid, and apigenin ranging from 0.21 ± 0.02 to 0.07 ± 0.00 mg/g, 4.03 ± 0.40 mg/g to trace, 7.84 ± 0.07 to 3.96 ± 0.53 mg/g, and 0.42 ± 0.04 to 0.01 ± 0.01 mg/g, respectively. Naringenin was identified only in savory (0.30 ± 0.01 mg/g) and Syrian oregano

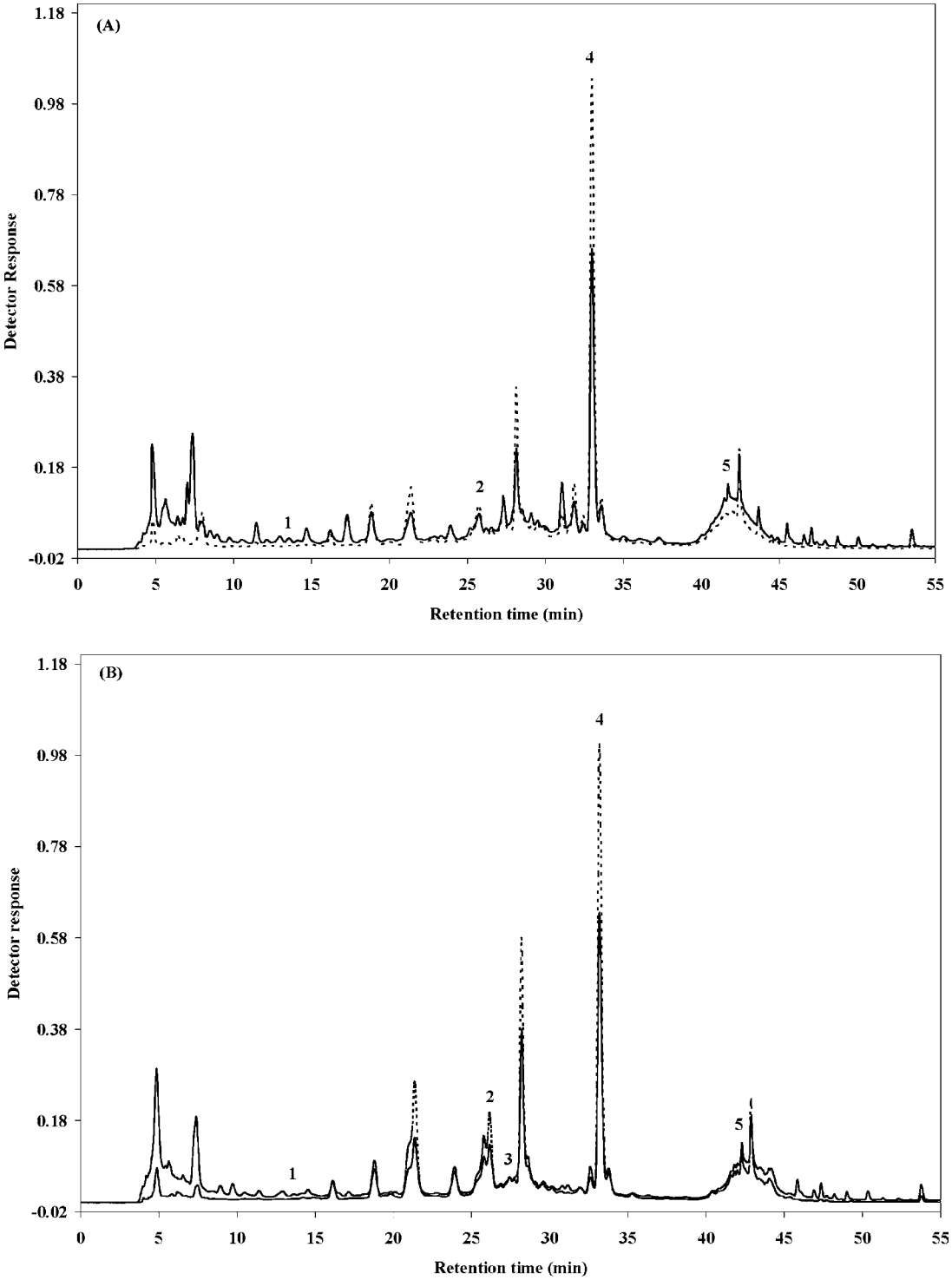

Figure 1. HPLC–PDA analyses of (A) black thyme and (B) savory water-soluble extracts with responses at 280 and 360 nm overlaid. **1**, caffeic acid; **2**, luteolin-7-*O*-glucoside; **3**, naringenin; **4**, rosmarinic acid; and **5**, apigenin.

(0.23 ± 0.00 mg/g) extracts. Amounts of unidentified hydroxybenzoates, hydroxycinnamates, and flavonoids ranged from 7.49 ± 0.09 to 0.57 ± 0.21 mg/g, 2.46 ± 0.00 to 0.34 ± 0.02 mg/g and 6.87 ± 0.05 to 2.46 ± 0.04 mg/g, respectively.

No significant association between extract yield ($r^2 = 0.068$, $p = 0.572$), Folin−Ciocalteu total phenols ($r^2 = 0.021$, $p = 0.756$), AscAE ($r^2 = 0.014$, $p = 0.800$), iron(II) chelation ($r^2 = 0.236$, $p = 0.269$), DPPH$^\bullet$ ($r^2 = 0.053$, $p = 0.619$), and hydroxyl ($r^2 = 0.167$, $p = 0.363$) radical scavenging and nonsite- ($r^2 = 0.200$, $p = 0.447$) and site- ($r^2 = 0.081$, $p = 0.538$) specific 2-deoxy-D-ribose degradation indices and the HPLC analyses was identified.

Iron(III) to Iron(II) Reducing Activity. The expression of antioxidant activity is thought to be concomitant with the development of reductones (*31*−*32*), which are reported to be terminators of free radical chain reactions (*33*); thus, any antioxidant properties of the water-soluble extracts may relate to their reductive activity. As can be seen in **Figure 3A**, all the extracts possessed the ability to reduce iron(III) and did so in a linear concentration-dependent fashion across the concentra-

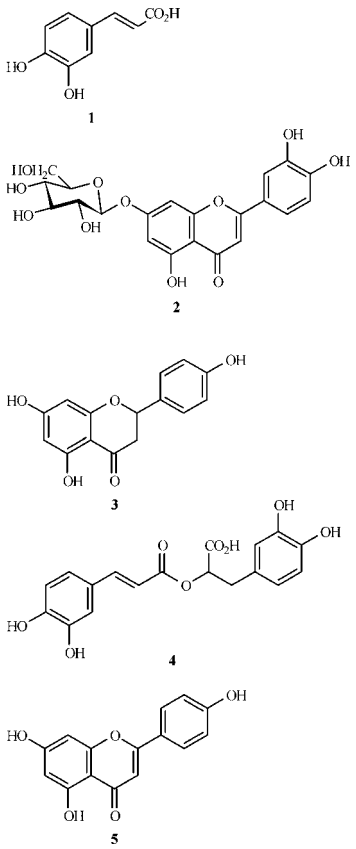

Figure 2. Structural formulas of identified components within the extracts: **1**, caffeic acid; **2**, luteolin-7-*O*-glucoside; **3**, naringenin; **4**, rosmarinic acid; **5**, apigenin.

tion range used in this study (data not shown). On the basis of the AscAE values, expressed as mmol ascorbic acid per gram dry weight of extract calculated from the plots of absorbance versus sample concentration, the savory extract was a significantly ($p < 0.05$) better iron(III) reducer than the other extracts. The Syrian oregano extract was the next most effective extract, followed by a group of significantly ($p > 0.05$) indistinguishable extracts comprised of black thyme, sweet marjoram, and Toka and Turkish oregano. The Spanish oregano extract was the least active extract in this assay system. A strong association between the AscAE data and DPPH• ($r^2 = 0.684$, $p = 0.022$) and hydroxyl ($r^2 = 0.823$, $p = 0.005$) radical scavenging activity was identified. This may be due to components within the extracts acting via a common redox mechanism. No such association existed between the AscAE values and iron(II) chelation ($r^2 = 0.352$, $p = 0.160$) and nonsite- ($r^2 = 0.126$, $p = 0.434$) and site- ($r^2 = 0.321$, $p = 0.185$) specific 2-deoxy-D-ribose degradation indices. Despite the extracts' ability to reduce iron(III) ions, none of the extracts were significantly ($p > 0.05$) better than the reference substance, ascorbic acid, or the positive controls, BHA, BHT, and Pycnogenol.

The extracts were capable of reducing iron(III), and thus, are capable of donating electrons. This property suggests that the extracts may act as free radical chain terminators, transforming reactive free radical species into more stable nonradical products.

Iron(II) Chelation Activity. Phenoxide groups of deprotonated phenolic compounds possess a high charge density, enabling them to bind suitably charged cations such as transition metal ions (*30*). Plant extracts enriched in phenolic compounds should therefore complex with and stabilize transition metal ions, rendering them unable to participate in metal-catalyzed initiation and hydroperoxide decomposition reactions (*33*). As metal chelation is an important antioxidant property (*34*, *35*), the aqueous extracts were assessed for their ability to compete with ferrozine for iron(II) ions in free solution.

All the aqueous extracts demonstrated an ability to chelate iron(II) ions in a dose-dependent fashion; i.e., as the extract concentration increased, the amount of iron(II) chelated similarly increased (data not shown). From the estimated IC_{50} values, the concentration of extract required to chelate 50% of the available iron(II) species, it can be seen in **Figure 3B** that the most effective iron(II) chelating extract was from Syrian oregano, followed by the extracts of Toka oregano and sweet marjoram (which were not significantly ($p > 0.05$) different), Spanish oregano (which was not significantly ($p > 0.05$) different in efficacy from the sweet marjoram extract), black thyme and Turkish oregano (which were not significantly ($p > 0.05$) different) and savory. Regression analysis between iron-(II) chelation and extract yield ($r^2 = 0.369$, $p = 0.147$), Folin−Ciocalteu ($r^2 = 0.299$, $p = 0.205$) and HPLC ($r^2 = 0.236$, $p = 0.269$) total phenols, AscAE ($r^2 = 0.352$, $p = 0.160$), DPPH• ($r^2 = 0.033$, $p = 0.694$), and hydroxyl ($r^2 = 0.454$, $p = 0.097$) radical scavenging, and nonsite- ($r^2 = 0.005$, $p = 0.884$) and site- ($r^2 = 0.013$, $p = 0.806$) specific 2-deoxy-D-ribose degradation indices suggest that there is no common underlying mechanism of action. The absence of a robust correlation between iron chelation and the indices dependent upon redox activity may be explained by the structural characteristics of the phenolic constituents within the extracts not possessing the optimum structural feature for metal chelation (*30*), while other redox active nonphenolic substances (e.g., ascorbate) are unable to participate in such reactions. None of the extracts appeared to be better chelators of iron(II) ions than the positive controls, EDTA, or Pycnogenol in this assay system.

From the presented data, one may conclude that the different Lamiaceae extracts may be able to afford protection against oxidative damage by sequestering free iron(II) ions, which may otherwise participate in hydroxyl radical-generating Fenton-type reactions or in metal-catalyzed hydroperoxide decomposition (*36*).

1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging. Free radicals are involved in the process of lipid peroxidation, are considered to play a cardinal role in numerous chronic diseases, and are implicated in the aging process. Phytochemicals recognized as possessing potent antioxidant activity are also strong scavengers of the synthetic nitrogen-centered free radical DPPH• (*36*). Therefore, the water-soluble extracts were assessed against DPPH• radicals to determine their free radical scavenging abilities. Substances capable of donating electrons or hydrogen atoms are able to convert 1,1-diphenyl-2-picrylhydrazyl radicals into 1,1-diphenyl-2-picrylhydrazine.

All the extracts were capable of scavenging DPPH• radicals in a concentration-dependent fashion (data not shown). From the estimated IC_{50} values, it can be seen that the savory extract was the most potent scavenger followed by Syrian and Toka oregano (which were not significantly ($p > 0.05$) different) > black thyme and sweet marjoram (which were not significantly ($p > 0.05$) different) > Turkish oregano > Spanish oregano (**Figure 3C**). There was no significant association between DPPH• free radical scavenging and extract yield ($r^2 = 0.304$, $p = 0.200$), HPLC total phenols ($r^2 = 0.053$, $p = 0.619$), iron(II) chelation ($r^2 = 0.033$, $p = 0.694$), and nonsite- ($r^2 = 0.137$, $p = 0.415$) and site- ($r^2 = 0.344$, $p = 0.166$) specific 2-deoxy-D-ribose degradation indices; however, there was a strong association with Folin−Ciocalteu total phenols ($r^2 = 0.835$, $p = 0.011$), AscAE ($r^2 = 0.684$, $p = 0.022$), and hydroxyl radical

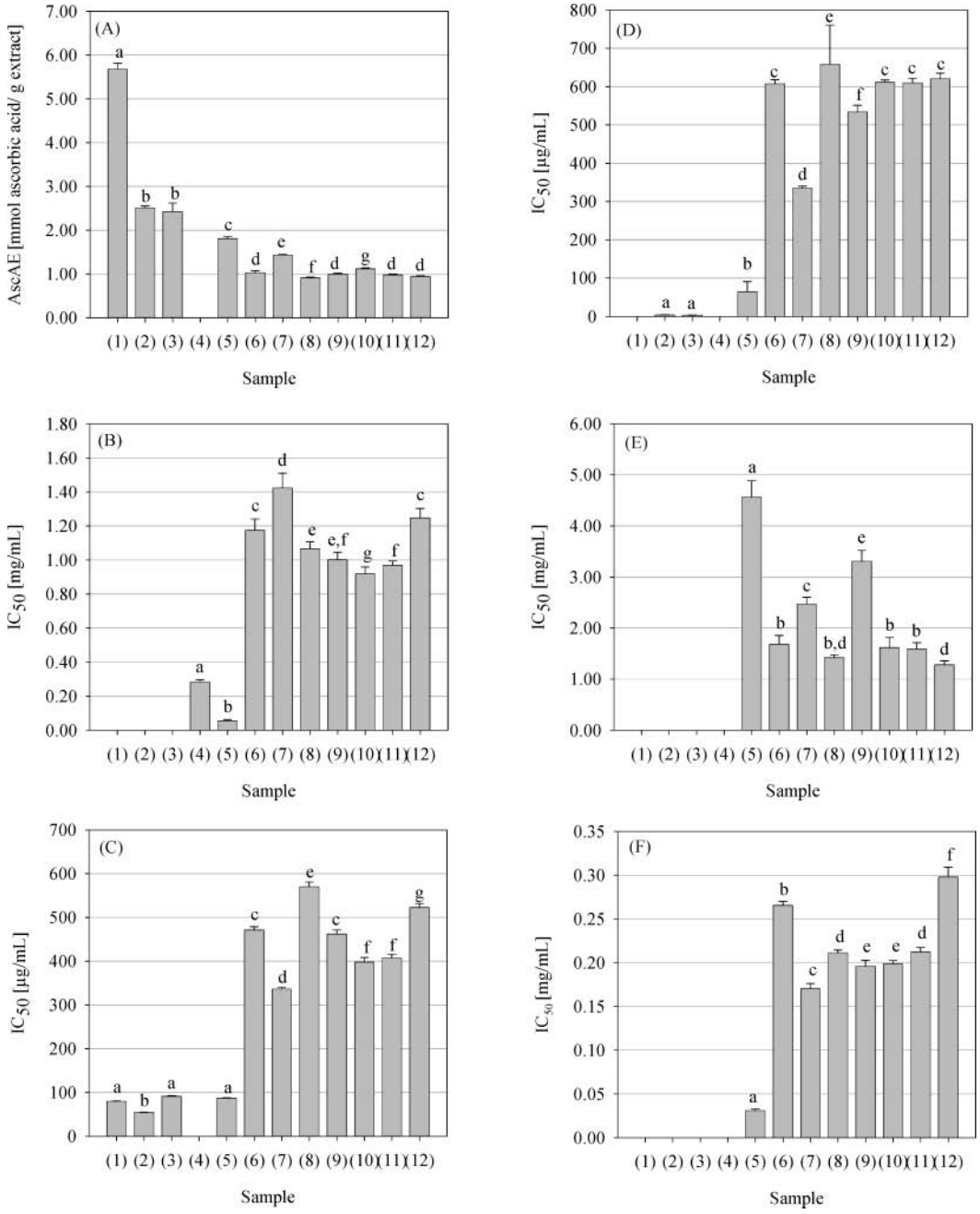

Figure 3. The effect of the extracts and positive controls upon (A) iron(III) reduction, (B) iron(II) chelation, (C) DPPH• radical scavenging, (D) hydroxyl radical scavenging, and (E) nonsite- and (F) site-specific hydroxyl radical scavenging. Values are presented as means ± 95% confidence interval. Bars with the same lowercase letter (a–g) are not significantly ($p > 0.05$) different. Samples: (1) ascorbic acid, (2) BHA, (3) BHT, (4) EDTA, (5) Pycnogenol, (6) black thyme, (7) savory, (8) Spanish oregano, (9) sweet marjoram, (10) Syrian oregano, (11) Toka oregano, and (12) Turkish oregano.

scavenging ($r^2 = 0.521$, $p = 0.067$) indices. None of the extracts were as effective DPPH• radical scavengers as the positive controls ascorbic acid, BHA, BHT, and Pycnogenol in this assay.

The results of the DPPH• free radical scavenging assay suggest that the extracts are capable of scavenging free radicals in solution at pH 7.4 and thus may be able to prevent the initiation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in deleterious reactions (e.g., the abstraction of hydrogens from susceptible polyunsaturated fatty acids).

Ascorbate-Iron(III)-Catalyzed Phospholipid Peroxidation. Free-radical-mediated degradation of phospholipids is considered to be responsible for the oxidative deterioration and off-flavor development of foods (*37*). Thus, phospholipids are regarded as a valuable substrate for the appraisal of potential food antioxidants and are considered to be an ideal model for the study of dietary components and drugs on membrane lipid peroxidation (*38*). Furthermore, iron(III) to iron(II) reduction and synthetic radical scavenging is only considered to indicate potential antioxidant activity as neither method utilizes a food or biologically relevant oxidizable substrate, thus no direct information on the extracts' protective properties can be determined (*23*). For these reasons, the extracts were assessed in a test system consisting of a complex, lipid-rich food/biologically relevant matrix (i.e., bovine brain-derived phospholipid liposome system using ascorbate-iron(III) as catalysts).

In the presence of iron(III) and ascorbic acid, phospholipid-based liposomes rapidly undergo hydroxyl radical-mediated

peroxidation, producing malondialdehyde and associated aldehydes (*39*). These species react with the reagent 2-thiobarbituric acid to produce a pink chromogen with an absorption maximum at 532 nm, under the experimental conditions used. By measuring the absorbance at 532 nm, it is possible to estimate the efficacy of an antioxidant against lipid peroxidation.

All the extracts demonstrated the ability to inhibit the formation of 2-thiobarbituric acid reactive species (TBARS) by scavenging hydroxyl radicals generated by ascorbate−iron(III)-dependent Fenton chemistry in a concentration-dependent fashion (data not shown). As can be seen from the estimated IC_{50} values presented in **Figure 3D**, the savory extract was the most active, followed by sweet marjoram, the significantly indistinct group comprising the extracts from black thyme, Syrian oregano, Toka oregano, and Turkish oregano. The least effective hydroxyl radical scavenging extract came from Spanish oregano. There was a strong association between the ability to inhibit hydroxyl radical-mediated phospholipid peroxidation and Folin−Ciocalteu total phenols ($r^2 = 0.720$, $p = 0.016$), AscAE ($r^2 = 0.823$, $p = 0.005$), and DPPH$^\bullet$ radical scavenging ($r^2 = 0.521$, $p = 0.067$) indices but not with extract yield ($r^2 = 0.175$, $p = 0.350$), HPLC total phenols ($r^2 = 0.167$, $p = 0.363$), iron(II) chelation ($r^2 = 0.454$, $p = 0.097$), and nonsite- ($r^2 = 0.377$, $p = 0.143$) and site- ($r^2 = 0.314$, $p = 0.191$) specific 2-deoxy-D-ribose degradation data. None of the extracts were as effective protectors of the phospholipid liposomes from hydroxyl radical-mediated degradation as the positive controls BHA, BHT, and Pycnogenol.

According to the data presented, the extracts were able to protect phospholipids by scavenging hydroxyl radicals before they reacted with susceptible components within the lipid matrix. Therefore, the extracts if incorporated into a lipid-rich matrix such as a foodstuff or biological membrane may protect such systems from the actions of the extremely reactive hydroxyl radical.

Nonsite- and Site-Specific Hydroxyl Radical-Mediated 2-Deoxy-D-ribose Degradation. When EDTA chelated iron(III) ions are incubated with a reducing agent and H_2O_2, hydroxyl radicals are generated in free solution. These hydroxyl radicals are capable of migrating to a substrate and fragmenting it into TBARS. This chemistry is used in the nonsite-specific hydroxyl radical-mediated 2-deoxy-D-ribose assay. A candidate compound/extract that inhibits the formation of TBARS in this system may be described as an antioxidant capable of scavenging hydroxyl radicals and protecting carbohydrates from oxidative degradation.

As can be seen from the estimated IC_{50} values from the nonsite-specific assay (**Figure 3E)**, the extracts were capable of inhibiting the formation of TBARS. The most effective extracts were obtained from the Turkish and Spanish oregano material. These extracts were not statistically significantly ($p > 0.05$) different in terms of their activity. A group composed of black thyme, Syrian oregano, Toka oregano, and Spanish oregano extracts were the next most effective, followed by savory and sweet marjoram extracts. There was no significant correlation between nonsite-specific 2-deoxy-D-ribose degradation and extract yield ($r^2 = 0.125$, $p = 0.437$), total phenol (Folin−Ciocalteu method) ($r^2 = 0.143$, $p = 0.403$), total phenol (HPLC method) ($r^2 = 0.200$, $p = 0.447$), AscAE ($r^2 = 0.126$, $p = 0.434$), iron(II) chelation ($r^2 = 0.005$, $p = 0.884$), DPPH$^\bullet$ ($r^2 = 0.137$, $p = 0.415$) and hydroxyl radical ($r^2 = 0.377$, $p = 0.143$) scavenging, and site-specific 2-deoxy-D-ribose degradation ($r^2 = 0.314$, $p = 0.191$) indices. All the extracts were statistically significantly ($p < 0.05$) more potent hydroxyl radical scavengers than the positive control Pycnogenol.

The extracts were capable of inhibiting TBARS formation at pH 7.4 by scavenging hydroxyl radicals before they reacted with the 2-deoxy-D-ribose substrate. Thus, the extracts contain compounds that are capable of protecting carbohydrate components in foods, cosmetics, and pharmaceutical preparations from oxidative damage mediated through hydroxyl radicals. This has implications for the durability of such products, as iron-EDTA is often added in the manufacturing process and H_2O_2 is widespread (*22*).

When EDTA is absent, iron(III) ions bind to 2-deoxy-D-ribose, and hydroxyl radicals are generated at the surface of the carbohydrate molecule and fragment it into TBARS. This chemistry is used in the site-specific hydroxyl radical-mediated 2-deoxy-D-ribose assay. The hypothesis that it is possible to intercept hydroxyl radicals thus formed before they fragment the 2-deoxy-D-ribose into TBARS is not plausible. Therefore, a candidate compound/extract that inhibits the formation of TBARS in this assay may be described as an antioxidant capable of preventing the formation of hydroxyl radicals by chelating and deactivating iron. Thus, it was considered necessary to repeat the aforementioned experiment without EDTA. Furthermore, it has been reported that the 2-deoxy-D-ribose assay when performed with EDTA is rather artificial when extrapolated to in vivo activity (*40*).

In the site-specific assay, the most effective extract was obtained from savory, followed by the significantly identically ($p > 0.05$) active group including sweet marjoram and Syrian oregano extracts, followed by Spanish and Toka oregano extracts (significantly ($p > 0.05$) indistinguishable), black thyme, and Turkish oregano extracts, **Figure 3F**. There was no association between site-specific 2-deoxy-D-ribose degradation and extract yield ($r^2 = 0.034$, $p = 0.691$), Folin−Ciocalteu ($r^2 = 0.291$, $p = 0.211$) and HPLC ($r^2 = 0.081$, $p = 0.538$) total phenols, AscAE ($r^2 = 0.321$, $p = 0.185$), iron(II) chelation ($r^2 = 0.013$, $p = 0.806$), DPPH$^\bullet$ ($r^2 = 0.344$, $p = 0.166$) and hydroxyl ($r^2 = 0.314$, $p = 0.191$) radical scavenging, and nonsite-specific 2-deoxy-D-ribose degradation ($r^2 = 0.314$, $p = 0.191$) indices.

The extracts were capable of preventing the oxidative degradation of 2-deoxy-D-ribose at pH 7.4 by interrupting the generation of hydroxyl radicals via the mechanism of iron(III) chelation and deactivation. Furthermore, it can be seen that the extracts were better at chelating iron than at scavenging hydroxyl radicals, as the extract IC_{50} values in site-specific assay (**Figure 3F**) are lower than the corresponding values generated in the nonsite-specific assay (**Figure 3E**). A similar finding has been reported previously (*41*).

The aerial parts of seven species grown in Turkey were extracted by the process of hydrodistillation to obtain deodorized water-soluble extracts. The extracts were screened in a battery of in vitro antioxidant assays. In addition, the Folin−Ciocalteu phenol content and qualitative−quantitative analysis of the analytes in each sample was determined. The qualitative−quantitative analysis revealed that the extracts contained varying amounts of phenolic derivatives such as hydroxybenzoic and hydroxycinnamic acids and flavonoids. Compounds identified within the extracts included caffeic acid, rosmarinic acid, luteolin-7-*O*-glucoside, and apigenin. Naringenin was identified in quantifiable amounts in the savory and Syrian oregano extracts only. The extract obtained from savory demonstrated the greatest efficacy in each assay, except for iron(II) chelation, where Syrian oregano was more potent, and in the nonsite-specific 2-deoxy-D-ribose assay, where the Turkish and Spanish

oregano extracts performed better. The greatest amount of Folin−Ciocalteu reactive substances were identified in the savory extract, which most probably contributed to its overall superior activity. Overall, one may conclude that the extracts possess exploitable in vitro antioxidant properties, especially the extract from savory. Further research should, however, be carried out to determine whether the components within the extracts are absorbed from the gastrointestinal tract, possess in vivo antioxidant activity, and ultimately are safe for human consumption. Otherwise, their potential use in the functionalization of foods or nutraceuticals cannot be confidently assumed.

LITERATURE CITED

(1) Martindale, W. H. Essential oils in relation to their antiseptic powers as determined by their carbolic coefficients. *Perf. Essent. Oil Res*. **1910**, *1*, 266−269.

(2) Chipault, J. R.; Mizuno, G. R.; Hawkins, J. M.; Lundberg, W. O. The antioxidant properties of natural spices. *Food Res*. **1952**, *17*, 46−55.

(3) American Institute of Cancer Research. *Food Nutrition and the Prevention of Cancer: A Global Perspective*; American Institute of Cancer Research: Washington, DC, 1997.

(4) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, J. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen elderly study. *Lancet* **1993**, *342*, 1007−1011.

(5) Joseph, J. A.; Shukitt-Hale, B.; Denisova, N. A.; Bielinski, D.; Martin, A.; McEwen, J. J.; Bickford, P. C. Reversal of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci*. **1999**, *19*, 8114−8121.

(6) Anon. *Major Groups, Families, and Genera*: *Lamiaceae* (*Labiatae*). Science & Horticulture, Royal Botanic Gardens, Kew, UK, 2003. http://www.rbgkew.org.uk/scihort/lamwhat.html.

(7) Anon. *Flowering Plant Families*: *Lamiaceae* (*Labiatae*). University of Hawaii, Department of Botany. 2003. http://www.botany.hawaii.edu/faculty/carr/lami.htm.

(8) Davis, P. H. *Flora of Turkey and the East Aegean Islands*. University Press: Edinburgh, UK, 1982; p 321.

(9) Kurkcuoglu, M.; Tumen, G.; Başer, K. H. C. Essential oil constituents of *Satureja boissieri* from Turkey. *Chem. Nat. Comp*. **2001**, *37*, 329−331.

(10) Englberger, W.; Hadding, U.; Etschenberg, E.; Graf, E.; Leyck, S.; Winkelmann, J.; Parnham, M. J. Rosmarinic acid: a new inhibitor of complement C3-convertase with antiinflammatory activity. *Int. J. Immunopharmacol*. **1988**, *10*, 729−737.

(11) Pizzale, L.; Bortolomeazzi, R.; Vichi, S.; Überegger, E.; Conte, L. S. Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. *J. Sci. Food Agric*. **2002**, *82*, 1645−1651.

(12) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol*. **2000**, *88*, 308−316.

(13) Sokovic, M.; Tzakou, O.; Pitarokili, D.; Couladis, M. Antifungal activities of selected aromatic plants growing wild in Greece. *Nahrung* **2002**, *46*, 317−320.

(14) Aruoma, O. I.; Spencer, J. P.; Rossi, R.; Aeschbach, R.; Khan, A.; Mahmood, N.; Muñoz, A.; Murcia, A.; Butler, J.; Halliwell, B. An evaluation of the antioxidant and antiviral action of extracts of rosemary and Provencal herbs. *Food Chem. Toxicol*. **1996**, *34*, 449−456.

(15) Koşar, M.; Dorman, H. J. D.; Bachmayer, O.; Başer, K. H. C.; Hiltunen, R. An improved on-line HPLC-DPPH• method for the screening of free radical scavenging compounds in water extracts of Lamiaceae plants. *Chem. Nat. Comp.* **2003**, *39*, 161−166.

(16) Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. In *Methods in Enzymology*; Packer, L., Ed.; Academic Press: San Diego, CA, 1999; Vol. 299, pp 152−315.

(17) Oyaizu, M. Studies on products of browning reaction: antioxidative activity of products of browning reaction. *Jpn. J. Nutr*. **1986**, *44*, 307−315.

(18) Carter, P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Anal. Biochem*. **1971**, *40*, 450−458.

(19) Gyamfi, M. A.; Yonamine, M.; Aniya, Y. Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally induced liver injuries. *Gen. Pharm*. **1999**, *32*, 661−667.

(20) Aruoma, O. I.; Spencer, J.; Warren, D.; Jenner, P.; Butler, J.; Halliwell, B. Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem*. **1997**, *60*, 149−156.

(21) Halliwell, B.; Gutteridge, J. M. C.; Aruoma, O. I. The deoxyribose method: A simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem*. **1987**, *165*, 215−219.

(22) Aeschbach, R.; Löliger, J.; Scott, B. C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O. I. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone, and hydroxytyrosol. *Food Chem. Toxicol*. **1994**, *32*, 31−36.

(23) Dorman, H. J. D.; Koşar, M.; Kahlos, K.; Holm, Y.; Hiltunen, R. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agric. Food Chem*. **2003**, *51*, 4563−4569.

(24) Gao, X.; Björk, L.; Trajkovski, V.; Uggla, M. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *J. Sci. Food Agric*. **2000**, *80*, 2021−2027.

(25) Benzie, I. F. F.; Szeto, Y. T. Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *J. Agric. Food Chem*. **1999**, *47*, 633−636.

(26) Peterson, D. M.; Emmons, C. L.; Hibbs, A. H. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. *J. Cereal Sci*. **2001**, *33*, 97−103.

(27) Jiménez-Escrig, A.; Rincón, M.; Pulido, R.; Saura-Calixto, F. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fibre. *J. Agric. Food Chem*. **2001**, *49*, 5489−5493.

(28) Fogliano, V.; Verde, V.; Randazzo, G.; Ritieni, A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem*. **1999**, *47*, 1035−1040.

(29) Yildirim, A.; Mavi, A.; Oktay, M.; Kara, A. A.; Algur, Ö. F.; Bilaloğlu, V. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf. Ex. D. C.), sage (*Salvia triloba* L.), and black tea (*Camellia sinensis* L.) extracts. *J. Agric. Food Chem*. **2000**, *48*, 5030−5034.

(30) Hider, R. C.; Liu, Z. D.; Khodr, H. H. Metal chelation of polyphenols. In *Methods in Enzymology*; Packer, L., Ed.; Academic Press: San Diego, CA, 2001; Vol. 335, pp 190−203.

(31) Duh, P. D. Antioxidant activity of budrock (*Arctium lappa* L.): Its scavenging effect on free radical and active oxygen. *J. Am. Oil Chem. Soc*. **1998**, *75*, 455−461.

(32) Tanaka, M.; Kuel, C. W.; Nagashima, Y.; Taguchi, T. Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi* **1988**, *54*, 1409−1414.

(33) Gordon, M. H. The mechanism of antioxidant action in vitro. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London, 1990; pp 1−18.

(34) Emerit, I. Reactive oxygen species, chromosome mutation, and cancer: possible role of clastogenic factors in carcinogenesis. *Free Radical Biol. Med*. **1994**, *16*, 99−109.

(35) Kehrer, J. P. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* **2000**, *149*, 43−50.

(36) Depkevicius, A.; van Beek, T. A.; Lelyveld, G. P.; van Veldhuizen, A.; de Groot, A.; Linssen, J. P. H.; Venskutonis, R. Isolation and structural elucidation of radical scavengers from *Thymus vulgaris* leaves. *J. Nat. Prod*. **2002**, *65*, 892−896.

(37) Sheldon, B. W. Influence of phospholipids on the development of oxidized off-flavors in cooked turkey rolls. *J. Food Sci*. **1988**, *53*, 55−61.

(38) Chatterjee, S. N.; Agarwal, S. Liposomes as a membrane model for study of lipid peroxidation. *Free Radical Biol. Med*. **1988**, *4*, 51−72.

(39) Esterbauer, H.; Schaur, R. G.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde, and related aldehydes. *Free Radical Biol. Med*. **1991**, *11*, 81−128.

(40) Lapenna, D.; de Gioia, S.; Mezzetti, A.; Ciofani, G.; di Ilio, C.; Cuccurullo, F. Prooxidant properties of captopril. *Biochem. Pharm*. **1995**, *50*, 27−32.

(41) Lee, J.-C.; Kim, H.-R.; Kim, J.; Jang, Y.-S. Antioxidant properties of an ethanol extract of the stem of *Opuntia ficus-indica* var. *saboten*. *J. Agric. Food Chem*. **2002**, *50*, 6490−6496.

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