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Antioxidant Properties and Composition of Aqueous Extracts from *Mentha* Species, Hybrids, Varieties, and Cultivars

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Water-soluble extracts from the *Mentha* species *M. aquatica* L. and *M. haplocalyx* Briq., the hybrids *M.* x *dalmatica* L. and *M.* x *verticillata* L., the varieties *M. arvensis* var. *japanensis* [*M. arvensis* L. var. *piperascens* Holmes ex Christ] and *M. spicata* L. var. *crispa* Benth, and *M.* x *piperita* L. "Frantsila", *M.* "Morocco", and *M.* "Native Wilmet" cultivars were screened for potential antioxidative properties. These properties included iron(III) reduction, iron(II) chelation, 1,1-diphenyl-2-picrylhydrazyl radical scavenging, and the ability to inhibit iron(III)−ascorbate-catalyzed hydroxyl radical-mediated brain phospholipid peroxidation. Total phenol content and qualitative and quantitative compositional analyses of each extract were also made. The extracts demonstrated varying degrees of efficacy in each assay, with the *M.* x *piperita* "Frantsila" extract being better than the other extracts, except for ferrous iron chelation. With the exception of iron chelation, it appeared that the level of activity identified was strongly associated with the phenolic content.

KEYWORDS: ***Mentha*; high-performance liquid chromatography; composition; antioxidants; water-soluble extracts; DPPH•; hydroxyl radicals**

INTRODUCTION

Since the pioneering work of Chipault and co-workers (*1*), who demonstrated that spices possess exploitable antioxidant properties, a large number of subsequent investigations have demonstrated that plant extracts, foodstuffs, and certain beverages can exert antioxidative actions in various in vitro models. The importance of natural antioxidants has been clarified by numerous studies which have demonstrated that the consumption of foods rich in such phytochemicals can exert beneficial effects upon human health (*2*−*4*), possibly by interfering in the processes involved in reactive oxygen and nitrogen species-mediated pathologies (*5*). This has resulted in a resurgence in phyto-pharmacognosy with extensive attention upon the role that plant secondary metabolites may have in preventative medicine.

The genus *Mentha* (Lamiaceae) comprises approximately 25−30 species which may be found in temperate regions of Eurasia, Australia, and South Africa (*6*). Although this is a distinct genus, it is notorious for its long history of cultivation, naturalization, and vegetative plasticity (*6*). Members of the genus are characterized by their volatile oils which are of great economic importance, being used by the flavor, fragrance, and pharmaceutical industries. A review of the literature reveals that the aerial materials of some members are used for herbal teas and condiments (*7*), as spasmolytics, antibacterial agents, and promoters of gas secretion (*8*), and for their analgesic and antigenotoxic properties (*9*).

The aim of this study was to assess the antioxidant properties of different species, hybrids, varieties, and cultivars from the genus *Mentha*, as members of this taxonomically complex genus have not been investigated previously except for specific species (*10*, *11*). Furthermore, a survey of the chemical literature reveals that, other than for volatile terpenes, the chemical composition of *Mentha* species is rarely reported. Therefore, the phenolic content, chemical composition, and in vitro antioxidant (iron reduction and chelation, 1,1-diphenyl-2-picrylhydrazyl radical and iron-ascorbate-generated hydroxyl radical scavenging) properties of water-soluble extracts from selected *Mentha* plants were examined. Such a study would contribute to the current knowledge relating to members of this important genus of the plant family Lamiaceae.

MATERIALS AND METHODS

Materials. *Mentha aquatica* was obtained from the Botanical Institute of Linz, Austria; *M. arvensis* var. *japanensis* and *M.* "Native Wilmet" were from the Agrifood Research North Ostrobotnia Research Station, Finland; *M.* x *dalmatica* was from the Agrifood Research Horticultural Institute, Finland; *M.* x *piperita* "Frantsila" was from the Frantsila Herb Garden, Finland; *M. haplocalyx* was from Baoding Agricultural College, China; *M.* "Morocco" was from Joroinen, Finland; *M. spicata* var. *crispa* was from the Botanical Gardens, University of Helsinki, Finland; and *M.* x *verticillata* was from the Botanical Gardens, University of Turku, Finland. Pycnogenol was obtained from Biolandes Arômes, France. Ultrapure water (18.2 MΩ cm) was prepared by using

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a Millipore Milli-RO 12 plus system (Millipore Corp., Bedford, MA). The chromatography standards were purchased from Extrasynthese (Genay, France). 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. Air-dried aerial material was suspended in ultrapure H_2O and deodorized by hydrodistillation, using a European Pharmacopoeian hydrodistillation apparatus. The water extract was then filtered, reduced in volume in vacuo at 45 °C, freeze-dried, and stored at 4 °C.

Total Phenolics. Total phenols were estimated as gallic acid equivalents according to the Folin−Ciocalteu method (*12*). First, 100 μL of sample was transferred to a 10.0-mL volumetric flask containing ca. 6.0 mL of H_2O, to which was subsequently added 500 μL of undiluted Folin−Ciocalteu reagent. After 1 min, 1.5 mL of 20% aqueous Na_2CO_3 was added, and the volume was made up to 10.0 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 phenolics were determined as gallic acid equivalents and are presented as the mean of duplicate analyses.

Chromatographic Fingerprint Analyses. The liquid chromatographic apparatus (Waters 600) consisted of an in-line degasser, pump, and controller coupled to a 2996 photodiode array (PDA) detector equipped with a Rheodyne injector (20 μL sample loop) interfaced to a PC running Millenium^{32} chromatography manager 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 0.7 mL/min. Detection was carried out with a sensitivity of 0.1 aufs between the wavelengths of 200 and 550 nm. Elution was effected using 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 B was increased from 15% to 30% in 15 min, increased to 40% in 3 min, held for 12 min, and increased to 100% in 5 min, and the composition of C was increased to 15% in 2 min, increased to 30% in 11 min, and then returned to the initial condition 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. 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-7-*O*-glucoside, eriodictyol, luteolin, isorhoifolin, and apigenin, 0.008−0.255 mg/mL for rosmarinic acid, and 0.02−0.50 mg/mL for eriocitrin 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 (*13*). One milliliter of each extract dissolved in distilled 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 a 30 min incubation at 50 °C, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.5 mL 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 milligrams of ascorbic acid per gram of extract. The bigger the AscAE, the greater the reducing power of the sample. BHA, BHT, and Pycnogenol were used as positive controls. Means are the average of at least nine measurements.

Iron(II) Chelation Activity. The chelation of iron(II) ions by the different extracts was studied as described by Carter (*14*). A 200 μL aliquot of each extract was added to 100 μL of 2.0 mM aqueous $FeCl_2 \cdot 4H_2O$ and 900 μL of methanol. After a 5 min incubation, the reaction was initiated by adding 400 μL of 5.0 mM ferrozine. The controls contained all the reaction reagents except the extract or positive control substance. After a 10 min equilibrium period, the absorbance at 562 nm was recorded. EDTA was used as a positive control. The Fe^{2+} chelating activity was calculated using eq 1. The values are presented as the mean of five measurements.

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

1,1-Diphenyl-2-picrylhydrazyl Scavenging Activity. The ability of the extracts to scavenge DPPH$^\bullet$ free radicals was determined by the method of Gyamfi et al. (*15*). 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 (50 mM, pH 7.4) 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 was calculated using eq 1, and the IC_{50} values were estimated by a nonlinear regression algorithm (SigmaPlot 2001 version 7.0, SPSS Inc., Chicago, IL). Ascorbic acid, BHA, BHT, and Pycnogenol were used as positive controls. The values are presented as the mean of nine measurements.

Ascorbate−Ferric Iron(III)-Catalyzed Phospholipid Peroxidation. The ability of the extracts to scavenge hydroxyl radicals was studied by the method of Aruoma et al. (*16*). 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 $FeCl_3$, and 0.1 mL of extract. The peroxidation was initiated by adding 0.1 mL of 1 mM ascorbate. The mixture was incubated at 37 °C for 60 min. After incubation, 50 μL of 2% butylated hydroxytoluene in EtOH was added to each tube, followed by 1 mL of 2.8% trichloroacetic acid and 1 mL of 1% 2-thiobarbituric acid (TBA) in 0.05 M NaOH. The solutions were heated in a water bath at 80 °C for 20 min. The resulting $(TBA)_2$−MDA chromogen was extracted into 2 mL of *n*-butanol, and the extent of peroxidation was determined in 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 of five measurements.

Statistical Analyses. 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 phenols data for the water-soluble *Mentha* extracts are shown in **Table 1**. The amount of extractable components from the different *Mentha* plants ranged from 285 mg/g (*M.* "Morocco") to 366 mg/g (*M.* x *verticillata*). No significant association between the amount of total extractable components and the AscAE ($r^2 = 0.100$, $p = 0.418$), iron(II) chelation ($r^2 = 0.017$, $p = 0.737$), DPPH$^\bullet$ radical scavenging ($r^2 = 0.235$, $p = 0.187$), or hydroxyl scavenging ($r^2 = 0.211$, $p = 0.213$) indices was identified by linear regression analysis. The order of increasing phenolic content, expressed as milligrams of gallic acid per gram of extract, was *M.* x *verticillata* < *M. aquatica* < *M. arvensis* var. *japanensis* < *M. haplocalyx* < *M.* "Morocco" < *M.* x *dalmatica* < *M.* "Native Wilmet" < *M. spicata* var. *crispa* < *M.* x *piperita* "Frantsila" (**Table 1**). There appeared to be a strong association between the total phenolic content and the AscAE ($r^2 = 0.818$, $p < 0.001$), DPPH$^\bullet$ radical scavenging ($r^2 = 0.902$, $p < 0.001$), and hydroxyl radical scavenging ($r^2 = 0.630$, $p < 0.01$) indices. Similar strong correlations between iron(III) reducing activity and total phenolic content have been reported in the literature (*17*, *18*); however,

Table 1. Extract Yield, Total Phenols, and HPLC Qualitative and Quantitative Data for Water-Soluble *Mentha* Extracts

			identified components[a]									
sample[b]	extract yield[c]	total phenols[d]	caffeic acid (13.4)[e]	eriocitrin (21.8)	luteolin-gluc[f] (25.8)	naringenin-gluc (26.7)	isorhoifolin (28.7)	rosmarinic acid (30.1)	eriodictyol (32.9)	luteolin (40.9)	apigenin (42.2)	Σ
(1)	334	152.5	0.02 ± 0.00	6.41 ± 0.07	6.62 ± 0.13	nd[g]	0.94 ± 0.02	9.33 ± 0.13	nd	nd	nd	23.3 ± 0.19
(2)	342	155.2	0.27 ± 0.00	0.37 ± 0.02	4.36 ± 0.04	0.30 ± 0.01	0.34 ± 0.01	5.26 ± 0.01	nd	0.03 ± 0.00	0.08 ± 0.00	11.0 ± 0.04
(3)	281	188.1	0.33 ± 0.01	8.78 ± 0.08	5.57 ± 0.03	nd	0.30 ± 0.00	12.11 ± 0.12	nd	nd	nd	27.1 ± 0.15
(4)	332	230.8	0.22 ± 0.00	40.27 ± 0.18	2.70 ± 0.01	nd	0.89 ± 0.03	8.44 ± 0.15	0.32 ± 0.00	nd	nd	52.8 ± 0.06
(5)	365	156.5	0.68 ± 0.01	0.40 ± 0.01	4.26 ± 0.04	0.19 ± 0.00	0.21 ± 0.01	4.76 ± 0.12	nd	0.28 ± 0.00	nd	10.8 ± 0.13
(6)	285	171.8	0.37 ± 0.01	0.37 ± 0.00	0.28 ± 0.01	nd	0.17 ± 0.00	0.66 ± 0.02	nd	nd	nd	1.85 ± 0.02
(7)	315	209.1	0.22 ± 0.00	23.39 ± 0.09	1.90 ± 0.02	nd	0.21 ± 0.01	4.77 ± 0.07	0.17 ± 0.00	nd	nd	30.7 ± 0.12
(8)	231	214.0	0.19 ± 0.01	10.70 ± 0.09	5.57 ± 0.03	nd	1.26 ± 0.02	4.60 ± 0.08	nd	0.54 ± 0.01	0.04 ± 0.00	22.9 ± 0.13
(9)	366	128.1	nd	6.66 ± 0.15	9.15 ± 0.11	0.07 ± 0.00	1.10 ± 0.04	6.45 ± 0.16	nd	nd	nd	23.4 ± 0.25

[a] Values (mg/g) are expressed as means ± standard deviation. [b] (1) *M. aquatica*, (2) *M. arvensis* var. *japanensis*, (3) *M.* x *dalmatica*, (4) *M.* x *piperita* "Frantsila", (5) *M. haplocalyx* (6) *M.* "Morocco", (7) *M.* "Native Wilmet", (8) *M. spicata* var. *crispa*, (9) *M.* x *verticillata*. [c] Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material. [d] Total phenols expressed as gallic acid equivalents, milligrams per gram (dry weight) of extract. [e] Retention time (min). [f] gluc, glucoside. [g] nd: not detected.

the correlation may not always be linear (*19*). A high correlation between free radical scavenging and the phenolic content has also been reported for cereals (*20*), fruits (*21*, *22*), beverages (*23*), and culinary herbs (*24*). There was no significant association with the iron(II) chelation index ($r^2 = 0.198$, $p = 0.231$). Flavonoids are known to chelate metals (*25*, *26*), but being phenolic does not per se mean that all such compounds are effective metal chelators. An explanation for the lack of correlation between the total phenol content and chelation may be that the phenolic components within each extract do not possess the optimum structural characteristics for metal chelation (*26*).

The total phenolic content:total extractable compounds (extract yield) ratio ranged from 35.0% (*M.* x *verticillata*) to 92.6% (*M. spicata* var. *crispa*). This means that there is a large amount (65.0%) of non-Folin−Ciocalteu-reagent-reactive substances in the extract of *M.* x *verticillata*, while the extract from *M. spicata* var. *crispa* contained only 7.4% of non-Folin−Ciocalteu-reagent-reactive substances.

Compositional Analysis. Species of the genus *Mentha* have been reported to contain a range of components, including cinnamic acids (*11*, *27*, *28*), aglycon, glycoside or acylated flavonoids (*27*−*31*), and steroidal glycosides (*29*). The qualitative−quantitative analysis of the *Mentha* 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, eriocitrin, luteolin-7-*O*-glucoside, naringenin-7-*O*-glucoside, isorhoifolin, rosmarinic acid, eriodictyol, luteolin, and apigenin (**Figure 2**) were identified by comparisons to the retention times and UV spectra of authentic standards, while the quantitative data were calculated from their calibration curves. Accordingly, eriocitrin (eriodictyol-7-*O*-rutinoside), luteolin-7-*O*-glucoside, and rosmarinic acid were identified as the major components in the extracts. The most active samples, *M.* x *piperita* "Frantsila" and *M.* "Native Wilmet", contained the largest amounts of eriocitrin (40.27 ± 0.18 and 23.39 ± 0.09 mg/g, respectively) and high levels of rosmarinic acid (8.44 ± 0.15 and 4.77 ± 0.07 mg/g, respectively). Areias et al. (*27*) have reported the main components in aqueous *Mentha* extracts to be the glycoside eriocitrin and the caffeic acid dimer rosmarinic acid. Triantaphyllou et al. (*11*) reported that water extracts from *Mentha* contain bound phenolic acids and flavonoid derivatives such as chlorogenic acid and rosmarinic acid (caffeic acid derivatives) and 3- or 5-position hydroxylated glycosidic flavonoids. This was a trend observed in these extracts, with relatively minor representation by flavone aglycons such as luteolin, apigenin, and eriodictyol. There was no significant association between the extract yields, total phenolic content, and the quantitative HPLC analysis.

Iron(III) to Iron(II) Reducing Activity. Different studies have indicated that the antioxidant effect is related to the development of reductones (*32*). Reductones are reported to be terminators of free radical chain reactions (*33*); thus, the antioxidant activity of an aqueous extract may be related to its reductive activity. As can be seen in **Figure 3**, all the extracts possessed the ability to reduce iron(III) and do so in a linear concentration-dependent fashion. On the basis of the AscAE values calculated from the plots of absorbance ($Abs_{700\ nm}$) versus sample concentration (mg/mL), the *M.* x *piperita* "Frantsila" cultivar extract was a significantly ($p < 0.05$) better iron(III) reducer than the other extracts, followed by the *M.* "Native Wilmet" cultivar. The hybrid *M.* x *verticillata* extract was the least active extract when compared to the others. The *M. aquatica* extract was the second least active extract; however, it was not possible to distinguish it from the potency of the *M. haplocalyx* extract ($p > 0.05$). Despite the lack of statistical difference between these two extracts, there was a significant difference between the performance of *M. aquatica* and the other *Mentha* plant extracts. There were no significant differences between the activities of the extracts of *M. arvensis* var. *japanensis*, *M.* x *dalmatica*, *M.* "Morocco", and *M. spicata* var. *crispa* or between this group of extracts and *M. haplocalyx*. None of the activities of the *Mentha* extracts were comparable to those of the positive controls BHA, BHT, and Pycnogenol in this assay.

There was a strong correlation between the calculated AscAE values and the estimated IC_{50} $DPPH^{\bullet}$ radical scavenging data ($r^2 = 0.924$, $p < 0.001$). This may be due to system solubility (both assays are polar in nature) and a common underpinning mechanism, i.e., electron/hydrogen donation. A high correlation has been reported between the results of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging (*34*). The correlation was less strong between the AscAE values and the estimated IC_{50} hydroxyl radical scavenging data ($r^2 = 0.549$, $p < 0.05$) but was still statistically significant. There was no apparent association between the AscAE values and the iron chelating properties of the extracts ($r^2 = 0.087$, $p = 0.441$).

The extracts demonstrated electron-donating properties and thus may act as free radical chain terminators, transforming reactive free radical species into more stable nonradical products.

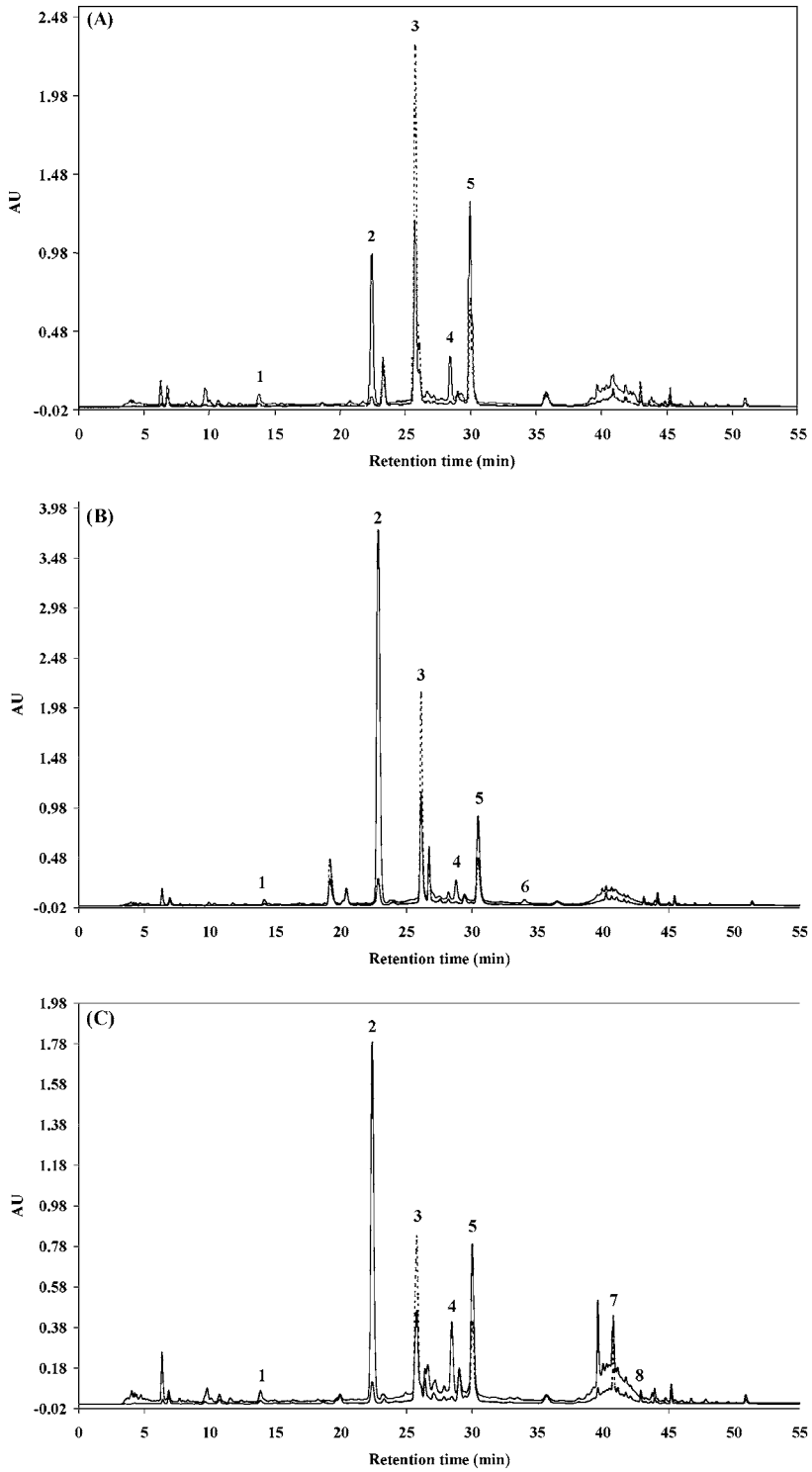

Figure 1. HPLC–PDA analyses of **(**A**)** *M*. x *dalmatica*, **(**B**)** *M*. x *piperita* "Frantsila", and **(**C**)** *M. spicata* var. *crispa* extracts with responses at 280 and 360 nm overlaid. **1**, Caffeic acid; **2**, eriocitrin; **3**, luteolin-7-*O*-glucoside; **4**, isorhoifolin; **5**, rosmarinic acid; **6**, eriodictyol; **7**, luteolin; and **8**, apigenin.

Iron(II) Chelation. The phenoxide group of a deprotonated phenolic compound possesses a high charge density which can bind a suitably highly charged cation (*26*). Extracts rich in such components should be able to complex metals ions and stabilize the form of the metal ion, thus hindering metal-catalyzed initiation and hydroperoxide decomposition reactions (*33*). Because of the importance of metal chelation as an antioxidant property (*35*, *36*), the ability of the *Mentha* extracts to compete with ferrozine for iron ions in free solution was studied. All the 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. As can be seen in **Figure 4A**, the extracts could be separated into two distinct groups on the basis of the estimated IC_{50} values: group 1, containing *M. spicata* var. *crispa*, *M.* x *piperita* "Frantsila", and *M. haplocalyx*; and group 2, containing *M. aquatica*, *M. arvensis* var. *japanensis*, *M.* x *dalmatica*, *M.* "Native Wilmet", *M.* "Morocco", and *M.* x *verticillata*. Group 2 members were significantly ($p < 0.05$) better chelators than the members of group 1. There were no statistically significant differences in potency between members within each group. Regression analysis between the IC_{50} chelation values and

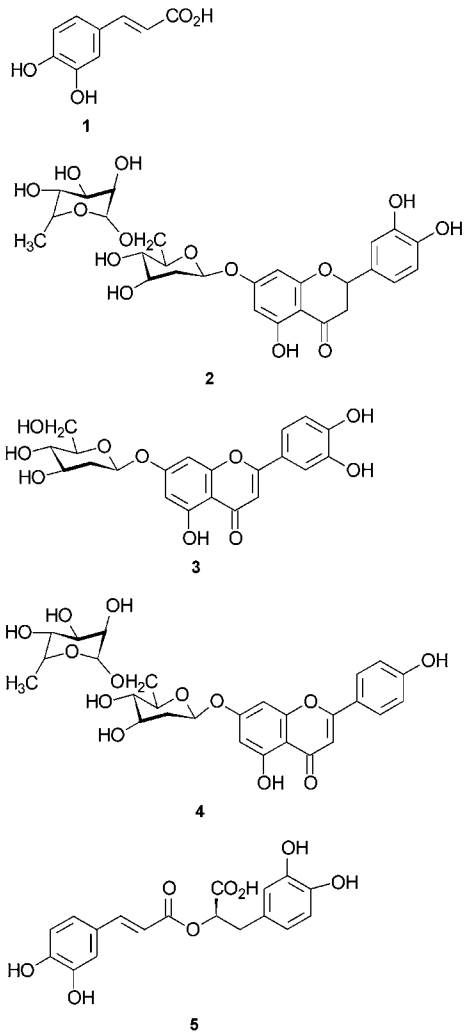

Figure 2. Structural formulas of the main compounds identified: **1**, caffeic acid; **2**, eriocitrin; **3**, luteolin-7-*O*-glucoside; **4**, isorhoifolin; **5**, rosmarinic acid.

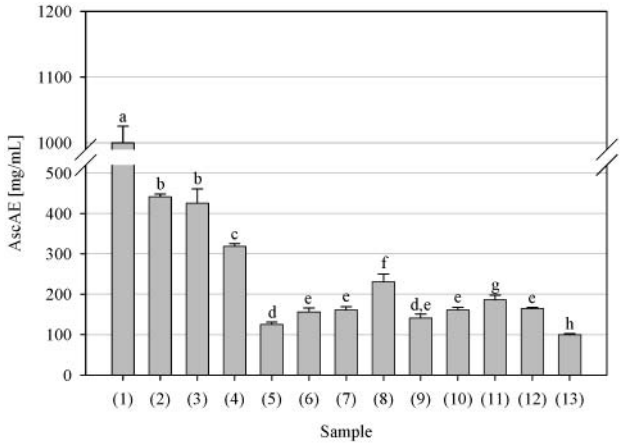

Figure 3. AscAE values calculated for the positive controls and *Mentha* extracts. Values are presented as mean values ± 95% confidence interval. Bars with the same lowercase letter (a–h) are not significantly ($p > 0.05$) different. Samples: (1) ascorbic acid, (2) BHA, (3) BHT, (4) Pycnogenol, (5) *M. aquatica*, (6) *M. arvensis* var. *japanensis*, (7) *M.* x *dalmatica*, (8) *M.* x *piperita* "Frantsila", (9) *M. haplocalyx*, (10) *M.* "Morocco", (11) *M.* "Native Wilmet", (12) *M. spicata* var. *crispa*, and (13) *M.* x *verticillata*.

AscAE ($r^2 = 0.087$, $p = 0.441$), DPPH• ($r^2 = 0.099$, $p = 0.041$), and hydroxyl radical scavenging ($r^2 = 0.060$, $p = 0.526$) values revealed that there was no common underlying mechanism of action between these different techniques when assessing the extracts.

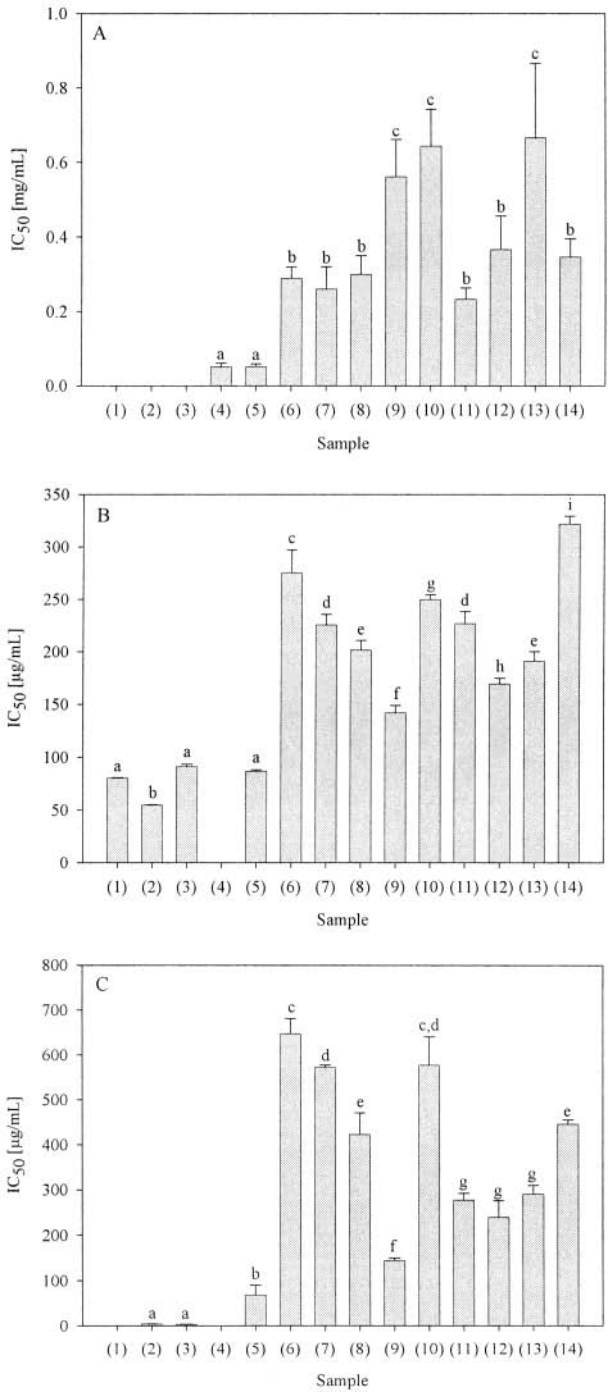

Figure 4. Effect of the different *Mentha* extracts upon (A) iron(II) chelation, (B) DPPH• radical scavenging, and (C) hydroxyl radical scavenging. Values are presented as mean values ± 95% confidence interval. Bars with the same lowercase letter (a–i) are not significantly ($p > 0.05$) different. Samples: (1) ascorbic acid, (2) BHA, (3) BHT, (4) EDTA, (5) Pycnogenol, (6) *M. aquatica*, (7) *M. arvensis* var. *japanensis*, (8) *M.* x *dalmatica*, (9) *M.* x *piperita* "Frantsila", (10) *M. haplocalyx*, (11) *M.* "Morocco", (12) *M.* "Native Wilmet", (13) *M. spicata* var. *crispa*, and (14) *M.* x *verticillata*.

From the data one may conclude that the *Mentha* extracts may be able to afford protection against oxidative damage by removing iron(II) ions which may otherwise participate in hydroxyl radical-generating Fenton-type reactions (*36*) or in

metal-catalyzed hydroperoxide decomposition reactions if available in a free unsequestered form.

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 pathologies, such as cancer and cardiovascular diseases among others, and are implicated in the aging process. Therefore, the water extracts were assessed against DPPH• radicals to determine their free radical scavenging properties. In this assay, the DPPH• radical serves as the oxidizing substrate, which can be reduced by an antioxidant compound to its hydrazine derivative via hydrogen donation, and as the reaction indicator molecule.

All the *Mentha* extracts were capable of scavenging DPPH• radicals at pH 7.4 in a dose-dependent fashion. From the estimated IC_{50} values, it can be seen that the *M.* x *piperita* "Frantsila" extract was the most potent scavenger, followed by those from *M.* "Native Wilmet" > *M. dalmatica* and *M. spicata* var. *crispa* (which were not statistically significantly different) > *M. arvensis* var. *japanensis* and *M.* "Morocco" (which were not significantly different) > *M. haplocalyx* > *M. aquatica* > *M.* x *verticillata* (**Figure 4B**). When the DPPH• scavenging activity is compared to that of identically prepared and assessed extracts from *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Thymus vulgaris* (335.0 ± 18.1, 236.5 ± 0.1, 265.8 ± 7.6, and 382.4 ± 28.3 μg/mL, respectively), Lamiaceae species recognized as antioxidative phytochemical-rich sources, the *Mentha* activities are very favorable. None of the extracts were as effective DPPH• radical scavengers as the positive controls ascorbic acid, BHA, BHT, or Pycnogenol in this assay. There was a strong association between iron(III) reducing activity of the *Mentha* extracts, expressed as AscAE values, and DPPH• radical scavenging ($r^2 = 0.924$, $p < 0.001$), but the association was less robust when calculated with the hydroxyl radical IC_{50} data ($r^2 = 0.506$, $p < 0.05$).

The results of the DPPH• radical scavenging assay reveal that the *Mentha* extracts are capable of scavenging free radicals in solution at pH 7.4 and may be able to prevent initiation of free radical-mediated chain reactions by preventing the abstraction of hydrogens from susceptible polyunsaturated fatty acids.

Ascorbate−Ferric Iron(III)-Catalyzed Phospholipid Peroxidation. The iron(III) to iron(II) reduction and DPPH• radical scavenging assays are potential indicators of antioxidant activity; however, neither method utilizes a food or biologically relevant oxidizable substrate, so no direct information on the extracts' protective properties can be determined. Furthermore, phospholipids are considered to play a major role in oxidative deterioration and off-flavor development in foods (*37*). Therefore, it was considered necessary to assess the *Mentha* extracts in a test system consisting of a complex, lipid-rich food/biologically relevant matrix, i.e., a brain-derived phospholipid liposome system, using iron(III) ions and ascorbate as catalysts.

Brain phospholipid liposomes rapidly undergo hydroxyl radical-mediated peroxidation in the presence of ferric ions and a reducing agent (*16*), producing malonaldehyde and associated aldehydes (*38*). These byproducts react with the reagent 2-thiobarbituric acid to produce a pink chromogen with an absorption maximum of 532 nm under the experimental conditions used in this assay. By measuring the absorbance at 532 nm, it is possible to estimate the properties of an antioxidant upon lipid peroxidation.

All the *Mentha* extracts were capable of preventing the formation of thiobarbituric acid-reactive substances (TBARS) by scavenging hydroxyl radicals generated in the presence of iron(III) ions and ascorbic acid in a dose-dependent manner. On the basis of the estimated IC_{50} values presented in **Figure 4C**, the most active extract was that obtained from the cultivar *M.* x *piperita* "Frantsila", followed by the statistically indistinct group comprising the extracts from the cultivars *M.* "Morocco" and *M.* "Native Wilmet" and the variety *M. spicata* var. *crispa*. The next most active extracts belonged to a group comprising the hybrids *M.* x *verticillata* and *M.* x *dalmatica*. The extract from the variety *M. arvensis* var. *japanensis* was significantly less potent than the aforementioned extracts but was better than the extract from *M. aquatica*. The estimated IC_{50} value obtained for the extract from *M. haplocalyx* was not significantly different from the values obtained for either the *M. aquatica* or *M. arvensis* var. *japanensis* extracts in this experimental model. None of the extracts were as effective at scavenging hydroxyl radicals in this assay as the positive controls BHA, BHT, and Pycnogenol.

The hydroxyl radical is an extremely reactive oxygen species which is capable of reacting with every biological molecule within its immediate vicinity. According to the presented data, the *Mentha* extracts were able to afford protection to the phospholipid liposomes by quenching hydroxyl radicals before they could react with susceptible components within the lipid substrate. Therefore, the *Mentha* extracts appear to be able to prevent the propagation of the lipid peroxidation process in a complex lipid matrix, such as a foodstuff or biological membrane.

Water-soluble extracts obtained from selected *Mentha* species, hybrids, varieties, and cultivars were screened to determine their reductive power, their chelating potency, their ability to scavenge the synthetic radical DPPH•, and their protective effects against hydroxyl radical-mediated phospholipid degradation. Furthermore, qualitative−quantitative analysis of the analytes in each extract was carried out using high-performance liquid chromatography with PDA detection. All the extracts demonstrated varying degrees of efficacy within each antioxidant assay. The cultivar *M.* x *piperita* "Frantsila" appeared to be a good source of natural antioxidants. It contained the highest levels of total phenols as determined using the Folin−Ciocalteu reagent and by HPLC, due to the high levels of eriocitrin and rosmarinic acid. The extract obtained from the hybrid *M.* x *verticillata* was the least active of all the extracts, except when assessed in the chelation and ascorbate−ferric iron(III)-catalyzed phospholipid peroxidation assays. Overall, the data suggest that the members of the genus *Mentha* used in this study possess exploitable antioxidant properties in vitro. Further work should be carried out to determine whether the water-soluble extracts possess in vivo activities; otherwise, their potential use in the functionalization of foods cannot be confidently assumed.

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