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Antioxidant Characterization of Some Sicilian Edible Wild Greens

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Epidemiological studies have demonstrated that many antioxidants and the total antioxidant capacity (TAC) of the diet may protect against cancers and cardiovascular disease. Common fruits and vegetables are good sources of antioxidants, although in some Mediterranean areas traditional wild greens are responsible for a significant percentage of total dietary antioxidant intake. In the European Prospective Investigation into Cancer and Nutrition cohort of Ragusa (Sicily), a high number of subjects were found to frequently eat wild greens, including *Sinapis incana* and *Sinapis nigra*, *Diplotaxis erucoides*, *Cichorium intybus*, *Asparagus acutifolius*, and *Borrago officinalis*. On the basis of these observations, detailed characterization of single antioxidant components (i.e., polyphenols, carotenoids, chlorophylls, and ascorbic acid) and the TAC of these edible wild traditional plants was performed. The wild plants examined were found to be very rich in antioxidants, such as flavonoids and carotenoids, with high TAC values, suggesting that the importance of these vegetables, not only in the traditional but even in the contemporary diet, needs to be emphasized.

KEYWORDS: Wild greens; antioxidant capacity; flavonoids; carotenoids; ascorbic acid

INTRODUCTION

A number of naturally occurring compounds in vegetables and herbs have antioxidant properties and, due to their putative protective effects against free radical-related pathologies emerging from the epidemiological studies, natural antioxidants have recently become a major area of research. Common fruits and vegetables are good sources of a large number of such compounds, although in some Mediterranean areas traditional wild greens are responsible for a significant percentage of total dietary antioxidant intake (*1*). In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort of Ragusa, Sicily, a high number of subjects were found to frequently eat wild vegetables such as *Sinapis incana* (shortpod mustard), *Sinapis nigra* (black mustard), *Diplotaxis erucoides* (white wallrocket), *Cichorium intybus* (wild chicory), *Asparagus acutifolius* (wood asparagus), and *Borrago officinalis* (common borage) (*2*). This dietary behavior was observed in the entire studied population, independent of age and sex, and contributed markedly to total fruit and vegetable intake. In this region, wild greens are generally consumed boiled and dressed with relatively large amounts of extra-virgin olive oil. This method of consumption has been considered a healthy nutritional habit typical of Mediterranean countries.

Dietary antioxidant intake has been associated with reduced risk of cardiovascular diseases (*3*), diabetes (*4*), cancer (*5*), and neurodegenerative diseases (*6*). Nevertheless, many studies have failed to observe remarkable effects of single classes of antioxidants in protecting the human body from these chronic diseases (*7*). It was recently demonstrated that the total antioxidant capacity (TAC) of the diet, an index taking into account the antioxidant capacity of single compounds present in food as well as their potential synergistic and redox interactions, may be related to the efficacy of protection against gastric cancer (*8*) or beneficial effects against inflammatory processes (*9*, *10*).

On the basis of these observations, detailed characterization of single antioxidant components and TAC values of edible wild traditional greens commonly consumed in southern Italy was performed to contribute to the understanding of the protective effects of the traditional Mediterranean diet.

MATERIALS AND METHODS

Samples. For this study, aerial parts of various wild plants (*A. acutifolius* L., *B. officinalis* L., *C. intybus* L., *D. erucoides* L., and two species of *Sinapis*, *S. incana* L. and *S. nigra* L.) were gathered at maturity in the Ragusa territory in three growth regions (mountain, countryside, and seaside). Within 24 h from collection, the edible

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portions of plants were boiled according to local recipes in uncovered pots with a minimum amount of water (1:10, grams of sample in milliliters of tap water) for 15 min (*Asparagus* for 10 min). The cooked vegetables were put in a domestic colander for 5 min to drain the water, cooled by means of an ice bath for 5 min, and weighed, and aliquots were immediately analyzed for total antioxidant capacity and contents of ascorbic acid (AA) and dehydroascorbic acid (DHAA). The remaining boiled vegetables were apportioned in plastic bags and stored at −80 °C until performance of other analyses. Each plant, collected in the three growth regions, was extracted in single and analyzed in duplicate.

Chemicals. Caffeic and *m*-coumaric acids, quercetin, chlorophyll *a*, chlorophyll *b*, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic) diammonium salt (ABTS), dithiothreitol (DTT), and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO). L-ascorbic acid was obtained from Merck (Darmstadt, Germany). *R*-Phycoerythrin (R-PE) was purchased from Prozyme (San Leandro, CA) and 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) from Wako Chemicals (Richmond, VA). Crystalline carotenoids used as standards were β-carotene and lutein obtained from Sigma-Aldrich. Canthaxanthin was a gift from F. Hoffman-La Roche (Basel, Switzerland). All chemicals and solvents used were of HPLC grade and purchased from Carlo Erba (Milan, Italy). High-purity water was produced in the laboratory using an Alpha-Q system (Millipore, Marlborough, MA).

Characterization and Quantification of Antioxidant Contents. *Determination of Carotenoids and Chlorophylls.* Precisely weighed amounts of frozen boiled vegetable (5 g) were homogenized with 10 mL of acetone, containing 0.2% 2,6-di-*tert*-butyl-4-methylphenol (BHT) (w/v), using an Ultra-Turrax homogenizer (Janke & Kunkel, Staufen, Germany) at a moderate speed for 1 min, keeping the sample refrigerated by means of an ice bath, and then sonicated for 30 s. The homogenized sample was centrifuged at 12000*g* for 5 min at 4 °C. The acetone was recovered and the pulp residue re-extracted with 5 mL of acetone four times. All extracts were pooled and diluted to 50 mL with acetone. Before injection, the extract was filtered through a 0.5 μm Fluoropore filter (Millipore, Bedford, MA).

Carotenoids and chlorophylls were evaluated using an HPLC system consisting of a 996 photodiode array detector (Waters, Milford, MA) and a 600E multisolvent delivery system (Waters) equipped with a 20 μL loop. HPLC analyses were performed using a Waters Nova-Pak column (4 μm, 150 × 3.9 mm i.d.) protected by a Waters Nova-Pak C18 guard column, maintained at 30 °C using a Waters column heater. The flow rate was maintained at 1 mL min^{-1}, and the gradient was 0−2 min 90% A, 3−5 min 70% A, 6−8 min 50% A, and 9−23 min 45% A, where solvent A was 80% aqueous methanol (v/v) and solvent B ethyl acetate. Data were recorded for 26 min, and the column was subsequently washed with 20% of solvent A and 80% of solvent B for 5 min before re-equilibration. Compounds were quantified at 445 nm (lutein), 453 nm (β-carotene), 663 nm (chlorophyll *a*), or 645 nm (chlorophyll *b*). A photodiode array detector (PAD) supported by the Millenium^{32} chromatography manager computing system (Waters) was used to assess or confirm the spectral identity of carotenoids and chlorophylls, registering the spectra in the range between 300 and 700 nm. In the absence of adequate standards, violaxanthin and neoxanthin were identified on the basis of retention time and characteristic spectra (λ_{max} at 450 nm) and quantified on the basis of β-carotene standard. The average relative standard deviations for this method were 2.9% for β-carotene and 3.3% for chlorophylls. Canthaxanthin was used as an internal standard, and recoveries were consistently between 92 and 96%.

Determination of Polyphenols. Extraction of phenolic compounds was performed according to the method of Escarpa et al. (*11*) with slight modifications. Briefly, 3 g of homogenized frozen boiled sample was extracted with 6 mL of 80% aqueous methanol (v/v), containing 1% BHT (w/v) as antioxidant and 5% formic acid (v/v), for 1 h in an ultrasonic bath at room temperature and in the absence of light. After centrifugation (12000*g* for 5 min at 4 °C), the solid phase was re-extracted with 6 mL for 30 min in the same conditions, and the procedure was repeated one more time with 3 mL for 30 min. The extracts were then combined and stored at −20 °C until analysis.

The extract was filtered on a 0.45 μm polypropylene Whatman filter (Brentford, Middlesex, U.K.), and an aliquot of it (4 mL) was dried under nitrogen flow, resuspended in 4 mL of distilled water, and pretreated on a Sep-Pak C_{18} cartridge (Waters). After brief washing of the cartridge with 0.4% tartaric acid, the polyphenol fraction was eluted with 2 mL of acidified methanol (0.1%, v/v). The collected fraction was then subjected to acid hydrolysis by adding 8 mL of 2 N HCl in a screw-capped tube flushed with nitrogen in the absence of light, with reaction allowed to proceed for 45 min in a boiling water bath. To eliminate acid and to concentrate the polyphenols in the hydrolyzed fraction, the sample was then treated with a new Sep-Pak C_{18} cartridge and the aglycones were eluted under the same conditions as described above. The eluted fraction was injected into the HPLC system previously described for carotenoid and chlorophyll analysis, with a flow rate of 0.8 mL min^{-1} and the column kept at 30 °C. A gradient of solvent A (water with 10% formic acid, v/v) and solvent B (10% formic acid/methanol, 60:40, v/v) was applied (0−15 min linear from 75 to 55% A, 16−50 min linear to 10% A, 51−55 min isocratic 10% A). The PAD registered wavelengths from 260 and 600 nm. All of the phenolic acids were quantified as caffeic acid equivalents and all of the flavonoids as quercetin equivalents. Recoveries, which were checked using *m*-coumaric acid as internal standard, ranged between 92 and 96%.

Determination of Ascorbic and Dehydroascorbic Acid. Using the method proposed by Dürust et al. (*12*), a homogenized portion of the cooked vegetable was added to an equivalent weight of oxalic acid solution (0.4%, w/v). The mixture was homogenized in a high-speed blender. A portion of the homogenized sample (∼1 g) was subsequently diluted with 2 mL of oxalic acid solution, shaken, and centrifuged at 1000*g* for 5 min.

Dehydroascorbic acid was determined as the difference between the total ascorbic acid obtained after DHAA reduction and the original AA content of the sample. Complete reduction of DHAA to AA was achieved by reaction with a 1 mg mL^{-1} DTT solution for 90 min at room temperature in the dark. All samples were immediately analyzed in the HPLC system as described by Gokmen et al. (*13*).

Determination of Total Antioxidant Capacity. A precisely weighed amount of the homogenized cooked sample (∼1 g) was extracted with 4 mL of water for 15 min at room temperature and centrifuged at 1000*g* for 10 min, and the supernatant was collected. The extraction was repeated with 2 mL of water, and the two supernatants were combined. The pulp residue was re-extracted with 4 mL of acetone for 15 min at room temperature and centrifuged at 1000*g* for 10 min, and the supernatant was collected. The extraction was repeated with 2 mL of acetone, and the two supernatants were combined. All extracts were adequately diluted in appropriate solvent (depending on their activity) and immediately analyzed for antioxidant capacity with three different methods, as follows.

(a) Trolox Equivalent Antioxidant Capacity (TEAC) Assay. This method is based on the ability of antioxidant molecules to quench long-lived ABTS$^{\bullet+}$, a blue-green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble vitamin E analogue. The addition of antioxidants to the preformed radical cation reduces it to ABTS, determining a decolorization. A stable stock solution of ABTS$^{\bullet+}$ was produced by reacting a 7 mmol L^{-1} aqueous solution of ABTS with 2.45 mmol L^{-1} potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use (*14*). At the beginning of the day of analysis, an ABTS$^{\bullet+}$ working solution was obtained by dilution in ethanol of stock solution to an absorbance of 0.70 (± 0.02) AU at 734 nm, checked by a Hewlett-Packard 8453 diode array spectrophotometer (HP, Waldbronn, Germany), and used as mobile phase in a flow injection system, according to the method of Pellegrini et al. (*15*). Results were expressed as TEAC in millimoles of Trolox per kilogram of wet weight.

(b) Total Radical-Trapping Antioxidant Parameter (TRAP) Assay. TRAP was determined according to the method of Ghiselli et al. (*16*) on the basis of the protection provided by antioxidants against the fluorescence decay of R-PE (lag-phase) during a controlled peroxidation reaction. Briefly, 120 μL of diluted sample was added to 2.4 mL of phosphate buffer (pH 7.4), 375 μL of bidistilled water, 30 μL of diluted

Table 1. Contents of Chlorophylls and Carotenoids in the Samples Examined[a]

component	*A. acutifolius*	*B. officinalis*	*C. intybus*	*D. erucoides*	*S. incana*	*S. nigra*
carotenoids						
lutein	27.4 ± 5.6a	38.1 ± 11.2a	42.5 ± 5.9a	70.2 ± 25.7a	53.4 ± 26.8a	68.4 ± 19.8a
neoxanthin[b]	1.7 ± 3.0b	11.3 ± 0.5a	14.4 ± 2.9a	13.3 ± 3.0a	10.3 ± 4.2a	11.7 ± 2.1a
unidentified 1[b]	5.8 ± 0.7a			9.0 ± 3.8a	5.4 ± 0.2a	7.3 ± 0.8a
unidentified 2[b]						7.3 ± 0.4
violaxanthin[b]	1.6 ± 2.7b	11.5 ± 1.2a	17.0 ± 3.1a	11.3 ± 1.4a	11.3 ± 2.8a	14.2 ± 3.5a
β-carotene	11.9 ± 2.0b	28.6 ± 3.6ab	40.2 ± 4.3a	47.5 ± 13.7a	30.3 ± 13.7b	37.0 ± 6.7ab
total carotenoids	48.4b	89.5ab	114.1ab	151.3a	110.7ab	145.9a
chlorophylls						
chlorophyll *a*	63.6 ± 2.1b	278.1 ± 72.1a	382.4 ± 50.2a	428.3 ± 88.4a	250.7 ± 136.7a	471.5 ± 74.5a
chlorophyll *a*′[c]		84.3 ± 13.3a	108.6 ± 17.9a	121.2 ± 24.7a	75.5 ± 36.5a	128.7 ± 13.3a
chlorophyll *a* derivative[c]		13.5 ± 23.4a	28.1 ± 27.4a			
chlorophyll *b*		90.2 ± 18.6a	125.5 ± 19.8a	138.1 ± 55.0a	85.4 ± 61.4a	135.1 ± 32.3a
chlorophyll *b*′[d]		9.6 ± 16.7b	23.3 ± 20.7ab	19.4 ± 16.9ab		38.4 ± 7.8a
pheophitin *a*[c]	40.3 ± 8.3a	25.0 ± 21.7a	65.0 ± 7.4a	60.9 ± 22.7a	51.5 ± 19.4a	53.8 ± 12.5a
total chlorophylls	103.9b	500.7a	732.9a	767.9a	463.1b	827.5a
total components	152.3b	590.2a	847.0a	919.2a	573.8b	973.4a

[a] Values are expressed as mg/kg of wet weight. Values with the same letter are not statistically different at the 5% level. [b] Quantified as β-carotene. [c] Quantified as chlorophyll *a*. [d] Quantified as chlorophyll *b*.

R-PE, and 75 μL of ABAP, and the reaction kinetics at 38 °C were recorded for 45 min (or longer, if necessary) by an LS-55 luminescence spectrometer (Perkin-Elmer Corp., Wellesley, MA). TRAP values were calculated by comparison of the length of the lag phase due to the sample with that due to Trolox and expressed as millimoles of Trolox per kilogram of wet weight.

(c) Ferric Reducing Antioxidant Power (FRAP) Assay. FRAP was assessed according to the method of Benzie and Strain (*17*) using a Hewlett-Packard 8453 diode array spectrophotometer. The method is based on the reduction of the Fe^{3+}−TPTZ complex to the ferrous form at low pH. Reduction is monitored by measuring the change in absorption at 593 nm. Briefly, 3 mL of working FRAP reagent prepared daily was mixed with 100 μL of diluted sample and absorbance at 593 nm recorded after 30 min of incubation at 37 °C. FRAP values were obtained by comparing the change in absorption in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as millimoles of Fe^{2+} equivalents per kilogram of wet weight.

The variation in TEAC, TRAP, and FRAP values for replicates was always between 3 and 10% relative standard deviation (RSD). When the RSD was >10%, the analyses were repeated to confirm the value.

Statistical Analysis. Data were subjected to one-way analysis of variance for means comparison, and significant differences were calculated according to the post-hoc Tukey test using the facility of the statistical package SPSS 12.0 (SPSS Inc., Chicago, IL). Data are reported as mean ± standard deviation; P values of <0.05 were considered to be significant.

RESULTS

The wild greens examined in this study were collected at maturity in the Ragusa territory in three growth regions (mountain, countryside, and seaside). As already reported under Materials and Methods, each plant was extracted in single and the extracts were analyzed in duplicate. Because no remarkable difference was observed among samples grown in different regions, results are presented as mean values for the three growth regions for each analysis. The high standard deviation values observed for a few data are justifiable with this approach.

As the aim of this study was the characterization of edible wild greens as traditionally consumed, each analysis was performed on the sample boiled and carefully drained. The water content among the samples was homogeneous, ranging from 88.0 to 93.4% (RSD =2%). All of the results are reported on the basis of cooked vegetable weight (wet weight).

Carotenoids and Chlorophylls. The contents of carotenoids and chlorophylls, expressed as milligrams per kilogram of wet weight, are shown in **Table 1**. A total of 12 pigments were identified in and quantified for cooked wild vegetables. In order of chromatographic elution on the C_{18} reversed-phase column, 3 were xanthophylls (i.e., lutein, neoxanthin, and violaxanthin), 4 were chlorophyll *a*-related compounds (chlorophyll *a*, chlorophyll *a* derivative, chlorophyll *a*′, which represents the C-10 epimeric isomer of chlorophyll *a*, and pheophytin *a*, all quantified as chlorophyll *a*), 2 were chlorophyll *b*-related compounds (chlorophyll *b* and chlorophyll *b*′, which represents the C-10 epimeric isomer of chlorophyll *b*, quantified as chlorophyll *b*), and 1 was a carotene (β-carotene). In addition, two unidentified xanthophylls were observed in some wild vegetables and quantified as β-carotene equivalents.

The qualitative HPLC profiles of the lipophilic extracts of the wild vegetables examined in this study were almost identical, except for *A. acutifolius*, in which chlorophylls *b* and *b*′ were not present. Nevertheless, the concentration of each single compound varied among plants. The highest concentrations of carotenoids, chlorophylls, and total lipophilic compounds were found in *S. nigra* (145.9, 827.5, and 973.4 mg/kg, respectively) and *Diplotaxis erucoides* (151.3, 767.9, and 919.2 mg/kg, respectively), whereas the lowest were found in *S. incana* (110.7, 463.1, and 573.8 mg/kg, respectively) and *A. acutifolius* (48.4, 103.9, and 152.3 mg/kg, respectively).

Lutein and β-carotene, the predominant carotenoids in wild greens, ranged from 27.4 to 70.2 mg/kg and from 11.9 to 47.5 mg/kg, respectively, and were both found in all samples. All of the plants analyzed had similar amounts of lutein, whereas *D. erucoides* and *C. intybus* were the richest in β-carotene.

Chlorophyll *a* and its derivatives were the predominant chlorophylls in all samples analyzed, which had similar concentrations with the exception of *A. acutifolius*, which exhibited the lowest content of these compounds.

Polyphenols. The qualitative and quantitative profiles of phenolic acids and flavonoids in the samples examined are shown in **Tables 2** and **3**, respectively. The values are expressed as milligrams per kilogram of wet weight. A total of 20 different phenolic compounds were identified and quantified in the herbal samples. In particular, 8 components were recognized as phenolic acids (caffeic, chlorogenic, feruloyl-L-(+)-tartaric, ferulic, protocatechuic, *p*-coumaric, caftaric [monocaffeoyl-L-(+)-tartaric acid] and gallic acids) and 11 as flavonoids (procyanidin, quercetin, kaempferol, isorhamnetin, rhamnetin,

Table 2. Contents of Phenolic Acids in the Samples Examined[a]

phenolic acid	*A. acutifolius*	*B. officinalis*	*C. intybus*	*D. erucoides*	*S. incana*	*S. nigra*
caffeic	24.0 ± 0.6a	13.1 ± 0.4b	19.3 ± 4.1a	12.5 ± 0.7b	17.9 ± 0.8ab	13.4 ± 0.6b
caftaric			85.9 ± 29.8a	4.9 ± 8.5b	17.5 ± 6.1b	
chlorogenic		12.1 ± 0.9				
ferulic		16.2 ± 3.1b	40.4 ± 12.3a		14.1 ± 2.7b	
gallic					14.5 ± 1.1	
p-coumaric				14.8 ± 1.3a	12.8 ± 1.5a	13.0 ± 0.5a
protocatechuic	19.1 ± 12.7a		13.0 ± 11.3a	16.5 ± 3.8a	15.4 ± 5.5a	17.9 ± 4.4a
feruloyl-L-(+)-tartaric		22.6 ± 8.0				
total	43.1b	64.0b	158.6a	48.7b	92.2ab	44.3b

[a] Values are expressed as mg/kg of wet weight. All compounds are quantified as caffeic equivalents. Values with the same letter are not statistically different at the 5% level.

Table 3. Contents of Flavonoids in the Samples Examined[a]

flavonoid	*A. acutifolius*	*B. officinalis*	*C. intybus*	*D. erucoides*	*S. incana*	*S. nigra*
isorhamnetin	249.1 ± 108.7a			178.8 ± 101.0a	102.2 ± 78.4a	169.4 ± 84.8a
kaempferol	289.7 ± 136.5b		165.4 ± 123.7b	885.0 ± 219.8a	127.5 ± 93.6b	444.5 ± 398.3b
myricetin	63.4 ± 41.2a					163.3 ± 139.8a
myricetin-3-glucoside	235.4 ± 87.1a					64.1 ± 0.5b
procyanidin		189.4 ± 51.7a	173.5 ± 54.2a	185.8 ± 65.3a	169.7 ± 65.5a	
quercetin	1239.6 ± 264.1a		240.5 ± 270.5b	627.4 ± 407.6b	965.3 ± 347.8ab	374.2 ± 266.0b
quercetin variously glycosylated	185.7 ± 110.7					
quercetin-3-galattoside			286.6 ± 85.5			
quercetin-3-rhamnoside			200.0 ± 33.9			
quercetin-glucuronide						330.1 ± 339.5
rhamnetin				999.7 ± 414.5		
total flavonoids	2262.9a	189.4c	1066.0b	2876.7a	1364.7b	1545.6b
total polyphenols[b]	2306.0a	253.4c	1224.6b	2925.4a	1456.9b	1589.9b

[a] Values are expressed as mg/kg of wet weight. All compounds are quantified as quercetin equivalents. Values with the same letter are not statistically different at the 5% level. [b] The sum of phenolic acids (see **Table 2**) and flavonoids.

Table 4. Ferric Reducing Antioxidant Power (FRAP), Total Radical-Trapping Antioxidant Parameter (TRAP), Trolox Equivalent Antioxidant Capacity (TEAC), Ascorbic Acid, and Vitamin C in the Samples Examined[a]

sample	FRAP (mmol of Fe^{2+}/kg)	TRAP (mmol of Trolox/kg)	TEAC (mmol of Trolox/kg)	ascorbic acid (mg/kg)	vitamin C (mg/kg)
A. acutifolius	11.23 ± 1.90b	5.29 ± 1.63b	3.01 ± 0.46b	148.7 ± 22.9a	156.4 ± 24.5a
B. officinalis	14.31 ± 2.61b	5.06 ± 1.32b	3.17 ± 0.50b	2.3 ± 2.3b	4.2 ± 1.5b
C. intybus	19.03 ± 4.19a	10.52 ± 2.69a	4.59 ± 1.17a	6.7 ± 3.3b	6.8 ± 3.3b
D. erucoides	10.33 ± 1.92b	3.29 ± 0.82b	2.99 ± 0.42b	139.1 ± 31.7a	145.2 ± 29.0a
S. incana	11.48 ± 2.53b	4.78 ± 0.56b	3.19 ± 0.80b	119.2 ± 62.8a	121.3 ± 66.0a
S. nigra	10.74 ± 0.19b	2.92 ± 0.20b	3.20 ± 0.38b	110.7 ± 69.1a	111.6 ± 70.2a

[a] Values are referred to wet weight. Values with the same letter are not statistically different at the 5% level.

myricetin-3-glucoside, myricetin, quercetin glucuronide, quercetin-3-galactoside, quercetin-3-rhamnoside, and quercetin variously glycosylated). The identification process was based on coelution with pure standard or on spectrum and retention time comparison with previous work (*18*). All of the phenolic acids were quantified as caffeic acid equivalents and all of the flavonoids as quercetin equivalents.

The HPLC profiles of phenolic compounds varied markedly among samples. Some phenolic acids and some flavonoids were present only in one of the species analyzed, whereas quercetin, kaempferol, and caffeic acid were the most widespread polyphenols, detectable in almost all of the wild greens examined. Moreover, phenolic acid concentration was almost the same in each of the samples (ranging between 43.1 and 158.6 mg/kg), whereas concentrations of flavonoids varied more among the samples.

The highest concentration of total polyphenols, obtained as the sum of total phenolic acids and total flavonoids, was observed in *D. erucoides* (2925.4 mg/kg) and *A. acutifolius* (2306 mg/kg), whereas the lowest was observed in *B. officinalis* (253.4 mg/kg). The observed difference in polyphenol content, ~1 order of magnitude, was due to the great variation in flavonoid content among plants.

Ascorbic Acid, Total Vitamin C, and TAC. The amounts of total vitamin C (sum of AA and DHAA), ascorbic acid, and TAC, as evaluated by the three different methods, are shown in **Table 4**. The values are expressed as milligrams per kilogram of wet weight for vitamin C and ascorbic acid, as millimoles of Trolox equivalent per kilogram of wet weight for the TRAP and TEAC methods, and as millimoles of Fe^{2+} equivalents per kilogram of wet weight for the FRAP assay. No significant differences of total vitamin C and ascorbic acid concentrations were found among the wild plants examined except for *B. officinalis* and *C. intybus*, which had the lowest total vitamin C and ascorbic acid concentrations. *C. intybus* had the significantly highest TAC of wild greens examined for all three methods used, whereas other wild greens analyzed showed comparable TAC.

DISCUSSION

In the Mediterranean diet, which is considered a protective regimen aiding maintenance of health (*19*), fruits and vegetables rich in antioxidants are of central importance. Among vegetables, wild greens have recently been examined for protective effects against coronary heart disease (*20*) due to their large amounts of antioxidants in addition to their high content of essential micronutrients. Moreover, the European Commission has recently supported a project called "Local Mediterranean food plants: potential new nutraceuticals and their current role in the Mediterranean diet". In three years, this project has produced remarkable results (*21, 22*), and the attention toward wild greens as potential contributors to the benefits of the Mediterranean diet has grown significantly.

In the Mediterranean area, wild greens are traditionally collected throughout the country and usually consumed with virgin olive oil (*2*, *23*, *24*). In the Ragusa cohort of the EPIC study (*2*), the consumption of locally grown wild greens was surprisingly high, given the relatively low intake of cooked leafy vegetables in that region. Furthermore, consumption of the wild greens examined in that study (i.e., *A. acutifolius*, *B. officinalis*, *C. intybus*, *D. erucoides*, *S. incana*, and *S. nigra*) was high not only for older subjects, who are more prone to consume traditional recipes, but even for younger subjects, who had approximately the same frequency of consumption of these greens as the older subjects (*2*).

There are more than 150 edible wild green species present in the Mediterranean area, and in Greece daily consumption of them is 20 g per person, which represents 10% of the total daily intake of vegetables (*25*). Trichopoulou et al. (*25*) characterized the content of antioxidants of seven of these wild herbs; some of them were found to be astonishingly rich in flavonols, especially quercetin (*25*). In their study, the edible wild greens examined comprised only a small percentage of all wild plants consumed by the Greek population, whereas in Sicily the number of species consumed is restricted to the above-mentioned six plants and a very few others. Therefore, the antioxidant analysis performed in the present study covers almost the entire pattern of wild vegetable species actually consumed by the Sicilian population, and its findings will therefore have implications for future studies related to the health benefits of vegetable consumption.

The results obtained in this study demonstrated that the wild plants examined are considerably rich in antioxidants and total antioxidant capacity. This is particularly surprising because plants were examined after boiling, the domestic cooking method commonly used prior to their consumption. In particular, the wild plants examined had very high flavonol contents compared with regular fresh vegetables, commonly consumed fruits, and beverages (*26*−*29*) and even compared with food items known to be particularly rich sources of flavonoids, such as wine and tea (*30*). Among the wild greens examined, *A. acutifolius* contained 124.0 mg/100 g of quercetin, a remarkable concentration compared to the 34 mg/100 g in onion, which is considered to be the principal vegetable source of quercetin in European countries (*23, 26*). Moreover, the contents of quercetin and kaempferol of the wild vegetables examined in the present study were higher, ranging between 0 and 124.0 mg/100 g and between 0 and 88.5 mg/100 g, respectively, than those reported by Trichopoulou et al. (*25*) for Greek edible wild greens and green pies. Finally, the sum of phenolic acids and flavonoids in our samples varied between 25.3 and 292.5 mg/100 g. This phenolic content was higher than that found by Zeghichi et al. (*24*) for raw wild plants consumed in Crete, which ranged from 6.7 to 102.5 mg/100 g, and appears to be remarkably high.

To our knowledge, ours is the first study in which the lipophilic antioxidants (i.e., carotenoids and chlorophylls) of edible wild greens were determined. Due to the lack of reference values, the contents of carotenoids and chlorophylls of our samples could be compared only to those of similar green leafy vegetables such as spinach, which is the best source of carotenoids after carrots, and, in the case of *A. acutifolius*, to those of asparagus. In the wild greens examined, the most abundant carotenoids found were lutein and β-carotene. The content of lutein in particular ranged from 27.4 mg/kg for *A. acutifolius* to 70.2 mg/kg for *D. erucoides*, whereas β-carotene content varied from 11.9 mg/kg for *A. acutifolius* to 47.5 mg/kg for *D. erucoides*. These concentrations are similar to those observed in raw (*31*) and cooked spinach (*32*) but lower than those reported for raw spinach by Buratti et al. (*33*). In *A. acutifolius*, the content of principal carotenoids was higher than that reported for boiled asparagus (*32*). The wild greens examined had lower chlorophyll content than reported in the study by Buratti et al. (*33*), in which the contents of chlorophylls in raw spinach were found to be 927.2 and 436.6 mg/kg for chlorophylls *a* and *b*, respectively. In addition, Khachik et al. (*34*), who examined raw spinach and raw and boiled kale, observed higher chlorophyll content than measured in our samples.

As expected, due to their high content of lipid- and water-soluble antioxidants, the wild greens exhibited remarkably high values of TAC even if examined after boiling and regardless of the method used for analysis. In fact, the TAC values of the wild greens we examined can be placed in the first third in rank in the TAC database of raw Italian vegetables (*15*). Among them, *C. intybus* had the highest TAC; the only items in the Italian database with higher TAC are peppers and spinach for FRAP and TEAC assays, whereas the TRAP value for *Cichorium* is highest among all of the vegetables.

Ascorbic acid was the predominant form of vitamin C in the wild greens examined, and its content was relatively high, with the exception of *C. intybus* and *B. officinalis*, particularly considering that analysis was performed after boiling. In fact, it is known that cooking by boiling results in loss of ascorbate (∼40−60% of initial vitamin C content) due to its high solubility in water (*35, 36*).

Finally, some considerations on the principal contributors to TAC of the wild greens examined can be drawn. For instance, *C. intybus* has the highest TAC for all TAC assays; it was richest in β-carotene and in phenolic acids, particularly ferulic and caftaric acid, but almost devoid of vitamin C. The major role of phenolic acids in determining the TAC of *C. intybus* emerges also by comparing the antioxidant profile of *C. intybus* with that of *S. nigra*. The two herbs exhibited significantly different TAC values, but they had similar amounts of carotenoids, chlorophylls, and flavonoids, whereas *C. intybus* showed a significantly higher content of phenolic acids, even though it had a significantly lower content of vitamin C. This observation seems also confirmed by comparing the antioxidant composition of *C. intybus* to that of *B. officinalis*: the two herbs had similar contents of vitamin C, ascorbic acid, carotenoids, and chlorophylls, but the latter showed a significantly lower content of phenolic acids and flavonoids that likely justified its significantly lower TAC value compared to that of *C. intybus*.

In conclusion, the remarkably high contents of flavonoids and carotenoids, high TAC, and good content of vitamin C of

these wild vegetables suggest that they may have disease-preventing effects.

ABBREVIATIONS USED

EPIC, European Prospective Investigation into Cancer and Nutrition; TAC, total antioxidant capacity; AA, ascorbic acid; DHAA, dehydroascorbic acid; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic) acid; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DTT, dithiothreitol; TPTZ, 2,4,6-tripyridyl-*s*-triazine; R-PE, *R*-phycoerythrin; ABAP, 2,2′-azobis(2-amidinopropane) dihydrochloride; BHT, 2,6-di-*tert*-butyl-4-methylphenol; PDA, photodiode array detector; TEAC, Trolox equivalent antioxidant capacity; $ABTS^{\bullet+}$, ABTS radical cation; TRAP, total radical-trapping antioxidant parameter; FRAP, ferric reducing antioxidant power; RSD, relative standard deviation.

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