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Analysis of Antioxidative Phenolic Compounds in Artichoke (*Cynara scolymus* L.)

MINGFU WANG,† JAMES E. SIMON,*,† IRMA FABIOLA AVILES,† KAN HE,§ QUN-YI ZHENG,§ AND YAAKOV TADMOR†

New Use Agriculture and Natural Plant Products Program, Department of Plant Biology and Pathology, Rutgers University, 59 Dudley Road, New Brunswick, New Jersey 08901, and Pure World Botanicals, 375 Huyler Street, South Hackensack, New Jersey 07606

Artichoke leaf is an herbal medicine known for a long time. A systematic antioxidant activity-directed fractionation procedure was used to purify antioxidative components from the aqueous methanol extractions of artichoke heads and leaves in this study. Seven active polyphenolic compounds were purified from artichoke, and structural elucidation of each was achieved using MS and NMR. Two of these compounds, apigenin-7-rutinoside and narirutin, were found to be unique to artichoke heads, this represents the first report of these compounds in the edible portion of this plant. The contents of these antioxidants and total phenols in dried artichoke samples from leaves and immature and mature heads of three varieties, Imperial Star, Green Globe, and Violet, were then analyzed and compared by colorimetric and validated HPLC methods. Significant differences by variety and plant organ were observed.

KEYWORDS: Artichoke; *Cynara scolymus* L.; phenolic compounds; caffeoylquinic acids; flavonoids; antioxidant activity; apigenin-7-rutinoside; narirutin

INTRODUCTION

Artichoke (*Cynara scolymus* L.) is an ancient herbaceous perennial plant, originating from the southern Mediterranean parts of North Africa. Today, artichokes are widely grown around the world, with Italy and Spain being the world's leading producers. The buds of artichokes have been enjoyed as a vegetable all over the world. The leaves of artichoke have been widely used in herbal medicine as a choleretic since ancient times (*1*). The artichoke head, an immature flower, constitutes the edible part of this vegetable.

The chemical components of artichoke leaves have been studied extensively and have been found to be a rich source of polyphenolic compounds, with mono- and dicaffeoylquinic acids and flavonoids as the major chemical components (*2*−*5*). The chemical components in the edible portion of the artichoke head remain unknown.

In various pharmacological test systems, artichoke leaf extracts have shown antibacterial, antioxidative, anti-HIV, bile-expelling, hepatoprotective, urinative, and choleretic activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation (*3*, *6*−*9*). Recently, research has focused on the antioxidant activity of artichoke leaf extracts. Leaf extracts have been reported to show antioxidative and protective properties against hydroperoxide-induced oxidative stress in cultured rat hepatocytes (*10*), to protect lipoprotein from oxidation in vitro (*9*), to inhibit hemolysis induced by hydrogen peroxide, and to inhibit oxidative stress when human cells are stimulated with agents that generate reactive oxygen species: hydrogen, peroxide, phorbol-12-myristate-13-acetate, and *N*-formyl-methionyl-leucyl-phenylalanine (*11*). In contrast, there are no reports of bioactivity-directed fractionations to purify the antioxidants from artichoke, including from the heads, the edible and consumed part of the artichoke.

Various analytical methods have been used to analyze the phenolic compounds in artichoke leaves, including colorimetric methods, thin-layer chromatography (TLC), reverse-phase high-performance chromatography (RP-HPLC), and micellar electrokinetic capillary chromatography (*2*, *12*−*17*). Several HPLC methods have been developed to analyze the phenolic compounds in artichoke leaves, but the published results are confusing and the methods potentially suspect. The contents of mono- and dicaffeoylquinic acid derivatives, for example, have been reported to vary from 1 to 6% in dry artichoke leaves (*2*, *12*−*17*). Although this variation may reflect real genetic or developmental expression over time under a variety of environmental conditions, it appears that it could also be due to insufficient HPLC separation or incomplete extraction of the phenolic compounds. We initially used the reported analytical and extraction methods in our artichoke study but found that they are not good enough to accurately analyze artichoke samples. As part of this study, it therefore became necessary to develop a reliable HPLC method to clarify the extraction and analytical procedures.

* Author to whom correspondence should be addressed [telephone (732) 932-9711, ext. 355; fax (732) 932-9411; e-mail jesimon@aesop.rutgers.edu].
† Rutgers University.
§ Pure World Botanicals.

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In our screenings of antioxidants from vegetable, fruits, spices, and medicinal herbs, we recently have found that the leaves and heads of artichoke contained high contents of phenolic compounds and showed very strong antioxidant activity in our in vitro testing model. Here, we report the isolation and structural elucidation of antioxidants from artichoke leaves and heads and the analysis of these antioxidants in three varieties of artichoke: Green Globe, Imperial Star, and Violet.

MATERIALS AND METHODS

Plant Material. Three varieties of artichoke, Green Globe, Imperial Star, and Violet, were field-grown at the Snyder Agricultural Experiment Station, Pittstown, NJ, in the summer of 2001. Growth conditions and management practices were similar to those used in commercial cultivation. At least 10 plants of each variety were planted into a randomized complete block design with four replications. Fully matured leaves, young buds, and matured buds were collected from at least 10 different plants at two harvest dates, September 5 and October 3, 2001. The majority of the harvested plants were separated by plant tissue and dried in a 70 °C air-drying oven; a subsample of each was also freeze-dried utilizing a Labconco instrument (Kansas City, MO). Dried plant material was finely ground and kept in sealed bags at room temperature for further extractions.

General Procedures. Silica gel (130−270 mesh), RP-18 silica gel, and Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) were used for column chromatography. Radical 2,2-diphenylpicrylhydrazyl (DPPH) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Folin−Ciocalteu reagent was purchased from Sigma. All solvents used for chromatographic isolation were of analytical grade and purchased from Fisher Scientific (Springfield, NJ). Thin-layer chromatography was performed on Sigma-Aldrich silica gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in an ethanol solution. ^{1}H NMR and ^{13}C NMR spectra were obtained on an INOVA-400 instrument. Negative and positive ESI mass spectra were measured using an Agilent LC-MSD (1100 series LC/MSD trap). Analytical HPLC analyses were performed on a Hewlett-Packard 1100 modular system equipped with an autosampler, a quaternary pump system, a photodiode array detector, and an HP Chemstation data system. A prepacked 150 × 3.2 mm (5 μm particle size) Luna prodigy ODS3 column (Phenomenex, Torrance, CA) was selected for HPLC analysis. The absorption spectra were recorded from 200 to 400 nm for all peaks; quantification was carried out at a single wavelength of 330 nm.

Extraction and Isolation Procedures. *1. Isolation of Antioxidants from Artichoke Heads.* The dried Green Globe artichoke heads (300 g) were extracted in 2000 mL of 70% methanol twice. The extract was concentrated to dryness under reduced pressure, and the residue was dissolved in water (300 mL) and partitioned with chloroform (3 × 300 mL). The water layer was then extracted successively three times with ethyl acetate (3 × 300 mL) and *n*-butanol (3 × 300 mL). Among these four solvent extractions, the butanol extract exhibited the most significant activity in the DPPH scavenging assay (*25*). The butanol extract was evaporated under reduced pressure to afford 7 g of residue. The residue was dissolved into 70% methanol, applied to a Sephadex LH-20 column (100 g), and eluted with 70% methanol; fractions were collected and tested for scavenging DPPH free radical activity. Fractions 21−25, fractions 38−47, and fractions 50−61 showed strongest activity. Fraction 21 and 25 were pooled together and subjected to column chromatography on silica gel and eluted with ethyl acetate/methanol/water (8:1:1) to isolate and purify compounds **1** (60 mg) and **2** (60 mg). Fractions 38−47 were combined and subjected to a silica gel column eluted with ethyl acetate/methanol/water (7:1:1) to get 200 mg of compound **3**. Fractions 50−61 were pooled together and first purified with a silica gel column eluted with ethyl acetate/methanol/water (6:1:0.1) and then purified with a Sephadex LH-20 column (eluted with methanol) to get 45 mg of compound **4** as the active components in these fractions.

2. Isolation of Antioxidants from Artichoke Leaves. Fifty grams of dried leaves from the variety Violet was extracted twice in 1000 mL of 70% methanol. The residue were evaporated to dryness and suspended into 250 mL of water and partitioned with chloroform (3 × 250 mL). The water layer was then evaporated to 50 mL under reduced pressure and directly loaded onto a reverse-phase silica gel column (160 g) eluted with 2000 mL of water, 3000 mL of 15% acetonitrile and 3000 mL of 30% acetonitrile, and 500 mL of methanol. In total, 50 fractions were collected. Fractions 2, 3−16, 37−43, and 44−46 showed antioxidant activity scavenging DPPH free radicals. Fraction 2 was subjected to a normal-phase silica gel column and eluted by chloroform/methanol/water (5:1:0.1) to give 60 mg of compound **5**. Fractions 3−16 were pooled together and subjected to a Sephadex LH-20 column eluted by methanol to give 300 mg of compound **6**. Fractions 37−43 were first separated by a silica gel column using ethyl acetate/methanol/water (10:1:1) as mobile phase, and then we utilized a second chromatography on a silica gel column, eluted this time with chloroform/methanol/water (7:1:0.1), to give 30 mg of compound **7** and 40 mg of compound **8**. Fractions 44−46 were subjected to a Sephadex LH-20 column and eluted with pure methanol to get 40 mg of compound **9**.

Spectrometric Identification of Isolated Compounds. (2*R* and 2*S*)-Narirutin (compound **1**) was obtained as a yellow powder: positive ESI-MS, *m*/*z* 581 $[M + 1]^+$; ^{1}H NMR (DMSO, 400 MHz) δ 7.34 (d, J = 8.8 Hz, H-2′ and 6′), 7.33 (d, J = 8.8 MHz), 6.79 (2H, d, J = 8.8 Hz, H-3′ and H-5′), 6.13 (2H, m H-6 and H-8), 5.50 (m, H-2), 4.99 (d, J = 7.2 Hz), 4.98 (d, J = 7.2 Hz), 4.54 (1H, br s, rham-1), 3.0−3.84 (sugar protons), 2.74 (dd, J = 17.2, 3.2 Hz, H-3), 2.72 (dd, J = 17.2, 3.2 Hz, H-3), 3.26 (1H, m, H-3), 1.07 (d, J = 6.0 Hz, rham-6); ^{13}C NMR (DMSO, 100 MHz) δ 197.3 (s, C-4), 163.1 (s, C-5), 162.7 (s, C-9), 157.8 (s, C-4′), 128.6 (d, C-2′ and 6′), 128.4 (s, C-1′), 115.2 (d, C-5′ and C-3′), 103.3 (s, C-10), 103.1 (d, C-3), 100.6 (d, rham-1), 99.4 (d, glc-1), 96.4 (d, C-6), 95.4 (d, C-8), 78.7 (d, C-2), 78.6 (d, c-2), 76.3 (d, glc-3), 75.5 (d, glc-5), 73.0 (d, glc-2), 72.0 (d, rham-4), 70.7 (d, rham-3), 70.3 (rham-2), 69.6 (d, glc-4), 68.3 (d, rham-5), 66.0 (t, glc-6), 42.2 (t, C-3), 41.9 (t, C-3). 17.9 (q, rham-6) (*21*).

Apigenin 7-rutinoside (compound **2**) was obtained as a yellow powder: positive ESI-MS, *m*/*z* 579 $[M + 1]^+$; ^{1}H NMR (DMSO, 400 MHz) δ 7.95 (2H, d, J = 8.0 Hz, H-2′ and 6′), 6.96 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 6.87 (1H, s, H-3), 6.77 (1H, br s, H-8), 6.45 (1H, br s, H-6), 5.07 (1H, d, J = 6.8 Hz), 4.54 (1H, brs, rham-1), 3.0−3.84 (sugar protons), 1.07 (d, J = 6.0 Hz, rham-6); ^{13}C NMR (DMSO, 100 MHz) δ 182.1 (s, C-4), 164.4 (s, C-2), 162.9 (s, C-7), 161.4 (s, C-4′), 161.2 (s, C-5), 156.9 (s, C-9), 128.7 (d, C-2′ and 6′), 121.3 (s, C-1′), 119.3 (d, C-6′), 116.1 (d, C-5′ and C-3′), 105.4 (s, C-10), 103.1 (d, C-3), 100.6 (d, rham-1), 99.9 (d, C-6), 99.5 (d, glc-1), 94.8 (d, C-8), 76.3 (d, glc-3), 75.6 (d, glc-5), 73.1 (d, glc-2), 72.0 (d, rham-4), 70.7 (d, rham-3), 70.3 (rham-2), 69.5 (d, glc-4), 68.4 (d, rham-5), 66.0 (t, glc-6), 17.9 (q, rham-6) (*20*).

Cynarin (compounds **4** and **9**; 1,3-dicaffeoylquinic acid): negative ESI-MS, *m*/*z* 515 $[M - 1]^+$; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.46 (1H, d, J = 16.0 Hz), 7.42 (1H, d, J = 16.0 Hz), 7.07 (2H, d, J = 1.6 Hz), 6.96 (2H, m), 6.76 (2H, d, J = 8.0 Hz), 6.24 (1H, d, J = 16.0 Hz), 6.18 (1H, d, J = 16.0 Hz), 5.43 (1H, m), 4.23 (1H, m), 3.71 (1H, dd, J = 9.6, 3.2 Hz), 2.67 (2H, m), 2.24 (1H, dd, J = 15.6, 3.6 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 178.2 (s), 169.2 (s), 168.3 (s), 149,7 (s), 149.3 (s), 146.9 (d), 146.8 (d), 146.7 (s), 146.2 (s), 128.1 (s), 127.7 (s), 123.0 (d), 122.8 (d), 116.8 (d), 116.5 (d), 115.4 (d), 115.0 (d), 115.0 (d), 115.0 (d), 84.0 (s), 74.5 (d), 71.8 (d), 71.0 (d), 38.4 (t), 36.8 (t) (*23*).

1-Caffeoylquinic acid (compound *5*): negative ESI-MS, *m*/*z* 353 $[M - 1]^+$; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.50 (1H, d, J = 16.0 Hz), 7.03 (1H, d, J = 2.0 Hz), 6.91 (1H, dd, J = 8.0, 2.0 Hz), 6.76 (1H, d, J = 8.0 Hz), 6.26 (1H, d, J = 16.0 Hz), 4.05 (1H, m), 3.95 (1H, m), 3.62 (1H, m), 2.42 (2H, m), 2.16 (2H, m) (*22*).

Luteolin-7-*O*-α-L-rhamnosyl(1→6)-β-glucopyranoside (compound **7**; luteolin-7- rutinoside): yellow powder: positive ESI-MS, *m*/*z* $[M + 1]^+$, 595; ^{1}H NMR (DMSO, 400 MHz) δ 7.44 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 7.41 (1H, d, J = 2.0 Hz, H-2′), 6.89 (1H, d, J = 8.0 Hz, H-5′), 6.74 (1H, s, H-3), 6.73 (1H, d, J = 2.0 Hz, H-8), 6.45 (1H, d, J = 2.0 Hz, H-6), 5.08 (1H, d, J = 6.6 Hz, glc-1), 4.53 (1H, br s, rham-1), 3.10−3.85 (m, sugar protons), 1.07 (3H, d, J = 6.4 Hz, rham-6); ^{13}C NMR (DMSO, 100 MHz) δ 181.9 (s, C-4), 164.7 (s, C-2), 162.9

(s, C-7), 161.2 (s, C-5), 157.0 (s, C-9), 150.5 (s, C-4′), 146.0 (s, C-3′), 121.0 (s, C-1′), 119.3 (d, C-6′), 116.0 (d, C-5′), 113.4 (d, C-2′), 105.4 (s, C-10), 103.0 (d, C-3), 100.6 (d, rham-1), 99.9 (d, glc-1), 99.5 (d, C-6), 94.8 (d, C-8), 76.2 (d, glc-3), 75.6 (d, glc-5), 73.1 (d, glc-2), 72.0 (d, rham-4), 70.7 (d, rham-3), 70.3 (rham-2), 69.5 (d, glc-4), 68.4 (d, rham-5), 66.0 (t, glc-6), 17.9 (q, rham-6), identical with the literature (*19*).

Luteolin-7-*O*-β-glucopyranoside (compound **8**; cynaroside): yellow powder: positive ESI-MS, *m*/*z* $[M + 1]^+$, 449; ^{1}H NMR (DMSO, 400 MHz) δ 7.44 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 7.41 (1H, s, H-2′), 6.88 (1H, d, $J = 8.0$, H-5′), 6.80 (1H, d, $J = 2.0$ Hz, H-8), 6.75 (1H, s, H-3), 6.44 (1H, d, $J = 2.0$ Hz, H-6), 5.08 (1H, d, $J = 6.6$ Hz, glc-1), 3.10−3.70 (m); ^{13}C NMR (DMSO, 100 MHz) δ 181.9 (s, C-4), 164.6 (s, C-2), 162.9 (s, C-7), 161.1 (s, C-5), 157.0 (s, C-9), 150.6 (s, C-4′), 146.0 (s, C-3′), 120.9 (s, C-1′), 119.3 (d, C-6′), 116.0 (d, C-5′), 113.3 (d, C-2′), 105.3 (s, C-10), 103.0 (d, C-3), 99.8 (d, glc-1), 99.5 (d, C-6), 94.7 (d, C-8), 77.1 (d, glc-5), 76.4 (d, glc-3), 73.1 (d, glc-2), 69.5 (d, glc-4), 60.6 (t, glc-6), identical with the literature (*18*).

Preparation of Samples for HPLC Analysis, Colorimetric Analysis for Total Phenols, and DPPH Scavenging Activity of Artichoke Samples. We accurately weighed about 500 or 1000 mg of ground dried leaf or head, respectively, into a 100 mL volumetric flask. Seventy milliliters of 60% methanol solution was then immediately added, and samples were sonicated for 25 min. The flasks were allowed to cool to room temperature and then filled to volume with 60% methanol. Using a disposable syringe and a 0.45 μm filter, the samples were filtered into HPLC vials for HPLC analysis. Filtrates through no. 1 Whatman paper were utilized for the colorimetric analysis of total phenols and DPPH scavenging activity.

Colorimetric Analysis of Total Phenolics. The total phenols were estimated using the Folin−Ciocalteu method (*24*). One milliliter of clear extraction solution prepared from sample preparation was transferred to a 100 mL volumetric flask and swirled with 60−70 mL of HPLC grade water. Five milliliters of Folin−Ciocalteu's phenol reagent was added and swirled. After 1 min and before 8 min, 15 mL of sodium carbonate solution (20 g in 100 mL) was added and mixed. At this stage, this was recorded as time zero. Additional HPLC grade water was added to make the volume up to 100 mL exactly. The solution was mixed thoroughly by inverting the tube several times. After 2 h (within 1−2 min), the UV absorption, range at 550−850 nm, and maximum absorbance about 760 nm were recorded (HP model 8453 spectrophotometer), and the same solution but without the extraction solution served as the control or blank solution (*24*). All tests were conducted in triplicate and averaged. Differences among triplicates were always <5%.

Quantification was based on the standard curve generated with chlorogenic acid.

Preparation of Standards for HPLC Analysis. About 5 mg of each compound was accurately weighed and placed into a 25 mL volumetric flask. Fifteen milliliters of 60% methanol was added, and the solutions were sonicated for 15 min. The flasks were allowed to cool to room temperature and filled to full volume with 60% methanol solution. Five milliliters of the above solution was transferred to a new 25 mL volumetric flask and diluted to the full volume using 60% methanol (standard solution). Calibration curves were established on six data points covering concentration ranges of 1.08−216 μg/mL for chlorogenic acid, 1.10−220 μg/mL for 1-caffeoylquinic acid, 1.12−224 μg/mL for narirutin, 0.86−172 μg/mL for apigenen-7-rutinoside, 0.92−184 μg/mL for luteolin-7-*O*-α-L-rhamnosyl(1→6)-β-glucopyranoside, 1.06−212 μg/mL for luteolin-7-*O*-β-glucopyranoside, and 1.24−248 μg/mL for cynarin. Ten microliter aliquots were used for HPLC analysis.

Quantitative Determination of Antioxidants in Artichoke by HPLC. A Phenomenex prodigy (ODS3, 5 μm, 100A, 3.2 × 150 mm, 00F-4097-R0) was used in this analysis. The column temperature was ambient, and the mobile phase included water (containing 0.2% phosphoric acid, solvent A) and acetonitrile (solvent B) in the following gradient system: initial 6% B, linear gradient to 30% B in 20 min, hold at 30% for 5 min. The total running time was 25 min. The postrunning time was 10 min. The flow rate was 1.2 mL/min, the injection volume was 10 μL, and the detection wavelength was set at 330 nm.

Table 1. Total Phenols (Percent of Dry Weight) and Relative Antioxidant Activity (Percent Inhibition of DPPH Free Radicals) of Artichoke Samples As Determined by Spectrophotometric Analyses

variety	organ	total phenols[a]	relative activity[a]
Imperial Star	leaves[b,c]	9.806	0.465
Violet	leaves[b,c]	6.806	0.346
Violet	leaves[b,d]	6.897	0.287
Green Globe	leaves[b,c]	9.561	0.497
Green Globe	leaves[b,d]	8.760	0.405
Imperial Star	young heads[b,c]	2.806	0.189
Imperial Star	young heads[c,e]	2.733	0.161
Violet	young heads[c,e]	2.893	0.179
Green Globe	young heads[b,c]	3.106	0.192
Green Globe	young heads[c,e]	2.580	0.154
Imperial Star	mature heads[b,c]	1.784	0.076
Imperial Star	mature heads[c,e]	2.206	0.119
Green Globe	mature heads[b,c]	1.600	0.078
Green Globe	mature heads[c,e]	2.238	0.083

[a] Each number represents the mean of three replications with <5% difference among replications. [b] Harvested Oct 3, 2001. [c] Oven-dried (70 °C). [d] Freeze-dried. [e] Harvested Sept 5, 2001.

Determination of the Scavenging Effect on DPPH Radicals. DPPH radicals were prepared in ethanol to the final concentration of 1.0×10^{-4} M (*25*). Different concentrations of tested compounds were added and mixed with DPPH solution. The solutions with tested samples were then shaken vigorously and kept in the dark for 0.5 h. The absorbance of the samples was measured utilizing a spectrophotometer (HP model 8453) at 517 nm against a blank of ethanol without DPPH. All tests were run in triplicate and averaged. Differences among triplicates were always <5%.

RESULTS AND DISCUSSION

Total Phenols and DPPH Scavenging Activity of Artichoke Extracts. The total phenols in artichoke samples were estimated by using a Folin−Ciocalteu colorimetric method. This method assays for the content of all flavonoids, caffeic acid derivatives, and tannins (*24*). Results of the analysis indicated that overall the leaves contained the highest concentration of total phenols and that the "younger" heads have higher phenol contents than the mature heads (**Table 1**). Of the three artichoke varieties, Imperial Star leaves contained the highest concentration of phenols (11.2% expressed as chlorogenic acid), whereas the variety Violet contained the lowest (6.80% phenols from the dried leaves and expressed as chlorogenic acid equivalents).

The edible artichoke heads contained a significantly lower amount of phenols inside for dried mature heads at the range of 1.48−2.89% and for young dried buds at the range of 2.58−3.22% depending upon variety (**Table 1**). Different drying methods (oven-drying at 70 °C vs freeze-drying) were found to have no significant effect on the total phenol contents of the artichoke samples harvested on two different dates.

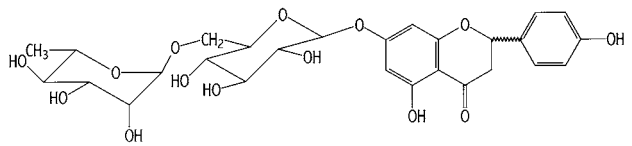
The antioxidant activities of artichoke samples were estimated by scavenging DPPH free radicals (**Table 1**). Samples that had low phenolic compound content also had lower antioxidant activity. For example, Green Globe and Imperial Star mature heads had the lowest phenolic content and the lowest antioxidant activity, whereas Green Globe and Imperial Star leaf extracts had the highest total phenolic compound content and the highest antioxidant activity. In general, our results indicate that the scavenging DPPH free radical activities of the different artichoke samples were highly correlated to the total phenols inside (r^2

= 0.96). These data show that most of the variation in antioxidant activity in the extract of artichoke can be accounted for by the variation in phenolic compounds content.

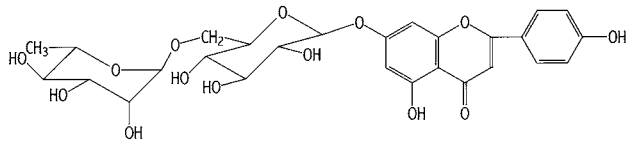
Isolation of Antioxidants from Artichoke Leaf and Head. In total, we purified four compounds from the 70% methanol extracts of artichoke heads. Compound **3** was identified as chlorogenic acid (**Figure 1**) by comparison with the authentic compound. Compounds **1**, **2**, and **4** were identified as (*R* and *S*)-narirutin, apigenin-7-rutinoside, and cynarin, respectively (**Figure 1**), by comparison with the reported spectral data in the literature (*20*, *21*, *23*). In total, five phenolic antioxidants were purified from the artichoke leaf extract; compounds **6** and **9** were found to be identical with compounds **3** and **4**, respectively. Compounds **5**, **7**, and **8** were elucidated as 1-caffeoylquinic acid, luteolin-7-*O*-α-L-rhamnosyl(1→6)-*β*-glucopyranoside (luteolin-7-rutinoside), and luteolin-7-*O*-*β*-glucopyranoside (cynaroside), respectively (**Figure 1**), by comparison with reported spectral data in the literature (*18*, *19*). During our structural elucidation process for cynarin, we found that the name "cynarin" is quite confusing, and many authors have erred in naming this compound (*26*, *27*). The major dicaffeoylquinic acid derivative in artichoke heads was identified by us as 1,3-dicaffeoylquinic acid. It is this compound that should be called cynarin and not 1,5-dicaffeoylquinic acid, as, for example, reported by Slanina et al. (*26*). We also found the compound (also called cynarin by the authors) in echinacea is not 1,3-dicaffeoylquinic acid, but actually 1,4-dicaffeoylquinic acid by comparison of the HPLC retention time with that of the authentic compound; this author inadvertently gave the wrong structure to this compound found in echinacea (*27*).

Antioxidant Activity of the Purified Compounds. The antioxidant activities of purified compounds were tested by utilizing the free radical scavenging activity of DPPH (*25*). The antioxidant activities of phenolic compounds are reported to be largely determined by the number of hydroxyl groups on the aromatic ring. The higher the number of hydroxyl groups, the greater the expected antioxidant activity. In addition, the presence of a second hydroxyl group on the ortho or para position will also increase the antioxidant activity (*18*). Our results are in full agreement with this observation (**Figure 2**). Cynarin, with two adjacent hydroxyl groups on each of its phenolic rings (**Figure 1**), showed the highest antioxidant activity (**Figure 2**), whereas cynaroside and luteolin-7-rutinoside, with two adjacent hydroxyl groups on one ring and only a single hydroxyl on the second ring (**Figure 1**), showed slightly less antioxidant activity, which was higher than that of chlorogenic acid or 1-caffeoylquinic acid with two adjacent hydroxyl groups on the same phenolic ring; apigenin-7-rutinoside and narirutin, with two hydroxyl groups on separate phenolic rings, were the least active antioxidants among the purified artichoke compounds (**Figure 2**).

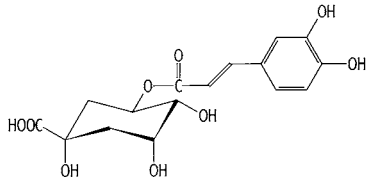
HPLC Analysis of Antioxidants in Artichoke. To accurately measure the antioxidants in artichoke, we developed a validated HPLC analytical method. A Luna ODS3 column (150 × 3.2 mm, 5 μm) was selected and used in this analysis. Various mobile phase systems were evaluated to achieve satisfactory separation of all of these compounds. Finally, we chose a water (0.2% phosphoric acid) and acetonitrile gradient. No interfering peaks were noted for artichoke samples, and good resolution was achieved among all compounds. The retention times for 1-caffeoylquinic acid, chlorogenic acid, luteolin-7-*O*-α-L-rhamnosyl(1→6)-*β*-glucopyranoside (luteolin-7-rutinoside), luteolin-7-*O*-*β*-glucopyranoside (cynaroside), narirutin, apigenin-7-rutinoside, and cynarin (1,3-dicaffeoylquinic acid) were approximately 3.75, 6.37, 12.20, 12.60, 13.1, 13.5, and 14.5 min, respectively (**Figure 3**). The major phenolic compounds in artichoke extracts are cynarin and chlorogenic acid.



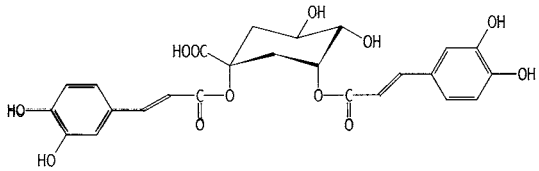
(Compound 1) (R&S)-narirutin



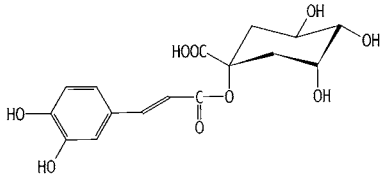
(Compound 2) apigenin-7-rutinoside



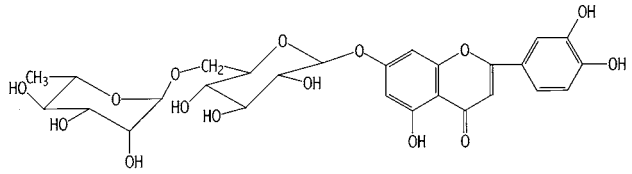
(Compounds 3 and 6) chlorogenic acid



(Compounds 4 and 9) 1,3-di-caffeoylquinic acid (cynarin)

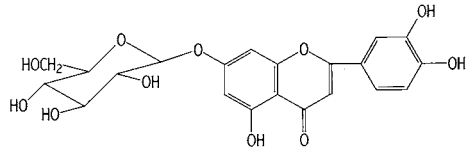


(Compound 5) 1-caffeoylquinic acid



(Compound 7) Luteolin-7-*O*-*α*-L-rhamnosyl (1→6)-β-glucopyranoside

(Luteolin-7-rutinoside)



(Compound 8) Luteolin-7-*O*-β-glucopyranoside (Cynaroside)

Figure 1. Structures of compounds purified from artichoke.

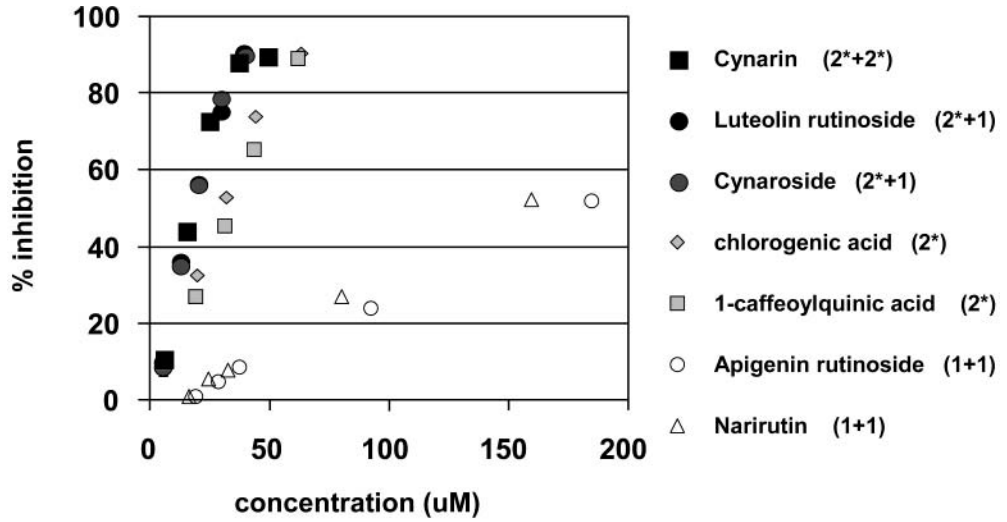


Figure 2. DPPH scavenging activity of artichoke's purified compounds. In parentheses appears the number of active hydroxyl groups on each phenolic ring; an asterisk (*) indicates two adjacent hydroxyls on the same phenolic ring.

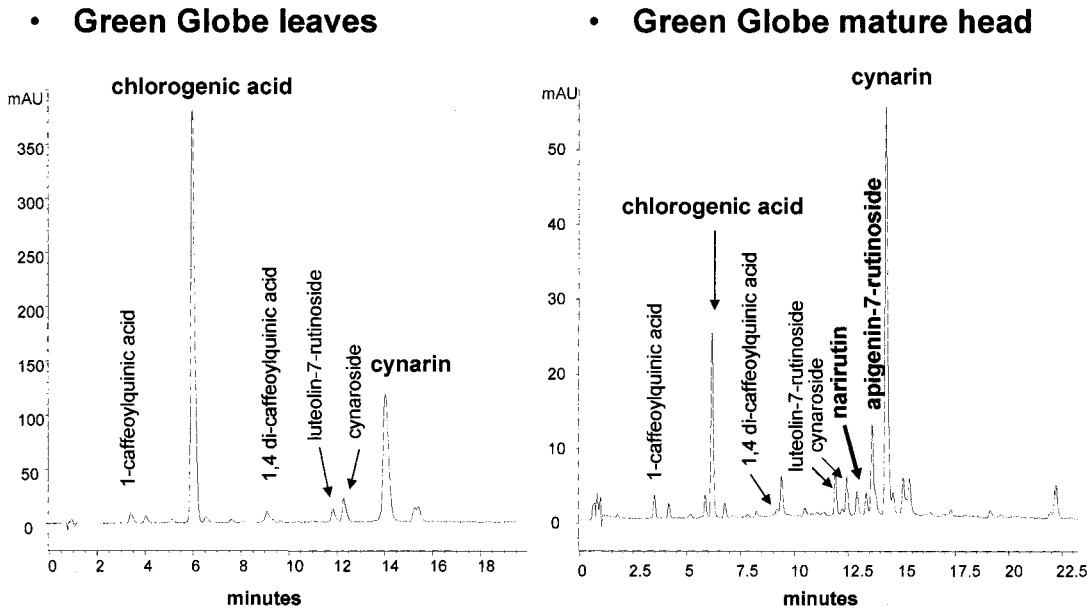


Figure 3. HPLC chromatograms of Green Globe artichoke's methanol extractions of leaves (left) and mature heads (right).

Extraction Solvent for HPLC Analysis. To achieve the highest recovery and extraction of antioxidants from artichoke, we first extracted artichoke leaves from one variety using a range of different concentration of methanol solution (0, 20, 40, 60, 80, and 100%) with the above-described procedure. We found that different methanol/water extraction solvents will yield different results and that 60% methanol was the best extraction solvent (**Figure 4**). Addition of 2 mL of acetic acid or 2 mL of 1 M NaOH to the extraction solvent did not increase the extraction efficacy (data not shown), and thus a 60% methanol aqueous solution was chosen as the final extraction solvent for sample analysis.

System Suitability for HPLC Analysis. System suitability was evaluated by performing 10 replicated analyses of one artichoke dried leaf sample within the same working day and then checking the percent relative standard deviation (%RSD) of the retention times and peak areas for 1-caffeoylquinic acid, chlorogenic acid, luteolin-7-rutinoside, luteolin-7-*O*-β-glucopyranoside, and cynarin. The %RSD values of the retention times for these five compounds were 0.32, 0.28, 0.76, 0.54, and 0.65%, respectively. The %RSD values for the peak areas of these five compounds were 0.45, 0.30, 0.72, 0.66, and 0.77%, respectively. These results indicated the method is suitable for the analysis of artichoke samples.

Precision of the Extraction Procedure. The precision of the extraction procedure was validated using one artichoke leaf sample. Six samples, weighing ~250 mg, were extracted as described above in 50 mL of 60% methanol. An aliquot of each sample was then injected and quantified. The average amounts of 1-caffeoylquinic acid, chlorogenic acid, luteolin-7-rutinoside, luteolin-7-*O*-β-glucopyranoside, and cynarin were 0.31% with an RSD of 1.50%, 1.52% with an RSD of 2.10%, 0.34% with an RSD of 1.10%, 0.21% with an RSD of 2.33%, and 1.47% with an RSD of 1.76% of the dry weight (DW), respectively. These results suggest that the method presented here has excellent precision.

Percent Recovery of HPLC Analysis. The recovery was determined by removing 25 mL of extraction solvent from all six extractions done in precision testing and replacing it with 25 mL of fresh extraction solvent. These samples were then re-extracted and reanalyzed for these five polyphenols. A value of exactly half of the original extraction value validates 100%

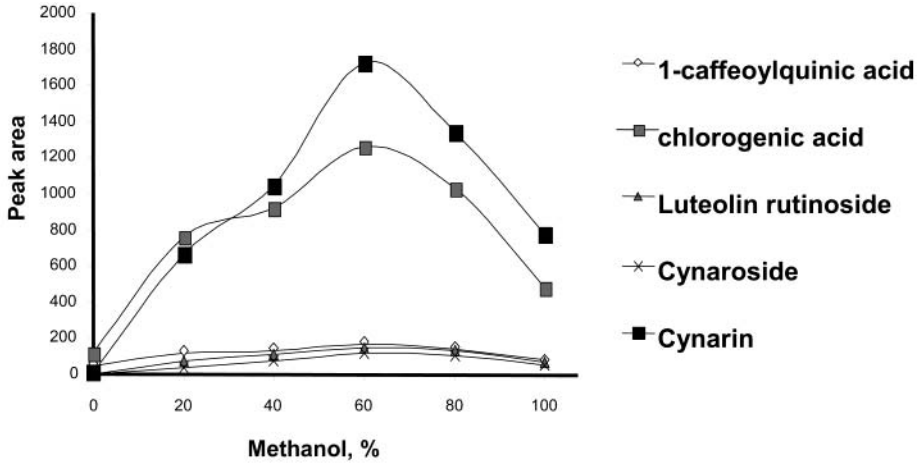


Figure 4. Extraction of phenolic compounds from artichoke leaves with different concentrations of methanol/water solutions. Dried leaf sample (500 mg) was extracted in the different solutions by 25 min of sonication, and then the extract was analyzed by HPLC.

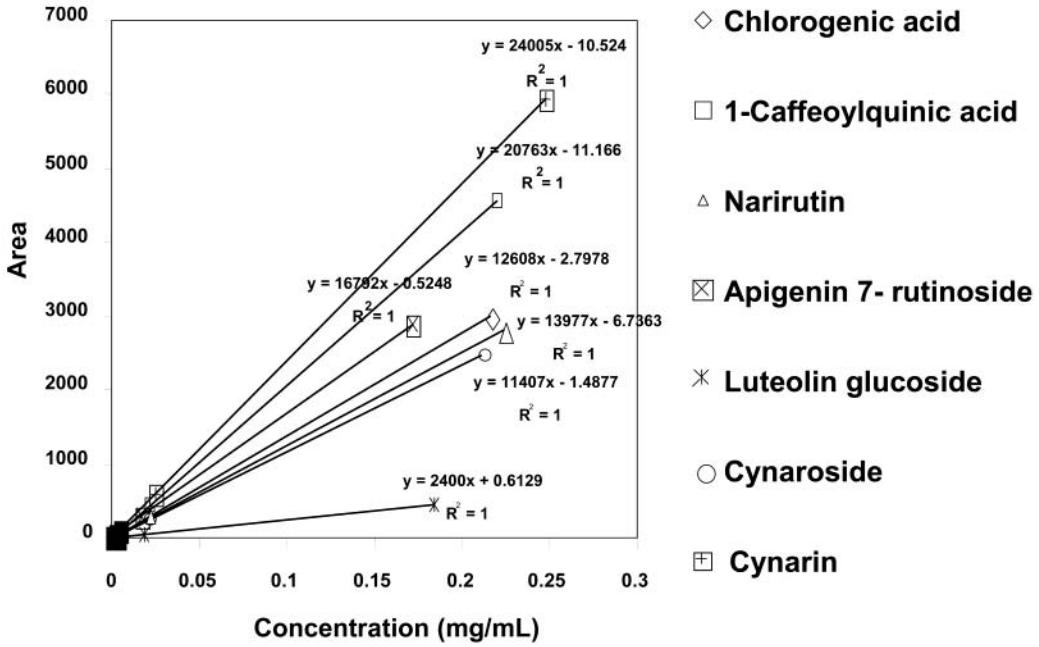


Figure 5. Calibration curves of purified compounds.

recovery. The average amounts of 1-caffeoylquinic acid, chlorogenic acid, luteolin rutinoside, luteolin-7-*O*-*β*-glucopyranoside, and cynarin recovered from the re-extraction of these samples multiplied by 2 were 0.31% with an RSD of 2.50%, 1.53% with an RSD of 1.19%, 0.33% with an RSD of 2.12%, 0.21% with an RSD of 2.37%, and 1.48% with an RSD of 2.76%, respectively. These numbers are highly in accordance with numbers calculated for the extraction precision. Assuming the average amount extracted in the re-extraction is 100%, the average amounts recovered in the original extraction were then validated to be 100.0, 100.6, 97.06, 100.0, and 100.7%, respectively. This study demonstrates the high extraction recovery of this method.

Calibration of HPLC Analysis. Calibration curves for all seven compounds were established by procedures as described above. The calibration curves showed excellent linearity covering the tested range described above with correlation coefficients of 1 for all seven compounds (**Figure 5**).

Ruggedness of HPLC Analysis. The ruggedness of the assay was evaluated by comparing the results obtained from two different independent working chemists. All extractions and standard preparations were carried according to the method described above. Both chemists got similar results for one artichoke sample (one got 4.15% total phenols; the second chemist reported 4.10% total phenols on a dry sample basis).

Content of Seven Isolated Antioxidants in Artichoke Leaves and Mature and Young Heads by HPLC Analysis. Three different varieties of artichokes (Green Globe, Imperial Star, and Violet) were tested by this validated HPLC method. The samples were divided into three groups: leaves, mature heads, and young heads (the edible stage). Samples collected on different dates were compared in this research, and sample drying methods (70 °C oven or freeze-drying) were also tested separately. We found that the leaves contained the highest antioxidants and the mature heads contained the least (**Table 2**). Two compounds, apigenin-7-rutinoside and (2*R* and 2*S*)-narirutin (**Figure 1**), were found only in the heads of all three varieties but not in the leaves. The two different drying processes (drying vs freeze-drying) using the specific conditions described by each method had no significant effects on the final testing results, although freeze-dried leaves of Green Globe had significantly lower amounts of phenolics (**Table 2**). Although

Table 2. Comparison of Purified Phenolic Compounds and Total Phenolic Content (Percent of Dry Weight) in Artichoke Varieties and Organs, Harvested on Two Dates and Oven- or Freeze-Dried, Based on HPLC Analysis of Methanol Artichoke Extracts

variety	organ	1-caffeoylquinic acid	chlorogenic acid	luteolin rutinoside	cynaroside	narirutin	apigenin-7-rutinoside	cynarin	total
Imperial Star	leaves[a,b]	0.125	4.713	0.420	0.252	0.000	0.000	1.617	7.127
Violet	leaves[a,b]	0.310	1.519	0.339	0.197	0.000	0.000	1.689	4.054
Violet	leaves[a,c]	0.740	1.429	0.345	0.186	0.000	0.000	1.471	4.170
Green Globe	leaves[a,b]	0.149	4.158	0.213	0.314	0.000	0.000	1.619	6.455
Green Globe	leaves[a,c]	0.064	4.654	0.275	0.114	0.000	0.000	0.924	6.031
Imperial Star	young heads[a,b]	0.092	0.540	0.015	0.045	0.150	0.042	0.918	1.802
Imperial Star	young heads[b,d]	0.028	0.210	0.011	0.050	0.109	0.067	0.792	1.268
Violet	young heads[b,d]	0.034	0.371	0.017	0.047	0.243	0.050	0.585	1.347
Green Globe	young heads[a,b]	0.070	0.459	0.011	0.035	0.085	0.032	0.748	1.440
Green Globe	young heads[b,d]	0.039	0.276	0.022	0.061	0.108	0.063	0.847	1.416
Imperial Star	mature heads[a,b]	0.045	0.229	0.023	0.025	0.146	0.054	0.424	0.945
Imperial Star	mature heads[b,d]	0.012	0.114	0.047	0.025	0.220	0.120	0.242	0.780
Green Globe	mature heads[a,b]	0.020	0.186	0.025	0.024	0.182	0.050	0.309	0.795
Green Globe	mature heads[b,d]	0.018	0.102	0.040	0.026	0.182	0.076	0.242	0.687

[a] Harvested Oct 3, 2001. [b] Oven-dried (70°C). [c] Freeze-dried. [d] Harvested Sept 5, 2001.

the cause for the lower phenolics in Green Globe following freeze-drying is unknown, in general our data demonstrate that proper oven-drying is at least as good for maintaining phenolic compounds content, composition, and activity, as compared to freeze-drying. Date of harvest had no significant effect on the phenolic composition and content of artichoke extracts based on the HPLC analysis, although we recognize that the entire developmental stage from bud initiation to seed maturity was not included in this study.

Comparison of the leaf's phenolic compound HPLC profile of three artichoke varieties showed differences among varieties. Imperial Star artichoke leaves contained the highest antioxidants (average = 7.2% totally), whereas the Violet variety contained an average of only 4.1%. The contents of cynarin (the most active antioxidant) were almost the same in the leaves of the three varieties. In contrast, the contents of chlorogenic acid were different in the varieties, with chlorogenic acid content in Imperial Star and Green Globe artichoke leaves ~3 times that found in Violet. Imperial Star artichoke also contained the highest content of flavonoids (luteolin-7-glucopyranoside and luteolin-7-*O*-α-L-rhamnosyl(1→6)-β-glucopyranoside) (**Table 2**). Total phenolic compounds as calculated by summarizing individual purified compounds (**Table 2**) is highly correlated ($R^2 = 0.986$) with total phenolic compounds calculated by the colorimetric method (**Table 1**). This is another indication for the precision of our HPLC method and for our success in purifying the majority of artichoke's phenolic compounds.

Conclusion. In this research, we found artichoke is a good source of phenolic compounds and antioxidants. The antioxidants of three varieties of artichoke have been purified, analyzed, and compared with each other. Imperial Star and Green Globe artichoke leaves contained significantly higher total phenols, antioxidant activity, and cynarin contents than the variety Violet. In this research, we also developed a rapid and quantitative method of quality assurance of antioxidant contents in artichoke products. By using a Luna ODS3 column with a mixture of phosphoric acid/water/acetonitrile as mobile phase, this method showed excellent linearity, accuracy, and precision. We also found a wide range of extraction recoveries can be achieved across a range of extraction solvents, and in this study we found the 60% methanol aqueous solution gave the highest recovery and will totally extract the phenolic compounds. Some of the earlier research on artichokes appears to have underestimated their caffeoylquinic acid derivatives (*2*, *16*) as the methods reported would not fully extract the targeted polyphenolic compounds. The contents of caffeoylquinic acid derivatives in dried artichoke leaves should be ~3−6.5%, on a dry weight basis, depending on the genetic background of the artichoke and the environment under which it is grown.

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