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Phenolic Compounds from the Leaf Extract of Artichoke (*Cynara scolymus* L.) and Their Antimicrobial Activities

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A preliminary antimicrobial disk assay of chloroform, ethyl acetate, and *n*-butanol extracts of artichoke (*Cynara scolymus* L.) leaf extracts showed that the *n*-butanol fraction exhibited the most significant antimicrobial activities against seven bacteria species, four yeasts, and four molds. Eight phenolic compounds were isolated from the *n*-butanol soluble fraction of artichoke leaf extracts. On the basis of high-performance liquid chromatography/electrospray ionization mass spectrometry, tandem mass spectrometry, and nuclear magnetic resonance techniques, the structures of the isolated compounds were determined as the four caffeoylquinic acid derivatives, chlorogenic acid (**1**), cynarin (**2**), 3,5-di-*O*-caffeoylquinic acid (**3**), and 4,5-di-*O*-caffeoylquinic acid (**4**), and the four flavonoids, luteolin-7-rutinoside (**5**), cynaroside (**6**), apigenin-7-rutinoside (**7**), and apigenin-7-*O*-β-D-glucopyranoside (**8**), respectively. The isolated compounds were examined for their antimicrobial activities on the above microorganisms, indicating that all eight phenolic compounds showed activity against most of the tested organisms. Among them, chlorogenic acid, cynarin, luteolin-7-rutinoside, and cynaroside exhibited a relatively higher activity than other compounds; in addition, they were more effective against fungi than bacteria. The minimum inhibitory concentrations of these compounds were between 50 and 200 μg/mL.

KEYWORDS: Artichoke; *Cynara scolymus* L.; antimicrobial activity; phenolic compounds; caffeoylquinic acid derivatives; flavonoids

INTRODUCTION

The use of natural products with therapeutic properties has a long history, and especially in China, plant, animal, and mineral products were the main source of medicines (*1*). Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration (*2*). In recent years, concern over pathogenic and spoilage microorganisms in foods has increased due to the increase in outbreaks of foodborne disease (*3*). There are growing interests in using natural antimicrobial compounds, especially extracted from plants, for the preservation of foods. In addition, there are more consumers who tend to question the safety of synthetic additives and would prefer natural foodstuffs (*4*, *5*).

Cynara scolymus L. (artichoke) is an ancient herbaceous plant, originating from the Mediterranean area, which today is widely cultivated all over the world. Its flower head is eaten as a vegetable and prepared for different value-added products such as salad, jam, concentrate, and canned beverages. Artichoke was first transplanted in China during the 1990s from Italy and Spain and has been planted commercially by the Yandi Agricultural Development Co. Ltd., Kunming, in southwest China since 2001. In China, artichoke can be used for alternative products such as tea and alcoholic beverages. Its leaves have been used for hepatoprotection and as a choleretic and diuretic in traditional European medicine since Roman times (*6*). In Germany, it is used today as a choleretic (*7*) for its lipid-lowering, hepato-stimulating, and appetite-stimulating actions. Recently, research has been carried out into the antioxidant, anti-HIV, liver protective, bile-expelling, and lipid-lowering effects of artichoke leaf extract (*8*−*11*). Although artichoke extract has been used for hundreds of years as a medicine, it is seldom used as an antimicrobial agent.

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers (*2*, *4*, *12*). In contrast, to date, there are few reports of antimicrobial activities of artichoke extracts except that Mossi and Echeverrigaray (*13*) reported the antimicrobial activities against three bacteria of artichoke leaf extract and its components. As their studies on the antimicrobial activities against three microorganisms only comprised bacteria, the species number of microorganisms tested was limited, and the components investigated were simple. To further study the antimicrobial properties of artichoke leaf extracts, we have made a detailed and extensive research of the antimicrobial properties

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of the extracts of artichoke leaf on three kinds of microorganisms, which included seven bacteria, four yeasts, and four molds. In addition, as the main antimicrobial activities were attributed mainly to certain chemical components, we have investigated the isolation and structural elucidation of the antimicrobial constituents from artichoke leaf extracts. At the same time, the minimum inhibitory concentration (MIC) of these compounds was examined by the agar and broth dilution method.

MATERIALS AND METHODS

Plant Materials. The leaves of artichoke were collected from the Yandi Agricultural Company Experiment Station, Kunming, China, in the summer of 2003, and then dried in a 60 °C air-drying oven. The dried materials were comminuted to a powder and kept in sealed bags at room temperature for further extractions. The plant was identified at the Research Center of Eco-Environmental Sciences, Chinese Academy of Sciences (CAS), where a voucher specimen was deposited.

Chemicals and General Procedures. Silica gel (130−270 mesh), RP-18 silica gel, and Sephadex LH-20 (Xin Jing Ke Biotechnology Co., Beijing, China) were used for column chromatography (CC). The solvents used for high-performance liquid chromatography (HPLC) and HPLC/electrospray ionization mass spectrometry (ESI-MS) analyses were of HPLC grade (Sigma Chemical Co.); antibiotics of ampicillin (Amp), streptomycin (Str), kanamycin sulfate (Kan), and nystatin (Nys) were of USP grade (Amresco Chemical Co.); and other solvents and chemicals were of analytical grade. Thin-layer chromatography (TLC) was performed using 8 cm × 2.5 cm, 0.25 mm, silica gel 60 F254 and RP-18 F254 plates (Huiyou Gel Co., Yantai, China). UV spectra were obtained on a Hitachi U-3010 spectrometer in MeOH. ^{1}H, ^{13}C, and HMBC (H-detected heteronuclear multiple-bond correlation) nuclear magnetic resonance (NMR) spectra were recorded with Bruker ARX 400 spectrometersin CD_3OD solution. Negative ESI-MS was measured using an Agilent 1100 LC/MSD system with an electrospray ion source. HPLC analyses were performed on an Agilent 1100 system equipped with an autosampler, a quaternary pump system, a photodiode array and DAD detector, and a Chemstation data system. A 250 mm × 9.4 mm i.d., 5 μm particle size Zorbax ODS column (Shimadzu) and a 250 mm × 4.6 mm i.d., 5 μm particle size Zorbax Eclipse XDB ODS column (Agilent) were selected for HPLC purification and analysis, respectively. 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 of Phenolic Compounds. The dried leaf powers of artichoke (1.5 kg) were extracted with 75% ethanol (5 L × 3). A solvent was evaporated in vacuo at 50 °C. The concentrated extract was partitioned successively with 60 mL of chloroform, ethyl acetate, and *n*-butanol. All three fractions were concentrated in vacuo to one-fifth volume in a centrifugal evaporator at 50 °C and then sterilized by filtration using a 0.22 μm membrane for antimicrobial assay. Other sets of the same fractions were evaporated to dryness to determine amounts of solids in concentrated materials. Among the three fractions, the *n*-butanol extract exhibited the most significant antimicrobial activities. The *n*-butanol extract (35 g) was subjected to Sephadex LH-20 CC (80 g, 47.5 cm × 2.4 cm i.d.). The column was continuously eluted with a gradient of methanol in water, and fraction 1 (10 mL) was collected with 25% methanol, fraction 2 (60 mL) was collected with 40−50%, fraction 3 (90 mL) was collected with 60−70%, fraction 4 (60 mL) was collected with 80%, fraction 5 (55 mL) was collected with 90%, and fraction 6 (15 mL) was collected with 100%. Fraction 2 was subjected to a silica gel CC eluted with ethyl acetate/methanol/water (7:1:1) to get 100 mg of compound **1**. Fraction 4 was subjected to silica gel CC (40 cm × 2.4 cm i.d.) and eluted with ethyl acetate/methanol/water (8:1:1) to isolate and purify compounds **5** (30 mg) and **6** (45 mg). Fraction 3 was first purified with a silica gel column eluted with ethyl acetate/methanol/water (8:1:0.2) and then repeatedly chromatographed over Sephadex LH-20 (30 g, 40 cm × 1.8 cm i.d.), eluting with MeOH to afford a mixture of caffeoylquinic acid derivatives. Further purification was achieved by semipreparative HPLC on the Zorbax ODS column (Shimadzu) with acetonitrile/0.1% acetic acid (1:4) as the mobile phase and a flow rate of 3.0 mL/min to yield compounds **2** (9 mg), **3** (32 mg), and **4** (14 mg). Fraction 5 was first separated by a silica gel CC using ethyl acetate/methanol/water (10:1:1) as mobile phase and then repeatedly chromatographed over a silica gel CC eluted with ethyl acetate/methanol/water (10:2:1). Further purification was by semipreparative HPLC with acetonitrile/0.1% acetic acid (2:5) as the mobile phase, a flow rate of 3.0 mL/min, and detection at 330 nm to afford compounds **7** (20 mg) and **8** (12 mg). Each fraction was analyzed with TLC developed in ethyl acetate/acetic acid/water (6:1:1) and observed under 330 nm UV illumination. Fractions showing similar TLC patterns were further analyzed by HPLC at 330 nm.

Spectrometric Identification of Isolated Compounds. *5-O-Caffeoylquinic Acid (Chlorogenic Acid) (Compound **1**).* White power. UV λ_{max} (MeOH): 244, 300sh, 328 nm. Negative ESI-MS: *m*/*z* 353 [M − H]$^-$. MS/MS fragments: *m*/*z* 190.8, 179.0. NMR data are consistent with the literature (*14*, *15*).

1,3-Di-O-caffeoylquinic Acid (Cynarin) (**2**). Yellow power. UV λ_{max} (MeOH): 242, 298sh, 327 nm. Negative ESI-MS: *m*/*z* 515.2 [M − H]$^-$. MS/MS fragments: *m*/*z* 353.0, 190.8, 178.9. NMR data are consistent with the literature (*16*−*18*).

3,5-Di-O-caffeoylquinic Acid (**3**). Yellow power. UV λ_{max} (MeOH): 245, 298sh, 330 nm. Negative ESI-MS: *m*/*z* 515.2 [M − H]$^-$. MS/MS fragments: *m*/*z* 352.9, 190.9, 178.9. NMR data are consistent with the literature (*19*−*23*).

4,5-Di-O-caffeoylquinic Acid (**4**). Yellow power. UV λ_{max} (MeOH): 246, 300sh, 330 nm. Negative ESI-MS: *m*/*z* 515.2 [M − H]$^-$. MS/MS fragments: *m*/*z* 353.0, 335.0, 190.8, 178.8. NMR data are consistent with the literature (*20*−*23*).

*Luteolin-7-O-α-L-rhamnosyl(1→6)-β-D-glucopyranoside (Luteolin-7-rutinoside) (Compound **5**) (18, 24, 27).* Yellow powder. UV λ_{max} (MeOH): 256, 266sh, 348 nm. Negative ESI-MS: *m*/*z* 593.3 [M − H]$^-$. MS/MS fragments: *m*/*z* 326.9, 284.9.

*Luteolin-7-O-β-D-glucopyranoside (Cynaroside) (Compound **6**) (18, 25*−*27).* Yellow powder. UV λ_{max} (MeOH): 255, 267sh, 349 nm. Negative ESI-MS: *m*/*z* 447.6 [M − H]$^-$. MS/MS fragments: *m*/*z* 284.9.

*Apigenin-7-O-α-L-rhamnosyl(1→6)-β-D-glucopyranoside (Apigenin-7-rutinoside) (Compound **7**) (18, 27).* Yellow powder. UV λ_{max} (MeOH): 254, 266sh, 348 nm. Negative ESI-MS: *m*/*z* 577.3 [M − H]$^-$. MS/MS fragments: *m*/*z* 268.9.

*Apigenin-7-O-β-D-glucopyranoside (Compound **8**) (27).* Yellow powder. UV λ_{max} (MeOH): 255, 267sh, 350 nm. Negative ESI-MS: *m*/*z* 431.6 [M − H]$^-$. MS/MS fragments: *m*/*z* 268.9.

HPLC/DAD Analysis of Artichoke Leaf Extract. The samples of the extracts were filtered through a 0.45 μm filter for each analysis. The Zorbax Eclipse XDB ODS column was used in this analysis. The column temperature was ambient, and the mobile phase included water (containing 0.1 acetic acid, solvent A) and acetonitrile (solvent B) in the following gradient system: initial 8% B; linear gradient to 15% B in 20 min; to 30% in 30 min; hold at 30% for 5 min. The total running time was 35 min. The postrunning time was 10 min. The flow rate was 1.0 mL/min, the injection volume was 10 μL, and the detection wavelength was set at 330 nm.

HPLC/ESI-MS for Characterizing Major Phenolic Compounds. The mobile phase was the same as the HPLC method described above. The ESI-MS was operated under a negative mode with a tube lens voltage of 60 V, a collision energy of 54%, a capillary temperature of 325 °C, and a capillary voltage of 4000 V. High-purity nitrogen (99.999%) was used as the drying gas, and the flow rate was at 9.00 L/min. Helium was used as the nebulizer at 35 psi. The trap scanned from *m*/*z* 80 to 1000. The ESI interface and MS parameters were optimized to obtain maximum sensitivity.

Test Microorganisms and Growth Media. Gram-positive and Gram-negative bacteria, yeasts, and molds were used for antimicrobial activities studies. Gram-positive bacteria: *Bacillus subtilis* (CGMCC 1.1849), *Staphylococcus aureus* (ATCC 6358P), *Agrobacterium tumefaciens* (CGMCC 1.1415), and *Micrococcus luteus* (CGMCC 1.880). Gram-negative bacteria: *Escherichia coli* (CGMCC 1.90), *Salmonella typhimurium* (CGMCC 1.1190), and *Pseudomonas aeruginosa* (CGMCC 1.2031). Yeasts: *Candida albicans* (ATCC 10231), *Candida lusitaniae* (ATCC 2201), *Saccharomyces cerevisiae* (IFFI 1611), and *Saccharomyces carlsbergensis* (ACCC 2166). Molds: *Aspergillus niger*

Table 1. Antibacterial and Antifungal Activities of Artichoke Leaf Extracts

	zones of inhibition (mm)[a,b]												
	chloroform (mg/mL)			ethyl acetate (mg/mL)			*n*-butanol (mg/mL)			controls (50 μg/mL)			
microorganisms	2.5	5.0	10.0	2.5	5.0	10.0	2.5	5.0	10.0	Amp	Str	Kan	Nys
B. subtilis	8	11	18	–[c]	9	11	12	14	18	32	15	22	–
S. aureus	–	8	11	–	–	9	12	16	25	31	13	26	–
A. tumefaciens	–	8	11	–	–	9	8	12	16	19	13	14	–
M. luteus	–	–	9	–	8	11	–	9	12	14	12	10	–
E. coli	–	8	13	–	11	16	8	12	25	40	18	12	–
S. typhimurium	–	9	12	–	9	12	–	11	14	14	–	9	–
P. aeruginosa	–	–	8	–	–	–	–	9	11	–	–	9	–
C. albicans	9	13	17	9	12	17	11	14	18	–	–	–	32
C. lusitaniae	–	–	–	–	–	–	8	10	13	–	–	–	31
S. cerevisiae	–	8	9	–	8	10	9	12	15	–	–	–	21
S. carlsbergensis	–	10	15	–	9	12	10	13	17	–	–	–	24
A. niger	9	13	17	–	9	12	10	13	19	–	–	–	32
P. oxalicum	8	11	14	–	9	11	9	12	16	–	–	–	33
M. mucedo	–	–	–	–	–	–	9	11	14	–	–	–	24
C. cucumerinum	–	9	12	–	–	–	8	11	15	–	–	–	18

[a] Values, including diameter of the disk (6.0 mm), are the mean of three replicates. [b] Twenty-five microliters of solution was applied to each disk. Amp, Str, and Kan served as the controls for bacteria. Nys served as the control for the fungi. [c] No inhibition or inhibition zone was <8 mm.

(CGMCC 3.316), *Penicillium oxalicum* (CGMCC 3.4022), *Mucor mucedo* (CGMCC 3.15), and *Cladosporium cucumerinum* (ATCC 11279) were used for antimicrobial activity tests. All microorganisms were provided by China General Microbiological Culture Collection Center (CGMCC), CAS. The bacterial strains were grown in Mueller−Hinton agar (MHA) plates at 37 °C, while the yeasts and molds were grown in Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28 °C.

Antimicrobial Disk Assay. Antibacterial and antifungal activities of three plant extracts were investigated by the disk diffusion method (*28*, *29*). The MHA plates, containing an inoculum size of 10^6 CFU/mL of bacteria or 2×10^5 CFU/mL yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L-shaped glass rod. Then, disks (6.0 mm in diameter) impregnated with 25 μL of each extract at a concentration of 10.0 mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk, with solvent only in the center to serve as a control, and antibiotic disks (6.0 mm in diameter) of 50 μg/mL Amp, Str, Kan (for bacteria), and Nys (for fungi) were also used as positive controls. All of the plates were incubated at 37 °C for 18−20 h for bacteria and at 28 °C for 48−96 h for fungi. The zones of growth inhibition around the disks were measured after 18−20 h of incubation at 37 °C for bacteria and 48−96 h for fungi at 28 °C, respectively. The sensitivity of the microorganisms species to the plant extracts was determined by measuring the sizes of the inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as nonactive against bacteria. All of the experiments were performed in triplicate. The results are reported as the average of three experiments.

Determination of MIC. MIC was determined by both agar and broth dilution methods (*30*). Two-fold serial dilutions (0−20.0 mg/mL) of the three extracts, with the appropriate antibiotic, were prepared as a positive control in Mueller−Hinton broth for bacteria and Saboraud glucose broth for fungi. For the agar dilution assay, previously prepared sensitivity plates, using serial 2-fold dilutions of the fractions and control antibiotics as above, were spot inoculated (10^6 CFU per spot for bacteria and 2×10^5 CFU cells or spores per spot for fungi). The inoculated plates were then incubated at either 37 °C for 24 h (bacteria) or 28 °C for 48−96 h (fungi). For broth dilution tests, 0.1 mL of standardized suspension of bacteria (10^6 CFU/mL) and fungal cell or spores (5×10^5 CFU/mL) was added to each tube (containing fractions of three extracts at a final concentration of 0−20.0 mg/mL) and incubated at 37 °C for bacteria for 24 h or at 28 °C for fungi for 48−96 h. The lowest concentration of the tube or plate that did not show any visible growth by macroscopic evaluation was considered as the MIC. Each assay was performed in triplicate.

Statistical Analysis. The triplicate data were subjected to an analysis of variance for a completely random design using Statistical Analysis System programs. Duncan's new multiple range test was used to compare the difference among means at the level of 0.05.

RESULTS AND DISCUSSION

Antimicrobial Activities of Artichoke Leaf Extracts. The preliminary disk assay of the three soluble fractions of artichoke leaf extracts, as shown in **Table 1**, showed that the *n*-butanol fraction exhibited the most significant antimicrobial activities against all of the tested microorganisms, followed by chloroform and ethyl acetate fractions.

As shown in **Table 1**, at least six kinds of the bacteria including four Gram-positive bacteria, *B. subtilis*, *S. aureus*, *A. tumefaciens*, and *M. luteus*, and two Gram-negative bacteria, *E. coli* and *S. typhimurium*, were susceptible to leaf extracts of artichoke. *P. aeruginosa* was only sensitive to the butanol fraction. There were five fungi including three yeasts, *C. albicans*, *S. cerevisiae*, and *S. carlsbergensis*, and two molds, *A. niger* and *P. oxalicum*, susceptible to all three artichoke leaf extracts except that *C. lusitaniae* and *M. mucedo* were sensitive only to the *n*-butanol fraction, and *C. cucumerinum* was sensitive only to *n*-butanol and chloroform fractions. Therefore, the *n*-butanol fraction was the most active to all of the tested microorganisms among the three artichoke leaf extracts. In addition, the more the concentration of fraction was, the more active to the tested microorganisms. In contrast, the inhibition zones of three solvent controls, chloroform, ethyl acetate, and *n*-butanol, were almost zero or below 8 cm, so that they were not active to all of the tested microorganisms. However, four antibiotics, Amp (50 μg/mL), Str sulfate (50 μg/mL), Kan (50 μg/mL), and Nys (50 μg/mL), were more effective than any of the soluble fractions of artichoke extracts, except that Amp had no activity to *P. aeruginosa* and Str sulfate had no effect on *S. typhimurium* and *P. aeruginosa*.

HPLC and HPLC/MS Analysis of Antimicrobial Compounds in Artichoke Leaf Extract. The major antimicrobial compounds in *n*-butanol extract of artichoke leaf were first identified by the TLC method, and five spots were detected by UV light. Further identification and measurement were subjected to HPLC and negative ESI-MS. A Zorbax Eclipse XDB ODS column was selected and used in this analysis. Various mobile

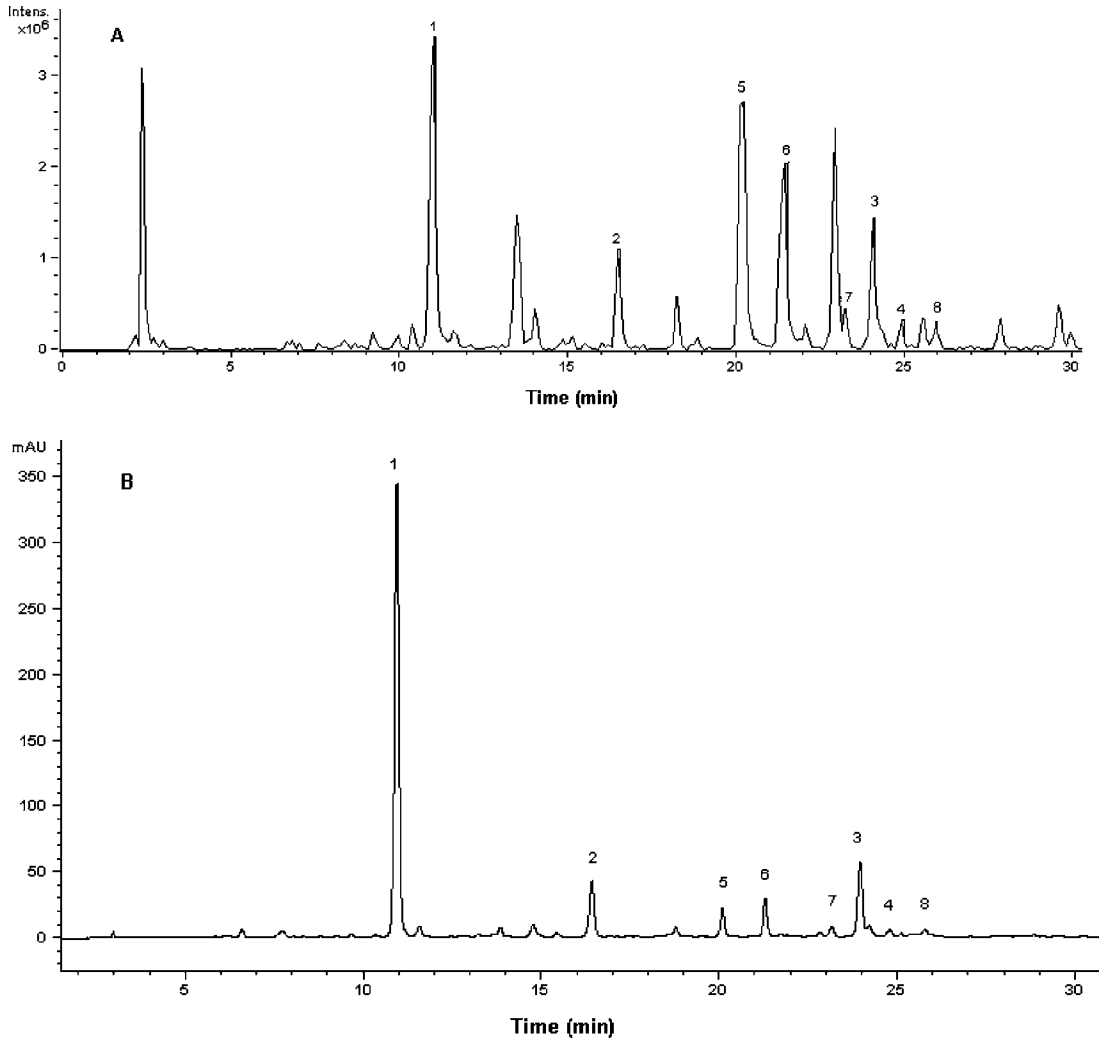

Figure 1. HPLC/MS chromatograms of the antimicrobial compounds from artichoke leaf extract. Total ion chromatogram under negative ion (**A**); HPLC chromatogram UV detected at 330 nm (**B**).

phase systems were evaluated to achieve satisfactory separation of all of these compounds. Finally, we chose a water (0.1% formic acid) and acetonitrile gradient. No interfering peaks were noted for artichoke extract samples, and good resolution was achieved among all compounds. The total of eight antimicrobial compounds was detected. The retention times for the eight active compounds were 10.83, 16.41, 20.08, 21.28, 23.15, 23.95, 24.78, and 25.77 min, respectively, detected by the total ion chromatogram (**Figure 1A**) and UV monitoring at 330 nm (**Figure 1B**). In addition, the MS and MS/MS spectrometric data of these phenolic compounds were obtained.

Isolation and Identification of the Antimicrobial Compounds from Artichoke Leaf Extract. The active *n*-butanol extract was separated into six fractions (fractions 1−6) by Sephadex LH-20 CC. Their antimicrobial activities were investigated by several microorganisms by the disk diffusion method. The zones of inhibition of fractions 1−6 to *B. subtilis* were 0, 12, 16, 20, 17, and 2 mm; the zones of inhibition of fractions 1−6 to *E. coli* were 0, 14, 22, 18, 16, and 4 mm; the zones of inhibition of fractions 1−6 to *C. albicans* were 0, 20, 22, 20, 14, and 0 mm; and the zones of inhibition of fractions 1−6 to *A. niger* were 16, 18, 20, 13, and 0 mm. All fractions except fractions 1 and 6 showed a strong activity to the tested microorganisms. Fractions 2−5 were purified by repeated chromatographies and preparative HPLC to afford eight active compounds.

Compounds **2**−**4** were found to have the same molecular formula ($C_{25}H_{24}O_{12}$) and to be isomeric compounds because of their same negative ESI-MS molecule ion peak at *m*/*z* 515.2 $[M - H]^-$, the same MS/MS fragment ion peaks and UV spectra, combined with the analysis of their ^{1}H NMR and ^{13}C NMR spectra. The MS/MS fragment ion peaks appearing at *m*/*z* 353 $[M -$ caffeoyl $- H]^-$, 191 $[M -$ 2caffeoyl $- H]^-$ (or $[$quinic acid $- H]^-$), and 179 $[$caffeoyl $- H]^-$ corresponded to the successive loss of two caffeoyl groups and a quinic acid moiety. The ^{1}H NMR spectra of the three isomeric compounds further exhibited signals for two caffeic acid moieties and a quinic acid moiety. Four doublets with coupling constants of 15.9 Hz appeared for the trans olefinic protons H-7′ (H-7″) and H-8′ (H-8″), and the coupling pattern of the three aromatic protons (H-2′ d; H-5′ d; H-6′ dd) indicated the presence of 1,3,4-trisubstituted benzenes. The signals of H-3 (equatorial), H-4 (axial), and H-5(axial) of the quinic acid moiety were able to be distinguished by their coupling pattern because of their different stereochemical configurations. The location of caffeoyl substitution on the quinic acid moiety was also deduced from the comparative analysis of ^{1}H NMR chemical shifts of the protons of the quinic acid moiety as compared to chemical shifts of the corresponding protons of quinic acid moieties of chlorogenic acid. Compound **2** was identified as 1,3-di-*O*-caffeoylquinic acid, because the signal for the proton at C-3 and C-1 shifted downfield as compared to the protons of C-3

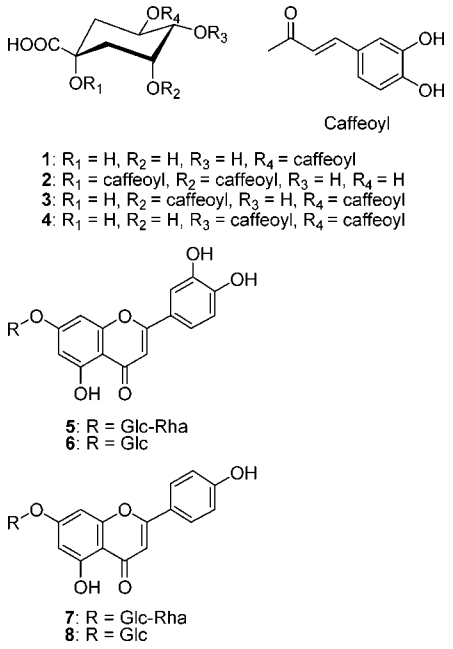

Figure 2. Structures of phenolic compounds of **1**–**8** isolated from the leaf extract of artichoke.

and C-1 of quinic acid moieties of chlorogenic acid. With a similar pattern, compound **3** was identified as 3,5-di-*O*-caffeoyl quinic acid and compound **4** was identified as 4,5-di-*O*-caffeoyl quinic acid because the signals for the C-3 and C-5 positions and signals for the C-4 and C-5 positions were shifted downfield as compared to the corresponding position of quinic acid moieties of chlorogenic acid, respectively (*17*, *22*). At the same time, the HMBC spectra confirmed the respective positions of the two caffeoyl groups on the ring of the quinic acid moiety by the observation of the specific HMBC correlations. Key correlations of H-3 and C-9′ of compound **2** showed caffeoyl groups linked to C-3; H-5/C-9′ and H-3/C-9″ of compound **3** showed two caffeoyl groups linked to C-5 and C-3; and H-5/C-9′ and H-4/C-9″of compound **4** showed two caffeoyl groups linked to C-5 and C-4, respectively (**Figure 2**).

1,3-Di-*O*-caffeoylquinic Acid (Cynarin) (2). Yellow power. ^{1}H NMR (CD_3OD, 400 MHz): δ 1.83−2.87 (4H, m, H-2, -6), 3.61 (1H, dd, J = 3.6, 9.6 Hz, H-4), 4.22 (1H, ddd, J = 4.4, 9.6, 11.2 Hz, H-5), 5.36 (1H, m, H-3), 6.11 and 6.18 (1H each, d, J = 15.9 Hz, H-8′, -8″), 6.50 and 6.63 (1H each, d, J = 8.2 Hz, H-5′, -5″), 6.58 and 6.74 (1H each, dd, J = 2.0, 8.2 Hz, H-6′, -6″), 6.81 and 6.92 (1H each, d, J = 2.0 Hz, H-2′, -2″), 7.46 and 7.48 (1H each, d, J = 15.9 Hz, H-7′, -7″). ^{13}C NMR (CD_3OD, 100 MHz): δ 32.9 (t, C-2), 41.3 (t, C-6), 67.8 (d, C-5), 73.0 (d, C-3), 75.3 (d, C-4), 81.1 (s, C-1), 115.1 (d, C-8′, -8″), 115.4 and 115.5 (d, C-2′, -2″), 116.1 and 116.6 (d, C-5′, -5″), 122.0 and 123.0 (d, C-6′, -6″), 127.4 and 127.5 (s, C-1′, -1″), 146.5 and 146.7 (s, C-3′, -3″), 147.2 and 147.8 (d, C-7′, -7″), 149.3 and 149.7 (s, C-4′, -4″), 167.8 and 168.9 (s, C-9′, -9″), 174.6 (s, C-7). Key HMBC correlation: H-3/C-9′ [identical to data in the literature (*17*−*18*)].

3,5-Di-*O*-caffeoylquinic Acid (3). ^{1}H NMR (CD_3OD, 400 MHz): δ 2.13−2.34 (4H, m, H-2, -6), 3.97 (1H, dd, J = 3.4, 7.6 Hz, H-4), 5.39 (1H, ddd, J = 5.9, 7.6, 10.8 Hz, H-5), 5.43 (1H, m, H-3), 6.26 and 6.34 (1H each, d, J = 16.0 Hz, H-8′, -8″), 6.77 and 6.78 (1H each, d, J = 8.2 Hz, H-5′, -5″), 6.96 and 6.97 (1H each, dd, J = 2.0, 8.2 Hz, H-6′, -6″), 7.06 and 7.07 (1H each, d, J = 2.0 Hz, H-2′, -2″), 7.57 and 7.61 (1H each, d, J = 16.0 Hz, H-7′, -7″). ^{13}C NMR (CD_3OD, 100 MHz): δ 36.1 (t, C-2), 37.8 (t, C-6), 70.8 (d, C-4), 72.2 (d, C-3), 72.6 (d, C-5), 74.6 (s, C-1), 115.2 and 115.3 (d, C-8′, -8″), 115.7 (d, C-2′, -2″), 116.5 (d, C-5′, -5″), 123.0 and 123.1 (d, C-6′, -6″), 127.9 and 128.0 (s, C-1′, -1″), 146.8 (s, C-3′, -3″), 147.1 and 147.3 (d, C-7′, -7″), 149.5 and 149.6 (s, C-4′, -4″), 168.4 and 168.9 (s, C-9′, -9″), 175.8 (s, C-7). Key HMBC correlations: H-5/C-9′, H-3/C-9″ [identical to data in the literature (*19*−*22*)].

4,5-Di-*O*-caffeoylquinic Acid (4). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.94−2.34 (4H, m, H-2, -6), 4.35 (1H, ddd, J = 3.2, 5.6, 6.4 Hz, H-3), 5.12 (1H, dd, J = 3.2, 8.8 Hz, H-4), 5.55 (1H, ddd, J = 4.5, 8.8, 10.8 Hz, H-5), 6.19 and 6.29 (1H each, d, J = 15.9 Hz, H-8′, -8″), 6.74 and 6.76 (1H each, d, J = 8.0 Hz, H-5′, -5″), 6.90 and 6.92 (1H each, dd, J = 2.0, 8.0 Hz, H-6′, -6″), 7.01 and 7.03 (1H each, d, J = 2.0 Hz, H-2′, -2″), 7.52 and 7.60 (1H each, d, J = 15.9 Hz, H-7′, -7″). ^{13}C NMR (CD_3OD, 100 MHz): δ 38.4 (t, C-2), 39.4 (t, C-6), 69.0 (d, C-5), 69.4 (d, C-3), 75.7 (d, C-4), 76.1 (s, C-1), 114.6 and 114.7 (d, C-8′, -8″), 115.2 (d, C-2′, -2″), 116.5 (d, C-5′, -5″), 123.2 (d, C-6′, -6″), 127.6 and 127.7 (s, C-1′, -1″), 146.8 (s, C-3′, -3″), 147.6 and 147.7 (d, C-7′, -7″), 149.7 (s, C-4′, -4″), 168.3 and 168.6 (s, C-9′, -9″), 175.3 (s, C-7). Key HMBC correlations: H-5/C-9′, H-4/C-9″ [identical to data in the literature (*20*−*22*)].

Table 2. Antibacterial and Antifungal Activities of Phenolic Compounds from Artichoke Leaf Extract

	MIC (μg/mL)											
microorganisms	1	2	3	4	5	6	7	8	Amp	Str	Kan	Nys
B. subtilis	200	200	200	200	200	100	200	200	5	20	10	
S. aureus	200	200	200	100	200	50	200	200	5	20	10	
A. tumefaciens	100	100	200	200	200	200	100	200	10	20	20	
M. luteus	100	100	100	50	100	200	200	100	20	20	40	
E. coli	200	100	200	100	100	200	200	–	5	10	20	
S. typhimurium	200	200	–[a]	–	–	200	–	–	20	–	40	
P. aeruginosa	200	100	–	–	100	100	–	–	–	–	40	
C. albicans	50	100	200	–	100	50	200	–				10
C. lusitaniae	50	100	200	200	50	50	200	–				10
S. cerevisiae	200	200	200	200	200	200	100	200				20
S. carlsbergensis	50	100	200	200	200	100	200	200				20
A. niger	100	100	100	200	100	50	200	200				10
P. oxalicum	100	100	100	100	100	50	100	200				10
M. mucedo	100	100	100	100	100	50	100	100				20
C. cucumerinum	50	100	100	100	100	50	100	100				20

[a] No inhibition or MIC > 200 μg/mL.

UV spectra of compounds **5**−**8** showed similar skeletons. The further ESI-MS molecular ion peak and MS/MS fragment ion peaks analysis of the four compounds, *m*/*z* 593.3 [M − H]$^-$, 284.9 [M − glc − rha − H]$^-$ (compound **5**); *m*/*z* 447.6 [M − H]$^-$, 326.9 [M − lut − H]$^-$, 284.9 [M − glc − H]$^-$ (compound **6**); *m*/*z* 577.3 [M − H]$^-$, 268.9 [M − glc − rha − H]$^-$ (compound **7**); and *m*/*z* 431.6 [M − H]$^-$, 326.9 [M − lut − H]$^-$, 268.9 [M − glc − H]$^-$ (compound **8**) suggested that compounds **5** and **6** have the same luteolin skeleton and compounds **7** and **8** have the same apigenin skeleton, respectively. In total, compounds **5**−**8** were elucidated as luteolin-7-*O*-α-L-rhamnosyl(1→6)-*β*-D-glucopyranoside (luteolin-7-rutinoside), luteolin-7-*O*-*β*-D-glucopyranoside (cynaroside), apigenin-7-*O*-α-L-rhamnosyl(1→6)-*β*-D-glucopyranoside (apigenin-7-rutinoside), and apigenin-7-*O*-*β*-D-glucopyranoside, respectively (**Figure 2**), by comparison with reported data in the literature (*18*, *24*, *27*).

Antimicrobial Activities of the Purified Compounds by MIC Determinations. As presented in **Table 2**, the MICs of eight phenolic compounds were tested against seven bacteria, four yeasts, and four molds. The results showed that most compounds exhibited strong activities against all of the tested microorganisms. Their MIC values ranged from 0.05 to 0.2 mg/mL. The effect of all compounds was very similar against all of the bacteria except that four compounds, 3,5-*O*-dicaffeoylquinic acid (**3**), 4,5-*O*-dicaffeoylquinic acid (**4**), apigenin-7-rutinoside (**7**), and apigenin-7-*O*-*β*-D-glucopyranoside (**8**), had no effect on *S. typhimurium* and *P. aeruginosa*. Chlorogenic acid (**1**), 1,3-*O*-dicaffeoylquinic acid (**2**), luteolin-7-rutinoside (**5**), and cynaroside (**6**) had a relatively higher activity than other compounds against all of the tested fungi; in addition, they were more effective against fungi than bacteria. Almost all compounds exhibited stronger activities against a bacterium, *M. luteus*, and four molds, *A. niger*, *P. oxalicum*, *M. mucedo*, and *C. cucumerinum*, whereas they showed weak activity against *S. cerevisiae*. This result maybe indicates that molds are more sensitive than other microorganisms.

In contrast, Mossi and Echeverrigaray (*13*) found that dichloromethane and ethanol extracts of artichoke could inhibit the growth of three bacteria, *S. aureus*, *B. cereus*, and *B. subtilis*, in concentrations of 5 mg/mL. However, they did not report about the effect on the growth of fungi, such as yeasts or molds. In our present study, a wide range of microorganisms was examined, not only including Gram-positive and Gram-negative bacteria but also four yeasts and four molds. This may indicate that artichoke leaf extracts have broad inhibitory activities to microorganisms and are promising for incorporation into various food products for which a natural antimicrobial additive is desired, although their antimicrobial activities are lower than many antibiotics at present. In conclusion, we have established that not only artichoke contains microbial inhibitors but also that the structures of the antimicrobial compounds isolated from artichoke leaf extract contain microbial inhibitors. The results of the present work indicate that artichoke leaf extracts may be an ideal candidate for further research into their uses for food preservation as well as pharmaceutical and natural plant-based products.

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