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), cyanarin (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, cyanarin, 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 choleric (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 (8–11). Although artichoke extract has been used
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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
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 (130–230 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 x 25 cm plates (Huiyou Gel Co., Yantai, China). UV spectra were recorded with a Hitachi U-3010 spectrometer in MeOH. 1H, 13C, and HMBC (H-detected heteronuclear multiple-bond correlation) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX 250 MHz spectrometer (Bruker). Chemical shifts are reported in δ (ppm) and coupling constants are in Hz. Mass spectra were recorded on a Bruker Daltonics microTOF mass spectrometer using ESI positive and negative ionization modes at the Peking University Floriculture Research Center, Beijing, China. HPLC/DAD analysis of artichoke leaf extract was performed on a Shimadzu LC-20A instrument with a DAD detector and a Chemstation data system. A 250 mm x 4.6 mm i.d., 5 μm particle size Zorbax Eclipse XDB ODS column (Shimadzu) and a 150 mm x 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 powders of artichoke (1.5 kg) were extracted with 75% ethanol (5 L x 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 x 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 1 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 x 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 x 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 acetone/tetrahydrofuran (99:1) as the mobile phase and a flow rate of 1.0 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) as the 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 powder. UV λmax (MeOH): 244, 298sh, 327 nm. Negative ESI-MS: m/z 355 [M – H]−. MS/MS fragments: m/z 190.8, 179.0. NMR data are consistent with the literature (14, 15).

1,3-Di-O-cafeoylquinic Acid (Cynarin) (2). Yellow powder. UV λmax (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-cafeoylquinic Acid (3). Yellow powder. UV λmax (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-cafeoylquinic Acid (4). Yellow powder. UV λmax (MeOH): 246, 300sh, 330 nm. Negative ESI-MS: m/z 515.2 [M – H]−. MS/MS fragments: m/z 353.0, 353.5, 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 λmax (MeOH): 256, 266sh, 348 nm. Negative ESI-MS: m/z 593.3 [M – H]−. MS/MS fragments: m/z 326.9, 284.9.


Apigenin-7-O-α-L-rhamnosyl(1→6)-β-D-glucopyranoside (Apigenin-7-rutinoside) (Compound 7) (18, 27). Yellow powder. UV λmax (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 λmax (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 (60:40) and acetonitrile (99.999%) as the drying gas. The flow rate was 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 6535), Agrobacterium tumefaciens (CGMCC 1.1415), and Micrococcus luteus (CGMCC 1.1880). Gram-negative bacteria: Escherichia coli (CGMCC 1.190), 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
Served as the controls for bacteria. Nys served as the control for the fungi. No inhibition or inhibition zone was <8 mm.

The bacterial strains were grown in Mueller−Hinton, using 200 mL of Mueller−Hinton broth for bacteria and Saboraud dextrose agar (SDA) and potato dextrose agar for fungi. For the agar dilution assay, previously prepared MHA plates, containing an inoculum size of 10^6 CFU per spot for bacteria and 10^5 CFU cells or spores per spot for fungi, were used. The inoculated plates were then incubated at either 37 °C for bacteria for 24 h or at 28 °C for fungi at 28 °C. For broth dilution tests, 0.1 mL of standardized suspensions of bacteria (10^6 CFU/mL) and fungal cells or spores (2 × 10^10 CFU/mL) were 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.

**Table 1.** Antibacterial and Antifungal Activities of Artichoke Leaf Extracts

<table>
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<tr>
<th>microorganisms</th>
<th>chloroform (mg/mL)</th>
<th>ethyl acetate (mg/mL)</th>
<th>n-butanol (mg/mL)</th>
<th>controls (50 µg/mL)</th>
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<tr>
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<td>M. luteus</td>
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<td>8</td>
<td>13</td>
<td>–</td>
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<tr>
<td>S. thymurium</td>
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<tr>
<td>P. aeruginosa</td>
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<td>17</td>
<td>11</td>
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<tr>
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<tr>
<td>C. cucumerinum</td>
<td>–</td>
<td>9</td>
<td>12</td>
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</table>

*4 Values, including diameter of the disk (6.0 mm), are the mean of three replicates. 5 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. 6 No inhibition or inhibition zone was <8 mm.

**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. thymurium,* 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, C. s. cerevisiae,* and *C. s. carlsbergensis,* and two molds, *A. niger* and *P. oxalicum,* susceptible to all three artichoke leaf extracts except that *C. lusitaniae* and *M. mucido* 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. thymurium* 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
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, 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 (C25H24O12) 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 1H NMR and 13C 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 1H 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 1H 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.
and C-1 of quinic acid moieties of chlorogenic acid. With a similar pattern, compound 3 was identified as 3,5-di-O-cafeoyl quinic acid and compound 4 was identified as 4,5-di-O-cafeoyl 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 caffeyl 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 caffeyl groups linked to C-3; H-5/C-9' and H-3/C-9'' of compound 3 showed two caffeyl groups linked to C-5 and C-3; and H-5/ C-9' and H-4/C-9'' of compound 4 showed two caffeyl groups linked to C-5 and C-4, respectively (Figure 2).

1,3-Di-O-cafeoylquinic Acid (Cynarin) (2). Yellow power. 1H NMR (CD3OD, 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'').

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

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<td>P. oxalicum</td>
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<td>M. mucudo</td>
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<td>C. cucumbarum</td>
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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 – gluc – 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-dicafeoylquinic acid (3), 4,5-O-dicafeoylquinic 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-dicafeoylquinic acid (2), luteolin-7-rutinoside (5), and cyanoroside (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. mucido, and C. cucumerinum, whereas they showed weak activity against S. cerevisiae. This result may indicate that molds are more sensitive than other microorganisms.

In contrast, Morsi and Echeverrigaray (13) found that dichloromethane and ethanol extracts of artichoke could inhibit the growth of three bacteria, S. aureus, B. cereus, and S. 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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