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Article

Inhibitory Activity of Caffeoylquinic Acids from the Aerial Parts of *Artemisia princeps* on Rat Lens Aldose Reductase and on the Formation of Advanced Glycation End Products

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Caffeoylquinic acids -3,4-di-*O*-caffeoylquinic acid (1); 1,3,5-tri-*O*-caffeoylquinic acid (2); and 3,4,5-tri-*O*-caffeoylqunic acid (3)- were isolated from an acetone-soluble fraction of the aerial parts of *Artemisia princeps*. Their structures were determined spectroscopically using 1D- and 2D-nuclear magnetic resonance (NMR) studies, as well as by comparing the NMR results with previously published structures. All the isolates were subjected to *in vitro* bioassays to evaluate their efficacy in inhibiting rat lens aldose reductase (RLAR) activity and the formation of advanced glycation end products (AGEs). We found 1,3,5-tri-*O*-caffeoylquinic acid (2) to be the most potent AGE inhibitor, and the concentration that resulted in 50% inhibition (IC_{50}) was 22.18 ±1.46 mM, as compared to the aminoguanidine and chlorogenic acid controls, which had IC_{50} values of 1,093.11±10.95 and 117.63±0.20 mM, respectively. In the RLAR assay, the three caffeoylquinic acids were found to have IC_{50} values in the range of 1.78-2.40 μM, demonstrating a 5- to 10-fold greater efficacy in RLAR inhibition as compared to the quercetin control, which had an IC_{50} value of 17.91 μM.

Key words: *advanced glycation end products,* Artemisia princeps, *caffeoylquinic acids, rat lens aldose reductase*

Diabetes mellitus is characterized by chronic hyperglycemia, and a deficiency in the production and secretion of insulin [DeFronzo *et al*., 1997]. Insulin-insensitive organs such as the lens, nerves, and kidneys are targets for complications such as cataracts, neuropathy, and nephropathy [Ureda *et al*., 2004]. Among the biochemical causes of these complications, hyperglycemia has attracted the most attention, and the polyol pathway, an alternate route of glucose metabolism, has been implicated [Alexiou *et al*., 2009]. It has been demonstrated that, in cells that take up glucose via an insulin-independent mechanism, hyperglycemia results in a marked rise in intracellular glucose concentration, consequently leading to hexokinase saturation. The excess substrate is acted upon by aldose reductase (AR, E.C. 1.1.1.21), an enzyme present in most tissues, which converts the glucose into sorbitol [Brownlee, 2001]. Increasing sorbitol accumulation is harmful to cells, and may contribute to diabetic complications [Chung and Chung, 2005]. Further, in hyperglycemia, body proteins undergo increased glycation, during which glucose reacts

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Abbreviations: AGEs, advanced glycation end products; *A. princeps, Artemisia princeps;* AR, aldose reductase; BSA-MGO, methylglyoxal-modified bovine serum albumin; ESI-MS, electron spray ionization-mass spectrophotometer; HMBC, heteronuclear multiple bond coherence; HMQC, heteronuclear multiple quantum coherence; IC_{50}, half maximal inhibitory concentration; MGO, methylglyoxal; NADPH, nicotinamide adenine dinucleotide phosphate; NMR, nuclear magnetic resonance; RLAR, rat lens aldose reductase; SD, Sprague-Dawley

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non-enzymatically with protein amino groups to form a labile Schiff's base that rearranges to a stable Amadori product [Peyroux and Sternberg, 2006]. This Amadori product undergoes further reactions involving reactive dicarbonyl intermediates such as 3-deoxyglucosone and methylglyoxal to form complex, heterogeneous, fluorescent, and cross-linked structures called advanced glycation end products [Ahmed, 2005; Vander, 2008]. These observations have led to the development of numerous AR and/or AGE inhibitors with diverse chemical structures for use as possible therapeutic agents. Recently, there has been a growing interest in alternative therapies, especially in the therapeutic use of plant-derived natural products for the management of diabetes [Yoshikawa and Matsuda, 2006]. This is because plant-derived products are usually considered to be less toxic and have fewer side-effects than synthetic ones. The genus *Artemisia* comprises about 500 plants [McArthur, 1979]. Most *Artemisia* herbs are perennials that grow in the Northern hemisphere and are used for various purposes such as medicine, food, spices, and ornamental plants. *Artemisia* herbs possess the following diverse medicinal effects: anti-atherosclerotic [Han *et al*., 2009], anti-inflammatory [Chang *et al*., 2009], anti-tumor [Sarath *et al*., 2007; Bang *et al*., 2008; Park *et al*., 2008], anti-oxidant [Kim *et al*., 2008], and anti-diabetic effects [AI-Waili, 1986; Jung *et al*., 2007; Kang *et al*., 2008]. Previous phytochemical investigations of this plant have resulted in the isolation of various flavonoids such as luteloin, apigenin, scoplletin, cinnamates, eupatilin, and jaceosidin [Okada, 1995; Jung *et al*., 2007]. Compounds such as davidigenin, 6-demethoxycapillarisin, 4,5-di-*O*-caffeoylquinic acid, and 2,4-dihydroxy-4-methoxydihydrochalcone from *Artemisia dracunculus* [Logendra *et al*., 2006] and capillarisin from *Artemisia capillaries* [Lee *et al*., 2008a] have been reported to exhibit an inhibitory effect on aldose reductase. Among *Artemisia* herbs, *A. princeps* has been widely used in Korean traditional medicine for treating colic, vomiting and diarrhea, and irregular uterine bleeding [Zhao *et al*., 1994]. Recently, Jung *et al*. [2007] reported that the ethanol extract from this plant exerted an anti-diabetic effect in type 2 diabetic mice. In this study, we investigated the AR-inhibitory activity of compounds isolated from *A. princeps* leaf extracts to evaluate their potential for treating diabetic complications that are caused by the enhanced activation of the polyol pathway and the formation of AGEs in hyperglycemic conditions. We isolated and identified three caffeoylquinic acids from *A. princeps* that inhibited both the activity of AR and the formation of AGEs. To our knowledge, this is the first report of the isolation of these compounds from the genus *Artemisia*.

Materials and Methods

Plant materials. The aerial parts of *A. princeps*, originally from Korea, were purchased from the herbal medicine market, Chuncheon, Korea. The plant was identified and authenticated by emeritus professor Hyung Jun Ji, Natural Products Research Institute, Seoul National University. A voucher specimen (No. RIC-0802) was deposited and maintained at the Herbarium of Regional Innovation Center, Chuncheon, Korea.

Instruments and chemicals. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX 400 spectrometer (Karlsrube, Germany) using tetramethylsilane as an internal standard at 400 and 100 MHz, respectively. FT-IR spectra data were taken with a JASCO (Hachioji, Tokyo, Japan) FT/IR-4100 spectrophotometer, and specific rotation $[\alpha]_D$ was measured with a Jasco P-2000 digital polarometer in a 3.5 mm i.d.×50 mm cell. UV–vis spectra were measured on a Uvikon Xs Secoman (Ales, France) spectrophotometer using quartz cell of 1 cm width. Melting points were determined with an electrothermal digital Büchi B-540 melting point apparatus. ESI mass spectra were obtained using a Finnigan LCQ Advantage Max ion trap mass spectrometer (ThermoQuest, San Jose, CA). DL-glyceraldehyde, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), quercetin, aminoguanidine hydrochloride (AG), methylglyoxal (MGO, 40% aqueous solution), and bovine serum albumin (BSA, essentially fatty acid free) were purchased from Sigma (St. Louis, MO).

Extraction and isolation. The aerial parts of *A. princeps* (1 kg) were ground in a blender, subjected twice to extraction with 95% ethanol (5 L) for 5 h, and then filtered (Advantec filter paper, type 2; Advantec, Tokyo, Japan). The combined filtrate was then concentrated *in vacuo* at 45°C (Büchi Rotavapor R-220, Flawil, Switzerland) to give 102.2 g of a total extract (Yield : 10.2% of the aerial parts). The extract was coated onto a filter aid (Celite 545 AW; Sigma-Aldrich, St. Louis, MO) and loaded on to a column (100 mm i.d.×200 mm). It was then eluted stepwise with organic solvents, which resulted in four fractions [*n*-hexane (16.6 g), methylene chloride (18.6 g), acetone (46.6 g), and methanol (18.2 g)], which were used for subsequent bioassays. The soluble fraction of the organic solvents was then concentrated to dryness using a rotary vacuum evaporator (Eyela N-N Series; Eyela, Tokyo, Japan) at 45°C. The acetone fraction was loaded onto a silica gel (70-230 mesh ASTM; Merck, Darmstadt, Germany) column (100 mm i.d.×1000 mm) and eluted stepwise with ethylacetate and methanol (2:0→0:2), and 18 fractions (A1-18) were obtained. The fractions A7-9 exhibited strong inhibitory

activity against both AR and the formation of AGEs. Therefore, these fractions were re-chromatographed on a 36 mm i.d.×460 mm column and were eluted with ethylacetate/methanol (2:1) to give the active A7-9:5 fraction. This fraction was further purified by preparative high-performance liquid chromatography (HPLC) (Japan Analytical Industry Co. Ltd., Tokyo, Japan), by applying it on to a JAI gel Silica Series column (DOS-BP-L, SP-120-15). A mixture of methanol/water (4/1, v/v) was used as the mobile phase at a flow rate of 4.0 mL/min, and detection was carried out at 245 nm; this yielded the active A7-9:5:6 fraction. All fractions were analyzed by HPLC (Agilent 1100 series; Agilent, Palo Alto, CA). Fractions with similar chromatography patterns were combined, and bioassays were performed using a concentration of 10 μg/mL to determine their effects on AR activity and 220 μg/mL to study the inhibition of AGE formation. The three active compounds from the A7-9:5:6 fraction were then isolated, and their structures were determined by instrumental analyses. Quercetin and chlorogenic acid, both known AR inhibitors, were used as positive controls for the RLAR enzyme activity assay and aminoguanidine, a known AGE inhibitor, was used as a positive control in the methylglyoxal-modified bovine serum albumin (BSA-MGO) assay. For the RLAR assay, DL-glyceraldehyde and NADPH were used as the substrate and cofactor, respectively.

3,4-di-*O*-caffeoylquinic acid (1). $[\alpha]_D^{25}$ -115.9° (c 0.05, MeOH); IR ν_{max} cm^{-1} 3364 (-OH), 2984 (C-H), 2947 (C-H), 2922 (C-H), 2868 (C-H), 2813 (C-H), 2782 (C-H), 1686 (aromatic C=C), 1647 (aromatic C=C), 1600 (aromatic C=C), 1550 (C-C), 1450 (C-C), 1267 (benzene); ESI-MS *m/z* 517 $[M+H]^+$; mp 154-156°C (lit. 155-158°C) [Hung *et al*., 2006]; ^{1}H-NMR (400 MHz, CD_3OD, ppm) δ 2.15 (brd m, 1H, *J*=13.43, 14.80 10.76 Hz, H-2), 2.31 (t, 2H, *J*=15.46, 7.73 Hz, H-6), 2.38 (d, 1H, *J*=14.09 Hz, H-2), 4.41 (ddd, 1H, *J*=2.65, 2.72, 2.67 Hz, H-5), 5.16 (dd, 1H, *J* =2.67, 10.2 Hz, H-4), 5.59 (ddd, 1H, *J*=3.1, 10.1, 10.7 Hz, H-3) (quinic acid moiety); 6.24 (dd, 1H, *J*=12.31, 15.56 Hz, H-8), 6.35 (dd, 1H, *J*=6.46, 15.82 Hz, H-8), 6.82 (m, 2H, *J*=4.75, 9.37 Hz, H-5 and 5), 6.98 (t, 2H, *J*=6.44, 11.83 Hz, H-2 and 2), 7.07 (t, 2H, *J*=9.2, 9.78Hz, H-6 and 6), 7.57 (dd, 1H, *J*=7.94, 15.78 Hz, H-7), 7.65 (d, 1H, *J*=15.74 Hz, H-7) (caffeoyl groups); ^{13}C-NMR (100 Hz, CD_3OD, ppm) δ 38.38 (C-6), 39.37 (C-2), 68.57 (C-3), 69.08 (C-5), 74.69 (C-4), 75.76 (C-1), 175.20 (C-7) (quinic acid moiety); δ 114.54 (C-2), 114.71 (C-2), 115.16 (C-8, 8), 116.48 (C-5, 5), 123.16 (C-6, 6), 127.54 (C-1), 127.70 (C-1), 146.80 (C-3, 3), 147.61 (C-4), 147.73 (C-4), 149.71 (C-7), 149.80 (C-7), 168.49, 168.56 (C-9, 9) (caffeoyl groups).

1,3,5-tri-*O*-caffeoylquinic acid (2). $[\alpha]_D^{25}$ -26.3° (c 0.6, MeOH); IR ν_{max} cm^{-1} 3470 (-OH), 2954 (C-H), 2936 (C-H), 2876 (C-H), 2848 (C-H), 2827 (C-H), 2757 (C-H), 1674 (C=C), 1618 (C=C), 1598 (C=C), 1526 (C-C), 1445 (C-C), 1273 (benzene); ESI-MS *m/z* 679 $[M+H]^+$; mp 179-183°C (lit. 178-181°C) [Agata *et al.* 1993]; ^{1}H-NMR (400 MHz, CD_3OD, ppm) δ 2.10 (dd, 1H, *J*=10.2, 13.3 Hz, H-6), 2.43 (dd, 1H, *J*=3.1, 16.5 Hz, H-2), 2.55 (dd, 1H, *J*=3.4, 13.7 Hz, H-6), 2.86 (d, 1H, *J*=16.2 Hz, H-2), 3.97 (dd, 1H, *J*=3.8, 8.4 Hz, H-4), 5.38 (d, 1H, *J*=3.41 Hz, H-3), 5.42 (m, 1H, H-5) (quinic acid moiety); δ 6.24, 6.29, 6.34 (d, 1H each, *J*=15.9 Hz, H-8), 6.55, 6.65, 6.75 (d, 1H each, *J*=8.2 Hz, H-5), 6.64, 6.81, 6.96 (dd, 1H each, *J*=2.1, 8.2 Hz, H-6). The signals at δ 6.64 and 6.96 are overlapped by other signals: 6.83, 6.95, 7.06 (d, 1H each, *J*=2.1 Hz, H-2), 7.51, 7.55, 7.62 (d, 1H each, *J*=16.0 Hz, H-7) (caffeoyl groups). ^{13}C-NMR (100 Hz, CD_3OD, ppm) δ 34.19 (C-2), 35.41 (C-6), 70.13 (C-5), 70.69 (C-4), 70.93 (C-3), 79.42 (C-1), 173.30 (C-7) (quinic acid moiety); δ 113.81, 113.70 (C-2, 2, 2'''), 114.17 (C-8, 8, 8'''), 115.06 (C-5, 5, 5'''), 121.61 (C-6, 6, 6'''), 126.37, 126.5 1 (C-1, 1, 1'''), 145.64, 145.40 (C-3, 3, 3'''), 145.90, 146.13 (C-4, 4, 4'''), 148.21 (C-7, 7, 7'''), 166.58, 167.19 (C-9, 9, 9''') (caffeoyl groups).

3,4,5-tri-*O*-caffeoylquinic acid (3). $[\alpha]_D^{25}$ -116.3° (c 0.09, MeOH); IR λ_{max} cm^{-1} 3378 (-OH), 2985 (C-H), 2934 (C-H), 2900 (C-H), 2876 (C-H), 2846 (C-H), 1684 (C=C), 1624 (C=C), 1600 (C=C), 1523 (C-C), 1453 (C-C), 1153 (benzene); ESI-MS *m/z* 679 $[M+H]^+$; mp 165-168°C; ^{1}H-NMR (400 MHz, CD_3OD, ppm) δ 2.18 (dd, 1H, *J*=10.5, 13.2 Hz, H-6), 2.27 (dd, 1H, *J*=3.2, 16.1 Hz H-2), 2.39 (dd, 1H, *J*=3.2, 13.3 Hz, H-6), 2.86 (d, 1H, *J*=16.2 Hz, H-2), 5.03 (dd, 1H, *J*=2.98, 9.7 Hz, H-4), 5.44 (ddd, 1H, *J*=2.97, 2.87, 2.62 Hz, H-5), 5.68 (ddd, 1H, *J*=2.65, 9.7, 11.8 Hz, H-3) (quinic acid moiety); δ 6.22, 6.29, 6.31 (d, 1H each, *J*=15.9 Hz, H-8), 6.51, 6.55, 6.61 (d, 1H each, *J*=8.2 Hz, H-5), 6.62, 6.74, 6.86 (dd, 1H each, *J*=2.1, 8.2 Hz, H-6). The signals at δ 6.62 and 6.86 are overlapped by other signals: 6.63, 6.85, 6.99 (d, 1H each, *J*=2.1 Hz, H-2), 7.47, 7.52, 7.60 (d, 1H each, *J*=16.0 Hz, H-7) (caffeoyl groups). ^{13}C-NMR(100 Hz, CD_3OD, ppm) δ 36.98 (C-6), 41.99 (C-2), 65.78 (C-5), 70.15 (C-3), 75.19 (C-4), 76.57 (C-1), 175.04 (C-7) (quinic acid moiety); δ 114.95, 115.26, 115.12 (C-2, 2, 2'''), 115.70 (C-8, 8, 8'''), 116.48 (C-5, 5, 5'''), 123.24, 123.11 (C-6, 6, 6'''), 123.11, 127.80 (C-1, 1, 1'''), 145.62 (C-3, 3, 3'''), 145.80, 146.13 (C-4, 4, 4'''), 149.63 (C-7, 7, 7'''), 168.33, 168.55 (C-9, 9, 9''') (caffeoyl groups).

Bovine serum albumin-methylglyoxal assay. Bovine serum albumin (10 mg/mL) was incubated with methylglyoxal (5 mM) in sodium phosphate buffer (0.1 M, pH 7.4). Dimethylsulfoxide used for dissolving samples was found to have no effect on the reaction. All of the reagent

and samples were sterilized by filtration through 0.2 μm membrane filters and the mixture was incubated at 37°C for 7 days. The fluorescence intensity was measured at an excitation wavelength of 330 nm and an emission wavelength of 410 nm with a Luminescence spectrometer LS50B (Perkin-Elmer Ltd., Buckinghamshire, England). Aminoguanidin hydrochloride (Sigma, St. Louis, MO) was tested as a known inhibitor. The concentration of each test sample giving 50% inhibition of the activities (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity [Lee *et al*., 2008b].

Assay for rat lens aldose reductase inhibitory activity. Crude aldose reductase was prepared as follows: Rat lenses were removed from Sprague-Dawley (SD) rats weighing 250-280 g and frozen until required. The rat lens homogenate was prepared according to the method of Hayman and Kinoshita [1965] with some modifications. A partially purified enzyme with a specific activity of 6.5 U/mg was routinely used to test the enzyme inhibition. The partially purified material was separated into 1.0 mL aliquots and stored at 40°C. RLAR activity was assayed spectrophotometrically by measuring the decrease in the absorption of NADPH at 340 nm over a 4-min period with DL-glyceraldehyde as the substrate. Each 1.0 mL cuvette contained equal units of the enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH, with or without 10 mM of the substrate and an inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity [Lee *et al*., 2008c].

Statistical analyses. The results are expressed as the mean±SD (n=3) of triplicate experiments.

Results and Discussion

The ethanol extract of *A. princeps* inhibited RLAR activity by 86.7% and the formation of AGEs in the BSA-MGO assay by 61.06%, whereas the inhibition by the quercetin and aminoguanidine controls was 70.17% and 95.06%, respectively (Table 1).

To identify the active compounds from the leaves of *A. princeps*, the leaf extract was systematically separated into four fractions, which were then tested for inhibitory activity against AR and protein glycation. The acetone fraction exhibited the strongest inhibitory activity against both RLAR and AGE formation in the BSA-MGO assay as shown in Table 1. Since this result suggested the likelihood of the presence of RLAR and/or AGE inhibitors in this fraction, we focused on isolating the active compounds from it. The acetone fraction was subjected to repeated chromatography on Si gel and reversed-phase Si gel to yield compounds **1-3**. The structures of these compounds were elucidated on the basis of infra-red (IR), electrospray ionization (ESI)-mass spectrometry, and 1-dimensional (^{1}H- and ^{13}C-NMR) and 2-dimensional NMR (heteronuclear multiple quantum coherence [HMQC] and heteronuclear multiple bond coherence [HMBC]) spectral data, and by comparison with published spectral data [Merfort 1992; Agata *et al*., 1993; Peluso *et al.,* 1995; Basnet *et al*., 1996; Pauli *et al*., 1998; Nakatani *et al*., 2000]. The three compounds were characterized as 3,4-di-*O*-caffeoylquinic acid (**1**), 1,3,5-tri-*O*-caffeoylquinic acid (**2**), and 3,4,5-tri-*O*-caffeoylquinic acid (**3**). Their chemical structures are shown in Fig. 1.

Table 1. Inhibitory activities of the extracts and solvent-soluble fractions obtained from the aerial parts of *A. princeps* on RLAR activity and on the formation of AGEs

Fractions	RLAR[a]	AGE[b]
	Inhibition (%)	Inhibition (%)
Ethanol ex.	86.7±0.53	61.06±1.44
n-Hexane fr.	25.17±0.59	26.22±1.32
Methyene chloride fr.	58.06±0.35	23.62±5.43
Acetone fr.	98.10±0.98	73.55±2.01
Methanol fr.	74.89±0.77	42.53±2.14
Quercetin[c]	70.17±0.63	-
Aminoguanidine[d]	-	95.06±0.01

[a]RLAR: The final concentration of the test samples and quercetin was 10 μg/mL in 100% dimethyl sulfoxide (DMSO). [b]AGE: The final concentration of the test samples and aminoguanidine was 200 μg/mL in 100% DMSO. [c]Quercetin and [d]aminoguanidine were used as positive controls for the RLAR and AGE assays, respectively.

In this study, we have demonstrated the inhibitory effect of caffeoylquinic acids from *A. princeps* on AR activity and AGE formation. Caffeoylquinic acids including 1,3-di-*O*-caffeoylquinic acid; 4,5-di-*O*-caffeoylquinic acid; and chlorogenic acid have been reported to be potent natural AR inhibitors [Fuente and Manzanaro, 2003; Logendra *et al*., 2006]. However, this is the first study to demonstrate that 3,4-di-*O*-dicaffeoylquinic acid (**1**), 1,3,5-tri-*O*-caffeoylquinic acid (**2**), and 3,4,5-tri-*O*-caffeoylqunic acid (**3**) possess similar inhibitory activity.

3,4-di-*O*-caffeoylquinic acid (**1**) is a well-known compound and has been previously isolated from many plant species [Yoshimoto *et al*., 2002; Satake *et al*., 2007; Shi *et al*., 2007; Timmermann *et al*., 1983], including *Flos Lonicerae* [Tong *et al*., 2008]. The reported biological activities of this compound include anti-

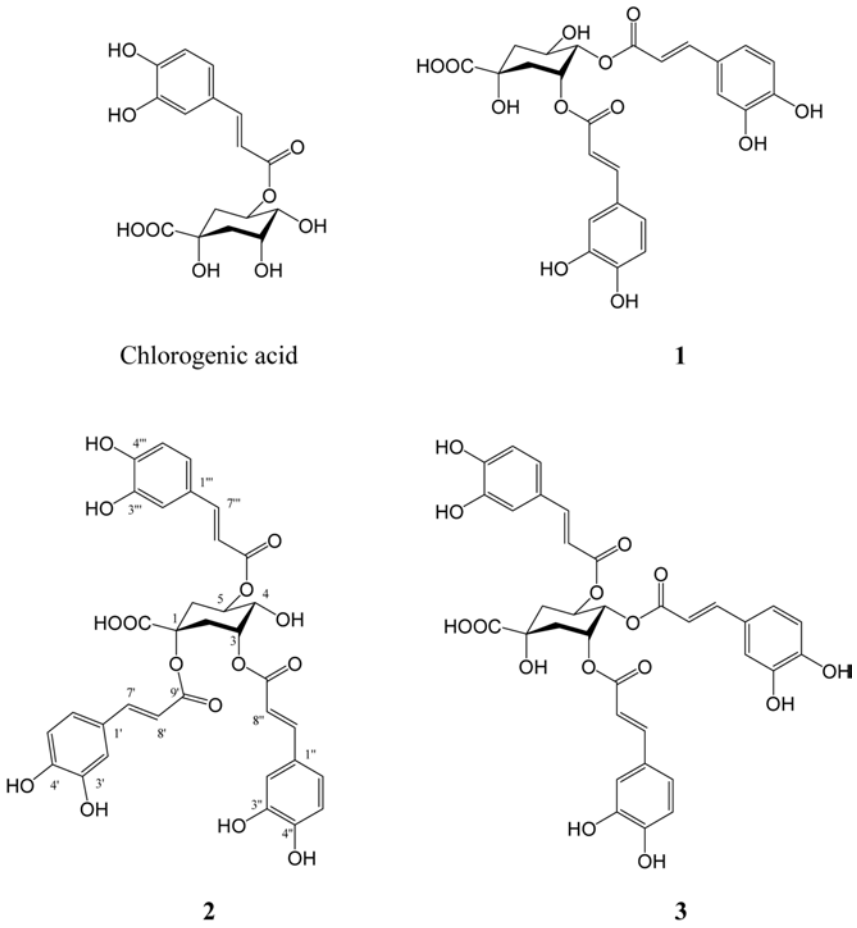

Fig. 1. Chemical structure of chlorogenic acid (3-*O*-caffeoylquinic acid) and caffeoylquinic acids isolated from the acetone-soluble fraction of the aerial parts of *A. princeps*. 3,4-di-*O*-caffeoylquinic acid, 1; 1,3,5-tri-*O*-caffeoylquinic acid, 2; 3,4,5-tri-*O*-caffeoylquinic acid, 3.

oxidant [Hung *et al*., 2006], xanthine oxidase inhibitory [Nguyen *et al*., 2006], anti-thrombotic [Satake *et al*., 2007], and antiviral activities [Li *et al*., 2005]. 1,3,5-tri-*O*-caffeoylquinic acid (**2**) has been previously isolated from *Xanthium strumarium* by Agata *et al.* [1993]. 3,4,5-tri-*O*-caffeoylqunic acid (**3**) has previously been isolated from sweetpotato (*Ipomoea batatas* L.) leaves, and this compound effectively inhibited the reverse mutation induced by Trp-P-1 in *Salmonella typhimurium* TA 98 [Yoshimoto *et al*., 2002]. However, this is the first study to test the efficacy of these three compounds on the inhibition of RLAR activity.

As shown in Table 2, all three caffeoylquinic acids exhibited potent inhibitory activity against RLAR with IC_{50} values of 2.40±0.58 μM (**1**), 1.78±0.94 μM (**2**), and 1.95±0.21 μM (**3**). They were 5- to 10-fold more potent than quercetin, which had an IC_{50} value of 17.91±0.69 μM. Therefore, we suggest that the number of caffeoyl groups and their position in the quinic acid moiety might not play a key role in their inhibitory activity against RLAR.

The inhibitory effects of compounds **1-3** on MGO-mediated protein glycation were investigated by observing the specific fluorescence generated during the course of AGE formation. In a BSA-MGO system, MGO readily reacts with lysine and arginine residues in proteins to produce high molecular weight, cross-linked, fluorescent products. As shown in Table 3, 1,3,5-tri-*O*-caffeoylquinic acid (**2**) exhibited the most potent inhibitory activity, with an IC_{50} value of 22.18±1.46 μM, as compared to aminoguanidine and chlorogenic acid controls, which had IC_{50} values of 1,093.11±10.95 and 117.63±0.20 μM, respectively. Interestingly, the inhibitory effect of 3, 4-di-*O*-caffeoylquinic acid (**1**) on AGE formation was similar to that of chlorogenic acid (3-*O*-caffeorylquinic acid), but was approximately two-fold higher than that of 3,4,5-tri-*O*-caffeoylqunic acid (**3**). This suggests that the number of caffeoyl groups and their position in the quinic acid moiety might be important for the inhibition of AGE formation.

These results demonstrate the potential therapeutic use of 3,4-di-*O*-caffeoylquinic acid (**1**), 1,3,5-tri-*O*-caffeoylquinic

Table 2. Inhibitory activities of the compounds isolated from *A. princeps* on RLAR activity

Samples	IC_{50}[a]	
	μg/mL	μM
Ethanol ext.	3.45±0.55	-
Acetone fr.	2.07±0.44	-
3,4-di-*O*-caffeoylquinic acid (**1**)	1.24±0.30	2.40±0.58
1,3,5-tri-*O*-caffeoylquinic acid (**2**)	1.21±0.64	1.78±0.94
3,4,5-tri-*O*-caffeoylqunic acid (**3**)	1.32±0.14	1.95±0.21
Quercetin[b]	5.41±3.23	17.91±0.69 (5.6)[d]
Chlorogenic acid[c]	0.92±0.18	2.60±0.51 (1.8)[e]

[a]The IC_{50} is expressed as the mean±SEM of triplicate experiments.
[b]Quercetin and [c]chlorogenic acid were used as positive controls for the RLAR assay.
[d]Fuente and Manzanaro, 2003. [e]Yoshikawa *et al.*, 1999

Table 3. Inhibitory activities of the compounds isolated from *A. princeps* on the formation of AGEs

Samples	IC_{50}[a]	
	μg/mL	μM
Ethanol ext.	108.36±0.48	-
Acetone fr.	192.87±1.44	-
3,4-di-*O*-caffeoylquinic acid (**1**)	51.10±1.14	99.03±2.21
1,3,5- tri-*O*-caffeoylquinic acid (**2**)	15.04±0.99	22.18±1.46
3,4,5- tri-*O*-caffeoylqunic acid (**3**)	149.19±0.57	220.04±0.84
Aminoguanidine[b]	80.89±0.81	1,093.11±10.95
Chlorogenic acid[c]	41.64±0.07	117.63±0.20

[a]The IC_{50} is expressed as the mean±SEM of triplicate experiments.
[b]Aminoguanidin and [c]chlorogenic acid were used as positive controls for the formation of AGEs assay.

acid (**2**), and 3,4,5-tri-*O*-caffeoylqunic acid (**3**) in the management of diabetes-related complications.

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