*Food Sci. Biotechnol.* 23(6): 1747-1752 (2014)
DOI 10.1007/s10068-014-0239-5

RESEARCH ARTICLE

# Inhibitory Efficacy of *Ligularia fischeri* against Aldose Reductase and Advanced Glycation End Products Formation

**Xing Fu Yin, Young Eun Jeon, Jae-Hoon Shim, and Il-Jun Kang**

Received January 21, 2014; revised May 8, 2014; accepted May 12, 2014; published online December 31, 2014


**Abstract** The efficacy of *Ligularia fischeri* (LF) for managing diabetic complications was evaluated by assessing inhibitory effects against advanced glycation end product (AGE) formation, rat lens aldose reductase (RLAR), and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging. The ethyl acetate fraction from 70% ethanol extracts of LF showed the highest DPPH radical scavenging activity of 53.90% at a concentration of 3.3 μg/mL. The ethyl acetate fraction exhibited the most potent AGE formation inhibition of 73.57% at a concentration of 55 μg/mL and showed the most potent RLAR inhibition of 88.97% at a concentration of 11.11 μg/mL. The ethyl acetate fraction exhibited the most potent antioxidant and anti-diabetic effects. Nuclear magnetic resonance via bioactivity-guided fractionation of the LF ethyl acetate fraction revealed that 3,4-dicaffeoylquinic acid was the bioactive compound. This compound from LF can be effective for prevention or treatment of diabetic complications.

**Keywords:** advanced glycation end products, diabetic complications, 3, 4-dicaffeoylquinic acid, *Ligularia fischeri*, rat lens aldose reductase

## Introduction

Diabetes mellitus is a disease characterized by hyperglycemia resulting from a deficiency of insulin, insulin secretion, insulin resistance, or a combination (1). Diabetes mellitus increases the risk of long term complications. Furthermore, diabetic complications appear to affect both micro and macrovessels, leading to blindness, neuropathy, nephropathy, and retinopathy (2). Chronic hyperglycemia plays an important role in the pathogenesis of diabetic complications (3), which involves activation of 4 major pathways, including increased flux through the polyol pathway (4), increases of advanced glycation end product (AGE) formation (5), activation of protein kinase C isoforms (6), and activation of the hexosamine pathway (7). Based on these pivotal mechanisms, particularly AGE formation, the polyol pathway, and oxidative stress, investigations of prospective agents to prevent and treat diabetic complications have been undertaken.

Aldose reductase (AR) plays an important role in the polyol pathway. Under normal glucose levels, this enzyme catalyzes reduction of toxic cellular aldehydes into inactive alcohols (8). However, glucose is converted into sorbitol by AR in the polyol pathway in the presence of increased glucose levels (9). Furthermore, AR activity has only been monitored based on consumption of NADPH, which is also required for regeneration of a reduced glutathione level, a critical intracellular antioxidant. Decreased concentrations of reduced glutathione lead to intracellular oxidative stress (10). Controlling high blood glucose levels is important for prevention of diabetic complications. AR inhibitors should be able to prevent and treat diabetic complications (11).

The classic pathway leading to AGE formation is a major pathway of diabetic complications. Non-enzymatic cross-links form freely reversible Schiff's bases between reducing sugars and the amino groups of long-lived proteins. Formation of irreversible AGEs occurs over a period of days and causes qualitative and quantitative changes in extracellular matrix components (12). These irreversible AGEs alter cell functions and induce diabetic retinopathy, nephropathy (13), and neuropathy. Once AGEs are formed, diabetic treatment is not dependent on control of blood glucose levels. AGE inhibitors are useful

Xing Fu Yin, Young Eun Jeon, Jae-Hoon Shim, Il-Jun Kang (✉)
Department of Food Science and Nutrition, Hallym University, Chuncheon, Gangwon 200-702, Korea
Tel: +82-33-248-2135; Fax: +82-33-255-4787
E-mail: ijkang@hallym.ac.kr

for prevention and/or reduction of certain diabetic complications (14). Excessive activation of the polyol pathway increases concentrations of sorbitol and reactive oxygen species, and enhances formation of AGE. These imbalances cause cell damage (15).

Gomchi (*Ligularia fischeri*, LF) is a wild vegetable mainly found in damp, shady areas near brooks and sloping fields in the eastern part of Korea. This plant is currently cultivated under glass and is traditionally used to treat jaundice, scarlet-fever, rheumatoid arthritis, and hepatic disease (16). Several studies have isolated novel terpenoids, spirosesquiterpenes, and fischelactone from LF (17,18). Hyperoside and 2'-acetylhyperoside have potent antioxidant effects against the DPPH radical and have also been isolated from LF (19). It was also reported that LF has anti-inflammatory and anti-ulcerogenic effects (20,21). Although there are numerous reports regarding LF extracts, there has not been any research about LF as an anti-diabetic drug. Therefore, to evaluate the potential efficacy of LF as an anti-diabetic drug, AGE formation and inhibition of rat lens aldose reductase (RLAR) were used as representative mechanisms to assess the effect of LF on anti-diabetic complications.

## Materials and Methods

**Plant materials** Dried LF was purchased from a local market in Yang-gu, Gangwon Do, Korea in April of 2012.

**Extraction, Fractionation, and Isolation**

***Extraction*:** Dried plant material (5 kg) was mixed with 70% ethanol for 3 h followed by filtration using filter paper (cellulose chromatography papers; Whatman, Little Chalfont, UK). After filtration, the 70% ethanol extract was concentrated under reduced pressure using a rotary vacuum evaporator (Rotavapor R-220; Büchi, Flawil, Switzerland) and lyophilized (Bondiro; Il Shin Lab Co., Seoul, Korea) to obtain 559.33 g of powder.

***Fractionation*:** The 70% ethanol extract was partitioned sequentially using *n*-hexane, methylene chloride, ethyl acetate, and *n*-butanol, leaving a residual water-soluble fraction. Each fraction was evaporated using a rotary vacuum evaporator (Rotavapor R-220; Büchi) *in vacuo* to yield fractionation residues (Table 1).

***Bioassay-guided isolation*:** The ethyl acetate fraction (25 g) was subject to chromatography on silica gel (ø5×60 cm) using a $CHCl_3$-$CH_3OH$ gradient from $CHCl_3$:MeOH (15:1, v:v) to $CHCl_3$:MeOH:$H_2O$ (5:5:1, v:v:v) to divide the ethyl acetate fraction into 12 subfractions (E01-E12). These subfractions were then tested for an inhibitory activity against AGE formation. The active fraction (E06, 11.23 g) was subjected to chromatography on silica gel (ø2.75×60 cm) using a $CHCl_3$-MeOH gradient from $CHCl_3$:MeOH (20:1, v:v) to $CHCl_3$:MeOH:$H_2O$ (5:5:1, v:v:v) to divide the active fraction into 6 additional fractions (E06F01-E06F06). These sub-fractions were then tested for an inhibitory activity against AGE formation. The active fraction (E06F03, 7.53 g) was subjected to chromatography on silica gel (ø2.75×60 cm) using a $CHCl_3$-MeOH gradient from $CHCl_3$:MeOH (10:1, v:v) to $CHCl_3$:MeOH:$H_2O$ (5:5:1, v:v:v) to divide the active fraction into 2 fractions (E06F03F01 and E06F03F02). These sub-fractions were tested for an inhibitory activity against AGE formation. The active fraction (E06F03F01, 4.46 g) was subjected to chromatography on silica gel (ø0.75×30 cm) using a $CHCl_3$-MeOH gradient from $CHCl_3$:MeOH (20:1, v:v) to $CHCl_3$:MeOH:$H_2O$ (5:5:1, v:v:v) to divide the active fraction into 3 fractions (E06F03F01F01-E06F03F01F03). These sub-fractions were tested for an inhibitory activity against AGE formation. The active fraction (E06F03F01F02, 101.09 mg) was then identified.

**Table 1. Extract yields and fractions from *Ligularia fischeri***

| Sample | Weight (g) | Yield (% dry basis) |
|---|---|---|
| *L. fischeri* | 5,000 | - |
| 70% ethanol extract | 559.33 | 11.19 |
| *n*-Hexane fraction | 157.21 | 28.11 |
| Methylene chloride fraction | 115.65 | 20.68 |
| Ethyl acetate fraction | 39.84 | 7.12 |
| *n*-Butanol fraction | 70.57 | 12.62 |
| Water fraction | 146.81 | 26.25 |

**Measurement of the DPPH radical scavenging activity** The DPPH radical scavenging activity was evaluated following the methods of Chon *et al*. (22) with slight modification. A sample (1 mg/mL) was dissolved in ethanol. Each sample (30 μL) was mixed with DPPH ($6.34\times10^{-5}$ M, 270 μL) in a 96-well microplate. The mixture was stored in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV-vis spectrometer against a blank. A lower absorbance value of the reaction mixture indicated a higher free radical scavenging activity. Samples were dissolved in dimethylsulfoxide (DMSO) at identical concentrations (3.3 μg/mL) for a DPPH inhibition assay, and inhibitory effects were measured. Ascorbic acid was used as a positive control. The half maximal inhibition concentration ($IC_{50}$) was determined using a semi-log plot of percentage of activity vs. test sample concentration.

**Bovine serum albumin-methylglyoxal assay** Inhibition of AGE formation was examined according to the method of Jung *et al*. (23) with modification. Bovine serum albumin (BSA; fatty acid free, 5 mg/mL) was incubated with methylglyoxal (2 mM) in a sodium phosphate buffer

(50 mM, pH 7.4). DMSO, used to dissolve the sample, had no effect on the reaction. All reagents and samples were sterilized by filtration through a 0.2 μm membrane filter, and the mixture was incubated at 37°C for 24 h. The fluorescence intensity was measured using a luminescence spectrometer (LS 50B; Perkin Elmer Ltd., Beaconsfield, UK) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using aminoguanidine hydrochloride (AG; Sigma-Aldrich, St. Louis, MO, USA) as a known inhibitor. The $IC_{50}$ value was estimated from a least-squares regression line of the logarithmic concentration plotted against the remaining activity.

**Assay for inhibition of rat lens AR** Inhibition of AR activity was assayed using a modified method (24). Crude AR was prepared. Rat lenses were removed from Sprague-Dawley rats (weight, 250-280 g) and frozen until use. All operations were performed at 4°C. Rat lens homogenates were prepared according to a method described previously (25). A partially purified enzyme with a specific activity of 6.5 U/mg was routinely used for enzyme inhibition. RLAR activity was assayed spectrophotometically by measurement of a decrease in the absorbance 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, a 50 mM sodium phosphate buffer (pH 6.2), and 1.6 mM NADPH with and without 100 mM of the substrate and an inhibitor. Quercetin, a well-known AR inhibitor, was used as a positive control. The $IC_{50}$ value was calculated from a least-squared regression line of logarithmic concentrations plotted against the residual activity.

**Nuclear magnetic resonance and mass spectroscopic analyses** $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) spectra were obtained using a Bruker DPX-400 spectrometer (Bruker, Ettlingen, Germany) with tetramethylsilane as an internal standard at 400 and 100 MHz.

**Statistical analysis** Results were expressed as mean± standard deviation (SD) of triplicate experiments. An analysis of variance (ANOVA) and Duncan's multiple range test were used to detect differences between means. A $p<0.05$ value was considered significant. A least-squared regression was also used. The SPSS software package was used for all statistical testing (SPSS, version11; Chicago, IL, USA).

## Results and Discussion

**Yield of the LF extract fraction** The yield of the 70% ethanol extract of LF was 11.19% (w/w). The fraction was obtained using *n*-hexane, methylene chloride, ethyl acetate, *n*-butanol, and water, then dried using an evaporator. Percentage values of extracts for each fraction were 28.11, 20.68, 7.12, 12.62, and 26.25%, respectively (Table 1).

**DPPH radical scavenging activity** The DPPH radical is commonly used to evaluate the antioxidant ability due to relative stability. Free radical plays an important role in both microvascular and macrovascular complications in type 1 and 2 diabetes mellitus (15). DPPH, which contains stable free radicals, loses its violet color when the DPPH is neutralized by other radicals. Results for the DPPH radical scavenging activity of the 70% ethanol extract and several solvent fractions are shown in Table 2. The LF ethyl acetate fraction showed the highest DPPH radical scavenging activity (53.90%) at a concentration of 3.3 μg/mL, followed in a decreasing order by the *n*-butanol (42.20%), methylene chloride (40.39%), water (24.30%), and *n*-hexane (23.88%) fractions. The ethyl acetate fraction exhibited a DPPH radical scavenging activity with an $IC_{50}$ value of 3.08 μg/mL, compared to ascorbic acid (1.73 μg/mL) (Table 2). These results were in agreement with previous reports. Choi *et al*. (26) reported that extracts from LF leaves showed a strong free radical scavenging effect against DPPH, superoxide, and peroxy radicals, and significantly inhibited the lipid peroxidation. Shang *et al*. (27) isolated 4 major caffeoylquinic acid compounds from LF using an online HPLC-ABTS system and reported significant antioxidant activities.

**Table 2. DPPH radical scavenging effects of different fractions from *L. fischeri***

| Sample | Concentration (μg/mL) | Inhibition (%) | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Ascorbic acid[1)] | 3.3 | 77.57±1.37[a] | 1.73±0.21 |
| 70% ethanol extract | 3.3 | 29.83±0.58[d] | - |
| *n*-Hexane fraction | 3.3 | 23.88±1.57[e] | - |
| Methylene chloride fraction | 3.3 | 40.39±1.23[c] | - |
| Ethyl acetate fraction | 3.3 | 53.90±2.14[b] | 3.08±0.33 |
| *n*-Butanol fraction | 3.3 | 42.20±0.63[c] | - |
| Water fraction | 3.3 | 24.30±1.64[e] | - |

[1)]A reference compound

**Table 3. Inhibition of formation of advanced glycation end products by different fractions of *L. fischeri***

| Sample | Concentration (μg/mL) | Inhibition (%) | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Aminoguanidine[1)] | 55 | 25.36±0.89[c] | 90.79±1.34 |
| 70% ethanol extract | 55 | 31.34±2.82[c] | - |
| *n*-Hexane fraction | 55 | 5.48±3.40[d] | - |
| Methylene chloride fraction | 55 | 6.17±1.86[d] | - |
| Ethyl acetate fraction | 55 | 73.57±4.21[a] | 39.89±1.97 |
| *n*-Butanol fraction | 55 | 70.97±2.12[a] | - |
| Water fraction | 55 | 46.49±4.80[b] | - |

[1)]A reference compound

**Table 4. Inhibition of aldose reductase activity by different fractions of *L. fischeri***

| Sample | Concentration (μg/mL) | Inhibition (%) | $IC_{50}$ (μg/mL) |
|---|---|---|---|
| Quercetin[1)] | 11.11 | 72.18±1.44[b] | 2.14±0.05 |
| 70% ethanol extract | 11.11 | 69.89±1.44[b] | - |
| *n*-Hexane fraction | 11.11 | 11.03±0.69[d] | - |
| Methylene chloride fraction | 11.11 | 13.56±2.79[d] | - |
| Ethyl acetate fraction | 11.11 | 88.97±1.82[a] | 0.32±0.05 |
| *n*-Butanol fraction | 11.11 | 86.89±3.01[a] | - |
| Water fraction | 11.11 | 49.20±2.87[c] | - |

[1)]A reference compound

**Inhibition of AGE formation** A significant rise in the intracellular sugar level occurs under high levels of free radicals due to oxidative stress and chronic hyperglycemia (28). Reducing sugars, such as glucose and fructose, react non-enzymatically with amino groups in proteins, followed by formation of Schiff's bases and Amadori products to form reversible AGEs through cross-linking (29). Therefore, the function of the antioxidant defense system is compromised by increased tissue and plasma concentrations of AGEs (30). In the pathogenesis of diabetic complication-related AGE formation, the BSA-glucose system can be monitored by detection of fluorescence at excitation and emission wavelengths of 350 and 450 nm, respectively. Several solvent fractions from the 70% ethanol extract of LF were evaluated based on inhibitory activities against AGE formation. The ethyl acetate fraction of LF showed the highest inhibition of 73.57% at a concentration of 55 μg/mL, followed by the *n*-butanol fraction (70.97%) (Table 3). The $IC_{50}$ value of the ethyl acetate fraction was 39.89 μg/mL and the value of aminoguanidine was 90.79 μg/mL (Table 3).

**Inhibition of RLAR** Activation of the polyol pathway by AR leads to oxidative and other stresses, such as protein kinase activation and glycation reactions, which lead to serious diabetic complications (31). The 70% ethanol extract and different LF solvent fractions were tested for inhibition of RLAR. Crude AR was prepared using rat lenses, and NADPH and DL-glyceraldehyde were used as a coenzyme and a substrate, respectively. Inhibition of RLAR was measured as a decrease in NADPH absorption at 340 nm over 4 min. The highest inhibition value of 88.97% was observed in the ethyl acetate fraction, followed by the *n*-butanol (86.89%), and the water (49.20%) fractions (Table 4). The $IC_{50}$ value of the ethyl acetate fraction was 0.32 μg/mL and the value of quercetin was 2.14 μg/mL (Table 4).

**Structural analysis of the compound isolated from the ethyl acetate fraction** The ethyl acetate fraction exhibited the most potent antioxidant and anti-diabetic effects. In order to identify the active compound in the ethyl acetate fraction, the crude fraction was divided into 12 fractions that were tested individually for inhibitory activities against AGE formation. Fraction 6 showed a significantly ($p<0.05$) higher inhibition of 98.68% at 60 μg/mL than the other fractions. This active fraction was named E06. Fractionation of the E06 fraction, yielded 6 sub-fractions. An inhibition of AGE formation assay allowed identification of the most active sub-fraction, named E06F03, with an inhibition value 48.78% at 20 μg/mL. Further purification of the active E06F03sub-fraction yielded 2 sub-fractions. The most active sub-fraction, identified using an inhibition of AGE formation assay, was named E06F03F01 and showed an inhibition value of 63.04% at 20 μg/mL. Sub-fraction E06F03F01 was purified to yield 3 fractions. An inhibition of AGE formation assay allowed identification of the most active sub-fraction, named E06F03F01F02 with an inhibition value of 90.04% at 20 μg/mL. The active compound in E06F03F01F02 was identified based on NMR.

**Table 5. Inhibition of formation of advanced glycation end products by purified compounds**

| Sample | Concentration (μM) | Inhibition (%) | $IC_{50}$ (μM) |
|---|---|---|---|
| Aminoguanidine[1)] | 498 | 25.36±0.89 | 822.02±10.38 |
| | 996 | 65.15±0.52 | |
| | 1992 | 83.73±0.25 | |
| 3,4-Dicaffeoylquinic acid | 9.68 | 33.29±2.15 | 18.39±1.11 |
| | 19.36 | 51.23±3.26 | |
| | 38.72 | 90.04±1.17 | |

[1)]A reference compound

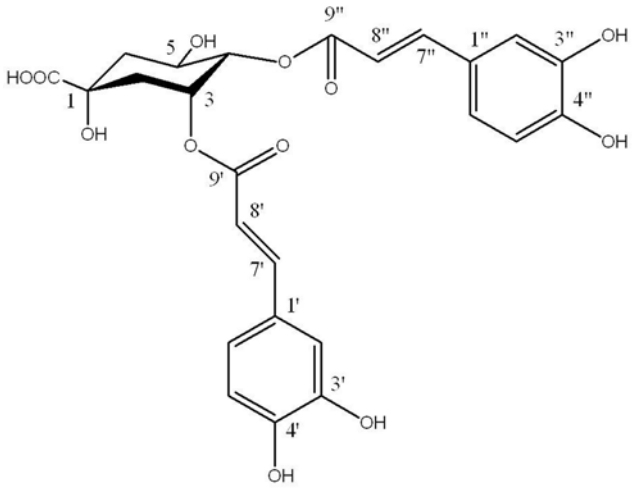


**Fig. 1. Chemical structure of 3, 4-dicaffeoylquinic acid isolated from the ethyl acetate-soluble fraction of *L. fischeri***

The active compound in the ethyl acetate fraction of LF was purified using repeated chromatography via bioassay-guided fractionation. The purified compound had an $IC_{50}$ value of 18.39 μM (Table 5), which was comparable to the AG-positive control with an $IC_{50}$ value of 822.02 μM. Chemical shifts of the purified compound from $^{1}H$ and $^{13}C$ NMR analysis were $^{1}H$-NMR (400 MHz, MeOD): δ 7.58 (2H, t, J=15.4 Hz, H-7, 7), δ 7.03 (2H, d, J=1.9 Hz, H-2, 2), δ 6.93 (2H, dd, J=1.9, 8.2 Hz, H-6, 6), δ 6.77 (2H, d, J=8.2 Hz, H-5, 5), δ 6.29 (2H, t, J=16.3 Hz, H-8, 8), δ 5.63 (1H, m, H-3), δ 4.99 (1H, m, H-4), δ 4.37 (1H, m, H-5), δ 2.11, 2.36 (4H, m, H-2,6). $^{13}C$-NMR (400 MHz, MeOD): δ 176.54 (COOH), δ 167.30 (C-9, 9), δ 149.65 (C-4, 4), δ 147.63 (C-7, 7), δ 146.96 (C-3, 3), δ 127.89 (C-1, 1), δ 123.11 (C-6, 6), δ 116.60 (C-5, 5), δ 115.20 (C-2, 2), δ 114.73 (C-8, 8), δ 75.79 (C-4), δ 73.61 (C-1), δ 71.36 (C-3), δ 69.15 (C-5), δ 39.9 (C-2), δ 35.99 (C-6). The $^{1}H$-NMR spectrum of the compound showed the presence of 2 trisubstituted aromatic ring protons [δ 7.03 (2H, d, J=1.9 Hz, H-2, 2), δ 6.93 (2H, dd, J=1.9, 8.2 Hz, H-6, 6), δ 6.77 (2H, d, J=8.2 Hz, H-5, 5)] and 4 protons in *trans*-configurations [δ 7.58 (2H, t, J=15.4 Hz, H-7, 7), δ 6.29 (2H, t, J=16.3 Hz, H-8, 8)] in a caffeoyl group, 4 methylene protons [δ 2.11, 2.36 (4H, m, H-2,6)], and 3 oxygenated methine protons [δ 5.63 (1H, m, H-3), δ 4.99 (1H, m, H-4), δ 4.37 (1H, m, H-5)] in quinic acid. The $^{13}C$-NMR spectrum of the compound contained a carbon resonance for the carboxylic acid [δ 176.54 (COOH)] in quinic acid and a carbon signal for the single ester carbonyl [δ 167.30 (C-9, 9)] in a caffeoyl group. Therefore, the compound was identified as 3, 4-dicaffeoylquinic acid on the basis of NMR spectral data and comparison with previous reports (32-34). The chemical structure of the compound is shown in Fig. 1.

In conclusion, the *in vitro* anti-diabetic and antioxidant effects of different solvent fractions from LF were evaluated. The ethyl acetate fraction demonstrated the strongest inhibition against AR and AGE formation, and showed a strong radical scavenging activity. The most effective compound in the ethyl acetate fraction was analyzed using NMR and identified as 3, 4-dicaffeoylquinic acid. This compound from LF can be of use for development of therapeutic drugs, and prevention or treatment of diabetic complications.

**Acknowledgments** This study was supported by the Hallym University Research Fund (HRF-201311-008).

**Disclosure** The authors declare no conflict of interest.

## References

1. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet. Med. 15: 539-553 (1998)
2. Williamson JR, Chang K, Frangos M, Hasan KS, Ido Y, Kawamura T, Nyengaard JR, van den Enden M, Kilo C, Tilton RG. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes 42: 801-813 (1993)
3. de Groot M, Anderson R, Freedland KE, Clouse RE, Lustman PJ. Association of depression and diabetes complications: A meta-analysis. Psychosom. Med. 63: 619-630 (2001)
4. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. J. Am. Soc. Nephrol. 14: S233-S236 (2003)
5. Reddy VP, Beyaz A. Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. Drug Discov. Today 11: 646-654 (2006)

6. Park JY, Ha SW, King GL. The role of protein kinase C activation in the pathogenesis of diabetic vascular complications. Perit. Dial. Int. 19: S222-S227 (1999)
7. Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: Current status. Am. J. Physiol. Endocrinol. Metab. 290: E1-E8 (2006)
8. Yabe-Nishimura C. Aldose reductase in glucose toxicity: A potential target for the prevention of diabetic complications. Pharmacol. Rev. 50: 21-33 (1998)
9. Dunlop M. Aldose reductase and the role of the polyol pathway in diabetic nephropathy. Kidney Int. Suppl. 58: S3-S12 (2000)
10. Brownlee M. The pathobiology of diabetic complications: A unifying mechanism. Diabetes 54: 1615-1625 (2005)
11. Costantino L, Rastelli G, Gamberini MC, Vinson JA, Bose P, Iannone A, Staffieri M, Antolini L, Del Corso A, Mura U, Albasini A. 1-Benzopyran-4-one antioxidants as aldose reductase inhibitors. J. Med. Chem. 42: 1881-1893 (1999)
12. Ahmed N. Advanced glycation endproducts-role in pathology of diabetic complications. Diabetes Res. Clin. Pract. 67: 3-21 (2005)
13. Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Care 15: 1835-1843 (1992)
14. Wirasathien L, Pengsuparp T, Suttisri R, Ueda H, Moriyasu M, Kawanishi K. Inhibitors of aldose reductase and advanced glycation end products formation from the leaves of *Stelechocarpus cauliflorus* R.E. Fr. Phytomedicine 14: 546-550 (2007)
15. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: A review. J. Biochem. Mol. Toxicol. 17: 24-38 (2003)
16. Choi J, Park JK, Lee KT, Park KK, Kim WB, Lee JH, Jung HJ, Park HJ. *In vivo* antihepatotoxic effects of *Ligularia fischeri* var. *spiciformis* and the identification of the active component, 3,4-dicaffeoylquinic acid. J. Med. Food 8: 348-352 (2005)
17. Lee KT, Koo SJ, Jung SH, Choi J, Jung HJ, Park HJ. Structure of three new terpenoids, spiciformisins a and b, and monocyclosqualene, isolated from the herbs of *Ligularia fischeri* var. *spiciformis* and cytotoxicity. Arch. Pharm. Res. 25: 820-823 (2002)
18. Zhang WJ, Li XH, Shi YP. A pair of epimeric spirosesquiterpenes from the roots of *Ligularia fischeri*. J. Nat. Prod. 73: 143-146 (2010)
19. Piao XL, Mi XY, Tian YZ, Wu Q, Piao HS, Zeng Z, Wang D, Piao X. Rapid identification and characterization of antioxidants from *Ligularia fischeri*. Arch. Pharm. Res. 32: 1689-1694 (2009)
20. Choi EM, Kim YH. A preliminary study of the effects of an extract of *Ligularia fischeri* leaves on type II collagen-induced arthritis in DBA/1J mice. Food Chem. Toxicol. 46: 375-379 (2008)
21. Lee BI, Nugroho A, Bachri MS, Choi J, Lee KR, Choi JS, Kim WB, Lee KT, Lee JD, Park HJ. Anti-ulcerogenic effect and HPLC analysis of the caffeoylquinic acid-rich extract from *Ligularia stenocephala*. Biol. Pharm. Bull. 33: 493-497 (2010)
22. Chon SU, Kim YM, Kim DK, Heo BG, Cho JY. Phytotoxic effect, DPPH radical scavenging activity and chlorogenic acid level of methanol extracts from aerial parts of several Korean salad plants. Koren J. Plant Res. 19: 405-410 (2006)
23. Jung HA, Jung YJ, Yoon NY, Jeong DM, Bae HJ, Kim DW, Na DH, Choi JS. Inhibitory effects of *Nelumbo nucifera* leaves on rat lens aldose reductase, advanced glycation endproducts formation, and oxidative stress. Food Chem. Toxicol. 46: 3818-3826 (2008)
24. Kim HS, Park UY, Seong YH. Inhibitory effects of ginseng components on rat lens aldose reductase activities. Korean J. Ginseng Sci. 20: 106-110 (1996)
25. Hayman S, Kinoshita JH. Isolation and properties of lens aldose reductase. J. Biol. Chem. 240: 877-882 (1965)
26. Choi EM, Ding Y, Nguyen HT, Park SH, Kim YH. Antioxidant activity of Gomchi (*Ligularia fischeri*) Leaves. Food Sci. Biotechnol. 16: 710-714 (2007)
27. Shang YF, Kim SM, Song DG, Pan CH, Lee WJ, Um BH. Isolation and identification of antioxidant compounds from *Ligularia fischeri*. J. Food Sci. 75: C530-C535 (2010)
28. Peyroux J, Sternberg M. Advanced glycation endproducts (AGEs): Pharmacological inhibition in diabetes. Pathol. Biol. (Paris) 54: 405-419 (2006)
29. Singh R, Barden A, Mori T, Beilin L. Advanced glycation end products: A review. Diabetologia 44: 129-146 (2001)
30. Lee HS, Hong CO, Lee KW. Advanced glycation endproduct-induced diabetic complications. Food Sci. Biotechnol. 17: 1131-1138 (2008)
31. Logendra S, Ribnicky DM, Yang H, Poulev A, Ma J, Kennelly EJ, Raskin I. Bioassay-guided isolation of aldose reductase inhibitors from *Artemisia dracunculus*. Phytochemistry 67: 1539-1546 (2006)
32. Arbiser JL, Li XC, Hossain CF, Nagle DG, Smith DM, Miller P, Govindarajan B, DiCarlo J, Landis-Piwowar KR, Dou QP. Naturally occurring proteasome inhibitors from mate tea (Ilex paraguayensis) serve as models for topical proteasome inhibitors. J. Invest. Dermatol. 125: 207-212 (2005)
33. Wu C, Chen F, Wang X, Wu Y, Dong M, He G, Galyean RD, He L, Huang G. Identification of antioxidant phenolic compounds in feverfew (*Tanacetum parthenium*) by HPLC-ESI-MS/MS and NMR. Phytochem. Anal. 18: 401-410 (2007)
34. Wan C, Yu Y, Zhou S, Tian S, Cao S. Isolation and identification of phenolic compounds from Gynura divaricata leaves. Pharmacogn. Mag. 7: 101-108 (2011)