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Protein Glycation Inhibitory and Antioxidative Activities of Some Plant Extracts in Vitro

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The protein glycation inhibitory activity of aqueous ethanolic extracts from 25 plant tissues was evaluated in vitro using the model system of bovine serum albumin and fructose. The most bioactive plant tissue was *Allium cepa* (skin), followed by *Illicium religiosum* (bark and wood), *Fagopyrum esculentum* (hull), *Origanum officinalis* (leaf), *Rosmarinus officinalis* (leaf), *Pyrus pyrifolia* (bark), *Acanthopanax senticosus* (bark), *Eugenia caryophllata* (leaf), and *Erigeron annuus* (whole). The extracts with glycation inhibitory activity also showed antioxidative activity when a micellar linoleic acid peroxidation system was applied followed by 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization and 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assays. The glycation inhibitory activity was significantly correlated with the antioxidative potency of the extracts. The positive glycation inhibitory and antioxidative activities of these plants might suggest a possible role in targeting aging and diabetic complications.

KEYWORDS: Plants; protein glycation inhibition; antioxidation

INTRODUCTION

The accumulation of the reaction products of protein glycation (nonenzymatic reaction of proteins with glucose and other reducing sugars) in living organisms leads to structural and functional modifications of tissue proteins. Many studies have shown a significant role for glycation in the progress of normal aging and the pathogenesis of age-related diseases, such as diabetes, atherosclerosis, end-stage renal disease, rheumatoid arthritis, and neurodegenerative diseases. Therefore, targeting glycation should have a broad and beneficial effect on aging and age-related diseases. Aminoguanidine, a small hydrazine-like compound, has been synthesized and become one of the most promising pharmacological interventions for glycation inhibition (*1*−*3*).

Besides synthesized compounds, plants might offer a new source of glycation inhibitory agents. The extracts of *Garcinia indica* (*4*), spices (*5*), and green tea (*6*, *7*) have been reported to inhibit glycation. Some medicinal plants and common botanicals were collected from Korea. Aqueous ethanolic extracts of the plants were tested in a model system. Albumin was selected as a model protein, because it is a key protein found abundantly in human plasma, lymph, and humors throughout the body's essential organs. Under normal conditions albumin is glycated, and this process has been promoted by aging or diabetes. The nature of the reducing sugars influences the rate and extent of the glycation. Fructose instead of glucose was included in our study, because fructose is present in tissues at a concentration comparable to that of glucose and reacts with protein ∼10 times more rapidly than glucose (*8*).

Protein glycation has been associated with the presence of increased oxidative damage in tissues (*9*−*13*). However, it has not been known exactly how glycation affects oxidative stress and vice versa. Recently, it has been reported that glycation generated active centers for catalyzing one-electron oxidation−reduction reactions and that glycated proteins accumulated in vivo provided stable active sites for catalyzing the formation of free radicals (*14*). It has also been suggested that inhibitors of mitochondrial superoxide generation completely prevented hyperglycemia-induced glycation in cultured bovine aortic endothelial cells and that mitochondrial superoxide initiated intracellular glycation (*15*). In our study, the antioxidative activity of the plant extracts was also tested in vitro, and the relationship to glycation was discussed.

MATERIALS AND METHODS

Plant Materials. Some medicinal plants (*Illicium religiosum*, *Pyrus pyrifolia*, *Erigeron annuus*, *Euonymus allata*, *Phellodendron amjrense*, *Torreya nucifera*, and *Thuja orientalis*) were collected from wild forests throughout Korea during the months of April−October of 2000, botanically identified, and air-dried in a sunless place. Voucher specimens have been deposited in the laboratory of the Korea Food Research Institute. The other medicinal plants cultivated in Korea (*Acanthopanax senticosus*, *Paeonia suffruticosa*, *Paeonia lactiflora*, *Eucommia ulmoides*, *Cornus officinalis*, *Saururus chinensis*, and *Schizandra chinensis*), some common botanicals cultivated in Korea (*Allium cepa*, *Origanum saltiva*, and *Camellia sinensis*), some imported spices (*Origanum officinalis*, *Rosmarinus officinalis*, *Eugenia caryophllata*, *Thymus vulgaris*, *Laurus nobilis*, and *Cinamomum cassia*) were

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Table 1. Effects of Plant Extracts on Fluorescence Formation in the BSA–Fructose Reaction in Vitro

no.	family	plant name	tissue used	concn (μg/mL)	RFI[a] (AU)	inhibition[b] (%)	IC_{50}[c] (μg/mL)
	control (d water)				1.08 ± 0.02		
	control (DMSO)				1.00 ± 0.00		
1	Liliaceae	*Allium cepa*	skin	50	31 ± 0.01**	69.5 ± 4.0	16.8 ± 5.0s
				100	0.28 ± 0.02**	71.0 ± 3.9	
				200	0.11 ± 0.01***	89.8 ± 3.5	
2	Magnoliaceae	*Illicium religiosum*	bark	50	0.40 ± 0.02**	66.2 ± 1.0	25.6 ± 4.7rs
				100	0.19 ± 0.02***	83.8 ± 1.4	
				200	0.12 ± 0.04**	90.0 ± 2.4	
3	Polygonaceae	*Fagopyrum esculentum*	hull	50	0.48 ± 0.01**	53.0 ± 0.92	39.0 ± 3.02q
				100	0.34 ± 0.02**	66.9 ± 1.89	
				200	0.05 ± 0.02**	94.5 ± 1.02	
4	Labiatae	*Origanum officinalis*	leaf	50	0.48 ± 0.01***	52.9 ± 0.65	41.4 ± 1.74pq
				100	0.20 ± 0.04***	80.6 ± 3.17	
				200	0.06 ± 0.04***	94.3 ± 3.67	
5	Magnoliaceae	*Illicium religiosum*	wood	50	0.60 ± 0.06**	49.7 ± 3.7	46.5 ± 4.80opq
				100	0.33 ± 0.05**	72.5 ± 2.8	
				200	0.19 ± 0.02***	84.3 ± 1.2	
6	Labiatae	*Rosmarinus officinalis*	leaf	50	0.41 ± 0.08**	53.1 ± 0.15	48.5 ± 0.35opq
				100	0.35 ± 0.01***	65.5 ± 0.43	
				200	0.08 ± 0.01***	92.2 ± 0.72	
7	Rosaceae	*Pyrus pyrifolia*	bark	50	0.67 ± 0.02**	46.0 ± 1.1	49.6 ± 16.5nop
				100	0.47 ± 0.05**	62.2 ± 2.8	
				200	0.42 ± 0.03**	66.6 ± 3.5	
8	Araliaceae	*Acanthopanax senticosus*	bark	50	0.37 ± 0.02***	47.3 ± 4.1	50.8 ± 3.4no
				100	0.32 ± 0.09**	60.0 ± 3.2	
				200	0.47 ± 0.09**	65.9 ± 1.4	
9	Myrtaceae	*Eugenia caryophllata*	leaf	50	0.54 ± 0.01***	46.6 ± 0.79	55.9 ± 0.54mn
				100	28 ± 0.00***	72.2 ± 0.33	
				200	0.00 ± 0.01***	100 ± 3.43	
10	Compositae	*Erigeron annuus*	whole	50	0.53 ± 0.08**	45.8 ± 6.1	56.6 ± 6.5mn
				100	0.29 ± 0.001***	70.3 ± 0.1	
				200	0***	100 ± 0.0	
11	Ranunculaceae	*Paeonia suffruticosa*	root	50	0.76 ± 0.05**	36.5 ± 2.9	63.9 ± 0.8lm
				100	0.30 ± 0.13*	74.7 ± 7.6	
				200	0***	100 ± 0.0	
12	*Labiatae*	*Thymus vulgaris*	leaf	50	0.70 ± 0.10**	29.6 ± 9.87	85.0 ± 13.9k
				100	0.42 ± 0.04***	58.0 ± 3.66	
				200	0.22 ± 0.01***	78.8 ± 0.60	
13	Gramineae	*Oryza sativa* var. Suwon 415	seed	50	0.63 ± 0.2*	38.3 ± 0.7	92.5 ± 3.2jk
				100	0.47 ± 0.01**	54.0 ± 0.3	
				200	0.26 ± 0.02*	73.6 ± 0.7	
14	Paeoniaceae	*Paeonia lactiflora*	root	50	0.85 ± 0.02**	28.7 ± 1.1	94.5 ± 3.2j
				100	0.66 ± 0.05**	45.1 ± 3.0	
				200	0.20 ± 0.01***	83.3 ± 0.5	
15	Theaceae	*Camellia sinensis*	leaf	50	0.67 ± 0.03	31.1 ± 10.8	97.9 ± 18.9ij
				100	0.47 ± 0.06	52.4 ± 4.7	
				200	0.21 ± 0.08*	79.7 ± 2.2	
16	Lauraceae	*Laurus nobilis*	leaf	50	0.67 ± 0.05***	33.6 ± 5.25	105 ± 8.19hi
				100	0.52 ± 0.01***	48.4 ± 0.56	
				200	0.36 ± 0.00***	64.4 ± 0.09	
17	Eucomimiaceae	*Eucommia ulmoides*	leaf	50	0.85 ± 0.02*	16.6 ± 1.0	109.7 ± 2.5h
				100	0.42 ± 0.31	58.4 ± 4.6	
				200	0.34 ± 0.02**	65.5 ± 2.9	
18	Celastraceae	*Euonymus alata*	root	50	0.54 ± 0.08*	40.0 ± 3.7	118.9 ± 1.4g
				100	0.42 ± 0.07**	47.2 ± 5.4	
				200	0.35 ± 0.04**	56.5 ± 5.4	
19	Cornaceae	*Cornus officinalis*	fruit	50	0.92 ± 0.03*	40.0 ± 3.7	158.0 ± 10.9ef
				100	0.64 ± 0.00**	47.2 ± 5.4	
				200	0.44 ± 0.05*	56.5 ± 5.4	
20	Rutaceae	*Phellodendron amjrense*	leaf	50	1.06 ± 0.12	14.6 ± 7.0	160.0 ± 0.8ef
				100	1.02 ± 0.01*	17.7 ± 0.8	
				200	0.38 ± 0.02**	69.2 ± 1.4	
21	Taxaceae	*Torreya nucifera*	leaf	50	0.83 ± 0.05*	18.5 ± 3.5	166.1 ± 0.8de
				100	0.53 ± 0.003**	47.7 ± 0.3	
				200	0.51 ± 0.001*	50.2 ± 0.1	
22	Cypressaceae	*Thuja orientalis*	leaf	50	0.71 ± 0.05**	27.8 ± 3.5	170.0 ± 2.0d
				100	0.63 ± 0.05**	36.1 ± 3.9	
				200	0.42 ± 0.05**	56.8 ± 3.6	
23	Saururaceae	*Saururus chinensis*	leaf	50	0.75 ± 0.19	25.2 ± 0.9	174.5 ± 8.1cd
				100	0.70 ± 0.2	27.8 ± 1.7	
				200	0.41 ± 0.08	58.6 ± 2.0	

Table 1 (Continued)

no.	family	plant name	tissue used	concn (μg/mL)	RFI[a] (AU)	inhibition[b] (%)	IC_{50}[c] (μg/mL)
24	Lauraceae	*Cinamomum cassia*	bark	50	0.80 ± 0.02	19.2 ± 1.6	182.7 ± 10.1c
				100	0.58 ± 0.23	31.6 ± 4.0	
				200	0.43 ± 0.03	53.7 ± 1.3	
25	Magnoliaceae	*Schizandra chinensis*	fruit	50	0.83 ± 0.03*	17.5 ± 1.5	352.4 ± 2.5a
				100	0.65 ± 0.27	25.2 ± 8.2	
				200	0.54 ± 0.24	36.5 ± 8.2	
	aminoguanidine			50	0.43 ± 0.00***	60.6 ± 0.2	27.7 ± 3.2r
				100	0.29 ± 0.1**	72.9 ± 0.0	
				200	0.024 ± 0.04***	97.8 ± 2.2	

[a] Relative fluorescence intensity. The fluorescence intensity was measured at ex 370 nm and em 440 nm. The intensity of each blank (without fructose) was subtracted from the intensity of each sample. AU means arbitrary unit. [b] Percentage inhibition was determined as follows: inhibition (%) = 100 − [fluorescence intensity (sample) − fluorescence intensity (blank of sample)] × 100/[fluorescence intensity (control) − fluorescence intensity (blank of control)]. [c] Concentration of an inhibitor required to inhibit 50% of the control. Calculated from linear regression equation in semilogarithmic manner. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ vs control. Values with different letters in a column are significantly different ($p < 0.05$).

purchased in dried form at commercial markets. All of the plant tissues were powdered with a grinder.

Preparation of Extract. The powdered tissue was extracted with ethanol/water (50:50, v/v) at a ratio of 20 mL/g for 2 h at room temperature three times, followed by filtration with filter papers (Toyo no. 2 and 4, Advantec). The combined filtrate was concentrated in a rotary vacuum evaporator ($T < 40$ °C) until all extraction solvent was completely removed so that solid residue was obtained. The residue was dissolved in dimethyl sulfoxide (DMSO) and used for subsequent bioassays.

Protein Glycation. The procedure followed that of McPherson et al. (*16*) and was modified. Bovine serum albumin (BSA; 10 mg/mL) was incubated with D-fructose (250 mM) in potassium phosphate buffer (200 mM; pH 7.4). DMSO used for dissolving the residue was found to have no effect on the reaction at <2% (v/v). All of the reagents and extracts were sterilized by filtration through 0.2 μm membrane filters, and the mixture was incubated at 37 °C in 5% CO_2 in air for 6 days. The fluorescence intensity was measured at an excitation of 370 nm and an emission of 440 nm with a spectofluorometer (Aminco Bowman). Aminoguanidine (Sigma Chemical Co.) dissolved in distilled water was also tested as a known inhibitor.

Antioxidant Activity. *Fluorescent Lipid Peroxidation.* One hundred millimolar methyl linoleate were emulsified in a 40 mM glycine/0.3% (w/v) Tween 20 solution by sonication under ice-cold conditions. Twenty microliters (a $^1/_{50}$ volume) of the dried residue in DMSO was added to 1 mL of the emulsion. The mixture was incubated at 50 °C for 1 week, after which time the fluorescent product was extracted with a 6-fold volume of ethanol/diethyl ether (3:1) and the fluorescence intensity measured (excitation at 335 nm, emission at 430 nm) with a spectrofluorometer (*17*).

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation ($ABTS^+$) Decolorization Assay. The preformed $ABTS^+$ was generated by oxidation of 7 mM ABTS with 2.45 mM potassium persulfate and reduced in the presence of the plant extracts. After the addition of 990 μL of diluted $ABTS^+$ solution ($A_{734nm} = 0.700 \pm 0.020$) to 10 μL of the plant extracts in ethanol, the absorbance reading was taken at 30 °C exactly 1 min after initial mixing and up to 6 min (*18*).

Free Radical Scavenging Activity on 1,1-Diphenyl-2-picrylhydrazyl (DPPH). For 20 μL of each extract residue in methanol, 98 μM DPPH was added in methanol of a total volume of 1 mL. Five minutes later, the absorbance was measured at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH decolorization compared to the control (*19*).

Analysis of Total Phenolic Content. The concentration of total phenolics in the plant extract was estimated using the Folin−Ciocalteu reagent (*20*). Aliquots of 0.1 mL of the extract residue dissolved in DMSO (0.1 mg/mL) was added in a test tube with 0.5 mL of Folin−Ciocalteu reagent and mixed thoroughly. After an interval of 3 min, 0.5 mL of 10% Na_2CO_3 solution was added, and the mixture was allowed to stand for 1 h at room temperature. The absorbance of the mixture was measured at 760 nm. A standard curve using gallic acid was also prepared. Results were expressed as milligrams per gram of extract of gallic acid equivalents.

Analysis of Total Flavonoid Content. Aliquots of 0.5 mL of the extract residue dissolved in DMSO (0.1 mg/mL) were added to equal volumes of a solution of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 mL of ethanol). The mixture was vigorously shaken, and absorbance was read at 365.7 nm after 10 min at room temperature. Flavonoid contents were expressed in milligrams of quercetin equivalent per gram of extract (*21*).

Analysis of Ascorbic Acid Content. The ascorbic acid contents in the plant extract residue were analyzed using HPLC with a 900 series binary pump, an autosampler, and a diode array detector (Jasco Corp.) linked to a Borwin data handling system. Reversed phase separations were carried out at room temperature using a 4.5 × 150 mm i.d., 5 μm XTerra RP_{18} column (Waters, Milford, MA). Solvent A was 100% methanol, and solvent B was 50 mM potassium phosphate buffer (pH 6.7). Initial condition was 0% A; 0−6 min, 0% A; 6−13 min, 20% A; 13−15 min, 20% A; 15−17 min, 80% A; and back to the initial condition eluted at flow rate of 1 mL/min. L-Ascorbic acid was detected at 270 nm and identified according to the retention times and UV spectra of standards (*22*).

Analysis of Organic Acid Content. The standard materials of such organic acids, that is, oxalic acid, citric acid, malic acid, malonic acid, succinic acid, formic acid, and acetic acid, were purchased from Sigma (St. Louis, MO). All organic acids were determined by ion exchange HPLC analysis system (Jasco Corp.). Conditions for the ion exchange column were as follows: Bio-Rad Aminex HPX-87H, 300 × 7.8 mm; elution, 0.01 N sulfuric acid solution; flow rate, 0.6 mL/min; volume of sample solution injected, 20 μL; detector, UV 210 nm (*23*).

Statistical Analysis. Experiments were performed in duplicate and replicated three times. All values were expressed as mean and standard deviation (SD). Student's *t* test, Duncan's multiple-range test, and correlation analysis program in the SAS (*24*) were used for statistical analysis.

RESULTS AND DISCUSSION

In this study we tested extracts of 25 plant tissues for their inhibitory activity on protein glycation (**Table 1**). Twenty-two extracts inhibited the reaction >50% at 200 μg of dried extract residue/mL, inhibited significantly at concentrations of 50, 100, and 200 μg/mL, and sustained dose dependency. The most active extract was that of *A. cepa* (skin). Its IC_{50} value was 16.8 μg/mL and lower than that of aminoguanidine at 27.7 μg/mL. *I. religiosum* (bark) was the second most bioactive extract followed by *Fagopyrum esculentum* (hull), *O. officinalis* (leaf), *I. religiosum* (wood), *R. officinalis* (leaf), *P. pyrifolia* (bark), *A. senticosus* (bark), *E. caryophllata* (leaf), and *E. annuus* (whole). The IC_{50} values of these were below 60 μg/mL.

Some spices (*O. officinalis*, *T. vulgaris*, and *E. caryophllata*) and green tea (*C. sinensis*) have been reported to inhibit glycation in vitro. The methanolic extracts of three spices were reported to inhibit, respectively, ∼20% at 1 mg/mL the

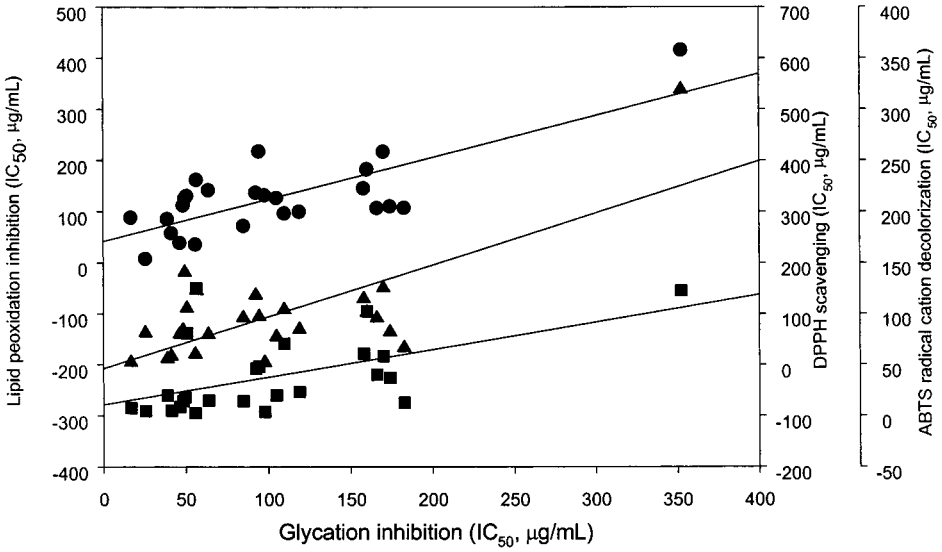

Figure 1. Relationship between glycation inhibition and antioxidative activity [lipid peroxidation inhibition (●), DPPH scavenging activity (▲), and ABTS decolorization (■)] of plant extracts.

Table 2. Antioxidative Activity of Plant Extracts

plant no.	IC_{50}[a] (μg/mL) lipid peroxidation inhibition	ABTS decolorization	DPPH scavenging
1	88.2 ± 5.23efghi	7.60 ± 0.90lm	4.49 ± 0.59k
2	8.00 ± 1.40j	4.48 ± 0.43mn	62.7 ± 8.20g
3	85.3 ± 6.00efghi	19.8 ± 0.13hi	11.7 ±0.70jk
4	58.2 ± 2.78ghif	5.13 ± 0.26lmn	16.8 ± 0.76jk
5	39.7 ± 11.4hij	8.78 ± 0.65l	60.3 ± 0.74g
6	112 ± 8.06defg	14.4 ± 1.19jk	68.4 ± 0.21fg
7	126 ± 2.81cdefg	17.9 ± 0.52ij	182 ± 6.01b
8	130± 3.30 cdef	80.9 ± 5.56c	111 ± 1.72de
9	36.1 ± 1.29ij	2.75 ± 0.89n	20.6 ± 1.92jk
10	162 ± 24.8bcd	125 ± 1.06a	146 ± 14.3bc
11	143 ± 12.4cdef	15.0 ± 1.00jk	59.9 ± 5.00g
12	72.2 ± 14.1fghij	14.3 ± 1.04jk	91.9 ± 0.45ef
13	137 ± 20.0cdef	46.2 ± 0.62f	137 ± 0.00bc
14	217 ± 39.0b	48.2 ± 0.52f	94.4 ±0.30ef
15	132 ± 4.73cdef	3.89 ± 0.89mn	4.75 ± 0.05k
16	126 ± 5.23cdefg	20.2 ± 3.31hi	55.2 ± 1.27gh
17	96.6 ± 3.50defghi	70.6 ± 1.22d	108 ± 18.2de
18	99.9 ± 2.30defghi	23.4 ± 0.45h	69.3 ± 6.54fg
19	145 ± 22.1cde	61.0 ± 3.07e	129 ± 3.20cd
20	183 ± 23.5bc	102 ± 0.19b	103 ± 3.99e
21	107 ± 2.35defgh	40.2 ± 2.20g	91.6 ± 0.72ef
22	217 ± 20.0b	58.3 ± 1.34e	151 ± 14.7bc
23	110 ± 3.51defgh	37.3 ± 0.39g	64.5 ± 2.30g
24	107 ± 3.22defgh	12.7 ± 1.21k	33.3 ± 21.1hij
25	416 ± 87.4a	122 ± 3.94a	539 ± 21.7a
A[b]	6.45 ± 0.27j	1.97 ± 0.07n	64.5 ± 0.11g
B[c]	16.2 ± 0.00j	3.91 ± 0.47mn	24.5 ± 1.25ijk
C[d]	8.42 ± 0.12j	4.79 ± 0.64mn	170 ± 0.60b

[a] Concentration of plant extract residue required to inhibit 50% of the control calculated from linear regression equation in semilogarithmic manner. Values with different letters in a column are significantly different ($p < 0.05$). [b] A, L-ascorbic acid. [c] B, DL-α-tocopherol. [d] C, Trolox.

Table 3. Total Polyphenol, Flavonoid, and Ascorbate Contents (Milligrams per Gram of Residue) of Plant Extracts

plant no.	total polyphenol[a]	total flavonoid[b]	L-ascorbic acid
1	472.62 ± 14.34d	323.18 ± 3.95a	0.00 ± 0.00g
2	423.00 ± 15.89fg	186.31 ± 7.90b	0.05 ± 0.00g
3	438.90 ± 7.62ef	92.74 ± 5.93f	0.88 ± 0.07efg
4	257.41 ± 2.50h	66.20 ± 3.95j	1.09 ± 0.14ef
5	502.57 ± 14.34c	41.06 ± 3.95kl	0.02 ± 0.00g
6	114.97 ± 5.74lmn	150.00 ± 0.00d	0.12 ± 0.01fg
7	460.45 ± 2.92de	38.27 ± 3.95klm	0.00 ± 0.00g
8	138.01 ± 8.12kl	144.41 ± 3.95d	2.34 ± 0.32d
9	532.89 ± 5.45b	113.69 ± 7.90e	2.33 ± 0.29d
10	187.12 ±11.14j	43.85 ± 3.95kl	0.23 ± 0.06fg
11	639.74 ± 2.25a	81.56 ± 1.98gh	8.83 ± 0.59b
12	195.95 ± 6.26j	112.29 ± 1.98e	0.01 ± 0.00g
13	223.10 ± 8.03i	70.39 ± 1.98ji	0.60 ± 0.04efg
14	110.29 ± 4.26mn	31.28 ± 1.98m	6.79 ± 0.58c
15	405.67 ± 3.09g	75.98 ± 1.98hi	12.44 ± 1.91a
16	148.23 ± 7.62k	45.25 ± 5.93k	0.14 ± 0.01fg
17	100.94 ± 3.71n	85.75 ± 3.95fg	0.08 ± 0.00g
18	190.61 ± 12.91j	38.27 ± 0.00klm	0.00 ± 0.00g
19	99.47 ± 1.70n	35.47 ± 0.00lm	0.66 ± 0.12efg
20	132.18 ± 6.36klm	110.89 ± 0.00e	1.32 ± 0.14e
21	95.12 ± 8.94no	42.46 ± 1.98kl	0.71 ± 0.05efg
22	132.33 ± 3.72klm	161.17 ± 0.00c	0.01 ± 0.00g
23	222.13 ± 4.46i	78.77 ± 5.93ghi	0.13 ± 0.02fg
24	420.99 ± 4.13fg	84.36 ± 5.93fgh	0.04 ± 0.00g
25	73.11 ± 3.09o	29.89 ± 3.95m	0.45 ± 0.04efg

[a] Expressed as mg of gallic acid equiv/g of dry weight of residue. Values within each column followed by the same letters are not significantly different ($p < 0.05$). [b] Expressed as mg of quercetin equiv/g of dry weight of residue. Values within each column followed by the same letters are not significantly different ($p < 0.05$).

fluorescence formed by BSA and glucose (*25*). A boiled water extract of green tea was reported to inhibit ~50% at 100 μg/mL the fluorescence formed by human serum albumin and glucose (*6*).

The edible parts of *A. cepa* and *F. esculentum* have been reported to exhibit a wide range of biological effects, including antioxidative, antimutagenic, and cardioprotective actions due to the high content of flavonoids (*26*, *27*). However, their inedible parts have not been studied much. *I. religiosum* grows wild in southern coastal areas and on the island of Cheju in Korea and has not been utilized industrially.

The formation of fluorescent lipid peroxidation products was suppressed by the addition of the plant extracts (**Table 2**). *I. religiosum* (bark) inhibited most potently the peroxidation comparable to L-ascorbic acid and Trolox, followed by *E. caryophllata* (leaf) and *I. religiosum* (wood). In the $ABTS^+$ radical cation assay, *C. sinensis* (leaf) was the most potent product, comparable to L-ascorbic acid, followed by *E. caryophllata* (leaf), *I. religiosum* (bark), *O. officinalis* (leaf), and *A. cepa* (skin). In the DPPH assay, *A. cepa* (skin) exhibited the most potent radical scavenging activity at an extent almost 5 times higher than that of DL-α-tocopherol. Following were *C. sinensis*

Table 4. Composition of Nonvolatile Organic Acids in Plant Extracts[a]

no.	acetic acid	citric acid	succinic acid	oxalic acid	malic acid	malonic acid	total
1	nd	nd	nd	nd	nd	nd	nd
2	nd	nd	nd	nd	nd	nd	nd
3	nd	nd	201.29 ± 3.50	nd	51.09 ± 1.30	nd	252.38 ± 5.11
4	nd	nd	nd	nd	nd	nd	nd
5	nd	nd	nd	nd	nd	nd	nd
6	nd	nd	nd	nd	nd	nd	nd
7	nd	nd	nd	nd	nd	nd	nd
8	nd	10.81 ± 2.30	nd	nd	45.11 ± 3.36	nd	55.92 ± 6.41
9	nd	nd	15.81 ± 2.19	nd	nd	nd	15.81 ± 2.19
10	nd	2.85 ± 0.13	103.06 ± 3.31	nd	nd	4.56 ± 0.36	110.47 ± 4.90
11	nd	nd	nd	nd	nd	nd	nd
12	nd	nd	12.02 ± 3.56	nd	nd	nd	12.02 ± 3.56
13	nd	nd	nd	nd	nd	nd	nd
14	nd	24.61 ± 3.64	nd	nd	nd	nd	24.61 ± 3.64
15	nd	nd	227.01 ± 5.91	49.01 ± 0.36	14.14 ± 0.24	nd	290.16 ± 7.00
16	nd	nd	nd	nd	nd	nd	nd
17	44.33 ± 5.64	nd	12.78 ± 0.50	nd	17.22 ± 0.73	nd	74.33 ± 7.09
18	nd	nd	18.14 ± 4.48	nd	nd	nd	18.14 ± 4.48
19	nd	nd	nd	nd	110.98±11.79	nd	110.98 ± 11.79
20	nd	nd	nd	nd	nd	nd	nd
21	nd	nd	nd	nd	nd	nd	nd
22	nd	nd	nd	nd	nd	nd	nd
23	nd	nd	nd	nd	nd	nd	nd
24	nd	nd	nd	nd	n.d.	nd	nd
25	nd	272.99 ± 9.56	nd	nd	68.32 ± 4.08	nd	341.31 ± 12.00

[a] Data are expressed as mg/g dry weight of residue ± SD ($n = 3$). nd, not detected.

(leaf), *F. esculentum* (hull), *O. officinalis* (leaf), and *E. caryophllata* (leaf). Spices such as *E. caryophllata* and *O. officinalis* and green tea (*C. sinensis*) have been well-known to possess antioxidaive activity (*5*, *25*). The antioxidative activity of *I. religiosum* has never been reported.

Attempts to relate glycation inhibition with antioxidation have been reported. Several kinds of tea extracts showed protein glycation inhibitory activity and DPPH radical scavenging activity (*6*). Spice constituents scavenging free radicals inhibited protein glycation, but the order of potency was not exactly consistent (*5*). Garcinol isolated from *G. indica* fruit rind showed antioxidative and glycation inhibitory activities (*4*). However, analysis for statistical correlation between the two activities has not been reported.

The statistical significance of the association between the glycation inhibition and the antioxidation by the plant extracts was analyzed in our study. The IC_{50} values for the glycation inhibition were significantly correlated with those of lipid peroxidation inhibition ($r = 0.7592$ and $p = 0.0001$), those of $ABTS^+$ antioxidation ($r = 0.5010$ and $p = 0.0001$), and those of DPPH radical scavenging effect ($r = 0.7604$ and $p = 0.0001$), respectively (**Figure 1**). The IC_{50} for glycation inhibition was correlated significantly with all three IC_{50} values for antioxidation ($r = 0.7956$ and $p = 0.0001$). These associations between the glycation inhibitory activity and the antioxidative activity by the plant extracts might support indirectly the suggestion that protein glycation generates free radicals (*14*) and that superoxide formation initiates protein glycation (*15*).

Most of the glycation inhibitory phytochemicals contained in plants or isolated from plants have been reported to be polyphenolic compounds. The active glycation inhibitory constituents of green tea have been regarded as polyphenols (*28*). Quercetin, eriodictyol, 5,6,4′-trihydroxy-7, 8,3′-trimethoxyflavone, and cirsilineol were isolated from *T. vulgaris* as glycation inhibitors (*25*). Garcinol, which is a polyisoprenylated benzophenone derivative and has phenolic hydroxyl groups, was isolated from *G. indica* fruit as a glycation inhibitor (*4*). The three most potent plants in our results contain high contents of polyphenols. *A. cepa* skin contains quercetin at 5000−12000 mg/kg of dry weight (*29*), *I. religiosum* bark contains quercitrin at 50000 mg/kg of dry weight (our unpublished data), and *F. esculentum* hull contains rutin at 130−350 mg/kg of dry weight (*27*). The potent glycation inhibitory activity of these plants might be related to their high content of polyphenols.

For common compounds responsible for the glycation inhibitory and antioxidative activities of the plant extracts, the contents of total polyphenol, total flavonoid, and ascorbic acid were determined (**Table 3**). Attempts to correlate statistically the levels of the common compounds with the glycation inhibitory and the antioxidative activities were less successful. Although correlation with total polyphenols was detected with glycation inhibition ($r = -0.4955$, $p = 0.0118$), lipid peroxidation ($r = -0.4768$, $p = 0.0160$), $ABTS^+$ ($r = -0.6262$, $p = 0.0008$), and DPPH ($r = -0.4247$, $p = 0.0343$), the other individual correlations were lower. It seems likely that the glycation inhibitory and antioxidative activities of the plant extracts are partly due to total polyphenol content, but solely due to common compounds such as total flavonoids and ascorbic acid.

In the glycation assay, chelating agents such as organic acids inhibit the reaction. The content of citric acid and other multibasic organic acids that commonly occur in plants was measured in the extracts (**Table 4**). The correlation of total organic acid level with the glycation inhibitory activity was not statistically significant. It seems likely that the glycation inhibitory activity of the extracts is not due to chelating agents such as organic acids.

The potent glycation inhibitory and antioxidative activities of *A. cepa* (skin), *I. religiosum* (bark and wood), and *F. esculentum* (hull) suggest their possible role in targeting diabetic complications and aging.

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