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Effect of *Alpinia officinarum* on the Formation of Advanced Glycation Endproducts and Aldose Reductase Activity

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Abstract The inhibition effects of an *Alpinia officinarum* (AO, Zingiberaceae) on the formation of advanced glycation end products, aldose reductase, and scavenging effect on 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical for the prevention and/or treatment of diabetic complications were investigated. The ethyl acetate fraction of AO was the most effective among all fractions. Through the tests with electron impact-mass spectrometry and nuclear magnetic resonance, two compounds (compound 1 and 2) finally obtained from the ethyl acetate fraction of AO were identified as galangin (1) and kaempferide (2), respectively. In addition, the compound 1 and 2 and the ethyl acetate fraction were compared for the prevention effect on advanced glycation end products, aldose reductase, and the scavenging effect on DPPH radical. The ethyl acetate fraction was significantly more effective than the 2 compounds for those preventive activities.

Keywords: *Alpinia officinarum*, advanced glycation endproduct, aldose reductase, diabetic complication

Introduction

Abnormal glucose metabolism from insulin deficiency in diabetic patients causes their blood glucose levels to be elevated. Chronic hyperglycemia plays an important role in the pathogenesis of diabetic vascular complications, leading to atherosclerosis, a variety of neuropathies, blindness, and end-stage renal failure, all of which account for disabilities and high mortality rates in these patients (1). High glucose level in the blood or other body fluids is known to cause oxidative damage, followed by an imbalance between the reactive oxygen species (ROS) formation and the antioxidant mechanism in organism (2). Diabetics are more influenced by oxidation than normal subjects, because of their higher production of ROS and lower content of antioxidants (3). Several mechanisms are thought to be involved in hyperglycemia-related oxidative stress, such as glucose autoxidation, glycation, and the formation of advanced glycation endproducts (AGEs)(4).

Glycation or Maillard reaction is a series of non-enzymatic reaction between carbonyl groups of reducing sugars with amino groups of proteins, enzymes, nucleic acids, or phospholipids. In the later stages of the reaction, the products convert into dicarbonyl intermediates, forming AGEs (5). AGEs are formed at accelerated rates in diabetes, but the amount of AGEs also increases with aging (6). AGEs are not only markers, but also important causes of diabetes, cataracts, atherosclerosis, diabetic nephropathy, and neurodegenerative diseases, including Alzheimer's disease (7). Compounds that inhibit the formation of AGEs are thought to be effective against the pathogenesis, therefore aminoguanidine (AG) hydrochloride, D-penicillamine, aspirin, α-tocopherol, thiamine pyrophosphate, and a thiazolidine derivative have been investigated for the reason (8,9).

Aldose reductase (AR) has been appeared to play important roles not only in cataract formation in the lens, but also in the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy (10). AR is an enzyme in the polyol pathway that catalyzes the reduction of glucose to the polar sugar alcohol, sorbitol, using NADPH as a cofactor (11). In diabetes mellitus, a marked increase in intracellular glucose occurring in some cells possessing insulin independent glucose transport results in a marked increase in sorbitol amount. The increased accumulation of sorbitol has been linked to cellular damage. Evidence suggests that compounds which inhibit AR could be effective in the prevention of diabetic complications (12).

Methyl cinnamate, 1,8-cineole, α-cadinene, galangin, 3-*O*-methyl galangin, kaempferide, alpinin, galangol, and some diarylheptanoids have been reported as constituents of *Alpinia officinarum* (AO) (13-15). The rhizome of AO has been known to be effective for stomachic and carminative, and its other biological effects, such as antiemetic, antioxidant, antigenotoxic activities, inhibitions of prostaglandin, and leukotriene biosynthesis, pancreatic lipase, and 5α-reductase have been reported (16). Galangin and 3-*O*-methyl galangin among various constituents of AO are known as the major bioactive constituents. Galangin as a flavonol, which was present in high concentrations in AO, exhibits anticlastogenic (17), antimutagenic (18), and radical scavenging activities (19). 3-*O*-Methyl galangin is probably effective as a hypolipidemic agent, due to its inhibition of pancreatic lipase (15).

Eight % of aqueous acetone extract from the rhizomes of AO containing 2 diarylheptanoids and galangin substantially was reported to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production with IC_{50} values of 33-62 μM (20). 8-(1,1)-Dimethylallyl-kaempferide have shown an

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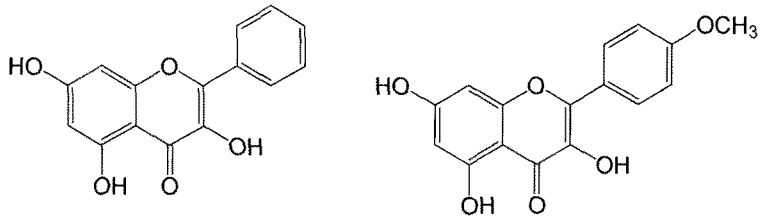

Fig. 1. Structure of flavonoids.

antiplasmodial activity *in vitro* on chloroquine-sensitive and -resistant *Plasmodium falciparum* strains (21). Some isolated compounds from both *n*-butanol and chloroform fractions of green propolis have also been assessed, including kaempferide, isosakuranetin, aromadendrine-4'-methyl-ether, and 3-prenyl-*p*-coumaric acid. Kaempferide presented the highest inhibitory effect on the lucigenin-enhanced chemiluminescence in comparison with the other compounds (22). A kaempferide triglycoside was also found as a constitutive component in an uninfected carnation (*Dianthus caryophyllus*) of the cultivar Novada from *Fusarium oxysporum* f. sp. *Dianthi*, the most important fungal parasite affecting carnation (23). Figure 1 shows the structures of galangin and kaempferide, respectively, which have attracted our attention through the present investigation.

Despite of some previous reports about the biological effect of AO, its effect on the prevention of diabetic complications has not been reported so far. Therefore, some effect of an AO on AGEs formation and AR activity was investigated *in vitro* in the present study for any possibility of the prevention and/or treatment of diabetic complications.

Materials and Methods

Extraction and isolation ***Plant material*****:** The dried rhizomes of *Alpinia officinarum* (AO) (family Zingiberaceae) were purchased from Dea-Gang Herb, Chuncheon, Gangwon, Korea in Sep. 2006 and were identified by Dr. Y.D. Kim, professor of the department of biology, Hallym University, Korea. A voucher specimen (HLP-00123) was deposited at Center for Efficacy Assessment and Development of Functional Foods and Drugs, Hallym University.

Extraction**:** The dried rhizomes of AO (100 g) were extracted with 95% methanol by refluxing at 80°C for 3 hr. The extract was concentrated under reduced pressure by using rotary vacuum evaporator (Rotavapor R-220; Büchi, Switzerland) and lyophilized (Bondiro; Il Shin Lab Co., Seoul, Korea) to obtain powder (12.3 g).

Isolation**:** Ordinary-phase column chromatography; thin layer chromatography (TLC), silica gel 60 F254 Al sheets (Merck, Darmstadt, Germany) using *n*-hexane-EtOAc (1:1); reversed phase high performance liquid chromatography (HPLC) was carried out using an HPLC system HP-1100 (Hewlett-Packard, Palo Alto, CA, USA) equipped with a binary pump. Agilent Prep-C18 Scalar column (250×4.6 mm i.d., Agilent, Santa Clara, CA, USA) and a guard column (4×3.0 mm i.d., Phenomenex, Torrence, CA, USA) were used. Semi-preparative HPLC was performed to fractionate the active fraction on a JAI-908-C60 HPLC (Japan Analytical Industry Co., Tokyo, Japan) equipped with a JAI RI and JAI UV detector operating at 280 nm. A commercially available prepacked ODS-BP column (JAI-GEL 50×2.1 cm i.d., Japan Analytical Industry Co.) was employed. A Hewlett-Packard 1100 series on-line photodiode array detector was used for detection. The instrumentation was controlled by HP Chemstation software.

Physical data**:** UV spectra, Shimadzu UV-1600 spectrometer; infrared (IR) spectra, Shimadzu FTIR-8100 spectrometer; mass spectrometry (MS) and high-resolution MS, Jeol JMS-GCMATE mass spectrometer; ^{1}H-nuclear magnetic resonance (NMR) spectra, Bruker DPX 400 (400 MHz) spectrometer; ^{13}C-NMR spectra, Bruker DPX 400 (100 MHz) spectrometer with tetramethylsilane as an internal standard.

AGEs formation in the BSA/glucose-fructose system AO extracts were incubated with bovine serum albumin (BSA, 10 mg/mL) and 50 μL of the mixture of 0.1 M glucose and 0.1 M fructose in 50 mM phosphate buffered saline (PBS, pH 7.4) containing 0.02% sodium azide at 37°C for 30 days. Aminoguanidine was used as a positive standard inhibitor. Each solution was kept in a capped tube, and incubation was carried out in triplicate tubes. The formation of AGEs was assessed by the characteristic fluorescence (excitation wavelength of 360 nm, emission wavelength of 460 nm) (26).

Measurement of aldose reductase (AR) activity *in vitro* Crude AR was prepared as follows: Rat lenses were removed from Sprague-Dawley (SD) rats weighing 250-280 g and frozen until used. The rat lens homogenate was prepared according to the method of Hayman and Kinoshita (27). A partially purified enzyme was separated into 1.0 mL aliquots and stored at –40°C for the test of enzyme inhibition. AR 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 (28). Each 1.0-mL cuvette contained equal units of enzyme, 0.1 M sodium phosphate buffer (pH 6.2), and 0.3 mM NADPH, both with and without 10 mM of the substrate and AO extracts. The % inhibition of test samples was calculated by subtracting the AR activity measured in the absence of inhibitors to that assessed with the use of inhibitors.

Measurement of DPPH radical scavenging activity The hydrogen atom or electron donation abilities of AO

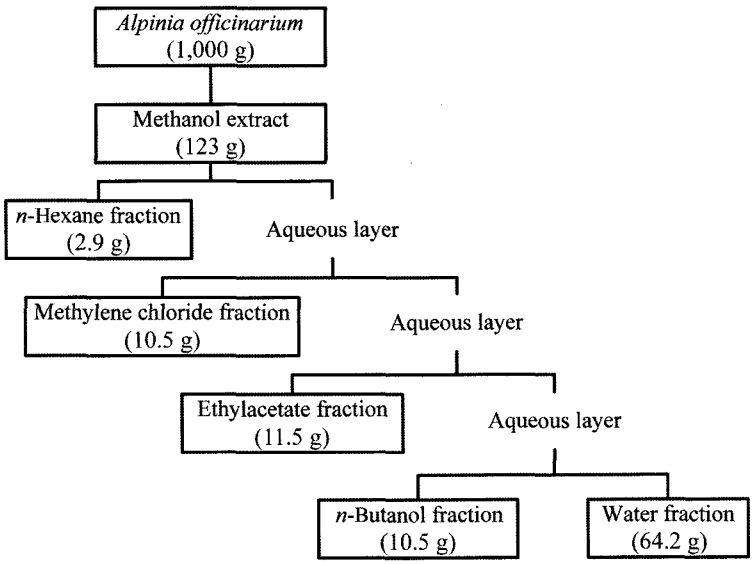

Fig. 2. Extraction and isolation procedures of *Alpinia officinarum*.

extracts were measured from bleaching of the purple-colored ethanol solution of 1,1-diphenyl-2-picryl-hydrazil (DPPH) (29,30). First, the pre-mixture of 400 mM DPPH, 20% ethanol, and 0.2 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer was made, and then a 0.75 mL aliquot of each solvent extract was added to 2.25 mL of the pre-mixture, producing the final mixture. The final mixture was shaken vigorously for 10 sec, and left in the dark at room temperature for 20 min. The absorbance of the mixture was measured spectrophotometrically at 520 nm. DPPH radical of scavenging effect was shown as inhibition (%) obtained from the following equation: Inhibition (%)= $[(OD_{control}-OD_{sample})/OD_{control}]\times 100$.

Measurement of the conjugated malondialdehyde (MDA) with BSA The powder of MDA (1,1,3,3-tetramethoxy-propane) was produced, according to the method of Gomez-Sanchez (31). One-hundred μL BSA (2 mg/mL), 100 μL MDA (20 mM), and 2 different amounts of 0.1 M PBS were added to the lyophilized methanol extract of AO, making the final concentrations of samples 10 and 20 mg/mL, respectively, and then reacted for 24 hr at 37°C. In order to remove free MDA from MDA-bound BSA, 500 μL of the incubated sample was centrifuged for 2 hr at 125×g with centricon (Centricon YM-10; Amicon, Danvers, MA, USA). In addition, the extra salt was removed from the sample by 3 washes of distilled water containing 0.05% sodium azide. Sodium dodecyl sulfate-polyacrylamide gel electro-phoresis (SDS-PAGE) was done with the sample on 12% acrylamide gel, and stained with Coomassie. The density resulting from the conjugated MDA with BSA was measured with densitometer (Vilber Lourmat; BIO-1D Image Analysis, Vilber Lourmat, France) and finally the inhibition effect of AO on conjugated MDA with BSA was calculated.

Statistical analysis Analysis of variance (ANOVA) and Duncan's multiple-range test were used to determine the significance of differences among means, and $p<0.05$ was considered to be statistically significant.

Results and Discussion

Fractionation and isolation of compounds As Fig. 2 shows, the methanol extract powder, 123 g, was suspended in 1,000 mL distilled water and extracted with 1,000 mL of the following solvents stepwise; *n*-hexane, methylene chloride, ethyl acetate, and *n*-butanol. Each extraction was obtained by shaking each mixture of sample dissolved in distilled water and solvent at 20°C for 1 hr 3 times. Each extract was filtered, concentrated under reduced pressure by using rotary vacuum evaporator, lyophilized, and stored at 4±3 °C until used.

The ethylacetate soluble fraction (10 g) which had the most potent anti-glycation activity was subjected to silica gel column [1.0 kg, *n*-hexane→*n*-hexane-ethylacetate (9:1→8:2→7:3→1:1→2:5)→ethylacetate→methanol] to give 8 fractions [Fr. 1 (0.2 g), Fr. 2 (0.8 g), Fr. 3 (1.5 g), Fr. 4 (4.2 g), Fr. 5 (0.4 g), Fr. 6 (0.9 g), Fr. 7 (0.1 g), Fr. 8 (1.8 g)]. Fraction 4 (4.2 g) was subjected to ODS column chromatography [MeOH-water (1:1→6:4→9:1)→MeOH] to give compounds **1** (1.2 g, 0.12%) and **2** (0.5 g, 0.05%). The structures of these compounds were characterized galangin (**1**) and kaempferide (**2**) by UV, IR, MS, ^{1}H- and ^{13}C-NMR, and 2D-NMR methods. Their structures were also confirmed by comparing the previously reported spectral data (24,25).

Compound **1** (galangin): Light yellow crystals (methanol); m.p. 220-222°C; $C_{15}H_{10}O_5$, Mw: 270, UV (λ_{max}, nm) (MeOH): 264, 310, 358. IR ν (1/cm, KBr): 3,353 (br.,-OH), 1,660 (flavone >CO), 1,610, 1,517 (aromatic). ^{1}H-NMR (300 MHz, DMSO-d_6): 6.22 (1H, d, J=2.2, H-6), 6.46 (1H, d, J=2.1 Hz, H-8), 7.48-7.59 (3H, m, H-3', 4', 5'), 8.12-8.18 (2H, m, H-2',6'). ^{13}C-NMR (100 MHz, DMSO-d_6): 92.5 (C-8), 97.3 (C-6), 102.4 (C-10), 126.1 (C-2', C-6'),126.4 (C-4'), 129.8 (C-3', C-5'), 130.9 (C-1'), 137.9 (C-3), 147.0 (C-2), 157.2 (C-9), 162.8 (C-5), 165.9 (C-7), 176.9 (C-4). EI Mass *m/z* (rel. Int., %): 270 $[M]^+$ (100), 269 $[M-H]^+$ (40), 242 $[M-38]^+$ (15), 213 $[M-57]^+$ (15), 167 [M-102 (hydroxyl phenol)]$^+$ (10), 105 [M-165 (hydroxyl phenol)]$^+$ (25), 77 [benzene]$^+$ (30) (Fig. 1).

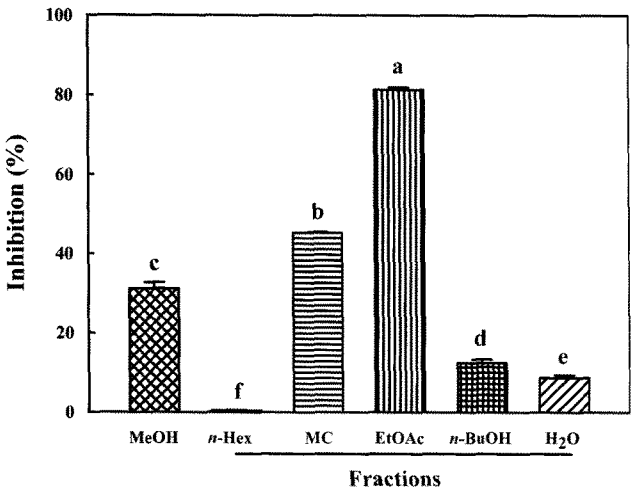

Fig. 3. Inhibition effect of *Alpinia officinarum* fractions on advanced glycation end products (AGEs) formation. Each bar represents the mean±SD (n=5). Values with different letters are significantly different (p<0.05). MeOH, methanol extract; n-Hex, n-hexane fraction; MC, methylene chloride fraction; EtOAc, ethylacetate fraction; n-BuOH, n-butanol fraction; H_2O, water fraction.

Compound **2** (kaempferide): Yellow crystals (methanol); m.p. 225-226°C; $C_{16}H_{12}O_6$, Mw: 300; UV (λ_{max}, nm) (MeOH): 268, 315, 352; IR ν (1/cm, KBr): 3,350 (br.,-OH), 1,662 (flavone >CO), 1,612, 1,518 (aromatic), 822 (p-substituted phenyl ring); ^{1}H-NMR (300 MHz, DMSO-d_6): 3.83 (3H, s, OCH_3), 6.18 (1H, d, J=2.1 Hz, H-6), 6.44 (1H, d, J=2.1 Hz, H-8), 7.13 (2H, d, J=9.3 Hz, H-3', 5'), 8.13 (2H, d, J=9.3 Hz, H-2', 6'); ^{13}C-NMR (100 MHz, DMSO-d_6): 56.2 (OCH_3), 93.5 (C-8), 99.7 (C-6), 105.4 (C-10), 115.3 (C-3', C-5'), 128.1 (C-2', C-6'), 131.6 (C-1'), 136.9 (C-3), 147.3 (C-2), 158.2 (C-9), 161.7 (C-4'), 162.4 (C-5), 165.5 (C-7), 177.5 (C-4); EI Mass *m/z* (rel. Int., %): 300 $[M]^+$ (100), 285 $[M\text{-}CH_3]^+$ (18.4), 272 $[M\text{-}CO]^+$ (5), 257 $[M\text{-}CH\text{-}CO]^+$ (15), 229 $[M\text{-}CH_3\text{-}2CO]^+$ (20), 77 $[benzene]^+$ (40) (Fig. 1).

Inhibition effect of AO on AGEs formation Methanol extract and various solvent fractions of AO were tested for any possible inhibitory effect on non-enzymatic glycation process with the BSA/sugars model. Fluorescence, which increases during glycation, was used as a parameter to study the influence of AO extracts on the formation of AGEs in the BSA/sugars model.

As shown in Fig. 3, the ethyl acetate fraction of AO has shown the significantly highest activity with 81.01% inhibition, and then followed by methylene chloride, 46.54%; methanol, 30.10%; n-butanol, 13.58%; and water fraction, 8.00%, respectively. However, n-hexane fraction did not show any inhibitory activity on AGEs formation.

In view of the result with each fraction on AGEs formation, ethyl acetate fraction and its isolated compounds, galangin and kaempferide, were subsequently tested with the BSA/glucose-fructose system. Table 1 also shows inhibition effects of ethylacetate fraction, galangin, and kaempferide with various concentrations on AGEs formation. IC_{50} (μg/mL) values represented the concentration that caused 50% inhibition of AGEs formation, and aminoguanidine, the typical inhibitor of AGEs formation, was used as a reference. The ethyl acetate fraction has shown the significantly highest activity, IC_{50}, 77.73 μg/mL, 8 times as high as aminoguanidine. However, the both compounds isolated from ethyl acetate fraction, galangin and kaempferide, did not show comparable inhibition activities on AGEs formation, as much as the ethyl acetate fraction showed.

Table 1. Inhibition effect of ethylacetate fraction and isolated compounds from *Alpinia officinarum* on advanced glycation end products (AGEs) formation

	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)[1)]
Ethylacetate fraction	294	81.01	77.73
	58.8	56.14	
	5.88	31.76	
Galangin	588	62.27	330.01
	294	39.2	
	58.8	46.38	
Kaempferide	588	57.84	438.5
	294	34.07	
	58.8	33.1	
Aminoguanidine[2)]	638	52.03	613.84
	588	45.91	
	294	3.19	

[1)]IC_{50} values representing the concentration that caused 50% inhibition of AGEs formation were calculated from least-square regression equations in the plot of the logarithm of 3 graded concentrations vs. % inhibition.
[2)]A reference compound and typical inhibitor of AGEs formation.

Inhibition effect of AO on aldose reductase Methanol extract and various solvent fractions of AO were tested for any possible inhibitory effect on rat lens aldose reductase. Figure 4 shows the result of inhibition test on AR activity with methanol extract and various solvent fractions of AO. All they except n-hexane fraction showed AR inhibition ability with various degrees of effectiveness. The highest inhibition effect, 84.36% was observed on n-butanol fraction, and followed by ethyl acetate fraction, 63.21%, and then water fraction, 32.93%. However, the activity of water fraction was not significantly different from that of methylene chloride fraction. As the case of the inhibition effect on AGEs formation, n-hexane fraction did not also have any significant effect on aldose reductase activity. In the same way of the inhibition on AGEs formation, the aldose reductase inhibitory activity was compared among the ethyl acetate fraction, galangin and kaempferide, and the result was displayed in Table 2. The inhibitory effects increased with their amounts, showing the following decreasing order: ethyl acetate fraction (IC_{50}, 10.2 μg/mL), galangin (34.6 μg/mL), kaempferide (43.4 μg/mL). Interestingly, ethyl acetate fraction showed the similar activity to quercetin (IC_{50}, 9.7 μg/mL), the control of AR inhibitor. However, each isolated compound, galangin and kaempferide, did not also display comparable inhibitory activity on AR, as much as the ethyl acetate fraction.

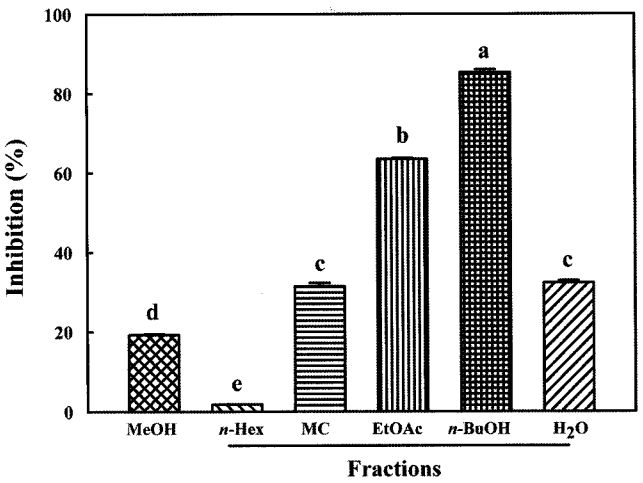

Fig. 4. Inhibition effect of *Alpinia officinarum* fractions on aldose reductase activity. Each bar represents the mean±SD (n=5). Values with different letters are significantly different (p<0.05). MeOH, methanol extract; n-Hex, n-hexane fraction; MC, methylene chloride fraction; EtOAc, ethylacetate fraction; n-BuOH, n-butanol fraction; H_2O, water fraction.

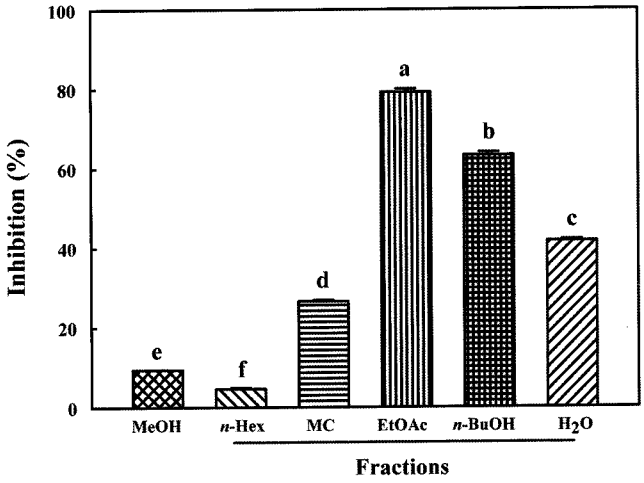

Fig. 5. DPPH radical of scavenging effects by *Alpinia officinarum* fractions. Each bar represents the mean±SD (n=5). Values with different letters are significantly different (p<0.05). MeOH, methanol extract; n-Hex, n-hexane fraction; MC, methylene chloride fraction; EtOAc, ethylacetate fraction; n-BuOH, n-butanol fraction; H_2O, water fraction.

Table 2. Inhibition effect of ethylacetate fraction and isolated compounds from *Alpinia officinarum* on aldose reductase activity

	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)[1)]
Ethylacetate fraction	28	78.5	10.2
	14	52.4	
	3	38.9	
Galangin	70	82.5	34.6
	28	42.9	
	14	25.1	
Kaempferide	70	60.1	43.4
	28	32.2	
	14	20.6	
Quercetin[2)]	28	80.5	9.7
	14	62.7	
	2.8	20.7	

[1)]IC_{50} values representing the concentration that caused 50% inhibition of enzyme activity were calculated from least-square regression equation in the plot of the logarithm of 3 graded concentrations vs. % inhibition.
[2)]A reference compound and typical aldose reductase inhibitor.

Table 3. DPPH radical scavenging effects of ethylacetate fraction and isolated compounds from from *Alpinia officinarum*

	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)[1)]
Ethylacetate fraction	28	63.21	22
	14	34.19	
	3	4.99	
Galangin	140	84.14	65
	28	40.43	
	14	33.29	
Kaempferide	140	70.01	83.4
	28	33.54	
	14	21.78	
Ascorbic acid[2)]	28	90.53	16.6
	14	42.70	
	2.8	2.7	

[1)]IC_{50} values representing the concentration that caused 50% inhibition of DPPH radical were calculated from least-square regression equation in the plot of the logarithm of 3 graded concentrations vs. % inhibition.
[2)]A reference compound and typical DPPH radical scavenger.

DPPH radical scavenging activity of AO Radical scavenging capacity or electron donating activity of 0.75 mL of solvent extracts of AO was evaluated. The free radical scavenging activity was shown as the inhibition rate by inversing proportion to the absorbance, as shown in Fig. 5. Ethyl acetate fraction of AO showed the highest radical scavenging activity (78.52% inhibition), i.e., the lowest level of absorbance, and then followed in order by n-butanol fraction (62.54%), water fraction (41.27%), and methylene chloride fraction (26.30%). However, both methanol extract and n-hexane fraction displayed less than 10% inhibition effect on DPPH radical.

After comparing all fractions, finally 3 samples (ethyl acetate fraction, galangin, and kaempferide) were also reevaluated with various concentrations on DPPH radical scavenging activity, as displayed in Table 3. IC_{50} values represented the concentration that caused 50% inhibition of DPPH radical, and ascorbic acid as a reference compound and typical DPPH radical scavenger was used. The ethyl acetate fraction showed the significantly highest inhibition activity on DPPH radical, IC_{50}, 22 μg/mL, and was comparable to the reference. However, 2 isolated compounds, galangin and kaempferide, did not show effective inhibition activity on DPPH radical as much as ethyl acetate fraction.

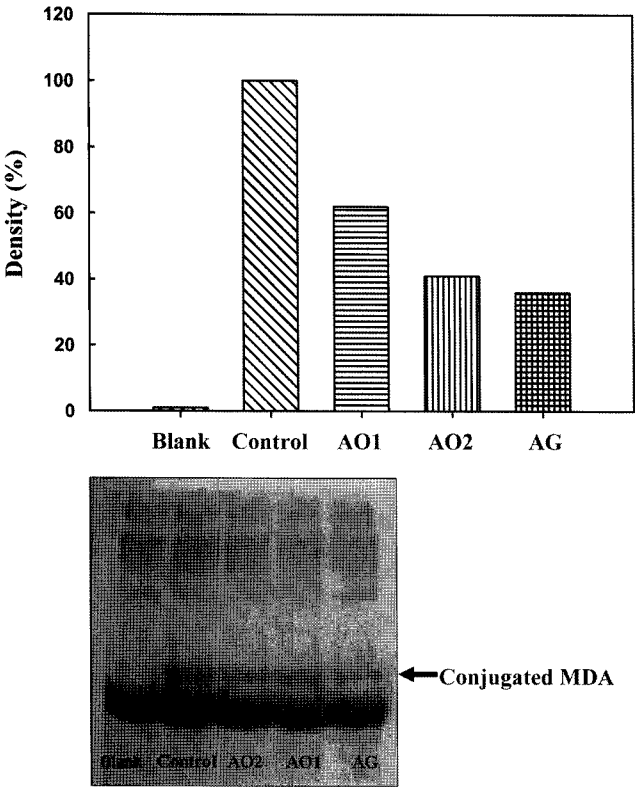

Fig. 6. Inhibition effects of *Alpinia officinarum* on conjugated MDA with BSA in 12% SDS-PAGE. Blank, BSA; control, BSA+MDA; AO1, BSA+MDA+10 mg/mL methanol extract of *Alpinia officinarum*; AO2, BSA+MDA+20 mg/mL methanol extract of *Alpinia officinarum*; AG, BSA+MDA+10 mg/mL amonoguanidine.

Inhibitory effect of AO on conjugation between MDA and BSA MDA is a highly reactive 3 carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. MDA readily combines with several functional groups on molecules including proteins, lipoproteins, and DNA. MDA-modified proteins may show altered physicochemical behavior and antigenicity (32).

As the amount of AO increased, the band density, meaning the degree of conjugation between MDA (an end product of lipid peroxidation) and BSA, reduced from 100% density of control (Fig. 6) to 61% with 10 mg/mL methanol extract, and to 42% density with 20 mg/mL methanol extract, respectively. The addition of 20 mg/mL methanol extract to the mixture of MDA and BSA showed statistically similar inhibition activity to that of 10 mg/mL aminoguanidine, the reference.

We have shown in the present study that ethylacetate fraction prepared from AO strongly inhibited the formation of AGEs and the activity of aldose reductase, suggesting that AO might contribute significantly in treating diabetic complications, when it is processed and consumed appropriately as health-promoting food ingredients. Those activities might be mainly attributed to 2 compounds, galangin and kaempferide, isolated from ethylacetate fraction, but separate comparison among ethylacetate fraction, galangin and kaempferide with various concentrations suggests that further investigation be needed if there is any synergy effect between 2 compounds, or any contribution of possible unknown compounds in ethyl acetate fraction on the inhibition of AGEs formation and aldose reductase activity.

Oxidative stress has been suggested to be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes (33). There are many investigations which have studied the effects of the antioxidative plants and their antioxidant ingredients on diabetes and its complications and achieved good results (34,35). Therefore, antioxidant flavonoids (galangin and kaemfpride) and ethylacetate fraction prepared from AO possess therapeutic potential in the protection and improvement of diabetic complications.

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