

Research Article

The Inhibitory Effect of *Prunella vulgaris* L. on Aldose Reductase and Protein Glycation

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To evaluate the aldose reductase (AR) enzyme inhibitory ability of *Prunella vulgaris* L. extract, six compounds were isolated and tested for their effects. The components were subjected to *in vitro* bioassays to investigate their inhibitory assays using rat lens aldose reductase (rAR) and human recombinant AR (rhAR). Among them, caffeic acid ethylene ester showed the potent inhibition, with the IC₅₀ values of rAR and rhAR at $3.2 \pm 0.55 \mu\text{M}$ and $12.58 \pm 0.32 \mu\text{M}$, respectively. In the kinetic analyses using Lineweaver-Burk plots of 1/velocity and 1/concentration of substrate, this compound showed noncompetitive inhibition against rhAR. Furthermore, it inhibited galactitol formation in a rat lens incubated with a high concentration of galactose. Also it has antioxidative as well as advanced glycation end products (AGEs) inhibitory effects. As a result, this compound could be offered as a leading compound for further study as a new natural products drug for diabetic complications.

1. Introduction

Aldose reductase (AR) is a key enzyme in the polyol pathway known to play important roles in the cataract formation and the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy [1].

Several naturally occurring and synthetic AR inhibitors with diverse structures have been studied in experimental animals and clinical trials to determine their effectiveness in preventing cataract formation and diabetic complications. Natural products are an excellent source of chemical structures with a wide variety of biological activities [2].

The development and progression of diabetic complications can be prevented by controlling blood glucose, but it is difficult to maintain normal blood glucose levels in a diabetic patient. AR inhibition is recognized as an important strategy for the prevention and attenuation of long-term diabetic complications. AR inhibitors are being studied as potential therapeutic agents for use in treating diabetic complications [3, 4].

Apart from diabetic microvascular disease, advanced glycation end products (AGEs) have also been implicated in a wide and seemingly disparate range of pathologies such as connective tissue diseases particularly in rheumatoid arthritis and neurological conditions such as Alzheimer's disease and end-stage renal disease. *In vitro* work has mostly shown AGE to be part of complex interactions within oxidative stress and vascular damage, particularly in atherosclerosis and in the accelerated vascular damage that occurs in diabetes [5]. Prolonged hyperglycemia results in the formation of AGEs in body tissues. The complex fluorescent AGE molecules formed during the Maillard reaction can lead to protein cross-linking and contribute to the development and progression of several diabetic complications, such as cataracts, atherosclerosis, nephropathy, and neuropathy [6]. Increased oxidative stress is a widely accepted contributor to the development and progression of diabetes and its complications. Diabetes is usually accompanied by an increased production of free radicals and impaired antioxidant defenses [7, 8]. Therefore, inhibitors of AR and

AGE formation provide an alternative mode of diabetes treatment, which is not dependent on the control of blood glucose level. These inhibitors should be useful in the prevention or reduction of certain diabetic complications [9].

Prunella vulgaris, with almost 15 known individual species, is widely distributed in Europe, Asia, northwest Africa, and North America, where it is generally known as self-heal herb [10]. *P. vulgaris* is used to cure high blood pressure, headaches, lymphatic system disorders, goiter, tuberculosis, and tumors [11–13]. More recently, a hot water infusion of this plant has been used to treat sores in the mouth and throat [11].

Various bioactive constituents have been identified in extracts of *P. vulgaris*. Phytochemical studies indicate that *P. vulgaris* contains oleanolic, betulinic, ursolic, 2 α , 3 α -dihydroxyurs-12-en-28-oic, and 2 α , 3 α -ursolic acids, triterpenoids, flavonoids, tannins, and anionic polysaccharide called prunellin [11, 14]. The current study aimed to evaluate the AR and AGE inhibitory of *P. vulgaris* extract. These studies are important in understanding the inhibitory effects of *P. vulgaris* on diabetic complications.

2. Materials and Methods

2.1. Apparatus and Reagents. DL-Glyceraldehyde, the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), bovine serum albumin (BSA), sodium phosphate, and quercetin used in this study were purchased from Sigma (St. Louis, MO, USA). Human recombinant aldose reductase was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals and reagents used were of analytical grade.

2.2. Plant Materials. *P. vulgaris* was purchased from Dae Kwang Herb Medicine Co., Ltd., Chuncheon, Republic of Korea and the voucher specimen (No. RIC-1021) was deposited at Regional Innovation Center, Hallym University, Republic of Korea.

2.3. Extraction and Isolation. The air-dried *P. vulgaris* (1 Kg) was extracted with water (5 L \times 2 times) for 2 h at 100°C. The total filtrate was concentrated to dryness *in vacuo* at 40°C. The extract was suspended in distilled water and partitioned sequentially with *n*-hexane, methylene chloride (CH₂Cl₂), ethyl acetate (EtOAc), and *n*-butanol (BuOH), respectively. CH₂Cl₂ fraction was further purified by using a medium pressure liquid chromatography (LiChroprep RP-18 Glass column (36 \times 460 mm; 25–40 μ M particle size); mobile phase MeOH/H₂O (40 to 50%); flow rate 10 to 5 mL/min) resulted in the isolation of compounds **5** (10.9 mg) and **6** (13.5 mg). Since the EtOAc fraction showed the AR inhibitory activity, this fraction (2.2 g) was further purified by using a medium pressure liquid chromatography (LiChroprep RP-18 Glass column (36 \times 460 mm; 25–40 μ M particle size); mobile phase MeOH/H₂O (40 to 50%); flow rate 10 to 5 mL/min) resulted in the isolation of compounds **1** (102.8 mg), **2** (53.3 mg), **3** (10.6 mg), and **4** (27.9 mg).

2.4. Preparation of Aldose Reductase. Crude rat lens aldose reductase (rAR) was prepared as follows: lenses were removed from Sprague-Dawley rats weighing 250–280 g and frozen at –70°C until use. The rat lens homogenate was prepared according to the method of Hayman and Kinoshita with some modifications [15–17]. Noncataractous transparent lenses were pooled and homogenate was prepared in 0.1 M phosphate buffer saline (pH 6.2). After centrifugation at 10,000 rpm for 20 min in a refrigerated centrifuge, the supernatant containing the rAR was collected. All procedures were carried out at 4°C.

2.5. Determination of Aldose Reductase Inhibition In Vitro. AR activity was assayed spectrophotometrically by measuring the decrease in the absorption of NADPH at 340 nm over a 4 min period according to the method of Hayman and Konoshita with some modifications, using 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 [18, 19]. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC₅₀) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.

2.6. Kinetics of Recombinant Human Aldose Reductase by Active Compound. Reaction mixtures consisted of 0.1 M potassium phosphate, 0.16 mM NADPH, 2 mM of recombinant human aldose reductase (rhAR) with varied concentrations of substrate DL-glyceraldehyde and AR inhibitor in a total volume of 200 μ L. Concentrations were ranged from 0.1 to 1 mM for DL-glyceraldehyde, and from 0.1 to 1 mM for active compound. Recombinant human aldose reductase activity was assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm after substrate addition using Bio Tek Power Wave XS spectrophotometer (Bio Tek Instruments, VT, USA) [16].

2.7. Lens Culture and Intracellular Galactitol Measurement. Lenses isolated from 10-week-old male rats were cultured for 6 d in TC-199 medium that contained 15% fetal bovine serum 100 units/mL penicillin and 0.1 mg/mL streptomycin under sterile conditions in an atmosphere of 5% CO₂ and 95% air at 37°C. Samples were dissolved in dimethyl sulfoxide. The lenses were divided into 5 groups and cultured in medium containing 5 mM glucose, 30 mM galactose, and rosmarinic acid or caffeic acid ethylene ester. Each lens was placed in well containing 1.0 mL of medium. Galactitol was determined by HPLC after its derivatization by reaction with benzoic acid to a fluorescent compound [20].

2.8. Blood Culture and Intracellular Galactitol Measurement. Blood sample was collected in heparin-containing polypropylene tube from 10-week-old male rats. For sugar and sugar alcohol analysis, erythrocytes from heparinized blood were separated from the plasma and buffy coat by centrifuging at 2000 \times g for 10 min. The cells were washed thrice with normal saline (0.9% NaCl) at 4°C. In the

TABLE 1: Inhibitory effects of the *P. vulgaris* L. on rat lens aldose reductase (rAR).

| Fraction | Concentration ($\mu\text{g/mL}$) | Inhibition (%) | IC ₅₀ ($\mu\text{g/mL}$) |
|------------------------|------------------------------------|------------------|---------------------------------------|
| Quercetin ^a | 10 | 75.33 \pm 0.55 | 1.53 \pm 0.16 |
| | 5 | 64.72 \pm 1.56 | |
| | 1 | 44.7 \pm 1.39 | |
| Water extract | 10 | 36.18 \pm 1.13 | — |
| <i>n</i> -Hexane fr. | 10 | 33.94 \pm 0.49 | — |
| Methylene chloride fr. | 10 | 32.49 \pm 0.54 | — |
| Ethyl acetate fr. | 10 | 87.33 \pm 2.39 | 2.99 \pm 0.10 |
| | 5 | 62.79 \pm 1.48 | |
| | 1 | 18.34 \pm 0.59 | |
| <i>n</i> -Butanol fr. | 10 | 59.56 \pm 2.34 | — |
| Water fr. | 10 | — | — |

Inhibition rates were calculated as percentages with respect to the control value. ^aQuercetin was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

final washing, the cells were centrifuged at 2000 \times g for 10 min to obtain a consistently packed cell preparation. The packed cells (1 mL) were then incubated in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 30 mM galactose in the presence or absence of samples at 37°C in 5% CO₂ for 3 h. The erythrocytes were washed with cold saline by centrifuging at 2000 \times g for 10 min, precipitated by adding 6% of cold perchloric acid (3 mL), and centrifuged again at 2000 \times g for 10 min. The supernatant was neutralized with 2.5 M K₂CO₃ at 4°C and used for galactitol determination [21]. HPLC analysis for sugar and sugar alcohol in blood was performed with this supernatant of red blood cell homogenate after being benzoylated.

2.9. Effects on AGEs Formation. The modified procedure of Lee et al. [22] was followed. Bovine serum albumin (10 mg/mL) was incubated with 5 mM methylglyoxal in sodium phosphate buffer (100 mM; 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 LS50B fluorescence spectrometer (Perkin-Elmer Ltd., USA). Aminoguanidine was also tested as a known inhibitor.

2.10. ABTS^{•+} Assay. The method of Re et al. [23] was used with slight modification. The ABTS diammonium salt (2 mM) and potassium persulfate (3.5 mM) were mixed and diluted in distilled water kept in the dark at room temperature for 24 h before use. After addition of ABTS^{•+} solution to 10 μL of antioxidant compounds were recorded at after 10 min reaction. The percentage inhibition of absorbance at 750 nm is calculated and potted as a function

TABLE 2: Inhibitory effects of the compounds isolated from the *P. vulgaris* L. on rat lens aldose reductase (rAR) and human recombinant aldose reductase (rhAR).

| Compounds | IC ₅₀ (μM) ^a | |
|------------------------|---|------------------|
| | rAR | rhAR |
| Quercetin ^b | 5.66 \pm 1.05 | 19.23 \pm 0.74 |
| 1 | — | — |
| 2 | — | — |
| 3 | 8.35 \pm 0.51 | — |
| 4 | 2.77 \pm 0.48 | 18.62 \pm 0.40 |
| 5 | 3.20 \pm 0.55 | 12.58 \pm 0.32 |
| 6 | — | — |

^aInhibition rates were calculated as percentages with respect to the control value. The IC₅₀ values of each sample were estimated from the least-squares regression line of the logarithmic concentration plotted against inhibitory activity. ^bQuercetin was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

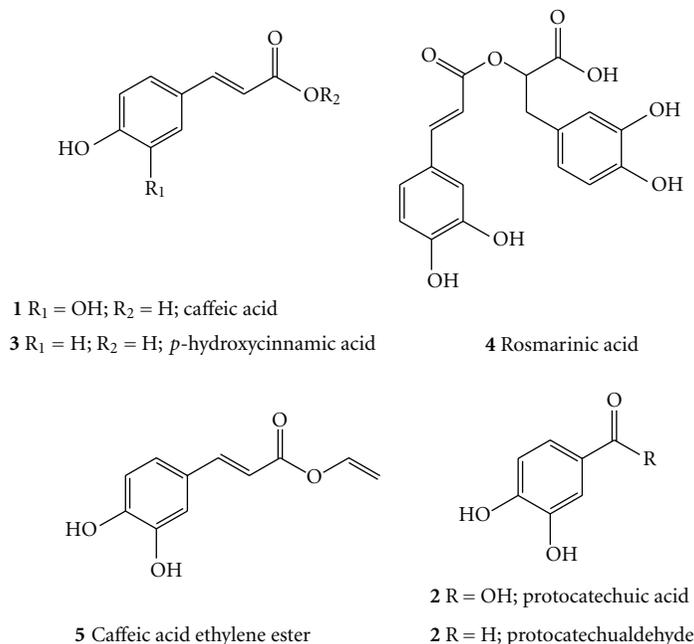
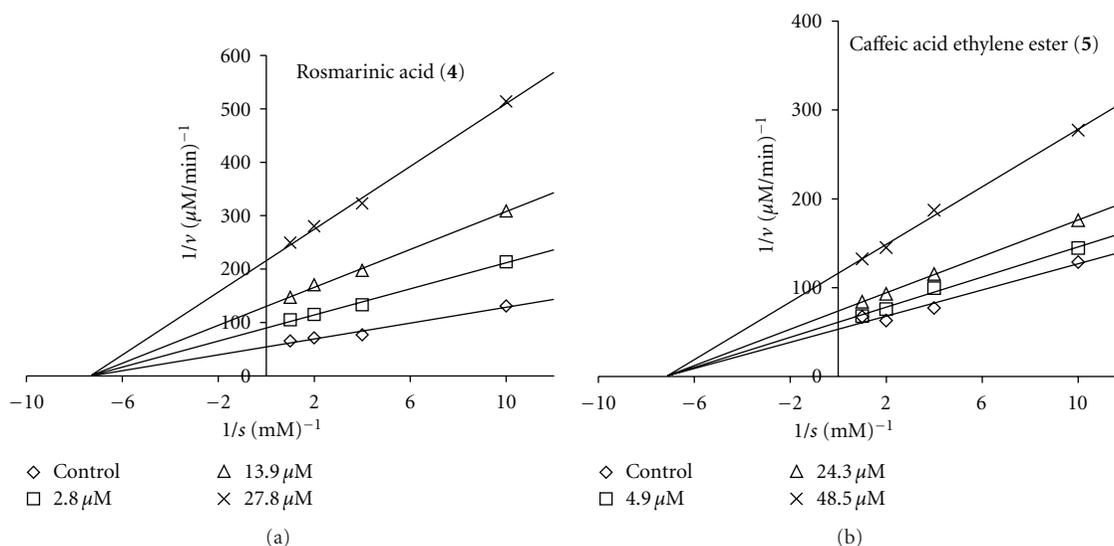
of concentration of antioxidants. Trolox was used as positive control.

3. Results and Discussion

3.1. Identification of the Isolated Compounds. The constituents of the dried *P. vulgaris* were extracted with water. In order to identify the active fractions from *P. vulgaris* the extract was divided into 5 systematic fractions. The CH₂Cl₂ soluble and EtOAc soluble fractions were isolated by using a medium pressure liquid chromatography. The chemical structures (Figure 1) of the 6 isolated compounds were identified. Their chemical structures were elucidated by chemical and spectral analysis as caffeic acid (**1**) [24], protocatechuic acid (**2**) [25], *p*-hydroxycinnamic acid (**3**) [26], rosmarinic acid (**4**) [27], caffeic acid ethylene ester (**5**) [28], and protocatechualdehyde (**6**) [29]. Compounds **2**, **3**, and **5** were isolated for the first time from this plant.

3.2. Aldose Reductase Inhibitory Activity of *P. vulgaris*. The present study was conducted to identify new potential rAR inhibitors from *P. vulgaris*, which might have potential uses in the treatment of diabetic complications. We showed that water extract of *P. vulgaris* inhibited the activity of rAR. In order to identify the active compounds from *P. vulgaris* the extract was divided into several fractions that were tested for their rAR inhibitory activity. The EtOAc fraction had high inhibitory activity against rAR with IC₅₀ value of 2.99 \pm 0.10 $\mu\text{g/mL}$. These results are shown in Table 1.

Previous studies used quercetin as a positive control when comparing the inhibitory activity of active compounds isolated from natural products against lens or human recombinant aldose reductase [9, 30, 31]. We compared the inhibition of rAR and rhAR by compounds **1–6** and quercetin, a natural aldose reductase inhibitor. Their inhibitory effects are shown in Table 2. Compounds **3–5** had IC₅₀ values of 8.35 \pm 0.51, 2.77 \pm 0.48, and 3.2 \pm 0.55 μM , respectively, for

FIGURE 1: Structures of compounds isolated from *P. vulgaris* L.FIGURE 2: Lineweaver-Burk plots showing the reciprocal of the velocity ($1/v$) of recombinant human aldose reductase versus the reciprocal of the substrate concentration ($1/s$) with DL-glyceraldehyde as the substrate concentration of 0.1 to 1 mM; rosmarinic acid (4), caffeic acid ethylene ester (5).

AR while compounds 4 and 5 had IC_{50} values of 18.62 ± 0.40 and $12.58 \pm 0.32 \mu\text{M}$, respectively, for rhAR. Of the compounds tested, rosmarinic acid had the most potent rAR inhibitory activity, while caffeic acid ethylene ester showed almost the same activity at the same concentration. *p*-Hydroxycinnamic acid showed weak inhibitory activity against rAR. Rosmarinic acid was demonstrated to possess strong AR inhibitory activity [32]. Compounds isolated from *P. vulgaris* had potent inhibitory effects on rhAR, especially caffeic acid ethylene ester.

3.3. Kinetics of Recombinant Human Aldose Reductase Inhibitory Activity by Active Compounds. To determine the type of inhibition activity produced by compounds 4 and 5, a kinetic study was conducted using DL-glyceraldehyde as a substrate (concentration: 0.1–1 mM) at 3 different concentrations for each compound. The Lineweaver-Burk plots of $1/\text{velocity}$ and $1/\text{concentration}$ for compounds 4 and 5 are shown in Figure 2. Changes in the concentration of the substrate DL-glyceraldehyde resulted in different slopes and different x axis intercepts being obtained with the

TABLE 3: Inhibitory effect of the compounds on the galactitol accumulation in rat lenses and erythrocyte.

| Compounds | Rat erythrocyte galactitol content (μM) ^a | Galactitol content $\mu\text{g}/\text{lens}$ wet weight (g) ^b |
|---------------------------------|---|--|
| Galactitol-free | 0.98 \pm 0.04 | — |
| Control | 22.8 \pm 0.35 | 844.05 \pm 6.89 |
| Quercetin ^c | 16.15 \pm 0.39 | 448.81 \pm 3.38 |
| Rosmarinic acid (4) | 15.05 \pm 0.30 | 532.38 \pm 6.56 |
| Caffeic acid ethylene ester (5) | 15.89 \pm 0.41 | 653.76 \pm 4.68 |

^aErythrocyte was incubated in a Krebs-Ringer bicarbonate buffer containing 30 mM galactose and in the presence or absence of 5 $\mu\text{g}/\text{mL}$ compounds. ^bMean of 3 duplication analyses of rat lens with compounds at a concentration of 5 $\mu\text{g}/\text{mL}$. ^cQuercetin was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

TABLE 4: Inhibitory effects of the *P. vulgaris* L. on advanced glycation end products (AGEs).

| Fraction | Concentration ($\mu\text{g}/\text{mL}$) | Inhibition (%) | IC ₅₀ ($\mu\text{g}/\text{mL}$) |
|-----------------------------|---|------------------|--|
| Aminoguanidine ^a | 196.08 | 66.85 \pm 0.13 | 143.92 \pm 0.62 |
| | 98.04 | 37.39 \pm 0.52 | |
| | 19.61 | 3.96 \pm 0.06 | |
| Water extract | 196.08 | 29.26 \pm 0.94 | — |
| <i>n</i> -Hexane fr. | 196.08 | 33.94 \pm 0.41 | — |
| Methylene chloride fr. | 196.08 | 54.03 \pm 1.00 | 186.72 \pm 2.05 |
| | 98.04 | 21.52 \pm 0.64 | |
| | 19.61 | 3.66 \pm 0.23 | |
| Ethyl acetate fr. | 196.08 | 68.31 \pm 1.06 | 141.34 \pm 1.27 |
| | 98.04 | 37.36 \pm 0.89 | |
| | 19.61 | 4.92 \pm 0.29 | |
| <i>n</i> -Butanol fr. | 196.08 | 40.47 \pm 0.68 | — |
| Water fr. | 196.08 | 30.24 \pm 1.01 | — |

Inhibition rates were calculated as percentages with respect to the control value. ^aAminoguanidine was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

uninhibited enzyme and the 3 different concentrations for each compound. The results indicated that the inhibition type of rhAR by rosmarinic acid (4) and caffeic acid ethylene ester (5) was noncompetitive, showing that the inhibitor was unable to bind to either the substrate-binding region or the NADPH-binding region of rhAR.

3.4. Galactitol Accumulation Inhibitory Activity by Active Compounds. We also studied the effects of compounds 4 and 5 on galactitol accumulation in rat erythrocytes and lenses (Table 3). Galactitol accumulation was 23-fold greater when incubated in a galactose-free medium. Rat lens incubation for 6 d in 30 mM galactose resulted in an increased intracellular accumulation of galactitol. Compounds 4 and 5 inhibited galactitol accumulation in rat erythrocyte by almost 35.5 and 31.7% at 5 $\mu\text{g}/\text{mL}$, respectively. Quercetin

TABLE 5: Inhibitory effects of the compounds isolated from the *P. vulgaris* L. on advanced glycation end products (AGEs).

| Compounds | Concentration ($\mu\text{g}/\text{mL}$) | Inhibition (%) | IC ₅₀ (μM) |
|-----------------------------|---|------------------|------------------------------------|
| Aminoguanidine ^a | 196.08 | 66.85 \pm 0.13 | 1944.86 \pm 8.39 |
| | 98.04 | 37.39 \pm 0.52 | |
| | 19.61 | 3.96 \pm 0.06 | |
| 1 | 19.61 | 9.33 \pm 0.27 | — |
| 2 | 19.61 | — | — |
| 3 | 19.61 | — | — |
| 4 | 19.61 | 20.67 \pm 0.37 | — |
| 5 | 9.8 | 74.81 \pm 1.41 | 33.16 \pm 0.54 |
| | 4.9 | 33.94 \pm 0.94 | |
| | 1.96 | 9.00 \pm 0.43 | |
| 6 | 98.04 | 88.69 \pm 0.56 | 304.36 \pm 3.41 |
| | 49.02 | 63.45 \pm 1.16 | |
| | 19.61 | 9.85 \pm 0.16 | |

Inhibition rates were calculated as percentages with respect to the control value. The IC₅₀ values of each sample were estimated from the least-squares regression line of the logarithmic concentration plotted against inhibitory activity. ^aAminoguanidine was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

TABLE 6: Antioxidant effects of the *P. vulgaris* L. on inhibition of the ABTS^{•+}.

| Fraction | Concentration ($\mu\text{g}/\text{mL}$) | Inhibition (%) | IC ₅₀ ($\mu\text{g}/\text{mL}$) |
|------------------------|---|------------------|--|
| Trolox ^a | 16.67 | 99.44 \pm 0.20 | 3.84 \pm 0.10 |
| | 3.33 | 45.48 \pm 1.20 | |
| | 1.67 | 21.76 \pm 1.15 | |
| Water extract | 33.33 | 84.60 \pm 1.17 | 12.42 \pm 0.47 |
| | 16.67 | 63.67 \pm 1.27 | |
| | 8.33 | 33.98 \pm 1.51 | |
| <i>n</i> -Hexane fr. | 33.33 | 14.73 \pm 0.39 | — |
| | 33.33 | 95.03 \pm 0.65 | |
| | 16.67 | 69.59 \pm 0.84 | |
| Methylene chloride fr. | 8.33 | 43.36 \pm 0.57 | 9.92 \pm 0.16 |
| | 8.33 | 96.99 \pm 0.32 | |
| | 3.33 | 62.39 \pm 0.83 | |
| Ethyl acetate fr. | 1.67 | 30.64 \pm 0.93 | 2.59 \pm 0.05 |
| | 16.67 | 97.66 \pm 0.16 | |
| <i>n</i> -Butanol fr. | 8.33 | 71.54 \pm 1.18 | 5.15 \pm 0.07 |
| | 3.33 | 31.36 \pm 0.62 | |
| Water fr. | 33.33 | 42.86 \pm 1.63 | — |

Inhibition rates were calculated as percentages with respect to the control value. ^aTrolox was used as positive control. Inhibitory effect was expressed as mean \pm SD of triplicate experiments.

(the positive control) inhibited galactitol accumulation in rat erythrocyte by 30.5% and in rat lenses by almost 36.9 and 22.5% at 5 $\mu\text{g}/\text{mL}$, respectively. Quercetin (the positive control) inhibited galactitol accumulation in rat lenses by 46.8%. The culture medium containing a high galactose concentration reduced the galactitol level.

TABLE 7: Antioxidant effects of the compounds isolated from the *P. vulgaris* L. on inhibition of the ABTS^{•+}.

| Compounds | Concentration (μg/mL) | Inhibition (%) | IC ₅₀ (μM) |
|---------------------|-----------------------|----------------|-----------------------|
| Trolox ^a | 16.67 | 99.44 ± 0.20 | 15.34 ± 0.40 |
| | 3.33 | 45.48 ± 1.20 | |
| | 1.67 | 21.76 ± 1.15 | |
| 1 | 3.33 | 75.14 ± 1.08 | 12.03 ± 0.24 |
| | 1.67 | 34.65 ± 1.03 | |
| | 0.33 | 11.33 ± 0.31 | |
| 2 | 3.33 | 93.42 ± 0.58 | 11.59 ± 0.22 |
| | 1.67 | 53.79 ± 0.92 | |
| | 0.33 | 14.90 ± 0.34 | |
| 3 | 8.33 | 74.05 ± 0.69 | 5.94 ± 1.01 |
| | 3.33 | 59.65 ± 1.39 | |
| | 1.67 | 47.60 ± 1.35 | |
| 4 | 3.33 | 78.40 ± 1.17 | 8.79 ± 0.10 |
| | 1.67 | 36.50 ± 0.78 | |
| | 0.33 | 9.93 ± 0.27 | |
| 5 | 3.33 | 84.68 ± 1.41 | 10.52 ± 0.28 |
| | 1.67 | 50.17 ± 1.75 | |
| | 0.33 | 13.00 ± 0.13 | |
| 6 | 3.33 | 92.80 ± 1.50 | 12.39 ± 0.33 |
| | 1.67 | 52.73 ± 0.93 | |
| | 0.33 | 13.73 ± 0.37 | |

Inhibition rate was calculated as percentage with respect to the control value. The IC₅₀ values of each sample were estimated from the least-squares regression line of the logarithmic concentration plotted against inhibitory activity. ^aTrolox was used as positive control. Inhibitory effect was expressed as mean ± SD of triplicate experiments.

3.5. Advanced Glycation End Production Inhibitory Activity of *P. vulgaris*. The present study tested for inhibitory effects on BSA glycation with a fluorescence method. The EtOAc fraction had high inhibitory activity against AGE with an IC₅₀ value of 141.34 ± 1.27 μg/mL, showing almost the same activity with positive control (Table 4). Inhibitory activity against AGE formation by the isolated compounds was tested using aminoguanidine as a positive control. Caffeic acid ethylene ester (5) exhibited potent inhibitory activity against AGE formation with an IC₅₀ value of 33.16 ± 0.54 μM, compared with the positive control IC₅₀ value of 1944.86 ± 8.39 μM (Table 5).

3.6. Antioxidant Activity of *P. vulgaris*. Oxidative stress might have a key role in the pathogenesis of vascular complications in diabetes, both microvascular and macrovascular, and it provides an early marker of such damage in the development of endothelial dysfunction [33, 34]. The EtOAc fraction showed high activity in the ABTS^{•+} assay. ABTS^{•+} inhibitory activity of the isolated compounds was tested using trolox as the positive control. Compounds 1–6 all exhibited potent antioxidant inhibitory activity. *p*-Hydroxycinnamic acid (3) had the strongest inhibitory activity with an IC₅₀ value of

5.94 ± 1.01 μM, and caffeic acid ethylene ester (5) also had potent inhibitory activity with an IC₅₀ value of 10.52 ± 0.28 μM, compared to a positive control IC₅₀ value of 15.34 ± 0.40 μM (see Tables 6 and 7).

4. Conclusions

The present study isolated six compounds from *P. vulgaris* by using a medium pressure liquid chromatography. Among these compounds, caffeic acid ethylene ester (5) has potent AR, AGE inhibitory activity, and antioxidant. Specifically, this compound exhibited the most potent inhibition with AGE, with an IC₅₀ of 33.16 ± 0.54 μM. The inhibitory effect of this compound was 58.0-fold stronger than that of the positive control aminoguanidine (IC₅₀ = 1944.86 ± 8.39 μM). These results suggest that this compound and *P. vulgaris* could potentially provide a new natural treatment for diabetic complications. However, further studies on the mechanism of AGE action of caffeic acid ethylene ester (5) and more *in vivo* evidence from diabetic animals are required.

Conflict of Interests

The authors declare no conflict of interests.

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References

- [1] H. I. Moon, J. C. Jung, and J. Lee, "Aldose reductase inhibitory effect by tectorigenin derivatives from *Viola hondoensis*," *Bioorganic and Medicinal Chemistry*, vol. 14, no. 22, pp. 7592–7594, 2006.
- [2] E. C. Leal, A. R. Santiago, and A. F. Ambrósio, "Old and new drug targets in diabetic retinopathy: from biochemical changes to inflammation and neurodegeneration," *Current Drug Targets: CNS and Neurological Disorders*, vol. 4, no. 4, pp. 421–434, 2005.
- [3] J. H. Kinoshita, "Mechanisms initiating cataract formation," *Investigative Ophthalmology*, vol. 13, no. 10, pp. 713–724, 1974.
- [4] D. R. Tomlinson, E. J. Stevens, and L. T. Diemel, "Aldose reductase inhibitors and their potential for the treatment of diabetic complications," *Trends in Pharmacological Sciences*, vol. 15, no. 8, pp. 293–297, 1994.
- [5] R. Singh, A. Barden, T. Mori, and L. Beilin, "Advanced glycation end-products: a review," *Diabetologia*, vol. 44, no. 2, pp. 129–146, 2001.
- [6] N. Ahmed, "Advanced glycation endproducts—role in pathology of diabetic complications," *Diabetes Research and Clinical Practice*, vol. 67, no. 1, pp. 3–21, 2005.

- [7] B. Halliwell and J. M. C. Gutteridge, "Role of free radicals and catalytic metal ions in human disease: an overview," *Methods in Enzymology*, vol. 186, pp. 1–85, 1990.
- [8] A. K. Saxena, P. Srivastava, R. K. Kale, and N. Z. Baquer, "Impaired antioxidant status in diabetic rat liver. Effect of vanadate," *Biochemical Pharmacology*, vol. 45, no. 3, pp. 539–542, 1993.
- [9] L. Wirasathien, T. Pengsuparp, R. Suttisri, H. Ueda, M. Moriyasu, and K. Kawanishi, "Inhibitors of aldose reductase and advanced glycation end-products formation from the leaves of *Stelechocarpus cauliflorus* R.E. Fr.," *Phytomedicine*, vol. 14, no. 7-8, pp. 546–550, 2007.
- [10] J. Pšotová, M. Kolář, J. Soušek, Z. Švagera, J. Vičar, and J. Ulrichová, "Biological activities of *Prunella vulgaris* extract," *Phytotherapy Research*, vol. 17, no. 9, pp. 1082–1087, 2003.
- [11] H. X. Xu, S. H. S. Lee, S. F. Lee, R. L. White, and J. Blay, "Isolation and characterization of an anti-HSV polysaccharide from *Prunella vulgaris*," *Antiviral Research*, vol. 44, no. 1, pp. 43–54, 1999.
- [12] X. Fang, M. M. Yu, W. H. Yuen, S. Y. Zee, and R. C. Chang, "Immune modulatory effects of *Prunella vulgaris* L. on monocytes/macrophages," *International Journal of Molecular Medicine*, vol. 16, no. 6, pp. 1109–1116, 2005.
- [13] L. Feng, X. B. Jia, F. Shi, and Y. Chen, "Identification of two polysaccharides from *Prunella vulgaris* L. and evaluation on their anti-lung adenocarcinoma activity," *Molecules*, vol. 15, no. 8, pp. 5093–5103, 2010.
- [14] S. Y. Ryu, M. H. Oak, S. K. Yoon et al., "Anti-allergic and anti-inflammatory triterpenes from the herb of *Prunella vulgaris*," *Planta Medica*, vol. 66, no. 4, pp. 358–360, 2000.
- [15] S. Hayman and J. H. Koshita, "Isolation and properties of lens aldose reductase," *The Journal of Biological Chemistry*, vol. 240, no. 2, pp. 877–882, 1965.
- [16] Y. S. Lee, Y. H. Kang, J. Y. Jung et al., "Inhibitory constituents of aldose reductase in the fruiting body of *Phellinus linteus*," *Biological and Pharmaceutical Bulletin*, vol. 31, no. 4, pp. 765–768, 2008.
- [17] S. S. Lim, Y. J. Jung, S. K. Hyun, Y. S. Lee, and J. S. Choi, "Rat lens aldose reductase inhibitory constituents of *Nelumbo nucifera* stamens," *Phytotherapy Research*, vol. 20, no. 10, pp. 825–830, 2006.
- [18] K. Kawanishi, H. Ueda, and M. Moriyasu, "Aldose reductase inhibitors from the nature," *Current Medicinal Chemistry*, vol. 10, no. 15, pp. 1353–1374, 2003.
- [19] J. A. de la Fuente and S. Manzanaro, "Aldose reductase inhibitors from natural sources," *Natural Product Reports*, vol. 20, no. 2, pp. 243–251, 2003.
- [20] S. Kwang-Hyok, P. Ui-Nam, C. Sarkar, and R. Bhadra, "A sensitive assay of red blood cell sorbitol level by high performance liquid chromatography: potential for diagnostic evaluation of diabetes," *Clinica Chimica Acta*, vol. 354, no. 1-2, pp. 41–47, 2005.
- [21] R. Shinohara, Y. Ohta, M. Yamauchi, and I. Ishiguro, "Improved fluorometric enzymatic sorbitol assay in human blood," *Clinica Chimica Acta*, vol. 273, no. 2, pp. 171–184, 1998.
- [22] C. Lee, M. B. Yim, P. B. Chock, H. S. Yim, and S. O. Kang, "Oxidation-reduction properties of methylglyoxal-modified protein in relation to free radical generation," *The Journal of Biological Chemistry*, vol. 273, no. 39, pp. 25272–25278, 1998.
- [23] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, no. 9-10, pp. 1231–1237, 1999.
- [24] W. H. Park, S. H. Kim, and C. H. Kim, "A new matrix metalloproteinase-9 inhibitor 3,4-dihydroxycinnamic acid (caffeic acid) from methanol extract of *Euonymus alatus*: isolation and structure determination," *Toxicology*, vol. 207, no. 3, pp. 383–390, 2005.
- [25] I. P. Gerathanassis, V. Exarchou, V. Lagouri, A. Troganis, M. Tsimidou, and D. Boskou, "Methodology for identification of phenolic acids in complex phenolic mixtures by high-resolution two-dimensional nuclear magnetic resonance. Application to methanolic extracts of two oregano species," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 10, pp. 4185–4192, 1998.
- [26] X. J. He and R. H. Liu, "Cranberry phytochemicals: isolation, structure elucidation, and their antiproliferative and antioxidant activities," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 19, pp. 7069–7074, 2006.
- [27] Y. Nakamura, Y. Ohto, A. Murakami, and H. Ohigashi, "Superoxide scavenging activity of rosmarinic acid from *Perilla frutescens* Britton var. *acuta* f. *viridis*," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 11, pp. 4545–4550, 1998.
- [28] X. M. Niu, S. H. Li, Z. Na, S. X. Mei, Q. S. Zhao, and H. D. Sum, "Studies on chemical constituents of *Isodon eriocalyx* var. *laxiflora*," *Chinese Traditional and Herbal Drugs*, vol. 34, no. 4, pp. 300–304, 2003.
- [29] T. S. Martin, H. Kikuzaki, M. Hisamoto, and N. Nakatani, "Constituents of *amomum tsao-ko* and their radical scavenging and antioxidant activities," *Journal of the American Oil Chemists' Society*, vol. 77, no. 6, pp. 667–673, 2000.
- [30] H. A. Jung, N. Y. Yoon, S. S. Kang, Y. S. Kim, and J. S. Choi, "Inhibitory activities of prenylated flavonoids from *Sophora flavescens* against aldose reductase and generation of advanced glycation endproducts," *Journal of Pharmacy and Pharmacology*, vol. 60, no. 9, pp. 1227–1236, 2008.
- [31] H. Matsuda, T. Morikawa, I. Toguchida, and M. Yoshikawa, "Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity," *Chemical and Pharmaceutical Bulletin*, vol. 50, no. 6, pp. 788–795, 2002.
- [32] C. Koukoulitsa, C. Zika, G. D. Geromichalos, V. J. Demopoulos, and H. Skaltsa, "Evaluation of aldose reductase inhibition and docking studies of some secondary metabolites, isolated from *Origanum vulgare* L. ssp. *hirtum*," *Bioorganic and Medicinal Chemistry*, vol. 14, no. 5, pp. 1653–1659, 2006.
- [33] H. Cai and D. G. Harrison, "Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress," *Circulation Research*, vol. 87, no. 10, pp. 840–844, 2000.
- [34] D. Giugliano, A. Ceriello, and G. Paolisso, "Oxidative stress and diabetic vascular complications," *Diabetes Care*, vol. 19, no. 3, pp. 257–267, 1996.



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