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Aldose Reductase Inhibition by Luteolin Derivatives from *Parasenecio pseudotaimingasa*

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Abstract – Effects of the extract and fractions from *Parasenecio pseudotaimingasa* on rat lens aldose reductase (AR) inhibition have been investigated. Among them, the *n*-BuOH fraction was exhibited good inhibitory potencies (IC_{50} value 1.42 μg/ml). Phytochemical constituents were isolated from the *n*-BuOH fraction by open column chromatography. Their structures were elucidated as luteolin-7-*O*-rutinoside (**1**) and luteolin-7-*O*-glucoside (**2**) on the basis of spectroscopic analysis. Compounds **1** and **2** exhibited strong AR inhibitory activity, with IC_{50} values of 2.37 and 1.05 μM, respectively. This is the first report on the isolation of compounds **1** and **2** from *P. pseudotaimingasa*. These results suggest that *P. pseudotaimingasa* could be a useful material in the development of a novel AR inhibitory agent against diabetic complications.
Keywords – *Parasenecio pseudotaimingasa*, Senecioneae, luteolin-7-*O*-rutinoside, luteolin-7-*O*-glucoside

Introduction

Aldose reductase (AR) is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. This reaction is vital for the function of various organs in the body and for the cataract formation in the lens (Van Heyningen, 1959). The enzyme is located in the eye (cornea, retina, and lens), kidney, myelin sheath, and also in other tissues less involved in the pathogenesis of diabetic complications such as neuropathy (Ward, 1973), nephropathy (Beyer-Mears and Cruz, 1985), and retinopathy (Engerman and Kern, 1984). AR inhibitors can prevent or reverse early abnormalities in diabetic complications. Among the AR inhibitors such as zopolrestat, ponalrestat, sorbinil, tolrestat, epalretat, and ranireatat *etc*., which have been developed with promising results in the past years (Constantino *et al*., 1999; Sun *et al*., 2006; Drel *et al*., 2008; Hotta *et al*., 2006; Matsumoto *et al*., 2008). These AR inhibitors, however, almost all have several problems such as side effects and decrease of effects *etc*. during human clinical trials causing hindrance to development of research (Ziegler, 2004; Chalk *et al*., 2007). Therefore, recently natural sources for AR inhibitors potential are spotlight for the treatment and prevention of diabetic complications due to safer and more effective phytochemicals (Jesús Ángel and Sonia, 2003; Kawanishi *et al*., 2003).

Parasenecio pseudotaimingasa, belonging to the family Senecioneae, is a perennial plant. About 80 species are distributed in Korea, Eastern Asia, Russia and America (Koyama, 1969; Lee *et al.*, 2011a). Young sprouts of *P. pseudotaimingasa* were used as food materials (Lee, 2006). *P. pseudotaimingasa* is designated endangered plant species and the Korean native plant from the Korea Forest Service. *P. pseudotaimingasa* grows to 60 - 100 cm in height, and has lines on the main stem and some hair on the upper part. A leaf on the stem and 27 - 32 cm in diameter circular surface has a few hairs. The leaf edges are divided into palmate, lobes divided into three split, and a petiole is short and like a sheath surrounding the stem. *P. pseudotaimingasa* is flowering in July-August, 7 mm in diameter, a capitulum on the compound raceme at the end of twigs, the peduncle length of 1-6 mm, and the involucres length of 5 and 8 mm width (Lee, 2003; Lee, 2006; Chung *et al.*, 2006).

In previous papers, there are reports on the ecological characteristics in *P. firmus* (Ahn and Kim, 2009; Jin and Ahn, 2010), HPLC analysis of constituents from *Cacalia firma* (Park *et al.*, 2009) and chemical composition of mountainous vegetables (Agung *et al.*, 2011; Lee *et al.*, 2011b). Up to now, there is no report on the isolation and AR activities of *P. pseudotaimingasa*. In the present paper, therefore, as a preliminary step for the evaluations of potential of naturally occurring AR inhibitors, we tested the effects of fraction from *P. pseudotaimingasa* on

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rat lens AR inhibition and isolated phytochemical constituents from the active fraction.

Materials and Methods

Plant materials – *Parasenecio pseudotaimingasa* was collected at Mt. Baekun, Korea. The specimen of *P. pseudotaimingasa* was botanically authenticated by Prof. Y. H. Ahn, Chung-Ang University, Korea. A voucher specimen (No. LEE 2009-08) was deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

Instruments and Reagents – The electron ionization mass spectrometry (EI-MS) was measured with a JEOL JMS-600W (Tokyo, Japan) mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra was recorded with a Bruker AVANCE 500 NMR (Rheinstetten, Germany) spectrometer in DMSO using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz (Hz). TLC analysis was conducted with Kieselgel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 followed by charring at 60 °C. Silica gel (200 - 400 mesh, Merck, Germany) and Sephadex LH-20 (25 - 100 μ, Amersham Biosciences, Sweden). All other chemicals and reagents were analytical grade. Fluorescence was measured with a Hitachi U-3210 spectrophotometer. Solvents such as DL-glyceraldehyde, β-NADPH, sodium phosphate buffer, potassium phosphate buffer, and DMSO (Sigma-Aldrich Chemical Co.) were used for rat lens AR assay.

Extraction and Isolation – The air-dried leaves of *P. pseudotaimingasa* (691.6 g) were extracted with 95% MeOH (10 liters × 3) under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 140.9 g of the residue. The 95% MeOH extract (140.9 g) was suspended in water and then partitioned successively with equal volumes of *n*-hexane (27.8 g), MC (2.9 g), and *n*-BuOH (21.0 g). A portion of the *n*-BuOH fraction (21.0 g) was chromatographed on a silica gel column eluting with a gradient of CH_2Cl_2 - MeOH (100% CH_2Cl_2 up to 100% MeOH) to afford subfraction 12. Subfraction 8 (CH_2Cl_2 - MeOH = 85 : 15) was recrystallization with MeOH to afford compound **1**. And subfraction 9 rechromatographed on sephadex LH-20 eluting with MeOH – water = 50 : 50 to afford compound **2**.

Compound **1** – yellow powder; FAB-MS *m/z*: 595 $[M + H]^+$; ^{1}H- and ^{13}C-NMR (500 MHz, DMSO-d_6): see Table 2.

Table 1. IC_{50} of the extract and fractions of *P. pseudotaimingasa* on rat lens AR inhibition

Sample	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μg/ml)
MeOH extract	10	75.71	3.35
	5	60.00	
	1	21.09	
n-Hexane fraction	10	27.44	–
MC fraction	10	24.20	–
n-BuOH fraction	10	88.15	1.42
	5	74.33	
	0.5	29.83	
Quercetin*	1	73.32	0.47
	0.5	47.91	
	0.1	35.68	

Each sample concentration was 1 mg/ml DMSO
[a] Inhibition rate was calculated as percentage with respect to the control value.
[b] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of at three graded concentrations *vs* % inhibition.
*Quercetin was used as a positive control.

Compound **2** – yellow powder; EI-MS *m/z*: 286 $[M-Glc]^+$; ^{1}H- and ^{13}C-NMR (500 MHz, DMSO-d_6): see Table 2.

Measurement of AR activity – Rat lens was removed from Sprague-Dawley rats (weighing 250 - 280 g) and preserved rat lens by freezing it until use. These were homogenized and centrifuged at 10,000 rpm (4 °C, 20 min) and the supernatant was used as an enzyme source. AR activity was spectrophotometrically determined by measuring the decrease in absorption of NADPH at 340 nm for a 4 min period at room temperature with DL-glyceraldehydes as a substrate (Sato and Kador, 1990). The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 0.1 M sodium phosphate buffer (pH 6.2), 1.6 mM NADPH, and test extract sample (in DMSO) with 0.025 M DL-glyceraldehyde as substrate in quartz cell. Total volume of assay mixture is 1 ml for the test. IC_{50} values, the concentration of inhibitors giving 50% inhibition of enzyme activity, were calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity. Quercetin known as one of typical AR inhibitors was used as a positive control. Each test sample of the MeOH extract and three fractions (each 1.0 mg) were dissolved in DMSO (1 ml).

Results and Discussion

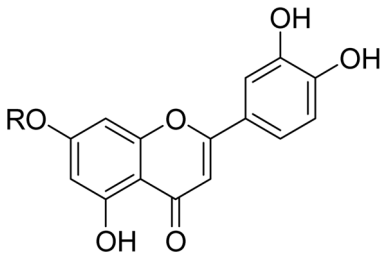
The MeOH extract and three fractions of *P. pseudotaimingasa* were tested for their inhibitory effects on rat

Table 2. ^{1}H- and ^{13}C-NMR spectral data for compounds **1** and **2** in DMSO-d_6

NO.	1		2	
	δ_H	δ_C	δ_H	δ_C
2	–	164.6	–	164.5
3	6.75 s	103.2	6.74 s	103.2
4	–	181.9	–	181.9
5	–	161.2	–	161.1
6	6.45 d (2.0)	99.5	6.44 d (2.0)	99.5
7	–	162.9	–	163.0
8	6.77 d (2.0)	94.8	6.79 d (2.0)	94.7
9	–	156.9	–	156.9
10	–	105.4	–	105.3
1'	–	121.4	–	121.4
2'	7.41 d (2.1)	113.6	7.42 d (1.9)	113.6
3'	–	145.8	–	145.8
4'	–	149.9	–	150.0
5'	6.91 d (8.4)	116.1	6.90 d (8.3)	116.0
6'	7.44 dd (2.1, 8.4)	121.4	7.45 dd (1.9, 8.3)	121.4
5-OH	12.97 s		12.98 s	
Glc-1	5.07 d (7.4)	100.5	5.08 d (7.5)	99.9
Glc-2	–	73.1	–	73.1
Glc-3	–	76.3	–	77.2
Glc-4	–	70.8	–	69.6
Glc-5	–	75.6	–	76.4
Glc-6	–	66.1	–	60.6
Rha-1	4.55 (br s)	99.9		
Rha-2	–	70.3		
Rha-3	–	69.6		
Rha-4	–	72.1		
Rha-5	–	68.3		
Rha-6	1.07 d (6.2)	17.8		

lens AR activity, and the results were summarized in Table 1. The rat lens AR inhibition percentages of the MeOH extracts and *n*-BuOH fraction were 75.21 and 88.15%, respectively. However, *n*-hexane and MC fractions of this plant showed below 50% degree of inhibition on rat lens AR that are supposed to be far less deserving of further consideration (27.44 and 24.20%, respectively). Following these results, we isolated phytochemical constituents from the *n*-BuOH fraction of *P. pseudo-taimingasa* leaves. A chromatographic separation of this active fraction led to the isolation of compounds **1** and **2**.

Compounds **1** and **2** were obtained as yellow powders from the *n*-BuOH fraction. In the ^{1}H-NMR spectra of **1** and **2**, typical 5-OH signals of flavonoid were observed at δ 12.97 - 12.98 (s, OH). Two signals at δ 6.44 - 6.45 (1H, d, J = 2.0 Hz, H-6) and δ 6.77-6.79 (1H, d, J = 2.0 Hz, H-8) indicated the methine signals of flavonoid A-ring. Furthermore, the proton resonances at δ 6.90-6.91 (1H, d, J = 8.3-8.4 Hz, H-5'), 7.41-7.42 (1H, d, J = 1.9-2.1 Hz, H-2') and 7.44-7.45 (1H, dd, J = 1.9-2.1, 8.3-8.4 Hz, H-6') were aromatic protons, suggesting the ABX splitting signals of the skeleton in the B-ring structure. The ^{1}H-NMR data of **1** were similar to those of **2** except for the presence of additional rhamnoside signals. The anomeric protons of glucoside and rhamnoside of **1** were observed at δ 5.07 (d, J = 7.4 Hz) and 4.55 (br s), respectively. The rutinoside position was β-linkage at C-7 of aglycone by HMBC analysis. On the other hand, the anomeric proton of glucoside of **2** was observed at δ 5.08 (d, J = 7.5 Hz). In the ^{13}C-NMR spectra, the carbonyl carbon signals at δ 181.9, anomeric carbon signals at δ 99.9 - 100.5, and typical carbon of rhamnose signal at δ 17.8 were observed. Compounds **1** and **2** had sugar moieties. In the ^{1}H-NMR spectrum of **1**, due to the anomeric proton of glucose and rhamnose at δ 5.08 (d, J = 7.5 Hz) and 5.07 (d, J = 7.4 Hz) of **1**, respectively, the rutinoside position was at C-7 of aglycone by HMBC analysis. On the other hand, due to the anomeric proton of glucose at δ 5.08 (d, J = 7.5 Hz), the glucose position was β-linkage at C-7 of the aglycone by HMBC analysis in compound **2** (Table 2). Accordingly, the structures of **1** and **2** (Fig. 1) were identified as luteolin-7-*O*-rutinoside and luteolin-7-*O*-glucoside, respectively, by comparison of the spectral data as described in the literature (Kim *et al*., 2000; Lee *et al*., 2011c). Luteolin derivatives have antimicrobial and anti-HBV activities (Sousa *et al*., 2006; Mukinda *et al*., 2010; Tian *et al*., 2010).



1: -Glc-Rha

2: -Glc

Fig. 1. Structures of compounds **1** and **2**.

These compounds were tested for AR inhibitory activity and results showed in Table 3. To evaluate the rat lens AR inhibitory activity, their inhibitory percentage and IC_{50} values were calculated. The IC_{50} values of luteolin-7-*O*-rutinoside (**1**) and luteolin-7-*O*-glucoside (**2**) were

Table 3. IC_{50} of the luteolin derivatives from *P. pseudotaimingasa* on rat lens AR inhibition

Compound	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μM)
Luteolin-7-*O*-rutinoside (**1**)	10	79.14	2.37
	5	72.59	
	1	43.81	
Luteolin-7-*O*-glucoside (**2**)	5	72.35	1.05
	1	63.54	
	0.5	46.49	
*Quercetin	1	61.10	1.56
	0.5	56.66	
	0.1	18.33	

Each sample concentration was 1 mg/ml DMSO
[a] Inhibition rate was calculated as percentage with respect to the control value.
[b] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of at three graded concentrations *vs* % inhibition.
*Quercetin was used as a positive control.

demonstrated 2.37 and 1.05 μM, respectively. Particularly, AR inhibition of luteolin-7-*O*-glucoside (**2**) was more potent than quercetin, a positive control (1.56 μM). It was reported that flavonoid, among the single compounds isolated from natural products, have a strong AR inhibitory activity (Lee *et al*., 2008; Jung *et al*., 2004). Previous investigations on the secondary metabolites of *P. pseudotaimingasa* have no revealed the presence of flavonoids.

In conclusion, the BuOH fraction of *P. pseudotaimingasa* was found to demonstrate good inhibitory activity based on *in vitro* data. We suggest that polar fraction of this plant may possibly provide a new natural resource for targeting the inhibition of AR, consequently. Therefore, luteolin-7-*O*-rutinoside (**1**) and luteolin-7-*O*-glucoside (**2**) were isolated from the BuOH fraction of *P. pseudotaimingasa*. Luteolin-7-*O*-glucoside (**2**) is far the better of the two compounds for AR inhibition. To the best of our knowledge, this is the first report on the isolation of compounds **1** and **2** from the plant. And, these results suggest that *P. pseudotaimingasa* could be a useful material in the development of a novel AR inhibitory agent against diabetic complications.

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References

Agung, N., Kim, M.H., Lee, J.H., Kim, J.D., Lee, K.R., Choi, J.S., Yoo, Y.M., and Park, H.J., Polyphenol analysis and peroxynitrite scavenging effect of the extracts from eight Korean mountainous vegetable. *Kor. J. Pharmacogn.* **42**, 38-45 (2011).

Ahn, Y.H. and Kim, Y.H., Distribution characteristics of native *Parasenecio firmus* and *Parasenecio pseudotaimingasa* on floristic region in Korea. *Pro. Kor. Soc. Env. Eco. Con.* **19**, 29-34 (2009).

Beyer-Mears, A. and Cruz, E., Reversal of diabetic cataract by sorbinil, an aldose reductase inhibitor. *Diabetes* **34**, 15-21 (1985).

Chalk, C., Benstead, T.J., and Moore, F., Aldose reductase inhibitors for the treatment of diabetic polyneuropathy. *Cochrane Database Syst. Rev.* **4**, CD004572 (2007).

Chung, G.Y., Nam, G.H., Park, M.S., and Jeong, H.J., Taxonomic study on the genus *Parasenecio* (Compositae) of Korea by the morphology and somatic chromosome numbers. *Korean J. Plant Res.* **19**, 323-330 (2006).

Constantino, L., Rastelli, G., Vianello, P., Cignarella, G., and Barlocco, D., Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. *Med. Res. Rev.* **19**, 3-23 (1999).

Drel, V.R., Pacher, P., Ali, T.K., Shin, J., Julius, U., El-Remessy, A.B., and Obrosova, I.G., Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. *Int. J. Mol. Med.* **21**, 667-676 (2008).

Engerman, R.L. and Kern, T.S., Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* **33**, 97-100 (1984).

Hotta, N., Akanuma, Y., Kawamori, R., Matsuoka, K., Oka, Y., Shichiri, M., Toyata, T., Nakashima, M., Yoshimura, I., Sakamoto, N., and Shigeta, Y., Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on diabetic peripheral neuropathy. *Diabetes Care* **29**, 1538-1544 (2006).

Jesús Ángel, de la F. and Sonia, M., Aldose reductase inhibitors from natural sources. *Nat. Prod. Rep.* **20**, 243-251 (2003).

Jin, Y.H. and Ahn, Y.H., Comparison of ecological characteristics of *Parasenecio firmus* population in Korea and China. *J. Environ. Sci.* **19**, 197-207 (2010).

Jung, S.H., Kang, S.S., Shin, K.H., and Kim, Y.S., Inhibitory effects of naturally occurring flavonoids on the rat lens aldose reductase. *Nat. Prod. Sci.* **10**, 35-39 (2004).

Kawanishi, K., Ueda, H., and Moriyasu, M., Aldose reductase inhibitors from the nature. *Curr. Med. Chem.* **10**, 1353-1374 (2003).

Kim, N.M., Kim, J., Chung, H.Y., and Choi, J.S., Isolation of luteolin-7-*O*-rutinoside and esculetin with potential antioxidant activity from the aerial parts of *Artemisia montana*. *Arch. Pharm. Res*. **23**, 237-239 (2000).

Koyama, H., Taxonomic studieson the tribe Senecioneae of Eastern Asia. II. Enumeration of the species of Eastern Asia., *Mem. Fac. Sci. Kyoto Univ., Ser. Biol*. **2**, 137-183 (1969).

Lee, H.C., Chekar, E.K., and Lim, D.O., The specific plant species and naturalized plants in the area of Naejangsan National Park, Korea. *Kor. J. Env. Eco.* **25**, 267-283 (2011a).

Lee, J.H., Park, A.R., Choi, D.W., Kim, J.D., Kim, J.C., Ahn, J.H., Lee, H.Y., Choe, M., Choi, K.P., Shin, I.C., and Park, H.J., Analysis of chemical compositions and electron-donating ability of 4 Korean wild sannamuls. *Korean J. Medicinal Crop Sci.* **19**, 111-116 (2011b).

Lee, S., Han, S., Kim, H.M., Lee, J.M., Mok, S.Y., and Lee, S., Isolation and identification of phytochemical constituents from *Taraxacum coreanum*. *J. Korean Soc. Appl. Biol. Chem.* **54**, 73-78 (2011c).

Lee, T.B., Coloured Flora of Korea. *Hyangmunsa, Seoul*. p. 344 (2003).

Lee, Y.M., Kim, N.H., Kim, J.M., Kim, Y.S., Jang, D.S., Kim, J.H., Bae,

K.H., and Kim, J.S., Screening of inhibitory effect on aldose reductase of Korean herbal medicines and preventive effect of *Catalpa bignonioides* against xylose-induced lens opacity. *Kor. J. Pharmacogn.* **39**, 165-173 (2008).

Lee, Y.N., New Flora of Korea. *Kyohak Publishing Co. Ltd., Seoul*. p. 314-315 (2006).

Matsumoto, T., Ono, Y., Kurono, M. Kuromiya, A., Nakamura, K., and Bril, V., Ranirestat (AS-3201), a potent aldose reductase inhibitor, reduces sorbitol levels and improves motor nerve conduction velocity in streptozotocin-diabetic rats. *J. Pharmacol. Sci.* **107**, 231-237 (2008).

Mukinda, J.T., Syce, J.A., Fisher, D., and Meyer, M., Effect of the plant matrix on the uptake of luteolin derivatives-containing *Artemisia afra* aqueous-extract in Caco-2 cells. *J. Ethnopharmacol.* **130**, 439-449 (2010).

Park, H.J., Nugroho, A., Lee, J., Kim, J.D., Kim, W.B., Lee, K.R., and Choi, J.S., HPLC analysis of caffeoylquinic acids in the extract of *Cacalia firma* and peroxynitrite scavenging effect. *Kor. J. Pharmacogn.* **40**, 365-369 (2009).

Sato, S. and Kador, P.F., Inhibition of aldehyde reductase by aldose reductase inhibitors. *Biochem. Pharmacol.* **40**, 1033-1042 (1990).

Sousa, A., Ferreira, I.C.F.R., Calhelha, R., Andrade, P.B., Valentão, P., Seabra, R., Estevinho, L., Bento, A., and Pereira, J. A. Phenolics and antimicrobial activity of traditional stoned table olives 'alcaparra'. *Bioorg. Med. Chem.* **14**, 8533-8538 (2006).

Sun, W., Oates, P.J., Coutcher, J.B., Gerhardinger, C., and Lorenzi, M., A selective aldose reductase inhibitor of a new structural class prevents or reverses early retinal abnormalities in experimental diabetic retino pathway. *Diabetes* **55**, 2757-2762 (2006).

Tian, Y., Sun, L.M., Liu, X.Q., Li, B., Wang, Q., and Dong, J.X., Anti-HBV active flavones glucosides from *Euphorbia humifusa* Willd. *Fitoterapia* **81**, 799-802 (2010).

Van Heyningen, R., Formation of polyol by the lens of the rat with sugar cataract. *Nature* **184**, 194-196 (1959).

Ward, J.D., Advance in metabolic disorders (suppl. 2): The polyol pathway in the neuropathy of early diabetes, *Academic Press, New York*. p. 425 (1973).

Ziegler, D., Polyneuropathy in the diabetic patient-up data on pathogenesis and management. *Nephrol. Dial. Transplant.* **19**, 2170-2175 (2004).

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