Natural Product Sciences
17(2) : 130-134 (2011)

Inhibition of Rat Lense Aldose Reductase by Flavonoids from Dandelions

So-Youn Mok, Sullim Lee, Hye Min Kim, Jeong Min Lee, Dong Gu Lee, Young Hee Ahn, Chun-Geon Park[1], Eun Ju Cho[2], and Sanghyun Lee*

Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea
[1]Department of Herbal Crop Research, National Institute of Horticultural & Herbal Science, Eumseong 369-873, Korea
[2]Department of Food Science and Nutrition, Pusan National University, Busan 609-735, Korea

Abstract – The purpose of this study was to evaluate the therapeutic potential of naturally occurring aldose reductase (AR) inhibitors isolated from Korean native plants. The MeOH extract and stepwise polarity fractions of dandelions were tested on rat lens AR inhibition *in vitro*. Of these, the EtOAc fractions from the leaves of dandelions (*Traxacum coreanum*, *T. officinale*, and *T. ohwianum*) exhibited an AR inhibitory activity (IC_{50} values, 2.37, 1.73 and 2.68 μg/ml, respectively). A chromatography of the EtOAc fraction from the leaves of *T. coreanum* led to the further isolation of two flavonoids identified as luteolin and luteolin 7-*O*-glucopyranoside. These compounds exhibited strong AR inhibitory activity, with IC_{50} values of 0.15 and 1.05 μM, respectively. These results suggested that luteolin is a potent AR inhibitor within dandelions and that it could be a useful lead compound in the development of a novel AR inhibitory agent against diabetic complications.
Keywords – Aldose reductase inhibitory activity, Dandelion, Diabetic complications, Luteolin, Luteolin 7-*O*-glucopyranoside

Introduction

Aldose reductase (AR) is the first enzyme in the polyol pathway. These enzymes catalyze the reduction of various sugars to sugar alcohols, including glucose to sorbitol (Carper *et al*., 1995). In a diabetic condition, sufficient glucose can enter the tissues, but this pathway operates to produce sorbitol and fructose. These abnormal metabolic products have been reported to be factors responsible for diabetic complications such as cataracts, retinopathy, neuropathy, and nephropathy (Kato *et al*., 2009). Aldose reductase inhibitors (ARIs) can prevent or reverse early stage diabetic complications. Nevertheless, no ARIs have achieved worldwide use because of limited efficacy or undesirable side-effects. Evaluating natural sources of ARIs potential may lead to the development of safer and more effective agents against diabetic complications (De la Fuente and Manzanaro, 2003).

Dandelions are herbaceous plants belonging to a family Asteraceae, distributed widely in the warmer temperate zones of the Northern hemisphere. These plants are found in fields, roadsides and rural sites (Ahn *et al*., 2003). Dandelions have long been used as medicinal herbs for the treatment of gout, diarrhea, blisters, spleen disorders, and liver complaints (Heo and Wang, 2008). Pogong-young, the root of *T. ohwianum*, has been used since ancient times to treat chronic indigestion and gastritis in countries, such as China and Korea (Yoon, 2008). In North American aboriginal medicine, dandelions are employed for treating arthritic and rheumatic complaints as well as eczema and other skin conditions, and as a mild laxative in popular medicine (Bisset *et al*., 1994). In Turkish popular medicine, dandelions are applied as a laxative, diuretic and potent anti-diabetic therapy (Önal *et al*., 2005). Phytochemical constituents from *T. officinale* include various sesquiterpenes, such as eudesmanolides (Hänsel *et al*., 1980), guaianolides and germacranolide esters (Kisiel and Barszcz, 2000). Furthermore, the presence of various triterpenes and phytosterols, such as taraxasterol, ψ-taraxasterol, taraxasterol acetates and their hydroxy derivatives arnidol and faradiol, α- and β-amyrins, β-sitosterol, β-sitosterol-β-D-glucoside and stigmasterol in *T. officinale* has been demonstrated (Hänsel *et al*., 1980; Akashi *et al*., 1994).

Several flavonoids such as quercetin and quercitrin have been reported to have inhibitory activity against AR (Andrew *et al*., 2008). The goal of this study was to take the preliminary step of evaluating the therapeutic potential

*Author for correspondence
Tel: +82-31-670-4688; E-mail: slee@cau.ac.kr

of naturally occurring ARIs. In doing so, we tested the effects of luteolin and its glycoside, luteolin 7-*O*-glucopyranoside, isolated from dandelions on AR inhibition in rat lenses.

Materials and Methods

Plant materials – *Taraxacum coreanum* and *T. ohwianum* were collected in 2007 from the Westcoast Express Highway and Anseong, Korea and *T. officinale* were collected in 2010 from Chung-Ang University and Anseong, Korea. Voucher specimens of *T. coreanum* (No. LEE 2007-01), *T. ohwianum* (No. LEE 2010-03) and *T. officinale* (No. LEE 2010-04) were deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

General instruments and reagents – Fluorescence was measured with a Hitachi U-3210 (Brisbane, CA). Solvents such as β-NADPH, sodium phosphate buffer, DL-glyceraldehyde potassium phosphate buffer, and DMSO (Sigma-Aldrich, St. Louis, MO) were used for the rat lens AR assay.

Fractionation and sample preparation – The aerial parts of *Taraxacum coreanum*, *T. officinale*, and *T. ohwianum* were extracted with MeOH under reflux (3 h × 5 times). This extract was suspended in distilled water and partitioned with $CHCl_3$, EtOAc, and *n*-BuOH, successively. Each sample (1.0 mg) of the MeOH extract, $CHCl_3$, EtOAc, and *n*-BuOH fractions were dissolved in DMSO (1 ml).

Isolation of compounds 1 and 2 – The aerial parts of *T. coreanum* (1970.2 g) were dried and finely powdered, then extracted with MeOH for 3 h (4 L × 8) under reflux at 65 °C - 75 °C. After filteration and removal of solvent *in vacuo*, the methanol extract (692.8 g) was collected. This extract was suspended in distilled water and partitioned with *n*-hexane (98.8 g), $CHCl_3$ (11.8 g), EtOAc (23.2 g), and *n*-BuOH (25.2 g), successively. A portion of the EtOAc fraction (7.0 g) was further separated by repeated column chromatography (6 × 80 cm, No.7734) with a gradient of *n*-hexane - EtOAc (*n*-hexane up to EtOAc) and EtOAc - MeOH (EtOAc - MeOH mixture of increasing polarity) to yield 16 subfractions. Among them, subfractions 7 (*n*-hexane:EtOAc = 7 : 3) and 12 (EtOAc) led to the isolation of compounds **1** and **2**.

Compound 1: EI-MS *m/z*: 286 $[M]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 6.18 (1H, d, *J* = 2.0 Hz, H-6), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 6.67 (1H, s, H-3), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 7.39 (1H, d, *J* = 1.7 Hz, H-2'), 7.40 (1H, dd, *J* = 1.7, 8.5 Hz, H-6'), 12.97 (1H, s, 5-OH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 163.9 (C-2), 102.9 (C-3), 181.6 (C-4), 161.4 (C-5), 98.8 (C-6), 164.1 (C-7), 93.8 (C-8), 157.3 (C-9), 103.7 (C-10), 119.0 (C-1'), 113.4 (C-2'), 145.7 (C-3'), 149.7 (C-4'), 116.0 (C-5'), 121.5 (C-6').

Compound 2: FAB-MS *m/z*: 449 $[M + H]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 5.08 (1H, d, *J* = 7.2 Hz, H-1", anomeric proton), 6.48 (1H, d, *J* = 2.1 Hz, H-6), 6.76 (1H, s, H-3), 6.79 (1H, d, *J* = 2.1 Hz, H-8), 6.91 (1H, d, *J* = 8.4 Hz, H-5'), 7.42 (1H, d, *J* = 2.4 Hz, H-2'), 7.44 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 12.99 (1H, s, 5-OH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 164.5 (C-2), 103.2 (C-3), 181.9 (C-4), 161.2 (C-5), 99.6 (C-6), 163.0 (C-7), 94.8 (C-8), 157.0 (C-9), 105.4 (C-10), 121.4 (C-1'), 113.6 (C-2'), 145.8 (C-3'), 150.0 (C-4'), 116.0 (C-5'), 121.4 (C-6'), 99.9 (C-1"), 73.1 (C-2"), 77.2 (C-3"), 69.6 (C-4"), 76.4 (C-5"), 60.6 (C-6").

Measurement of AR activity – Rat lenses were removed from Sprague-Dawley rats (weighing 250 - 280 g) and preserved by freezing until use. These were homogenized and centrifuged at 10,000 rpm (4 °C, 20 min) and the supernatant used as the enzyme source. AR activity was spectrophotometrically determined by measuring the decrease in absorption of β-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. Total volume of assay mixture is 1 ml for the test. The reaction occurred in a quartz cell. The concentrations of the test sample that produced 50% inhibition of enzyme activity (IC_{50}) were calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity. Quercetin, which is a known ARI, was used as a positive control.

Results and Discussion

The MeOH extracts of dandelions were tested for their inhibitory effects on rat lens AR activity. The rat lens AR inhibition of the leaf extracts from *T. coreanum*, *T. officinale*, and *T. ohwianum* were 46.50, 48.51 and 47.62%, respectively (Table 1). As shown in Table 1, the leaf extracts of dandelions exhibited a greater inhibitory effect on rat lens AR compared with those extracts from other plant parts. Therefore, stepwise polarity fractions of the MeOH extract from the dandelion leaves were investigated with an *in vitro* evaluation system detecting AR inhibitory activity. The EtOAc fraction of *T. coreanum*,

Table 1. Rat lens AR inhibitory activity for the MeOH extracts from dandelions

Species	Part tested	AR inhibition[a](%)
	Root	22.63
Taraxacum coreanum	Leaf	46.50
	Flower	15.27
	Root	21.50
T. officinale	Leaf	48.51
	Flower	22.95
	Root	9.73
T. ohwianum	Leaf	47.62
	Flower	9.53

Each sample concentration was 1 mg/ml DMSO.
[a]Inhibition rate was calculated as a percentage of the control value.

Table 2. IC_{50} of the fractions of dandelion leaves on rat lens AR

Samples	Fraction tested	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μg/ml)
	$CHCl_3$	10	46.70	-
Taraxacum coreanum	EtOAc	10	80.35	2.37
		5	67.24	
		1	31.03	
	n-BuOH	10	32.38	-
	$CHCl_3$	10	9.46	-
T. officinale	EtOAc	10	81.67	1.73
		5	72.10	
		1	39.08	
	n-BuOH	10	17.68	-
	$CHCl_3$	10	31.39	-
T. ohwianum	EtOAc	10	81.52	2.68
		5	74.12	
		1	20.78	
	n-BuOH	10	30.40	-
Quercetin[c]	-	5	63.10	2.52
		1	33.18	
		0.5	16.99	

[a] Inhibition rate was calculated as a percentage of the control value.
[b] IC_{50} calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.
[c] Quercetin was used as a positive control.

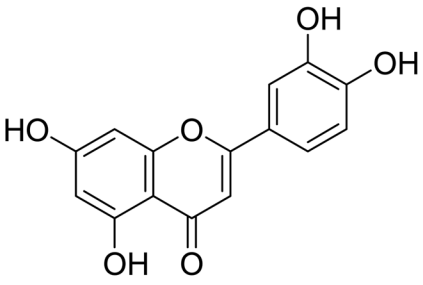
T. officinale, and *T. ohwianum* leaves exhibited significant rat lens AR inhibition, with IC_{50} values of 2.37, 1.73, and 2.68 μg/ml, respectively (Table 2). Quercetin, which is known as a very strong ARI in natural constituents, was used as the positive control.

Further fractionation of the EtOAc fraction from *T. coreanum* by repeated column chromatography gave 16 subfractions. Gradient elution of subfractions 7 and 12 by SiO_2 column chromatography gave two crystalline compounds. Compounds **1** and **2** had similar structural

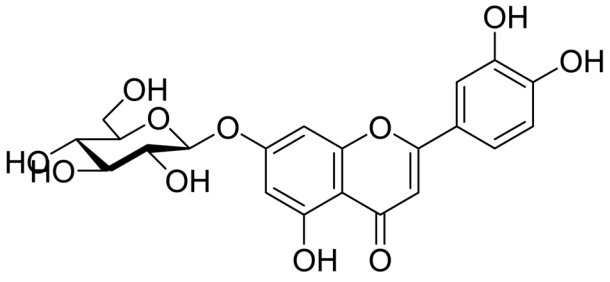
Table 3. IC_{50} of flavonoids from dandelion on rat lens AR

Compounds	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μM)
	1	67.86	
Luteolin	0.5	50.65	0.15
	0.1	23.87	
	5	72.35	
Luteolin 7-*O*-glucopyranoside	1	63.54	1.05
	0.5	46.49	
	5	63.10	
Quercetin[c]	1	33.18	8.35
	0.5	16.99	

[a] Inhibition rate was calculated as a percentage of the control value.
[b] IC_{50} calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.
[c] Quercetin was used as a positive control.



1



2

Fig. 1. Chemical structures of luteolin (**1**) and luteolin 7-*O*-glucopyranoside (**2**).

signals (Fig. 1). In the ^{1}H-NMR spectra of **1** and **2**, the typical flavonoid signals were observed. The proton resonances at δ 6.88 - 6.91 (d, H-5'), 7.39-7.42 (d, H-2') and 7.40-7.44 (dd, H-6') were aromatic protons, suggesting the ABX splitting signals of the skeleton. Two signals at δ 6.18 - 6.48 (d, H-6) and 6.37 - 6.79 (d, H-8) indicated methine signals in the structure. Signals for compound **2**

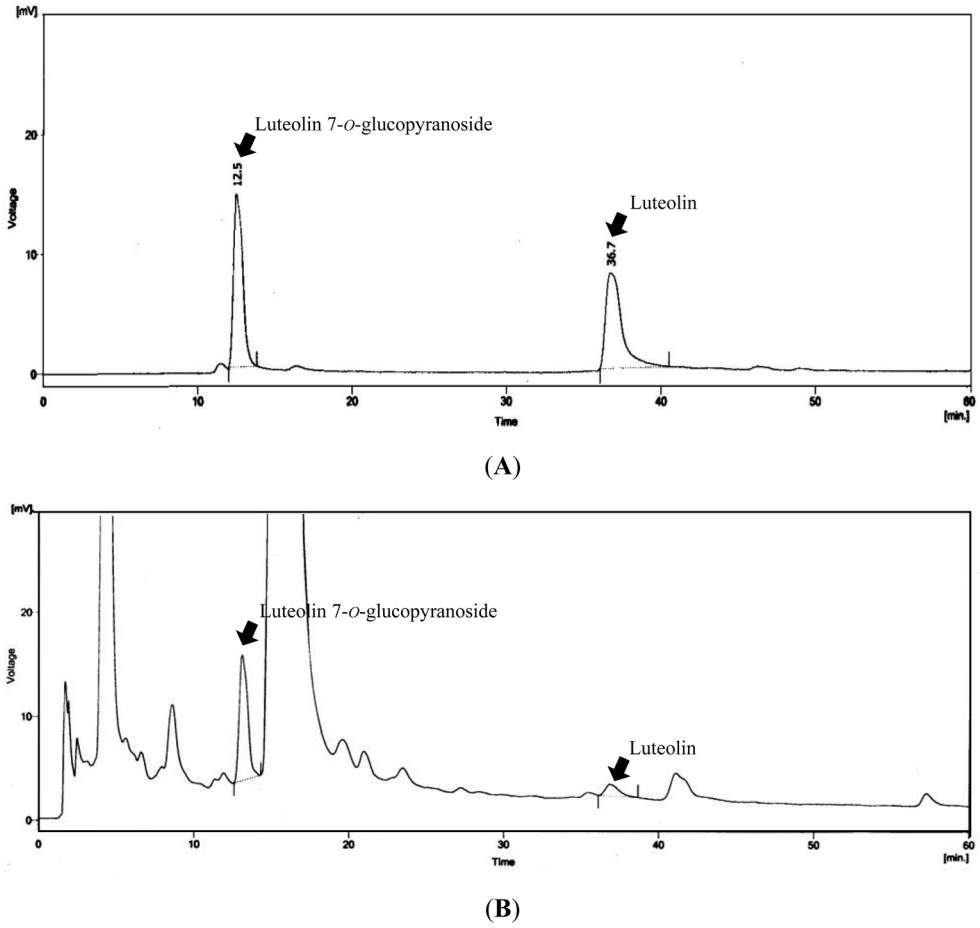


Fig. 2. HPLC Chromatograms of the standards (**A**) and the extract of *T. coreanum* leaves (**B**).

were analogous to the signals of **1**, with one exception; the signal differed by virtue of a glucoside. In the ^{1}H-NMR spectrum of **2**, due to the anomeric proton of glucose shown at δ 5.08 (d, J = 7.2 Hz), the glucose position was shown to be at C-7 (β-linkage) of the aglycon by HMBC. The EI-MS spectrum of **1** showed a molecular ion peak at *m/z* 286, corresponding to a molecular formula of $C_{15}H_{10}O_6$. Accordingly, the structures of **1** and **2** were elucidated to be luteolin (5,7,3',4'-tetrahydroxyflavone) and luteolin 7-*O*-glucopyranoside (5,7,3',4'-tetrahydroxyflavone-7-*O*-glucoside), respectively (Hartwig *et al*., 1990; Shin *et al*., 1995; Jung *et al*., 2004b; Wolbis *et al*., 1993). These flavonoids were confirmed by HPLC. The resolution of all the peaks was obtained using a gradient elution of 10% to 23% acetonitrile within 60 min. The retention times of luteolin 7-*O*-glucopyranoside and luteolin were 12.5 and 36.7 min, respectively. UV detection was obtained at 350 nm (Fig. 2).

These compounds were tested for AR inhibitory activity at three graded concentrations. Results showed that, particularly, AR inhibition of luteolin (**1**) was more potent than that of quercetin, a positive control (Table 3). In addition, luteolin 7-*O*-glucopyranoside (**2**), a flavonoid glycoside, showed lower inhibitory activities than luteolin, a flavonoid aglycon. It was reported that flavonoid and phenol constituents, among the single compounds isolated from natural products, have a strong AR inhibitory activity (Collins and Corder, 1977; De la Fuente and Manzanaro, 2003; Kawanishi *et al*., 2003; Jung *et al*., 2004a; Lee *et al*., 2008; Yawadio *et al*., 2007). Previous investigations on the secondary metabolites of dandelions have revealed the presence of flavonoids (Holiman *et al*., 1996; Williams *et al*., 1996) and phenolic acids (Wolbis *et al*., 1993).

In conclusion, the EtOAc fractions of *T. coreanum*, *T. officinale,* and *T. ohwianum* leaves were found to demonstrate good inhibitory activity based on *in vitro* data. Consequently, we suggest that dandelions such as *T. coreanum*, *T. officinale,* and *T. ohwianum* may possibly provide a new natural resource for therapies targeting the

inhibition of AR. It is postulated that luteolin (**1**) from *Taraxacum* species can also be effective in preventing and/or retarding cataractogenic or diabetic complications. These results demonstrate that dandelions containing luteolin (**1**) have the potential to prevent diabetic complications through the inhibition of AR.

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0023088).

References

Ahn, Y.H., Park, D.S., and Chung, K.Y., Analysis of genetic relationship among native *Taraxacum* and naturalized *Taraxacum* species using RAPD. *Korean J. Environment. Ecol.* **14**, 265-269 (2003).

Akashi, T., Furuno, T., Takahashi, T. and Ayabe, S.I., Biosynthesis of triterpenoids in cultured cells, and regenerated and wild plant organs of *Taraxacum officinale*. *Phytochemistry*. **36**, 303-308 (1994).

Andrew, G.M., Duchowicz, P.R., Fernandez, F.M., Castro E.A., Bennardi, D.O., Autino, J.C. and Romanelli, G.P., QSAR prediction of inhibition of aldose reductase for flavonoids. *Bioorg. Med. Chem.* **16**, 7470-7476 (2008).

Bisset, N.G., Phillipson, J.D., Czygan, F.C., Frohne, D., Höltzel, D., Nagell, A., Pfander, H.J., Willuhn, G. and Buff, W., Herbal drugs and phytopharmaceuticals: *A Handbook for Practice on a Scientific Basis. CRC Press, Boca Raton, Ann Arbor, London, Tokyo*. p. 486-489, (1994).

Carper, D.A., Hohman, T.C., and Old, S.E., Residues affecting the catalysis and inhibition of rat lens aldose reductase. *Biochim. Biophys. Acta*. **1246**, 67-63 (1995).

Collins, J.G. and Corder, C.N., Aldose reductase and sorbitol dehydrogenase distribution in substructures of normal and diabetic rat lens. *Invest. Ophthamol. Vis. Sci*. **16**, 242-243 (1977).

De la Fuente, J.A. and Manzanaro, S., Aldose reductase inhibitors from natural sources. *Nat. Prod. Rep*. **20**, 243-251 (2003).

Hänsel, R., Kartarahardja, M., Huang, J.T. and Bohlmann, F., Sesquiterpenlacton-β-D-glucopyranoside sowie ein neues eudesmanolide aus *Taraxacum officinale*. *Phytochemistry*. **19**, 857-861 (1980).

Hartwig, U.A., Maxwell, C.A., Joseph, C.M., and Phillips, D.A., Chrysoeriol and luteolin released from Alfalfa seeds induce nod genes in *Rhizobium meliloti*. *Plant Physiol*. **92**, 116-122 (1990).

Heo, S.I. and Wang, M.H., Antioxidant activity and cytotoxicity effect of extracts from *Taraxacum mongolicum* H. *Kor. J. Pharmacogn.* **39**, 255-259 (2008).

Holiman, P.C.H., Hertog, M.G.L., and Katan, M.B., Analysis and health effects of flavonoids. *Food Chem*. **57**, 43-46 (1996).

Jung, M.J., Kang, S.S., Jung, H.A., Kim, G.J., and Choi, J.S., Isolation of flavonoids and a cerebroside from the stem bark of *Albizzia julibrissin*. *Arch. Pharm. Res*. **7**, 593-599 (2004a)

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 (2004b).

Kato, A., Yasuko, H., Goto, H., Hollinshead, J., Nash, R.J., and Adachi, I., Inhibitory effect of rhetsinine isolated from *Evodia rutaecarpa* on aldose reductase activity. *Phytomedicine*. **16**, 258-261 (2009).

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

Kisiel, W. and Barszcz, B., Further sesquiterpenoids and phenolics from *Taraxacum officinale*. *Fitoterapia*. **71**, 269-273 (2000).

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).

Önal, S., Timur, S., Okutucu, B., and Zihnioglu, F., Inhibition of α-glucosidase by aqueous extract of some potent antidiabetic medicinal herbs. *Prep. Biochem. Biotechnol*. **35**, 29-36 (2005).

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

Shin, K.H., Kang, S.S., Seo, E.A., and Shin, S.W., Isolation of aldose reductase inhibitors from the flowers of *Chrysanthemum Boreale*. *Arch. Pharm. Res*. **18**, 65-68 (1995).

Williams, C.A., Goldstone, F., and Greenham, J., Flavonoids, cinnamic acids and coumarins from the different tissues and medicinal preparations of *Taraxacum officinale*. *Phytochemistry*. **42**, 121-127 (1996).

Wolbis, M., Krolikowska, M., and Bednarek, P., Polyphenolic compounds in *Taraxacum officinale*. *Acta Poloniae Pharm. Drug Res*. **50**, 153-159 (1993).

Yawadio, R., Tanimori, S., and Morita, N., Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. *Food Chem*. **101**, 1616-1625 (2007).

Yoon, T.J., Effect of water extracts from root of *Taraxacum officinale* on innate and adaptive immune responses in mice. *Kor. J. Food Nutr*. **21**, 275-282 (2008).

Received February 28, 2011
Revised May 11, 2011
Accepted May 15, 2011