Screening of aldose reductase inhibitory activity of white-color natural products

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화이트 칼라소재의 알도즈 환원효소 억제작용 탐색

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Abstract : The purpose of this study was to evaluate the potential of naturally occurring aldose reductase (AR) inhibitors from white-color natural products (*Aruncus dioicus* var. *kamtschaticus*, *Chionanthus retusa*, *Cosmos bipinnatus*, *Hibiscus syriacus*, *Hydrangea paniculata*, *Magnolia denudata*, *Prunus padus*, *Robinia pseudo-accacia*, *Rhododendron mucronulatum* for. *albiflorum*, *Spiraea blumei*, and *Spiraea prunifolia* var. *simpliciflora*). The MeOH extract of white-color natural products were tested on rat lens AR inhibition *in vitro*. Among them, the MeOH extract of *R. mucronulatum* for. *albiflorum* showed highest inhibition on AR (IC_{50} value, 1.07 μg/ml). These results suggested that *R. mucronulatum* for. *albiflorum*, a white-color natural product, could be a useful resource in the development of a novel AR inhibitory agent against diabetic complications.

Key words **:** Aldose reductase inhibition, Diabetic complications, *Rhododendron mucronulatum* for. *albiflorum*, White-color natural product

I. 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). 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 (Engerman and Kern, 1984), neuropathy (Ward, 1973), and nephropathy (Beyer-Mears *et al.*, 1984; 1985; Kato *et al.*, 2009). AR inhibitors (ARIs) can prevent or reverse early stage diabetic complications.The AR inhibitors such as zopolrestat, ponalrestat, sorbinil, tolrestat, epalretat, and ranireatat have been developed with promising results in the past years (Constantino *et al.*, 1999; Drel *et al.*, 2008; Hotta *et al.*, 2006; Matsumoto *et al.*, 2008; Sun *et al.*, 2006). However, no ARIs have achieved worldwide use because of limited efficacy or undesirable side-effects (Chalk *et al.*, 2007; Ziegler, 2004). 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).

There are many flowers from white-color natural

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products. White-color natural products such as *Magnolia denudata* and *Hibiscus syriacus* used as traditional medicine are commonly used as medicinal herbs with a long history of clinical application in many Asian countries for symptomatic management of allergic rhinitis, sinusitis and headache. Various biologically active compounds such as eudesmin, magnolin, epimagnoli, neolignans, lignans, phenyl propanoids, sesquiterpenes, saponarin, vitexin, rhamnosylvitexin, and alkaloids have been also isolated from white-color natural products (Kelm and Nair, 2000; Seo, 2010; Shen *et al.*, 2008; Yoo *et al.*, 1996).

In a series of investigations to evaluate potential ARIs from the natural products, we have shown that some MeOH extracts from herbal medicines exhibited a significant inhibition of AR *in vitro* (Kim *et al.*, 2010; Mok *et al.*, 2011a) and a number of flavonoids compounds were isolated and characterized as ARIs from natural products (Mok *et al.*, 2011a; Mok *et al.*, 2011b).

In the present paper, as a preliminary step for the evaluations of potential of naturally occurring ARIs, we tested the effects of the flowers from white-color natural products on rat lens AR inhibition.

II. Materials and methods

1. General instruments and reagents

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.

2. Plant materials

The flower of *Rhododendron mucronulatum* for. *albiflorum* was collected at Mt. Chilgap in 2009, Korea.

3. Sample preparation

The flowers of *R. mucronulatum* for. *albiflorum* (2.3 kg) were dried and finely powdered, then extracted with MeOH for 3 h (6 L × 5) under reflux at 65-75℃. After filteration and removal of solvent *in vacuo*, the MeOH extract (255.1 g) was collected. The MeOH extract of ten white color flowers (*Aruncus dioicus* var. *kamtschaticus*, *Chionanthus retusa*, *Cosmos bipinnatus*, *Hibiscus syriacus*, *Hydrangea paniculata*, *Magnolia denudata*, *Prunus padus*, *Robinia pseudo-accacia*, *Spiraea blumei*, and *Spiraea prunifolia* var. *simpliciflora*) were purchased from Korea Plant Extract Bank, KRIBB, Korea.

4. Measurement of AR activity

Rat lenses were removed from Sprague-Dawley male rats (weighing 250 - 280 g, 7 weeks) and preserved rat lenses by freezing it until use. These were hom-ogenized and centrifuged at 10,000 rpm (4℃, 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. Each sample (1.0 mg) of the MeOH extract was dissolved in DMSO (1 ml) for AR activity test. Total volume of assay mixture is 1 ml for the test. The reaction occurred in a quartz cell. 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.

III. Results and discussion

The MeOH extracts from white-color natural products were tested for their inhibitory effects on rat lens AR, and summarized in Table 1. Among white-color natural products tested, the MeOH extracts from *A. dioicus* var. *kamtschaticus*, *C. retusa*, *C. bipinnatus*, *M. deundata*, *P. padus*, *R. mucronulatum* for. *albiflorum* and *S. prunifolia* var. *simpliciflora* were demonstrated to show good inhibitory potencies on rat lens AR. Among them, the MeOH extracts of *A. dioicus* var. *kamtschaticus*, *C. bipinnatus*, *P. padus*, *R. mucronulatum* for. *albiflorum* were exhibited high degree of inhibition (〉 70% at 10 μg/ml) on rat lens AR, compared with other samples. The extract of *R. mucronulatum* for. *albiflorum*, *C. bipinnatus*, and *P. padus* was particularly exhibited highest inhibitory value of 85.1, 81.8 and 83.4 % on rat lens AR.

To evaluate the rat lens AR inhibitory activity, their inhibitory percentage and IC_{50} values were calculated. Quercetin known as a very strong AR inhibitor (IC_{50} value, 0.47 μg/ml) was used as a positive control and the results were indicated in Table 2. The IC_{50} values of the extracts of *A. dioicus* var. *kamtschaticus*, and *P. padus* were demonstrated 2.95 and 4.28 μg/ml, respectively. The extracts of *C. bipinnatus* also had inhibitory activity with IC_{50} value 1.87 μg/ml. In particular, *R. mucronulatum* for. *albiflorum* extracts had predominant inhibitory activities with IC_{50} value 1.07 μg/ml, comparable to that of the positive control, quercetin.

R. mucronulatum flower and their leaves have long been known to have high pharmacological potency such as tonic, diuretic and stomachic in Chinese medicine (Chung and Lee, 1991). Koreans have been using the *R. mucronulatum* flower to make wine, honeyed flower juice, and flower-patterned griddle cakes (Lee *et al.*, 2007). Home-made *R. mucronulatum* flower wine showed a significant antioxidant activity in an *in vitro* fatty acid peroxidation assay and which improves blood circulation and decreases cholesterol levels (An *et al.*, 2005; Cho *et al.*, 2008; Chung *et al.*, 1996). The flavonoid and simple phenol contents have already been reported for *R. mucronulatum* flowers, leaves, and stems (Cho *et al.*, 2008; Chung *et al.*, 1996; Lee *et al.*, 2005; Li *et al.*, 2008). In additions, several flavonoids such as quercetin and quercitrin have been reported to have inhibitory activity against AR (Andrew *et al.*, 2008).

To the best of our knowledge, the white-color flowers of *R. mucronulatum* for. *albiflorum* was found to demonstrate high inhibitory activities on AR from *in vitro* data. Therefore, we suggest that white-color natural products such as *R. mucronulatum* for. *albiflorum* has a possibility of new natural resources for the inhibition of AR. As a result, it can be used to study the preliminary data for new active substances. Further investigations on the bioactivity of constituents from

Table 1. Rat lens AR inhibitory activity of the MeOH extracts from white-color natural products.

Sample	Inhibition (%)
Aruncus dioicus var. *kamtschaticus*	72.6
Chionanthus retusa	65.5
Cosmos bipinnatus	81.8
Hibiscus syriacus	31.7
Hydrangea paniculata	35.6
Magnolia denudata	54.2
Prunus padus	83.4
Rhododendron mucronulatum for. *albiflorum*	85.1
Robinia pseudo-accacia	29.6
Spiraea blumei	40.0
Spiraea prunifolia var. *simpliciflora*	50.8

Inhibition rate was calculated as percentage with respect to the control value.

Table 2. IC_{50} values of the MeOH extract from white-color natural products on rat lens AR inhibition.

Sample	Concentration (μg/ml)	Inhibition (%)	IC_{50}[a)] (μg/ml)
Aruncus dioicus var. *kamtschaticus*	10	72.6	2.95
	5	60.1	
	1	29.7	
Chionanthus retusa	10	65.5	4.53
	5	52.4	
	1	19.8	
Cosmos bipinnatus	10	81.8	1.87
	5	67.9	
	1	38.4	
Magnolia denudata	10	54.2	8.29
	5	42.9	
	1	16.3	
Prunus padus	10	83.4	4.28
	5	58.7	
	1	27.5	
Rhododendron mucronulatum for. *albiflorum*	10	85.1	1.07
	5	78.9	
	1	48.0	
Spiraea prunifolia var. *simpliciflora*	10	50.8	9.58
	5	32.3	
	1	10.9	
*Quercetin	1	73.3	0.47
	0.5	47.9	
	0.1	35.7	

[a)]IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of three graded concentrations vs % inhibition.
*Quercetin was used as a positive control.

R. mucronulatum for. *albiflorum* may prove the use of new medicinal plants for the prevention of diabetic complications.

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