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# Aldose Reductase Inhibitory Activity of *Scrophularia* Species

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**Abstract** – Effects of the species *Scrophularia takesimensis*, *S. kakudensis*, *S. boreali-koreana*, and *S. buergeriana* on rat lens aldose reductase inhibition have been investigated. Among them, the extracts of *S. kakudensis* and *S. boreali-koreana* were exhibited good inhibitory potencies compared with those of *S. takesimensis* and *S. buergeriana*. The $IC_{50}$ values of the aerial part extracts from *S. kakudensis* and *S. boreali-koreana* were demonstrated 0.46 and 0.35 mg/ml, respectively.
**Keywords** – *Scrophularia* species, rat lens aldose reductase, diabetic complications

## 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 *et al*., 1984; 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 phyto-chemicals (Jesús Ángel and Sonia, 2003; Kawanishi *et al*., 2003).

The genus *Scrophularia* of the family Scrophulariaceae comprises about 300 species of herbaceous flowering plants and these are found throughout the Northern Hemisphere, but concentrated in Asia with only a few species in Europe and North America (Chung and Shin, 1990). The dried roots of *Scrophularia* species have been used in Asian medicine as a treatment for fever, laryngitis, swelling, constipation, neuritis, and pharyngitis (Qian *et al*., 1992; Park *et al*., 2003). Five types of *Scrophularia* species such as *S. takesimensis*, *S. kakudensis*, *S. koraiensis*, *S. boreali-koreana*, and *S. buergeriana* naturally grow in Korea (Ahn, 2005). Among these, *S. buergeriana* has been cultivated and used as a medicinal plant for diverse purposes while other species have been grown wild.

In present study, as a preliminary step for the evaluations potential of naturally occurring AR inhibitors, we tested the effects of *Scrophularia* species (*S. takesimensis*, *S. kakudensis*, *S. boreali-koreana*, and *S. buergeriana*) on rat lens AR inhibition.

## Experimental

**Plant materials** – The extracts of *S. kakudensis* and *S. boreali-koreana* were purchased from PEB (Plant Extract Bank) of KRIBB in Deajeon, Korea. *S. takesimensis* was collected at Ulleung Island, Korea and *S. buergeriana* was purchased from Kyungdong market, Seoul, Korea. The specimens of *S. takesimensis* and *S. buergeriana* were

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botanically authenticated by Prof. Y. H. Ahn, Chung-Ang University, Korea.

**General instruments and Reagents** – Fluorescence analysis 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 Sample preparation** – The MeOH extracts of *S. kakudensis* and *S. boreali-koreana* were purchased from PEB of KRIBB. *S. takesimensis* and *S. buergeriana* were extracted with MeOH under reflux (3 h × 5 times). Each sample of the MeOH extract (1.0 mg) was dissolved in DMSO (1 ml).

**Measurement of AR activity** – Rat lenses were removed from Sprague-Dawley rats (weighing 250 - 280 g) and preserved 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. $IC_{50}$ 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.

## Results and Discussion

The MeOH extracts of *Scrophularia* species were tested for their inhibitory effects on rat lens AR activity, and the results were summarized in Table 1. The rat lens AR inhibition percentages of the root extracts of *S. takesimensis*, *S. kakudensis*, *S. boreali-koreana*, and *S. buergeriana* were 39.50, 58.75, 24.79 and 11.41%, respectively. Except for *S. kakudensis*, however, the root extracts of other species showed below 50% degree of inhibition on rat lens AR that are supposed to be far less deserving of further consideration. The aerial part extracts of *S. takesimensis*, *S. kakudensis*, and *S. boreali-koreana* were subjected to test for rat lens AR activity, exclusive of *S. buergeriana*, which is generally used for medicinal purpose. As shown in Table 1, the aerial part extracts of *S. kakudensis* and *S. boreali-koreana* were exhibited good inhibitory potencies on rat lens AR compared with those of the root extracts. The aerial part extracts of *S. kakudensis* and *S. boreali-koreana* were exhibited highest rat lens AR inhibition (72.88 and 77.85%, respectively).

**Table 1.** Effects of the MeOH extracts of *Scrophularia* species on rat lens AR inhibition

| Species | Parts tested | AR inhibition[a] (%) |
|---|---|---|
| *Scrophularia takesimensis* | Aerial parts | 19.95 |
| | Roots | 39.50 |
| *S. kakudensis* | Aerial parts | 72.88 |
| | Roots | 58.75 |
| *S. boreali-koreana* | Aerial parts | 77.85 |
| | Roots | 24.79 |
| *S. buergeriana* | Roots | 11.41 |

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

**Table 2.** $IC_{50}$ of the aerial part extracts of *S. kakudensis* and *S. boreali-koreana* on rat lens AR inhibition

| Species | Concentration (mg/ml) | AR inhibition[a] (%) | $IC_{50}$[b] (mg/ml) |
|---|---|---|---|
| *Scrophularia kakudensis* | 1 | 72.88 | 0.46 |
| | 0.5 | 58.25 | |
| | 0.1 | 28.84 | |
| *S. boreali-koreana* | 1 | 77.85 | 0.35 |
| | 0.5 | 60.82 | |
| | 0.1 | 36.14 | |
| Quercetin* | 0.5 | 73.32 | 0.19 |
| | 0.1 | 47.91 | |
| | 0.05 | 35.68 | |

[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.

To evaluate the rat lens AR inhibitory activity potencies between active species extracts, *S. kakudensis* and *S. boreali-koreana*, more precisely, their inhibitory percentage and $IC_{50}$ values were tested. Quercetin known as a very strong AR inhibitor ($IC_{50}$ value, 0.19 mg/ml) in natural constituents was used as a positive control and the results were indicated in Table 2. The $IC_{50}$ values of the aerial part extracts of *S. kakudensis* and *S. boreali-*

*koreana* were demonstrated 0.46 and 0.35 mg/ml, respectively. The result demonstrated that *Scrophularia* species has new potential AR inhibitors for the preventive effect on diabetic complications. Specifically, the extracts of *S. kakudensis* and *S. boreali-koreana* were exhibited more potent inhibition effect on rat lens AR than that of *S. buergeriana*.

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; Jesús Ángel and Sonia, 2003; Kawanishi *et al*., 2003; Jung *et al*., 2004; Lee *et al*., 2008). According to previous report of Adisakwattana *et al*. (2005), *p*-methoxycinnamic acid (*p*-MCA) that was isolated from the roots of *S. buergeriana* has shown the anti-hyperglycemic effect in streptozotocin-induced diabetic rats. Furthermore, the effects of *p*-MCA on the activities of hepatic enzymes in diabetic rats were also investigated in an effort to reveal possible mechanisms underlying its anti-hyperglycemic effect. However, there are no rat lens AR inhibitors from *S. kakudensis* and *S. boreali-koreana*. The results above are necessary for specific studies about active constituents from *S. kakudensis* and *S. boreali-koreana* for the prevention and/or treatment of diabetic complications.

In conclusion, the AR inhibitory activities of *Scrophularia* species in Korea *via* the rat lens AR assay were revealed in this study for the first time. To the best of our knowledge, the aerial parts of *S. kakudensis* and *S. boreali-koreana* were found to demonstrate good inhibitory activities from *in vitro* data. Therefore, we suggest that two *Scrophularia* species such as *S. kakudensis* and *S. boreali-koreana* has a possibility of new natural resource 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 *S. kakudensis* and *S. boreali-koreana* may prove the use of new medicinal plants for the prevention of diabetic complications.

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