Aldose reductase inhibitory activity of quercetin from the stems of *Rhododendron mucronulatum* for. *albiflorum*

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**Abstract**

The methanol extract of *Rhododendron mucronulatum* for. *albiflorum* (RMFA) stems inhibited aldose reductase (AR) activity. The RMFA fractions obtained by stepwise extraction with solvents of different polarity were tested for AR inhibition in vitro using the lens of a rat. Among them, the ethyl acetate (EtOAc) fraction inhibited AR more than the other fractions. Quercetin (1) from the EtOAc fraction showed a high AR inhibition with IC₅₀ of 2.11 μM. The stems of RMFA contained the highest amount (5.12 mg/g extract) of quercetin. Our results suggest that RMFA, which contained quercetin, could be a useful material for the development of supplementary functional foods.

**Keywords**

Aldose reductase inhibition · High-performance liquid chromatography · Quercetin · *Rhododendron mucronulatum* for. *albiflorum*

**Introduction**

Aldose reductase (AR) is a member of the aldo-keto superfamily and accelerates the reduction of glucose to sorbitol. Accumulation of excessive sorbitol influences the development of disproportionate ratios of NADPH/NAD⁺ and NAD⁺/NADH cofactors and facilitates cell transformation (Kao et al. 1999). Thereby, AR promotes the generation of osmotic and oxidative stress. Among them, oxidative stress can cause diseases, including diabetes-related complication and disorders, including retinopathy, neuropathy, and nephropathy (Enomoto et al. 2004; Jung et al. 2007; Ha et al. 2009; Jung et al. 2011). The AR accumulation can cause numerous disorders and, therefore, the discovery of AR inhibitors is crucial.

*Rhododendron mucronulatum* (RM) is a vascular plant that is distributed widely worldwide, especially in the northern hemisphere. An ancient source reported that RM can cause toxic honey poisoning (Gunduz et al. 2007). Despite this observation, RM has been used as a folk medicine (Gunduz et al. 2008). e.g., as a tonic, diuretic, for stomach disorders, and gonorrhea while Koreans have used RM in cakes, wine, and as juice (Lee et al. 2007; Guleria et al. 2011). Among these products, the wine produced from the flowers exhibits significant antioxidant activity (An et al. 2005).

*R. mucronulatum* for. *albiflorum* (RMFA) is a sub-species of RM, which is shrub with white flowers, and is endemic in Korea. RMFA is a rare plant, that has been endangered by indiscriminate uprooting and cutting (Lee et al. 1991). Previous studies have reported that the flowers of RMFA contain flavonoids (Mok and Lee 2012; Mok et al. 2013). However, there are limited studies on RMFA and, therefore, additional investigations of this plant are needed.

Therefore, the aim of this investigation was to evaluate the AR inhibition of RMFA on the rat lens as well as compound isolation from the stems.

**Materials and Methods**

**Plant materials**

The RMFA and RM samples used in this study were collected from Chilgap Mountain, 2013, Chungnam, Republic of Korea. These voucher specimens of RMFA and RM were deposited at our Department.

**Apparatus and chemicals**

Nuclear magnetic resonance (NMR) and electron ionization-mass
Extraction, fractionation, and isolation of a flavonoid from RMFA stems

The dried, finely powdered RMFA stems (3.4 kg) were extracted with methanol (MeOH) for 3 h (8 L×4) under reflux (65-75 °C). After removal of solvent in vacuo, the extract (186.4 g) distilled in water was partitioned successively with n-hexane (40.0 g), CH₂Cl₂ (25.8 g), EtOAc (48.0 g), and n-BuOH (25.1 g). A part of the EtOAc fraction (20 g) from the RMFA sample was chromatographed using a silica gel column (6×80 cm, No. 7734) by a stepwise gradient of CHCl₃ and MeOH solvent systems to obtain 5 fractions. Compound 1 was isolated from sub-fraction 4 (CHCl₃:MeOH=9:1). Among them, sub-fraction 4 yielded compound 1 by recrystallization using MeOH. Then, compound 1 was subsequently isolated.

Measurement of AR activity

The rat lenses were harvested from Sprague-Dawley rats (weighing 250-280 g) and kept frozen before they were used. The homogenized lenses were centrifuged at 10,000 rpm (4°C, 20 min) and the supernatant was used as the enzyme source for the AR activity testing. The AR (EC 1.1.1.21) activity was spectrophotometrically determined by measuring the decrease in absorption of β-NADPH at 340 nm over a 4 min period at room temperature with D,L-glyceraldehyde as the substrate (Mok and Lee 2012; Mok et al. 2012). The AR activity testing. The AR (EC 1.1.1.21) activity was spectrophotometrically determined by measuring the decrease in absorption of β-NADPH at 340 nm over a 4 min period at room temperature with D,L-glyceraldehyde as the substrate (Mok and Lee 2012; Mok et al. 2012).

Result and Discussion

The extracts and fractions of RMFA were analyzed for their AR inhibitory effects, and the results are shown in Table 1. The EtOAc fraction exhibited a significantly higher inhibition of the AR than the other fractions and extracts did. In a previous study, the MeOH extracts of white-colored natural products including RMFA were shown to inhibit AR activity (Mok et al. 2012). There are few literature reports on the various biological activities of RMFA, and these results demonstrated that the EtOAc fractions showed AR inhibitory effects on the rat lens (Mok and Lee 2012).

Table 1 IC₅₀ of the extract and fractions from RMFA against rat lens AR

| Fraction | Concentration (µg/mL) | AR inhibition (%) | IC₅₀ (µg/mL) |
|----------|----------------------|------------------|-------------|
| MeOH ext. | 10                   | 45.83            | -           |
| n-Hexane | 10                   | 28.88            | -           |
| CH₂Cl₂   | 10                   | 58.38            | -           |
| EtOAc    | 5                    | 68.56            | 6.50        |
| n-BuOH   | 10                   | 37.88            | -           |
| TMG      | 10                   | 83.28            | -           |
|          | 0.1                  | 62.21            | 0.29        |

*IC₅₀ calculated from least-squares regression line of logarithmic concentrations plotted against residual activity
*TMG was used as a positive control
The EtOAc fraction of RMFA was repeatedly separated using silica gel and Sephadex LH-20 chromatography and led to the isolation of compound 1. The structure of compound 1 was confirmed by a combination of $^1$H-NMR and EI-MS. In the $^1$H-NMR spectra, the typical flavonoid signals of compound 1 were observed, and its molecular weight was at $m/z$ 302 [M]+. The presence of singlet signals at $\delta$ 12.49 showed a 5-OH of an A-ring in the structure while H-6 and -8 signals are observed at $\delta$ 6.18 (d, $J=2.0$ Hz, H-6) and $\delta$ 6.40 (d, $J=2.0$ Hz, H-8). Furthermore, $\delta$ 6.88–7.67 showed the ABX pattern of the B-ring: $\delta$ 7.67 (1H, d, $J=2.0$ Hz, H-2'), 6.88 (1H, d, $J=8.5$ Hz, H-5'), and 7.54 (1H, dd, $J=2.0$, 8.5 Hz, H-6'). From the spectroscopic comparison with values in the literature (Sato and Kador 1990), the chemical structure of purified compound 1 was elucidated as quercetin (Fig. 1). Numerous quercetin (1) derivatives have been isolated from RM sp. (Jung et al. 1996; Hong et al. 2007).

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### Table 2 IC$_{50}$ of compound 1 from RMFA against rat lens AR

| Compound | Concentration (µg/mL) | AR inhibition (%) | IC$_{50}$ (µM) |
|----------|-----------------------|-------------------|----------------|
| 1        | 10                    | 74.25             | 2.11           |
|          | 0.1                   | 18.06             | 3.01           |
| TMG      | 10                    | 83.28             | 1.52           |
|          | 0.1                   | 62.20             | 40.13          |

Table 3 Linearity of standard curves of compound 1

| Compound | $t_c$ | Calibration equation$^a$ | Correlation factor, $r^2$ $^b$ |
|----------|-------|--------------------------|-------------------------------|
| 1        | 21.68 | $Y=0.03223X-715.45$      | 1                             |

$^a$Y=peak area, X=concentration of standards (mg/mL) 
$^b$ $r^2$=correlation coefficient for 3 data points in calibration curves (n=5)

### Table 4 Quantities of compound 1 in each plant part of RMFA and RM

| Sample       | Content (mg/g extract) |
|--------------|------------------------|
| Flower of RMFA | 3.51±0.07              |
| Stem of RMFA  | 5.12±0.07              |
| Flowers of RM | 2.22±0.00              |
| Stem of RM    | 3.29±0.02              |
| Root of RM    | tr.                    |

Data are mean ± SD (n=3) in µg/g of dried samples 
tr., trace

Quercetin (1) from the EtOAc fraction of RMFA was evaluated for AR inhibitory activity (Table 2). Quercetin (1) exhibited

![Fig. 1 Structure of quercetin](image)

![Fig. 2 HPLC chromatograms of quercetin (A), flowers of RMFA (B), and stems of RMFA (C)](image)
significant AR inhibitory activity (IC$_{50}$ 2.11 μM) with TMG, as a positive control. There have been numerous reports of flavonoids and phenol constituents with significant AR inhibitory activity (Kawanishi et al. 2003; Jung et al. 2004; Lee et al. 2008). In addition, previous studies have demonstrated that flavonoids have various pharmaceutical activities including anti-ulcer, anti-viral, anti-inflammatory, and vasodilatory actions (Proestos and Komaitis 2006). Our study demonstrated that RMFA exhibits AR inhibitory effects. Recently, quercetin reduces manic-like behavior and brain oxidative stress (Kanazawa et al. 2016). Also it affects glutathione levels and redox in human aortic endothelial cells (Li et al. 2016).

The content analysis was performed to determine the concentration of quercetin (I) in the various parts of the RMFA and RM plants by using HPLC/UV analysis. The linear calibration equation of quercetin (I) was $Y=30223X-715.45$. The correlation coefficient ($r^2$) was 1 and shown in Table 3. The retention time of quercetin (I) was 21.68 min. The flowers and stems of RMFA contained high amounts of quercetin (I) at 5.12 and 3.51 mg/g extract, respectively, which was more than the other parts of the RM. The roots of RM showed a very low concentration of quercetin (I). The quercetin (I) content of the various parts of the RM and RMFA plants was quantified by using a calibration curve (Table 4). RMFA had more active than RM in a previous paper (Mok et al. 2012). We think that different concentrations of quercetin in RMFA and RM is main key for AR inhibition. The LOD and LOQ of compound I were 0.012 and 0.029 mg/mL, respectively (Table 5).

In conclusion, our study revealed that RMFA contains higher amount of quercetin (I) than RM. Furthermore, our results demonstrated that RMFA has the potential to be used as an AR inhibitory agent against diabetic complications.

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