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

Jaemin Lee¹ · Hyun-Sung Ryu² · Joyce P. Rodriguez¹ · Sanghyun Lee¹

Received: 19 October 2016 / Accepted: 1 November 2016 / Published Online: 31 March 2017
© The Korean Society for Applied Biological Chemistry 2017

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/NADP⁺ 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 gonorrhoea 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

Sanghyun Lee (✉)
E-mail: slee@cau.ac.kr

¹Department of Integrative Plant Science, Chung-Ang University, Anseong 17546, Republic of Korea

²Pyeongtaek High School, Pyeongtaek 17903, Republic of Korea

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

spectrometry (EI-MS) were recorded by using a Bruker AVANCE 500 NMR spectrometer (Rheinstetten, Germany) and a JEOL JMS-600W mass spectrometer (Tokyo, Japan), respectively. The reagents and solvents including 3,3-tetramethyleneglutaric acid (TMG), β -NADPH, DL-glyceraldehyde, potassium phosphate buffer, and sodium phosphate buffer were obtained from Sigma-Aldrich (St. Louis, MO, USA).

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 $\times$ 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 $\times$ 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 DL-glyceraldehyde as a substrate. 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 the test extract sample (in dimethyl sulfoxide) with 0.025 M DL-glyceraldehyde as the substrate (Mok and Lee 2012; Mok et al. 2012).

Sample preparation for high-performance liquid chromatography (HPLC)

To analyze the components of compound **1** from the various parts of the RMFA and RM plants, the extracts were dissolved in MeOH, filtered by a Whatman 0.45- μ m syringe filter (Cat No. 6779, Piscataway, NJ, USA), and then, the solution was analyzed by using HPLC.

Quantitative analysis of parts of RMAF and RM

The HPLC analysis of compound **1** from the different parts of the RMFA and RM was performed. A Waters Spherisorb[®] INNO C18 (4.6 $\times$ 250 mm, 5 μ m) reverse phase column was used for the determination. The mobile phase was 0.5 % acetic acid and MeOH (reagents A and B, respectively). The aliquot volume was

10 μ L. The flow rate was at 1 mL/min. The gradient system was run as follows: initially, reagent A and B at 80:20, the linear gradient was increased to 0:100 for 35 min and maintained for 5 min, and then finally the gradient was increased to 80:20 for 5 min again and maintained for 5 min for a total run time of 55 min. The UV wavelength absorbance was detected at 330 nm for quantification of the flavonoids.

Limit of detection and quantification (LOD and LOQ) of compound 1 from RMFA and RM

The validation of compound **1** as a standard was conducted by using LOD and LOQ. The LOD and LOQ were calculated based on a linear regression equation, and the values were determined separately at a signal to noise ratios of 3 and 10, respectively.

Calibration curve

The stock solution (1 mg/1 mL) of compound **1** was dissolved in MeOH and mixed while the same solvent was used repeatedly to compare the integrated peak area of the individual compound. The calibration function of compound **1** were calculated by peak area (Y), concentration (X, mg/mL) and mean values (n=5).

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 significant 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 ^a (%)	IC ₅₀ ^b (μg/mL)
MeOH ext.	10	45.83	-
<i>n</i> -Hexane	10	28.88	-
CH ₂ Cl ₂	10	58.38	-
	10	68.56	
EtOAc	5	24.75	6.50
	1	24.75	
<i>n</i> -BuOH	10	37.88	-
	10	83.28	
TMG ^c	1	62.21	0.29
	0.1	40.13	

^aInhibition rate was calculated as a percentage of the control value

^bIC₅₀ calculated from least-squares regression line of logarithmic concentrations plotted against residual activity

^cTMG was used as a positive control

Fig. 1 Structure of quercetin

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 ^1H -NMR and EI-MS. In the ^1H -NMR spectra, the typical flavonoid signals of compound **1** were observed, and its molecular weight was at m/z 302 $[\text{M}]^+$. The presence of singlet signals at δ 12.49 showed a 5-OH of an A-ring in the structure while H-6 and -8 signals are observed at δ 6.18 (d, $J=2.0$ Hz, H-6) and δ 6.40 (d, $J=2.0$ Hz, H-8). Furthermore, δ 6.88–7.67 showed the ABX pattern of the B-ring: δ 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).

Table 2 IC_{50} of compound **1** from RMFA against rat lens AR

Compound	Concentration ($\mu\text{g/mL}$)	AR inhibition (%)	IC_{50} (μM)
1	10	74.25	2.11
	1	18.06	
	0.1	3.01	
TMG	10	83.28	1.52
	1	62.20	
	0.1	40.13	

Same as in Table 1

Table 3 Linearity of standard curves of compound **1**

Compound	t_R	Calibration equation ^a	Correlation factor, r^2 ^b
1	21.68	$Y=0.30223X-715.45$	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 $\pm$ SD ($n=3$) in $\mu\text{g/g}$ 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. 2 HPLC chromatograms of quercetin (A), flowers of RMFA (B), and stems of RMFA (C)

Table 5 LOD and LOQ values of compound **1**

Compound	Calibration equation ^a	r^2 ^b	Linear range (mg/mL)	LOD (mg/mL)	LOQ (mg/mL)
1	Y=33335X-177.23	1	0.1–1.000	0.012	0.029

^aY=peak area, X=concentration of standard (mg/mL)^b r^2 =correlation coefficient for 3 data points in calibration curve

significant AR inhibitory activity (IC₅₀, 2.11 μ M) with TMG₁ 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 (**1**) in the various parts of the RMFA and RM plants by using HPLC/UV analysis. The linear calibration equation of quercetin (**1**) was Y=30223X-715.45. The correlation coefficient (r^2) was 1 and shown in Table 3. The retention time of quercetin (**1**) was 21.68 min. The flowers and stems of RMFA contained high amounts of quercetin (**1**) 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 (**1**). The quercetin (**1**) 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 **1** were 0.012 and 0.029 mg/mL, respectively (Table 5).

In conclusion, our study revealed that RMFA contains higher amount of quercetin (**1**) than RM. Furthermore, our results demonstrated that RMFA has the potential to be used as an AR inhibitory agent against diabetic complications.

Acknowledgment This work was supported by a Grant from Pyeongtaek High School, Pyeongtaek, Korea. We would like to thank the National Center for Inter-University Research Facilities (Seoul National University, Republic of Korea) for the NMR and MS measurements.

References

- An BJ, Lee CE, Son JH, Lee JY, Choi GH, Park TS (2005) Antioxidant, anticancer and tyrosinase inhibition activities of extracts from *Rhododendron mucronulatum* T. J Kor Soc Appl Biol Chem 48: 280–284
- Enomoto S, Okada Y, Güvenc A (2004) Inhibitory effect of traditional Turkish folk medicines on aldose reductase (AR) and hematological activity, and on AR inhibitory activity of quercetin-3-O-methyl ether isolated from *Cistus laurifolius* L. Biol Pharm Bull 27: 1140–1143
- Guleria S, Jaitak V, Saini R, Kaul VK, Lal B, Babu GDK, Singh B, Singh RD (2011) Comparative studies of volatile oil composition of *Rhododendron anthopogon* by hydro distillation, supercritical carbon dioxide extraction and head space analysis. Nat Prod Res 25: 1271–1277
- Gunduz A, Durmus I, Turedi S, Nuhoglu I, Ozturk S (2007) Mad honey poisoning-related asystole. Am Med J 24: 592–593
- Gunduz A, Turedi S, Russell RM, Ayaz FA (2008) Clinical review of grayano-toxin/mad honey poisoning past and present. Clin Toxicol 46: 437–442
- Ha DT, Ngoc TM, Lee I (2009) Inhibitors of aldose reductase and formation of advanced glycation end-products in moutan cortex (*Paonia suffruticosa*). J Nat Prod 72: 1465–1470
- Hong HS, Jeon SH, Kwon YS (2007) Cytotoxic constituents from the stem of *Rhododendron mucronulatum*. Kor J Pharmacogn 38: 227–233
- Jung HA, Islam MD, Kwon YS (2011) Extraction and identification of three major aldose reductase inhibitors from *Artemisia montana*. Food Chem Toxicol 49: 376–384
- Jung MJ, Kang SS, Jung HA, Kim GJ, Choi JS (2004) Isolation of flavonoids and a cerebroside from the stem bark of *Albizia julibrissin*. Arch Pharm Res 7: 593–599
- Jung SH, Lee JM, Lee HJ (2007) Aldose reductase and advanced glycation endproducts inhibitory effect of *Phyllostachys nigra*. Biol Pharm Bull 30: 1569–1572
- Jung TY, Kim MA, Daniel Jones A (1996) Antioxidant activity flavonoids isolated from Jindalrae flowers (*Rhododendron mucronulatum* Turcz.). Agric Chem Biotechnol 39: 320–326
- Kanazawa LK, Vecchia DD, Wendler EM, Hocayen PA, Dos Reis Lívero FA, Stipp MC, Barcaro IM, Acco A, Andreatini R (2016) Quercetin reduces manic-like behavior and brain oxidative stress induced by paradoxical sleep deprivation in mice. Free Radic Biol Med 99: 79–86
- Kao YL, Donaghue K, Chan A, Knight J, Silink M (1999) A novel polymorphism in the aldose reductase gene promoter region is strongly associated with diabetic retinopathy in adolescents with type 1 diabetes. Diabetes 48: 1338–1340
- Kawanishi K, Ueda H, Moriyasu M (2003) Aldose reductase inhibitors from the nature. Curr Med Chem 10: 1353–1374
- Lee KE, Lee WC, Cho HG, Yoo SC (1991) A study for use of wild *Rhododendron mucronulatum* for. *albiflorum* as landscape plant. J Korean Inst Landsc Archit 18: 73–85
- Lee SW, Choi SH, Hong YS, Lim SI (2007) Grayanotoxin poisoning from flower of *Rhododendron mucronulatum* in humans. Bull Environ Contam Toxicol 78: 132–133
- Lee YM, Kim NH, Kim JM, Kim YS, Jang DS, Kim JH, Bae KH, Kim JS (2008) Screening of inhibitory effect on aldose reductase of Korean herbal medicines and preventive effect of *Catalpa bignonioides* against xylose-induced lens opacity. Korean J Pharmacogn 39: 165–173
- Li C, Zhang WJ, Choi J, Frei B (2016) Quercetin affects glutathione levels and redox ratio in human aortic endothelial cells not through oxidation but formation and cellular export of quercetin-glutathione conjugates and upregulation of glutamate-cysteine ligase. Redox Biol 9: 220–228
- Mok SY, Kim HM, Lee SH (2013) Isolation of astragalin from flowers of *Rhododendron mucronulatum* for. *albiflorum*. Hort Environ Biotechnol 54: 450–455
- Mok SY, Lee SH (2012) Identification of flavonoids and flavonoid rhamnosides from *Rhododendron mucronulatum* for. *albiflorum* and their

-
- inhibitory activities against aldose reductase. Food Chem 136: 969–974
- Mok SY, Shin HC, Lee S (2012) Screening of aldose reductase inhibitory activity of white-color natural products. CNU J Agric Sci 39: 69–73
- Proestos C, Komaitis M (2006) Ultrasonically assisted extraction of phenolic compounds from aromatic plante: comparison with conventional extraction techniques. J Food Qual 29: 567–582
- Sato S, Kador PF (1990) Inhibition of aldehyde reductase by aldose reductase inhibitors. Biochem Pharmacol 40: 1033–1042