J Appl Biol Chem (2018) 61(2), 135–139
https://doi.org/10.3839/jabc.2018.020

Online ISSN 2234-7941
Print ISSN 1976-0442

Article: Bioactive Materials

Inhibitory effects of *Synurus excelsus* and *Weigela subsessilis* on aldose reductase and HPLC-UV analysis of scopolin, scopoletin, and quercetin

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Received: 22 March 2018 / Accepted: 14 April 2018 / Published Online: 30 June 2018

Abstract The inhibition of aldose reductase (AR) has been shown to prevent the progression of the many complications associated with diabetic hyperglycemia. Several compounds purified from various plant sources have exhibited potent inhibition against AR. In this study, the inhibitory effects of the methanol extracts of the flowers of *Synurus excelsus* and *Weigela subsessilis* on AR were determined *in vitro*. Scopolin and scopoletin are coumarins isolated from the flowers of *S. excelsus and W. subsessilis*; and quercetin is a known AR inhibitor present in many flowers. To determine and quantify their presence in both plants, HPLC-UV analysis of all three compounds was performed. *S. excelsus* and *W. subsessilis* showed potent inhibition against AR having IC_{50} values of 0.17 and 0.14 μg/mL, respectively. The concentration of scopolin in *S. excelsus* and *W. subsessilis* were 34.71 and 174.14 mg/g extract, respectively. Scopoletin was detected in *S. excelsus* at 3.41 mg/g extract, whereas quercetin was not detected in both plants. This study shows that *S. excelsus* and *W. subsessilis* exhibited promising AR inhibitory effects and are both sources of coumarins.

Keywords Aldose reductase · Quercetin · Scopoletin · Scopolin · *Synurus excelsus* · *Weigela subsessilis*

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Introduction

Aldose reductase (AR) is a cytosolic oxidoreductase that catalyzes the NADPH-dependent conversion of glucose to sorbitol [1]. AR is the rate-limiting enzyme of the polyol pathway, which is a metabolic pathway regarded as one of the key molecular mechanisms linked to the onset and progression of secondary diabetic complications (e.g., blindness, cardiovascular diseases, renal failure, and neurodegenerative disorders) [2]. Studies have shown that the inhibition of AR prevents the development of diabetic complications, thereby, making AR an attractive target for drug discovery [3-5]. Several synthetically produced AR inhibitors (ARIs) have shown promising results in animal models, however, they exhibit undesirable side effects or lesser efficacy during clinical trials [6]. Hence, there is an increased interest to identify novel and potent ARIs from natural sources because they offer safer alternative and potentially better results in treating and managing diabetic complications. For example, flavonoids, a class of polyphenolic compounds, are known to exhibit a variety of biological activities [7]. Particularly, quercetin, a flavonoid ubiquitously found in many flowers, is known to have strong inhibitory effects against AR [8].

Synurus excelsus (Asteraceae) and *Weigela subsessilis* (Caprifoliaceae) are perennial plants native to many East Asian countries such as Japan and Korea [9]. They are commonly grown as garden ornaments due to their beautiful foliage and inflorescence. Recent studies have shown that extracts from these plants are rich in bioactive compounds. Particularly, scopoletin and scopolin are coumarins isolated from the flowers of both these plants with various biological activities [10,11]. However, bioactive properties of *S. excelsus* and *W. subsessilis* are not fully explored.

Hence, this study aimed to identify the potential medicinal uses of both these plants. Particularly, we aimed to determine their *in vitro* inhibitory activity on AR obtained from rat lenses. Scopolin, scopoletin, and quercetin were also quantified by HPLC-UV.

Materials and Methods

Plant materials and animals

The methanol (MeOH) extracts of the flowers of *S. excelsus* and *W. subsessilis* were obtained from the Korea Plant Extract Bank. Seven-week-old Sprague-Dawley rats weighing 210-230 g were acquired from Core Tech Co. Ltd. (Pyeongtaek, Gyeonggi, Korea).

Chemicals and apparatus

Sodium phosphate buffer, potassium phosphate buffer, and MeOH, were obtained from Samchun Pure Chemical Co. (Pyeongtaek, Korea). Dimethyl sulfoxide (DMSO), 3,3'-tetramethyleneglutaric acid (TMG), DL-glyceraldehyde, β-NADPH, and standard compounds (scopolin and scopoletin) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Quercetin was previously isolated from the flowers of *Rhododendron mucronulatum* for. *albiflorum* [12]. An Allegra X-30R refrigerated benchtop centrifuge (Beckman Coulter™, Indianapolis, IN, USA) and an Optizen 2120 UV spectrophotometer (Mecasys Co., Daejeon, Korea) were used for the ARI assay.

Preparation of AR from rat lenses

AR was prepared from rat lenses following a previously described protocol [13]. Lenses from healthy Sprague-Dawley rats were resected and homogenized in 0.5 mL of 0.1 M sodium phosphate buffer (pH 6.2), for each lens. The homogenate was centrifuged at 10,000 rpm at 4°C for 20 min, and the resulting supernatant was collected and used as an enzyme source.

Measurement of ARI activity

The ARI activity of the flower extracts was determined by spectrophotometrically measuring the decrease in NADPH absorbance at 340 nm for a period of 4 min with DL-glyceraldehyde as a substrate. The total volume of the assay solution was 1 mL and it was composed of the rat lens AR, plant extract dissolved in DMSO, 25 mM DL-glyceraldehyde, 1.6 mM NADPH, 100 mM sodium phosphate buffer and 100 mM potassium phosphate buffer (pH 7.0). TMG was used as a positive control. The inhibitory activity of the samples is expressed as: (%) Inhibition = (normal enzyme activity – inhibited enzyme activity)/normal enzyme activity. The IC_{50} values were determined from the least-squares regression line of the logarithmic concentrations plotted against residual activity. Three trials were performed for every sample.

Standard and sample preparation for HPLC-UV analysis

HPLC samples were prepared by dissolving 10 mg of the flower extracts in 1 mL MeOH. Standard stock solutions (1 mg/mL) of scopolin, scopoletin, and quercetin were prepared in MeOH. All samples were filtered through a 0.45-μm filter prior to use.

HPLC-UV analysis of scopoletin and scopolin

The content of scopoletin and scopolin was measured with an Agilent HPLC system and an INNO C_{18} (4.6×250 mm, 5 μm) column. The mobile phase used was composed of 0.5 % acetic acid in water (solvent A) and MeOH (solvent B). The gradient elution program was as follows: 85% A at 0 min, 60% A at 25 min, 50% A at 40 min, and maintained until 55 min. The flowrate and injection volume were 1 mL/min and 10 μL, respectively. The UV detector was set at 330 nm.

Table 1 IC_{50} values for the inhibitory effects of the MeOH extracts of the flowers of *S. excelsus* and *W. subsessilis* on AR

Sample	Concentration (μg/mL)	Inhibition[a] (%)	IC_{50}[b] (μg/mL)
S. excelsus	10	92.82	0.17
	1	62.2	
	0.1	47.37	
W. subsessilis	10	94.4	0.14
	1	73.6	
	0.1	44.8	
TMG[c]	10	99.62	0.17
	1	67.31	
	0.1	45.38	

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

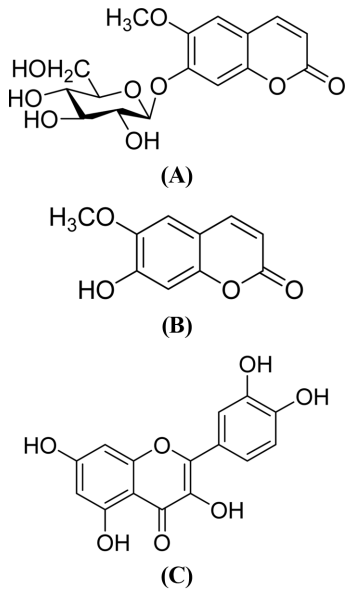

Fig. 1 Structures of scopolin (A), scopoletin (B), and quercetin (C)

HPLC-UV analysis of quercetin

An Agilent HPLC system was used for the analysis. Chromatographic separation was performed with a reverse-phase INNO C_{18} (4.6×250 mm, 5 μm) column. A gradient elution of 0.5% acetic acid in water: acetonitrile (90:10-50:50 for 50 min) was followed. The flowrate and injection volume were 1 mL/min and 10 μL,

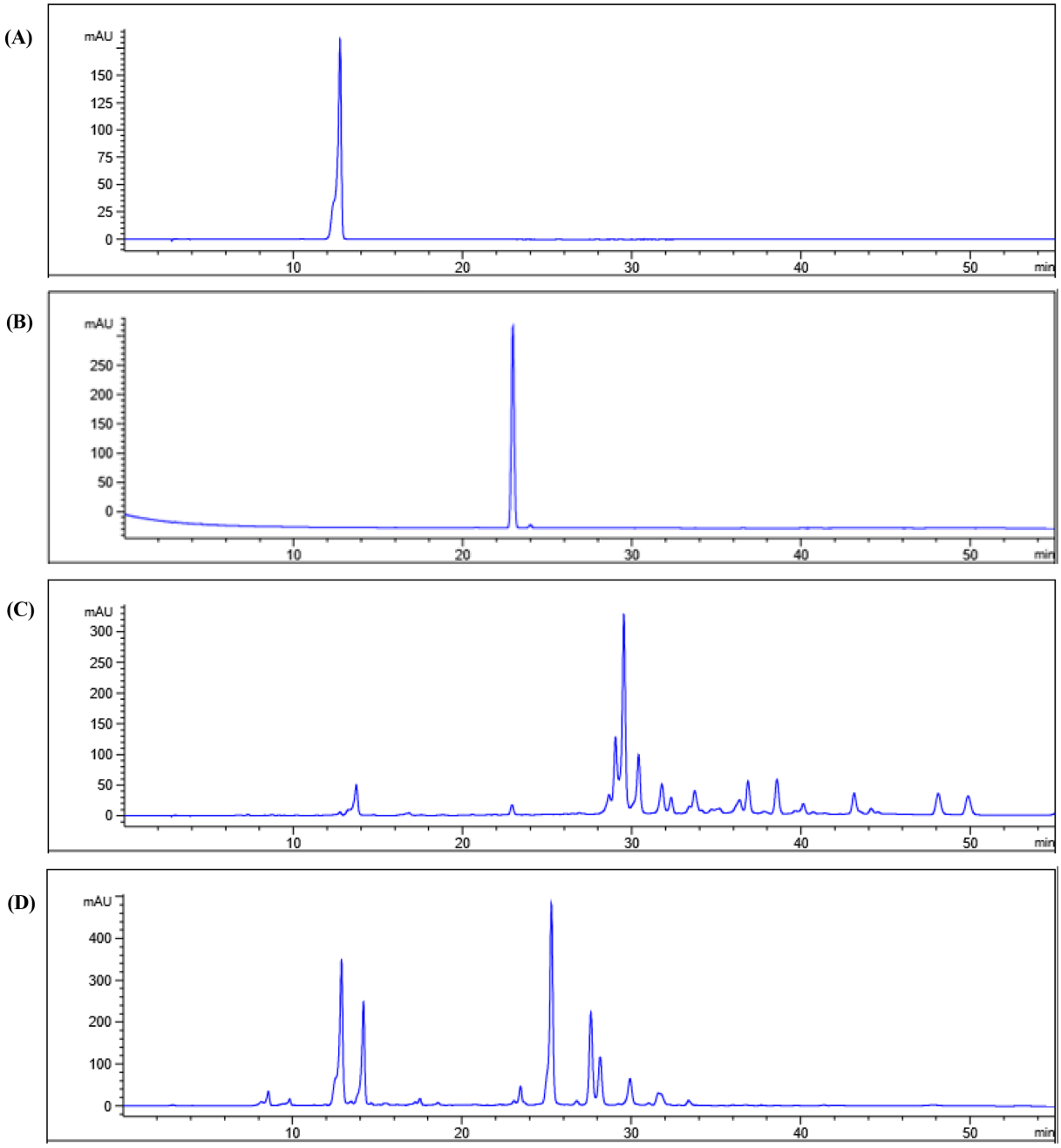

Fig. 2 HPLC chromatograms of scopolin (A), scopoletin (B), and the MeOH extracts of *S. excelsus* (C) and *W. subsessilis* (D)

respectively. The UV detector was set at 270 nm.

Calibration curve

The working solutions used to construct calibration curves for scopolin, scopoletin, and quercetin were obtained by diluting the respective stock solutions to desired concentrations. The calibration curves were used to determine the content of each reference compound in the samples. Linearity was assessed based on the correlation coefficient (r^2).

Results and Discussion

An increased activity of AR under chronic hyperglycemia leads to osmotic and oxidative stress in cells due to the accumulation of products of the polyol pathway (i.e., sorbitol and reactive oxygen species) and an imbalance in NADPH/NADH cofactors [14]. The secondary complications associated with diabetes have been postulated to be a consequence of prolonged oxidative stress as evident from the raised levels of oxidized DNA, proteins, and

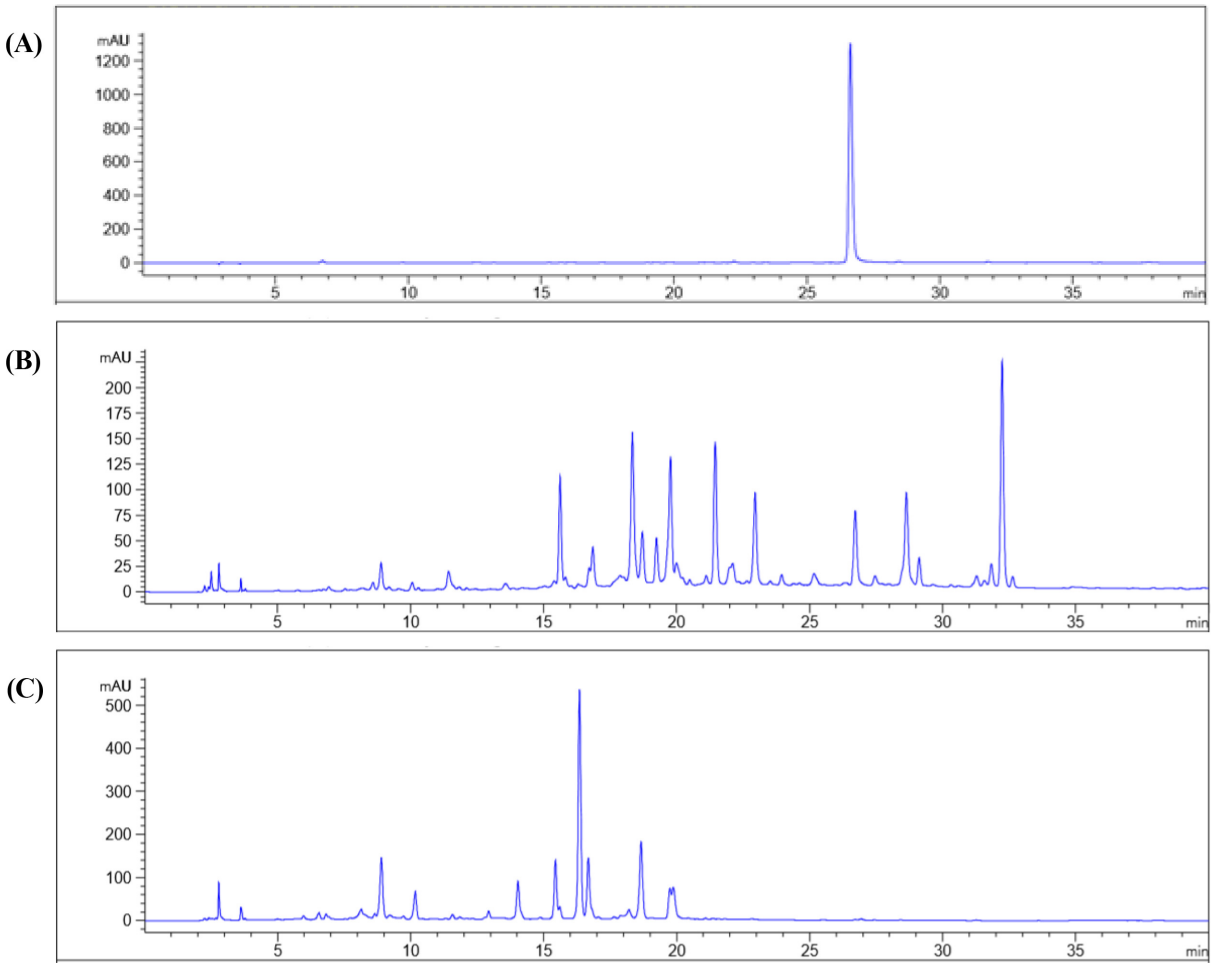

Fig. 3 HPLC chromatograms of quercetin (A), and the MeOH extracts of *S. excelsus* (B) and *W. subsessilis* (C)

lipids observed in diabetic individuals [15]. Accordingly, the polyol pathway is linked to the onset and progression of diabetic complications. Moreover, the inhibition of AR, the rate-limiting enzyme of the polyol pathway, prevents or arrests the progression of long-term complications associated with diabetes [16].

The ARI activity of the MeOH extracts of the flowers of *S. excelsus* and *W. subsessilis* was evaluated in this study. The results of the ARI assay are summarized in Table 1. Both *S. excelsus* and *W. subsessilis* exhibited potent ARI effects with IC_{50} values of 0.17 and 0. 14 μg/mL, respectively. *S. excelsus* extract showed similar and *W. subsessilis* extract exhibited better ARI activity than TMG (IC_{50}, 0.17 μg/mL). Lee et al. [17] reported that extracts of the leaves and stems of *W. subsessilis* exert three times more potent ARI effects than TMG. Nazaruk and Borzym-Kluczyk have reported that triterpenoid compounds exhibit potent ARI activities [18]. Interestingly, the MeOH extract of the aerial parts of *W. subsessilis* contains triterpenoids such as corosolic acid, which has been shown to exhibit strong ARI effects. Corosolic acid has a structure that is closely related to those of other known ARIs, such as ganoderic acid C2 and ganoderenic A [19,20,21]. These related studies support that *W. subsessilis* contains ARIs. Although the ARI activity of *S. excelsus* has not been reported, previous reports have shown that it is abundant in phenolic ARIs, such as as quercetin, lutein, rutin, and scopoletin which are reported to be ARIs [12,22]. The results of this study demonstrate the potential use of *W. subsessilis* and *S. excelsus* as a potential source of ARIs. Further research to characterize the bioactive components of these plants will provide insights into their inhibitory activity against AR.

Scopolin, scopoletin, and quercetin were quantified using a reverse-phase HPLC system (Fig. 1). The analytical method displayed high resolution for the chromatographic separation of all three compounds as shown in Fig. 2 and 3. The calibration curve for each compound showed good linearity (r^2=0.999) as seen in Table 2. Scopolin was present in *S. excelsus and W. subsessilis* at concentrations of 34.71 and 174.14 mg/g extract, respectively. Scopoletin was only detected in *S. excelsus* at 3.41 mg/g extract, whereas quercetin was not detected in both plants. Scopolin and its aglycone scopoletin are coumarins that were previously isolated from the flowers of *S.* excelsus and *W. subsessilis* [10,11]. These

Table 2 Calibration curves of scopolin, scopoletin, and quercetin

Compound	Linear range (mg/mL)	Linear regression equation Y=aX±b		Correlation coefficient (r^2)
		Slope (a)	Intercept (b)	
Scopolin	0.01-1.00	2290.4	224.8	0.9990
Scopoletin	0.01-1.00	3958.9	-61.6	0.9999
Quercetin	0.01-1.00	2513.3	-75.5	0.9999

Y = Peak area, X = Concentration of standard (mg/mL)
r^2 = Correlation coefficient for three data points from the calibration curve

Table 3. Scopolin, scopoletin, and quercetin contents in the MeOH extracts of *S. excelsus* and *W. subsessilis* flowers

Sample	Content (mg/g extract)		
	Scopolin	Scopoletin	Quercetin
S. excelsus	34.71±2.09	3.41±0.03	ND
W. subsessilis	174.14±0.01	ND	ND

Data are represented as mean±SD (n=3) in mg/g of the MeOH extracts of samples
ND=not detected

coumarins exhibit anti-inflammatory effects, and inhibit different enzymes, including nitric oxide synthase, prostaglandin synthase, monoamine synthase, and acetylcholinesterase [23,24]. Scopoletin has also been reported to exhibit moderate ARI activity [25]. Quercetin, a flavonoid that is commonly distributed in the flowers of different plant species, is widely-reported to exhibit strong antioxidant activity and ARI effects [8]. Hence, these compounds were analyzed in this study. To the best of our knowledge, this study is the first to describe an analytical method for the determination of all three compounds in *S. excelsus* and *W. subsessilis*.

The results of this study provide preliminary information regarding the ARI activities of the plants examined. Based on the results of our experiment, both *S. excelsus* and *W. subsessilis* can be potentially developed as natural therapies for managing diabetic complications.

Acknowledgments This research was supported by a grant from Seoul Science High School (2017), Republic of Korea.

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