

# Kaempferol Rhamnosides from *Geranium sibiricum* as Aldose Reductase Inhibitors and Their Content by HPLC Analysis

## **Authors:**

Norman G. Quilantang, Kyung Choi, Bog-Hieu Lee, Sanghyun Lee

*Date Submitted:* 2020-09-15

*Keywords:* flavonoid rhamnoside, HPLC-UV, aldose reductase, *Geranium sibiricum*

## **Abstract:**

The study aimed to assess the aldose reductase (AR) inhibition of selected *Geranium* species and determine the bioactive flavonoid constituents. Flavonoids are known to be good AR inhibitors. Among the species examined, *G. sibiricum* exhibited potent inhibition of AR (IC<sub>50</sub> value, 2.4 µg/mL). Further examination of *G. sibiricum*, after solvent extraction and fractionation, revealed that the ethyl acetate fraction (IC<sub>50</sub> value, 0.41 µg/mL) had a potent AR inhibitory effect. Kaempferol rhamnosides were the active compounds from this fraction. Moreover, *G. sibiricum* showed the highest content of kaempferol-7-O-rhamnoside and kaempferol-3,7-O-dirhamnoside among the samples examined with a concentration in the extracts of 28.1 and 2.2 mg/g, respectively. This study shows that *G. sibiricum* exhibits promising AR inhibitory activity, which can be explored further as a natural therapy for treating and managing complications associated with diabetes.

*Record Type:* Published Article

*Submitted To:* LAPSE (Living Archive for Process Systems Engineering)

*Citation (overall record, always the latest version):*

LAPSE:2020.0969

*Citation (this specific file, latest version):*

LAPSE:2020.0969-1

*Citation (this specific file, this version):*


LAPSE:2020.0969-1v1

*DOI of Published Version:* <https://doi.org/10.3390/pr8060694>

*License:* Creative Commons Attribution 4.0 International (CC BY 4.0)

Article

# Kaempferol Rhamnosides from *Geranium sibiricum* as Aldose Reductase Inhibitors and Their Content by HPLC Analysis

Norman G. Quilantang <sup>1</sup>, Kyung Choi <sup>2</sup>, Bog-Hieu Lee <sup>3</sup> and Sanghyun Lee <sup>1,\*</sup> 

<sup>1</sup> Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Korea; normanquilantang@gmail.com

<sup>2</sup> Division of Forest Biodiversity, Korea National Arboretum, Pocheon 11186, Korea; kchoi69@korea.kr

<sup>3</sup> Department of Food and Nutrition, Chung-Ang University, Anseong 17546, Korea; lbheelb@cau.ac.kr

\* Correspondence: slee@cau.ac.kr; Tel.: +82-31-670-4688

Received: 8 May 2020; Accepted: 11 June 2020; Published: 15 June 2020



**Abstract:** The study aimed to assess the aldose reductase (AR) inhibition of selected *Geranium* species and determine the bioactive flavonoid constituents. Flavonoids are known to be good AR inhibitors. Among the species examined, *G. sibiricum* exhibited potent inhibition of AR (IC<sub>50</sub> value, 2.4 µg/mL). Further examination of *G. sibiricum*, after solvent extraction and fractionation, revealed that the ethyl acetate fraction (IC<sub>50</sub> value, 0.41 µg/mL) had a potent AR inhibitory effect. Kaempferol rhamnosides were the active compounds from this fraction. Moreover, *G. sibiricum* showed the highest content of kaempferol-7-*O*-rhamnoside and kaempferol-3,7-*O*-dirhamnoside among the samples examined with a concentration in the extracts of 28.1 and 2.2 mg/g, respectively. This study shows that *G. sibiricum* exhibits promising AR inhibitory activity, which can be explored further as a natural therapy for treating and managing complications associated with diabetes.

**Keywords:** *Geranium sibiricum*; aldose reductase; HPLC-UV; flavonoid rhamnoside

## 1. Introduction

The polyol pathway is involved in glucose to fructose conversion via the formation of sorbitol as an intermediate [1]. It is one of the major cellular pathways linked to many complications associated with diabetic hyperglycemia, such as cataract formation, nephropathy, neuropathy, and other microvascular diseases [2]. Investigations in animal models have shown that diabetic complications can be prevented and delayed by inhibiting aldose reductase (AR) [3–5]. Currently, studies have aimed to identify potent agents that can effectively inhibit AR activity without causing adverse side effects, such as those observed when using synthetic aldose reductase inhibitors (ARIs) [6]. Inhibition of AR activity prevents and alleviates these complications. Thus, research on ARIs has been geared toward the use of novel agents from natural sources, as they may be a safer and more effective alternative. Among the different types of natural products, polyphenolic compounds, such as flavonoids, are the most widely reported to possess notable inhibitory activity against AR [7–10]. Furthermore, other compounds, such as capsaicin [11], pyrogallol [12], and bromophenol [13], have also shown good AR inhibition.

The genus *Geranium* is composed of herbaceous plants taxonomically classified under the Geraniaceae family, comprising 420 species distributed all over the world and mainly present in Southern Africa [14]. They have simple, glandular, and deeply lobed leaves and flowers that are composed of five carpels, sepals, petals, five to fifteen stamens, and yield five fruits. A distinct morphological feature shared by the Geraniaceae family is the beak-like shape of the fruits [15]; hence the etymology of the word *Geranium*, from “geranos” meaning crane [16]. Studies on the

phytochemical composition of different *Geranium* species have frequently reported on the abundance of polyphenolic compounds, such as flavonoids, tannins, catechins, and phenolic acids. Flavonoids and other polyphenolic compounds are classes of compounds that have been shown to exert AR inhibitory activities. As far as we know, there are no studies yet regarding the inhibitory effects of *Geranium* species against AR.

Therefore, the study aimed to assess the AR inhibitory activities of several species of *Geranium* and to isolate and characterize the flavonoids from *G. sibiricum* that showed the highest IC<sub>50</sub> values. Moreover, quantitative analysis was performed using high-performance liquid chromatography (HPLC).

## 2. Materials and Methods

### 2.1. Plant Materials

Six species of the genus *Geranium* (*G. eriostemon* var. *reinii*, *G. koreanum*, *G. kunthii*, *G. nepalense* subsp. *thunbergii*, *G. sibiricum*, and *G. wilfordii*) were tested for their in vitro activity against crude rat lens AR. The methanol (MeOH) extracts of the selected species were purchased from Korea Plant Extract Bank, KRIBB, South Korea. Aerial parts of *G. sibiricum* were collected from Korea National Arboretum, South Korea on 13 July 2016. A voucher specimen (K. Choi and H.J. Kim, 2016001) was deposited in Korea National Herbarium.

### 2.2. Chemicals and Instruments

Chloroform (CHCl<sub>3</sub>), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), *n*-hexane, and MeOH were used as solvents for extraction, fractionation, and open column chromatography (Samchun Pure Chemical Co., Seoul, Korea). Silica gel (200-400 mesh ASTM, Merck Co., Darmstadt, Germany) was used as the stationary phase. Dimethyl sulfoxide-*d*<sub>6</sub> (DMSO-*d*<sub>6</sub>) (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA) was used for nuclear magnetic resonance (NMR) solvent. HPLC-grade water and acetonitrile (ACN) were purchased from J.T. Baker Chemicals (Avantor, Radnor, PA, USA). 3,3'-tetramethyleneglutaric acid (TMG), DMSO,  $\beta$ -nicotinamide dinucleotide phosphate (NADPH), and DL-glyceraldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). A Jeol JMS-600W mass spectrometer (Jeol Ltd., Tokyo, Japan) was used for the detection of molecular weight, and a Bruker Avance 500 MHz spectrometer (Bruker, Rheinstetten, Germany) was used for NMR assignment with tetramethylsilane (TMS) as the internal standard. HPLC analysis was done by an Agilent 1260 Infinity II LC system (Santa Clara, CA, USA). An Optizen 2120 UV spectrophotometer (Mecasys Co., Daejeon, Korea) and an Allegra X-30R refrigerated benchtop centrifuge (Beckman Coulter™, Brea, CA, USA) were used for the AR inhibitory assay.

### 2.3. Extraction and Isolation of Flavonoids from *G. sibiricum*

Air-dried aerial parts of *G. sibiricum* (684 g) were extracted with MeOH under a reflux at 80 °C for 3 h. The resulting extract after 6 times of extraction was filtered with filter paper (185 mm) and evaporation was completed using a rotary evaporator to obtain a concentrated MeOH extract (82 g). The MeOH extract was subjected to water suspension and subsequently partitioned with solvents of varying polarity, namely *n*-hexane, CHCl<sub>3</sub>, EtOAc, and *n*-BuOH. The partitioned layer of organic solvent was then evaporated to afford the *n*-hexane, CHCl<sub>3</sub>, EtOAc, and *n*-BuOH fractions. Compounds from the EtOAc fraction were isolated using open column chromatography (CHCl<sub>3</sub>: MeOH, 1:0 to 0:1). In the EtOAc fraction, 35 sub-fractions were obtained. Compounds **1** and **2** were obtained from the recrystallization of Sub-fractions 10 and 15, respectively. Both compounds were prepared by the MeOH recrystallization method. NMR assignment of compounds **1** and **2** was in Table 1.

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR assignment of compounds **1** and **2**.

No.	1		2	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
2	-	155.7	-	157.5
3	-	136.0	-	134.4
4	-	176.1	-	178.4
5	-	160.4	-	161.9
6	6.42	98.8	6.35	100.8
7	-	161.4	-	162.5
8	6.83	94.3	6.67	95.2
9	-	147.5	-	156.1
10	-	104.7	-	107.2
1'	-	121.5	-	121.3
2',6'	8.09	129.6	7.78	131.4
3',5'	6.93	115.5	6.93	116.5
4'	-	159.4	-	161.4
3-O-Rh 1''	-	-	5.32	100.7
2''	-	-	3.87	70.9
3''	-	-	3.62	71.2
4''	-	-	3.55	72.8
5''	-	-	3.18	70.7
6''	-	-	0.82	18.9
7-O-Rh 1'''	5.54	98.4	5.55	99.2
2'''	-	70.1	3.87	70.6
3'''	-	70.2	3.65	71.3
4'''	-	71.6	3.32	71.9
5'''	-	69.8	3.45	70.4
6'''	1.13	17.9	1.15	18.4

#### 2.4. Preparation of AR from Rat Lenses

AR was prepared from the lenses of healthy Sprague Dawley rats (Koatech Co., Pyeongtaek, Korea) based on a previous literature [17]. Lenses were resected from the rats and 0.1 M sodium phosphate buffer was used for homogenization (pH 6.2) followed by centrifugation at 10,000 rpm (4 °C for 20 min). The supernatant collected was used as an enzyme source.

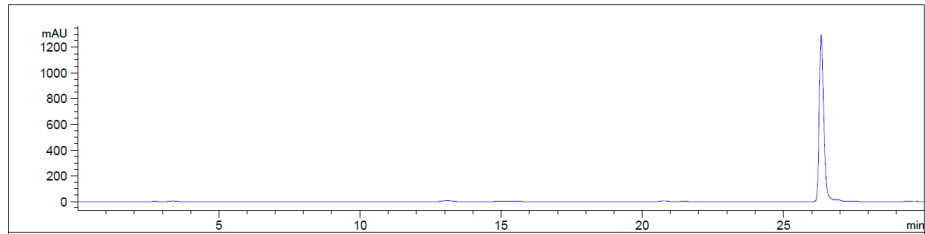
#### 2.5. Measurement of AR Inhibitory Activity

The AR inhibitory activities of *G. sibiricum* were measured by the decrease in the absorbance of  $\beta$ -NADPH at 340 nm for a period of 4 min using DL-glyceraldehyde as the substrate. The assay mixture was composed of the crude rat lens AR, 25 mM DL-glyceraldehyde, 1.6 mM NADPH, 100 mM sodium phosphate buffer, 100 mM potassium phosphate buffer (pH 7.0), and the sample dissolved in DMSO (1 mL). The AR inhibition of the samples was expressed as a percentage (Inhibition = (absorbance of control–absorbance of sample/absorbance of control)  $\times$  100). The  $\text{IC}_{50}$  values were obtained from the least-squares regression line of the log of concentration plotted against residual activity.

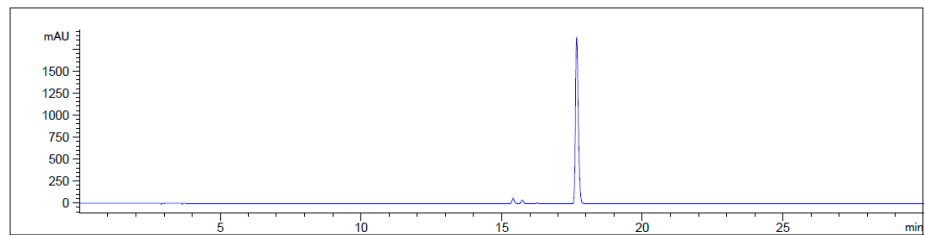
#### 2.6. Sample Preparation and HPLC Conditions

The MeOH extract of the *Geranium* species were dissolved in 20 mg/mL MeOH. Standard solutions of compounds **1** and **2** were prepared by 1 mg/mL MeOH. All samples were filtered through a 0.45  $\mu\text{m}$  membrane-filter before use. Chromatographic separation (Figure 1) of the *G. sibiricum* extract was performed on a  $\text{C}_{18}$  reversed-phase INNO column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ ). The mobile stationary was a mixture of water (Solvent A) and acetonitrile (Solvent B), and a gradient elution was followed (90% A to 60% A for 30 min). The flow rate and injection volume were set at 1 mL/min and 10  $\mu\text{L}$ , respectively. The column temperature was maintained at room temperature and UV absorbance was monitored at 270 nm. All injections were done in triplicate. In Figure 1, the arrows of compounds **1** and **2** in MeOH

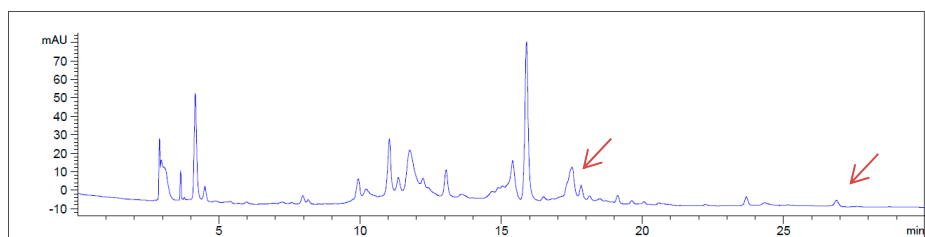
extracts were checked by spike tests. As can be observed in Figure 1, the retention time values of compounds 1 and 2 were similar to the retention time values of the spikes found in the chromatograms of the samples.



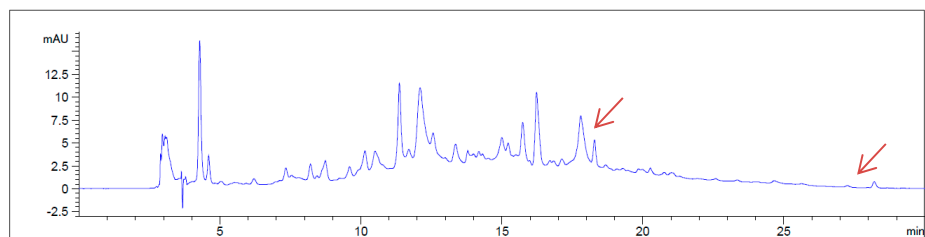
(A)



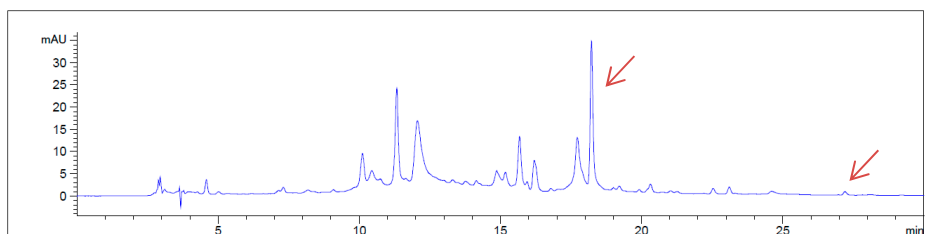
(B)



(C)

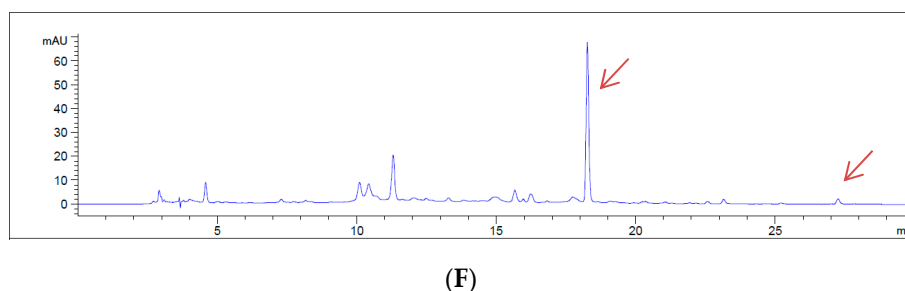


(D)



(E)

**Figure 1.** *Cont.*



**Figure 1.** HPLC chromatograms of compounds (A) **1** and (B) **2**, and the MeOH extracts of (C) *Geranium eriostemon*, (D) *G. koreanum*, (E) *G. nepalense*, and (F) *G. sibiricum*.

### 2.7. Limit of Detection and Quantification (LOD and LOQ)

LOD is the lowest amount of analyte having a signal that is three times greater than the noise level and LOQ is the lowest amount of analyte that can be quantitated with a signal-to-noise ratio of 10. The equations used to determine LOD and LOQ are as follows:  $LOD = 3.3 (\sigma/S)$  and  $LOQ = 10 (\sigma/S)$  where  $\sigma$  is the intercept and  $S$  is the slope (Table 2).

**Table 2.** Calibration curves, LOD, and LOQ of compounds **1** and **2**.

Compound	Linear Range ( $\mu\text{g/mL}$ )	Linear Regression Equation <sup>a</sup>		Correlation Coefficient ( $r^{2\ b}$ )	LOD ( $\mu\text{g/mL}$ )	LOQ ( $\mu\text{g/mL}$ )
		$Y = ax - b$				
		S (a)	$\sigma$ (b)			
<b>1</b>	0.01–1.00	16,047.7	45.833	0.9990	0.001	0.003
<b>2</b>	0.01–1.00	1,652.4	83.422	0.9992	0.001	0.003

<sup>a</sup>  $Y$  = peak area,  $X$  = concentration of standard compound ( $\mu\text{g/mL}$ ); <sup>b</sup>  $r^2$  = correlation coefficient for 6 data points in the calibration curve; LOD and LOQ were determined from the calibration curve of each standard compound using the values of the standard deviation of  $\sigma$  and  $S$ .

### 2.8. Calibration Curves

Different concentrations of compounds **1** and **2** were prepared by dilution of the standard solutions (0.1–1.0 mg/mL). The calibration curve (Table 2) for each standard was constructed using concentration ( $X$ ,  $\mu\text{g/mL}$ ) and peak area ( $Y$ ). The assessment of linearity was based on the correlation coefficient ( $r^2$ ). The contents of the analytes in the samples were estimated from the constructed calibration curves.

## 3. Results

To our knowledge, there are no studies regarding the investigation of the AR inhibitory activity of *Geranium* species. The study aimed to detect the inhibitory effects of selected *Geranium* species on AR and to characterize their bioactive constituents. The results revealed that among the *Geranium* species examined, the extracts of *G. sibiricum* exhibited potent AR inhibitory efficacy ( $IC_{50}$  value, 2.4  $\mu\text{g/mL}$ ) (Table 3).

**Table 3.** IC<sub>50</sub> values of MeOH extracts of the *Geranium* species.

Species	Conc. (µg/mL)	Inhibition <sup>a</sup> (%)	IC <sub>50</sub> <sup>b</sup> (µg/mL)
<i>Geranium eriostemon</i> var. <i>reinii</i>	10	87.2	3.4 ± 0.3
	1	40.1	
	0.1	27.8	
<i>G. koreanum</i>	10	45.9	>10
<i>G. kunthii</i>	10	84.5	4.2 ± 0.3
	1	32.6	
	0.1	24.6	
<i>G. nepalense</i> subsp. <i>thunbergii</i>	10	85.6	2.7 ± 0.1
	1	44.9	
	0.1	34.2	
<i>G. sibiricum</i>	10	70.6	2.4 ± 0.1
	1	34.8	
	0.1	11.8	
<i>G. wilfordii</i>	10	33.7	>10
TMG <sup>c</sup>	10	92	0.25 ± 0.0
	1	67.4	
	0.1	31	

<sup>a</sup> Inhibition was calculated as a percentage of the control value; <sup>b</sup> IC<sub>50</sub> values were calculated from the least-squares regression line of the log of the concentrations plotted against the residual activity; <sup>c</sup> 3,3'-tetramethyleneglutaric acid (TMG) was used as a positive control.

The MeOH extract and solvent fractions of *G. sibiricum* were tested for AR inhibition. The EtOAc fraction potently inhibited the enzyme (IC<sub>50</sub> value, 0.41 µg/mL) (Table 4). This means that the compounds from the EtOAc fraction were responsible for the inhibitory activity. Further examination of the bioactive components of the EtOAc fraction of *G. sibiricum* by open column chromatography yielded compounds **1** and **2** (Figure 1). The chemical structures of the compounds were elucidated by spectroscopic analysis, including <sup>1</sup>H- and <sup>13</sup>C-NMR and fast atom bombardment (FAB)-MS spectroscopy. The <sup>1</sup>H-NMR data for compounds **1** and **2** revealed signals typical of flavonoids (Table 1). The FAB-MS analysis of compounds **1** and **2** showed a molecular ion peak at *m/z* 433 [M + 1]<sup>+</sup> and *m/z* 579 [M + 1]<sup>+</sup>, respectively.

**Table 4.** IC<sub>50</sub> values of the fractions of *G. sibiricum*.

Fractions	Conc. (µg/mL)	Inhibition <sup>a</sup> (%)	IC <sub>50</sub> <sup>b</sup> (µg/mL)
<i>n</i> -hexane fraction	10	46	>10
CHCl <sub>3</sub> fraction	10	48.1	>10
EtOAc fraction	10	71.7	0.41 ± 0.04
	1	67.4	
	0.1	33.2	
<i>n</i> -BuOH fraction	10	81.8	0.79 ± 0.06
	1	51.9	
	0.1	25.1	
TMG <sup>c</sup>	10	92	0.25 ± 0.03
	1	67.4	
	0.1	31	

<sup>a,b,c</sup> Refer to Table 3 regarding the calculations.

Compounds **1** and **2** were identified as kaempferol-7-*O*-rhamnoside (KR) and kaempferol-3,7-*O*-dirhamnoside (KD), respectively [18,19] (Figure 2).

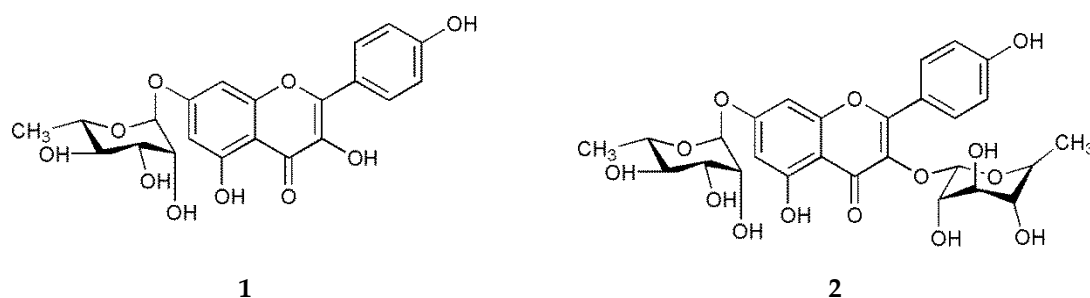


Figure 2. Structures of KR (1) and KD (2).

The  $IC_{50}$  values of KR and KD on AR were 1.23 and 0.55  $\mu$ M, respectively (Table 5). KD was more potent than KR, with  $IC_{50}$  value comparable to that of TMG (0.74  $\mu$ M).

Table 5.  $IC_{50}$  values of KR and KD.

Compound	Conc. ( $\mu$ g/mL)	AR inhibition <sup>a</sup> (%)	$IC_{50}$ <sup>b</sup>	
			( $\mu$ g/mL)	( $\mu$ M)
KR	10	80.37		
	1	68.1	0.53 $\pm$ 0.0	1.23 $\pm$ 0.0
	0.1	24.54		
KD	10	96.93		
	1	60.74	0.32 $\pm$ 0.0	0.55 $\pm$ 0.1
	0.1	36.81		
TMG <sup>c</sup>	10	92		
	1	67.4	0.25 $\pm$ 0.0	0.74 $\pm$ 0.0
	0.1	31		

<sup>a,b,c</sup> Refer to Table 3 regarding the calculations.

Quantitative analysis of KR and KD in six *Geranium* species was carried out using HPLC. Good separation was observed in the samples analyzed, as seen in Figure 1. The calibration curves for each reference compound showed high linearity (Table 2), and the results of the analysis are summarized in Table 6. KR and KD were not detected in *G. kunthii* and *G. wilfordii*, whereas both compounds were present in the four remaining species analyzed. *G. sibiricum* contained a high concentration of KR in the methanol extract (28.10 mg/g).

Table 6. Amount of KR and KD in the MeOH extract of *Geranium* species.

Species	Content (mg/g Extract)	
	KR	KD
<i>Geranium eriostemon</i> var. <i>reinii</i>	4.78 $\pm$ 0.05	0.04 $\pm$ 0.02
<i>G. koreanum</i>	1.33 $\pm$ 0.07	1.50 $\pm$ 0.10
<i>G. kunthii</i>	ND	ND
<i>G. nepalense</i> subsp. <i>thunbergii</i>	23.90 $\pm$ 0.50	0.14 $\pm$ 0.05
<i>G. sibiricum</i>	28.10 $\pm$ 0.40	2.20 $\pm$ 0.20
<i>G. wilfordii</i>	ND	ND

ND = not detected.

#### 4. Discussion

Studies on the phytochemistry of *Geranium* species have revealed that they are abundant in many phenolic constituents, including catechins, phenolic acids, flavonoids, and tannins [20]. The potent antioxidant and other bioactive properties of *Geranium* species have been attributed to the presence



of these polyphenolic compounds [21–23]. Moreover, polyphenolic compounds, such as flavonoids, potently inhibit AR [24,25]. Among the *Geranium* species examined, *G. sibiricum* exhibited potent AR inhibitory efficacy (IC<sub>50</sub> value, 2.4 µg/mL). *G. sibiricum* is an indigenous perennial herb in Europe and Asia, commonly referred to as the Siberian cranesbill.

It has been used in herbal preparations in traditional medicine for diarrhea treatment, eruptive skin diseases, wounds, and intestinal inflammation. Recent studies have reported that the beneficial effect of *G. sibiricum* on human health may be attributed to its potent anti-oxidant activity and to its high content of polyphenolic compounds [26–28]. Its extract has been shown to exert hair-promoting, xanthine oxidase inhibitory, anti-inflammatory, and anti-proliferative effects [26–29]. *G. nepalense* subsp. *thunbergii* is also used in ethnomedicinal applications for treating dysentery, influenza, and diabetes [30]. However, the ability of *G. sibiricum* to inhibit AR has never been examined to date, and as far as we know, this study is the first investigation regarding the inhibition of AR.

The tested EtOAc fraction for inhibitory efficacy against rat lens AR potently inhibited the enzyme (IC<sub>50</sub> value, 0.41 µg/mL). The EtOAc fraction of *G. sibiricum* has been previously reported to possess powerful anti-oxidant activity and xanthine oxidase inhibitory efficacy owing to its high phenolic concentration [28]. Similarly, in this study of AR inhibition, the EtOAc fraction was the bioactive fraction of *G. sibiricum* that showed the highest IC<sub>50</sub> value. KR and KD from the EtOAc fraction of *G. sibiricum* were tested for their inhibitory efficacy against rat lens AR. There have been no previous studies pertaining to the inhibition of the polyol pathway by KR and KD, and this study is the first to report on their AR inhibitory efficacies. KR and KD are flavonoid compounds. There are other AR inhibitory compounds, such as capsaicin [11], pyrogallol [12], and bromophenol [13] present in *G. sibiricum*. The potent AR inhibition showed by the *G. sibiricum* species can be attributed to its high concentrations of KR and KD, since these two compounds were the major components of the EtOAc fraction that showed the highest IC<sub>50</sub> value. This species also exhibited the best AR inhibition among all the species examined in this study. These results may have application in the development of novel therapeutic approaches to target complications associated with diabetic hyperglycemia.

**Author Contributions:** Investigation, N.G.Q.; methodology, K.C.; writing—original draft, B.-H.L.; writing—review and editing, S.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (NRF-2017R1D1A1B03033507).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Dunlop, M.E. Aldose reductase and the role of the polyol pathway in diabetic nephropathy. *Kidney Int.* **2000**, *58*, S3–S12. [[CrossRef](#)] [[PubMed](#)]
2. Tang, W.H.; Martin, K.A.; Hwa, J. Aldose reductase, oxidative stress, and diabetic mellitus. *Front. Pharmacol.* **2012**, *3*. [[CrossRef](#)] [[PubMed](#)]
3. Engerman, R.L.; Kern, T.S.; Larson, M.E. Nerve conduction and aldose reductase inhibition during 5 years of diabetes or galactosemia in dogs. *Diabetologia* **1994**, *37*, 141–144. [[CrossRef](#)]
4. Hotta, N.; Akanuma, Y.; Kawamori, R.; Matsuoka, K.; Oka, Y.; Shichiri, M.; Toyota, T.; Nakashima, M.; Yoshimura, I.; Sakamoto, N.; et al. Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on diabetic peripheral neuropathy: The 3-year, multicenter, comparative aldose reductase inhibitor-diabetes complications trial. *Diabetes Care* **2006**, *29*, 1538–1544. [[CrossRef](#)]
5. Drel, V.; Pacher, P.; Ali, T.K.; Shin, J.; Julius, U.; El-Remessy, A.B.; Obrosova, I.G. Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. *Int. J. Mol. Med.* **2008**, *21*, 667–676. [[CrossRef](#)]
6. Schemmel, K.E.; Padiyara, R.S.; D'Souza, J.J. Aldose reductase inhibitors in the treatment of diabetic peripheral neuropathy: A review. *J. Diabetes Its Complicat.* **2010**, *24*, 354–360. [[CrossRef](#)]

7. De la Fuente, J.A.; Manzanaro, S. Aldose reductase inhibitors from natural resources. *Nat. Prod. Rep.* **2003**, *20*, 243–251. [[CrossRef](#)]
8. Lee, J.; Rodriguez, J.P.; Lee, K.H.; Park, J.Y.; Kang, K.; Hahm, D.-H.; Huh, C.K.; Lee, S.C.; Lee, S. Determination of flavonoids from *Cirsium japonicum* var. *maackii* and their inhibitory activities against aldose reductase. *Appl. Biol. Chem.* **2017**, *60*, 487–496. [[CrossRef](#)]
9. Lee, J.; Rodriguez, J.P.; Quilantang, N.G.; Lee, M.-H.; Cho, E.J.; Jacinto, S.D.; Lee, S. Determination of flavonoids from *Perilla frutescens* var. *japonica* seeds and their inhibitory effect on aldose reductase. *Appl. Biol. Chem.* **2017**, *60*, 155–162. [[CrossRef](#)]
10. Rodriguez, J.P.; Lee, Y.K.; Woo, D.G.; Shim, J.S.; Geraldino, P.J.L.; Jacinto, S.D. Flavonoids from *Cirsium japonicum* var. *maackii* pappus as inhibitors of aldose reductase and their simultaneous determination. *Chem. Pap.* **2017**, *72*, 81–88. [[CrossRef](#)]
11. Alim, Z.; Kilinc, N.; Sengul, B.; Beydemir, S. Mechanism of capsaicin inhibition of aldose reductase activity. *J. Biochem. Mol. Toxicol.* **2017**, *31*, e21898. [[CrossRef](#)]
12. Demir, Y.; Işık, M.; Gulçin, I.; Beydemir, Ş. Phenolic compounds inhibit the aldose reductase enzyme from the sheep kidney. *J. Biochem. Mol. Toxicol.* **2017**, *31*, e21936. [[CrossRef](#)]
13. Demir, Y.; Taslimi, P.; Ozaslan, M.S.; Oztaskin, N.; Çetinkaya, Y.; Gulçin, I.; Beydemir, Ş.; Goksu, S. Antidiabetic potential: In vitro inhibition effects of bromophenol and diarylmethanones derivatives on metabolic enzymes. *Arch. Pharm. Chem. Life Sci.* **2018**, *351*, 1800263. [[CrossRef](#)] [[PubMed](#)]
14. Fiz, O.; Vargas, P.; Alarcón, M.; Aedo, C.; García, J.L.; Aldasoro, J.J. Phylogeny and historical biogeography of Geraniaceae in relation to climate changes and pollination ecology. *Syst. Bot.* **2008**, *33*, 326–342. [[CrossRef](#)]
15. Abraham, Y.; Elbaum, R. Hygroscopic movements in Geraniaceae: The structural variations that are responsible for coiling or bending. *New Phytol.* **2013**, *199*, 584–594. [[CrossRef](#)]
16. Ávila, M.B.; De Lúcio, J.A.G.; Mendoza, N.V.; González, C.V.; De la Arciniega, M.O.; Vargas, G.A. Chapter 5. Geranium Species as Antioxidants. In *Oxidative Stress and Chronic Degenerative Diseases—A Role for Antioxidants*; IntechOpen: London, UK, 2013; pp. 113–129.
17. Hayman, S.; Kinoshita, J.H. Isolation and properties of lens aldose reductase. *J. Biol. Chem.* **1965**, *240*, 877–882. [[PubMed](#)]
18. Kuçukislamoglu, M.; Yayli, N.; Senturk, H.B.; Genc, H. Flavonol glycosides from *Consolida armeniaca*. *Turk. J. Chem.* **2000**, *24*, 191–197.
19. Valente, L.M.M.; Bizarri, C.; Liechocki, S.; Barboza, R.; Da Paixão, D.; Almeida, M.B.S.; Benevides, P.J.C.; Magalhães, A.; Siani, A.C. Kaempferitrin from *Uncaria guianensis* (Rubiaceae) and its potential as a chemical marker for the species. *J. Braz. Chem. Soc.* **2009**, *20*, 1041–1045. [[CrossRef](#)]
20. Serkedjieva, J.; Ivancheva, S. Antiherpes virus activity of extracts from the medicinal plant *Geranium sanguineum* L. *J. Ethnopharmacol.* **1998**, *64*, 59–68. [[CrossRef](#)]
21. Ben Jemia, M.; Wannas, W.A.; Ouchikh, O.; Bruno, M.; Kchouck, M.E. Antioxidant activity of Tunisian *Geranium robertianum* L. (Geraniaceae). *Nat. Prod. Res.* **2013**, *2013*, 2076–2083. [[CrossRef](#)]
22. Graça, V.C.; Barros, L.; Calhelha, R.C.; Dias, M.I.; Carvalho, A.M.; Santos-Buelga, C.; Santos, P.F.; Ferreira, I.C.F.R. Chemical characterization and bioactive properties of aqueous and organic extracts of *Geranium robertianum* L. *Food Funct.* **2016**, *7*, 3807–3814. [[CrossRef](#)] [[PubMed](#)]
23. Neagu, E.; Paun, G.; Constantin, D.; Radu, G.L. Cytostatic activity of *Geranium robertianum* L. extracts processed by membrane procedures. *Arab. J. Chem.* **2017**, *10*, S2547–S2553. [[CrossRef](#)]
24. Lee, J.; Lee, D.G.; Rodriguez, J.P.; Park, J.Y.; Cho, E.J.; Jacinto, S.D.; Lee, S. Determination of flavonoids in *Acer okamotoanum* and their aldose reductase inhibitory activities. *Hortic. Environ. Biotechnol.* **2018**, *59*, 131–137. [[CrossRef](#)]
25. Quilantang, N.G.; Ryu, S.H.; Park, S.H.; Byun, J.S.; Chun, J.S.; Lee, J.S.; Rodriguez, J.P.; Yun, Y.-S.; Jacinto, S.D.; Lee, S. Inhibitory activity of methanol extracts from different colored flowers on aldose reductase and HPLC—UV analysis of quercetin. *Hortic. Environ. Biotechnol.* **2018**, *59*, 899–907. [[CrossRef](#)]
26. Shim, J.-U.; Lim, K.-T. Anti-oxidative and anti-proliferative character of glycoprotein isolated from *Geranium sibiricum* Linne in Chang liver cells. *Environ. Toxicol. Pharmacol.* **2008**, *26*, 320–324. [[CrossRef](#)] [[PubMed](#)]
27. Shim, J.-U.; Oh, P.-S.; Lim, K.-T. Anti-inflammatory activity of ethanol extract from *Geranium sibiricum* Linne. *J. Ethnopharmacol.* **2009**, *126*, 90–95. [[CrossRef](#)] [[PubMed](#)]

28. Wu, N.; Zu, Y.; Fu, Y.-J.; Kong, Y.; Zhao, J.; Li, X.; Li, J.; Wink, M.; Efferth, T. Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. *J. Agric. Food Chem.* **2010**, *58*, 4737–4743. [[CrossRef](#)]
29. Boisvert, W.A.; Yu, M.; Choi, Y.; Jeong, G.H.; Zhang, Y.-L.; Cho, S.; Choi, C.; Lee, S.; Lee, B.-H. Hair growth-promoting effect of *Geranium sibiricum* extract in human dermal papilla cells and C57BL/6 mice. *BMC Complement. Altern. Med.* **2017**, *17*, 109. [[CrossRef](#)]
30. Lu, C.H.; Li, Y.Y.; Li, L.J.; Liang, L.Y.; Shen, Y.M. Anti-inflammatory activities of fractions from *Geranium nepalense* and related polyphenols. *Drug Discov. Ther.* **2012**, *6*, 194–197. [[CrossRef](#)] [[PubMed](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).