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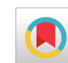

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Aldose reductase inhibition of the methanolic extracts of selected noxious and exotic plants

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Abstract The inhibition of aldose reductase (AR) by the extracts from sixteen noxious and exotic plants was examined. Among them, *Aster pilosus* showed the highest inhibition of AR (IC_{50} value 0.11 μg/mL). This study showed that *A. pilosus* with promising AR-inhibitory activities can be utilized for the development of natural therapies for treating and managing diabetic complications.

Keywords Aldose reductase · High-performance liquid chromatography · Noxious plant · Quercetin

Introduction

Korea has always been home to native plants with ethnobotanical importance. Currently, the native species' population in every country is considered to be a valuable source of dietary supplements, pharmaceutical products, and ornamental pieces. However, the introduction of exotic or noxious plant species can potentially disrupt a domestic ecosystem in the long run. When such species are brought into new regions, they can eventually form self-sustaining populations by overcoming survival barriers, proliferating, and competing with the native plant ecosystem. This phenomenon could be harmful and contribute to a decrease in the population of some of the native species. Some of these species could even produce toxins that inhibit the germination and growth of other neighboring species, and also deter herbivores, thus allowing the exotic plants to be more ecologically competitive [1]. Noxious species are primarily introduced to solve the pathological conditions plaguing a local ecosystem. However, if left unchecked, they can do more harm than good.

The inhibitory activity of selected noxious species against aldose reductase (AR) was measured in this study. AR is a major enzyme in the polyol pathway, which is implicated in diabetes and its complications. AR inhibition (ARI) has been previously proven to counteract the complications that occur in hyperglycemic animal models [2]. ARI is a good measure of the biological importance of a plant species as a source of bioactive natural products.

The aim of this study was to determine which of the selected noxious and exotic species have significant ARI. It also aimed to quantify the quercetin content of the selected plants.

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Materials and Methods

Plant materials and animal specimens

Noxious and exotic plant species (*Ambrosia trifida*, *Cirsium japonicum* var. *maackii*, *Lactuca serriola*, and *Sicyos angulatus*) were collected in Pyeongtaek and Imsil, Korea. The collected noxious plants were identified by Dr. K. Choi, the Korea National Arboretum, Pocheon, Korea. The voucher specimens (*A. trifida*: LEE2018-01, *C. japonicum* var. *maackii*: LEE2014-05, *L. serriola*: LEE2018-02, *S. angulatus*: LEE2018-03) were deposited at this department. Other extracts were purchased from the Korea Research Institute of Bioscience & Biotechnology (KRIBB), Daejeon, Korea. The samples were oven dried at 50 °C for 24 h. For the ARI assay, seven-week-old Sprague-Dawley rats weighing 210-230 g were supplied by Koatech Co.

Chemicals and apparatus

DL-glyceraldehyde, β-nicotinamide dinucleotide phosphate (NADPH), dimethyl sulfoxide (DMSO), and 3,3'-tetramethyleneglutaric acid

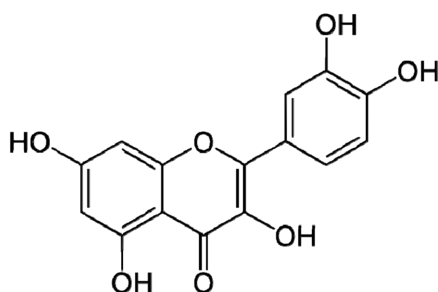

Fig. 1 Structure of quercetin

(TMG) were purchased from Sigma Aldrich (St. Louis, MO, USA). Methanol (MeOH), sodium buffer, and potassium buffer were purchased from Samchun Pure Chemical Co. (Pyeongtaek, Korea). An EYELA rotary evaporator system was used for sample extraction, while an Allegra X-30R refrigerated benchtop centrifuge (Beckman Coulter™, Brea, CA, USA) and an Optizen 2120 UV spectrophotometer (Mecasys Co., Daejeon, Korea) were used for the ARI assay. Quercetin (Fig. 1) isolated from *Rhododendron mucronulatum* for. *albiflorum* was used for analysis [3].

Sample preparation for ARI assay

Six grams of each plant sample was soaked in 300 mL MeOH at 80 °C in reflux for 3 h. The resulting extracts were filtered and concentrated *in vacuo*. The residues obtained were kept inside an oven for 24 h at 40 °C to allow further drying. The stock solutions used for the ARI assay were prepared by dissolving 1 mg extract in 1 mL DMSO. Three to four sample concentrations were prepared via serial dilution of the stock solution.

Preparation of rat lens AR

The protocol described by Hayman and Kinoshita [4] was followed to obtain crude rat lens AR. Lenses from healthy Sprague-Dawley rats were resected and homogenized in 0.1 M sodium buffer (pH 6.2) 0.5 mL was added per lens used. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was then collected and used as an enzyme source for the assay. The supernatant was kept cooled throughout the preparation and for the entire duration of the experiment.

Measurement of AR inhibitory activity

The different methanolic extracts were tested for their ARI activity. The bioactivity was determined by spectrophotometrically measuring the decrease in NADPH at 340 nm for a period of 4 min using DL-glyceraldehyde as a substrate. The assay solution (1 mL) contained the crude rat lens AR, 0.025 M DL-glyceraldehyde, 1.6 mM NADPH, 0.1 M sodium buffer, 0.1 M potassium phosphate buffer (pH 7.0), and the test samples. TMG, a known AR inhibitor, was used as a positive control. The inhibitory activity of the samples was expressed as (%) Inhibition = (normal enzyme activity − inhibited enzyme activity) / normal enzyme activity. The minimum concentration of the sample that inhibits 50% enzyme activity (IC_{50}) was determined from the least-squares regression line of the logarithmic concentrations plotted against the residual activity.

Standard and sample preparation for high-performance liquid chromatography with an ultraviolet detector (HPLC-UV)

Samples that were proven active in the ARI assay were then analyzed for their quercetin content. HPLC samples were prepared by dissolving 10-20 mg of the extracts in 1 mL MeOH. A standard stock solution of quercetin was also prepared by dissolving 1 mg quercetin in 1 mL MeOH. All samples were filtered through a 0.45-μm filter prior to analysis.

HPLC-UV analysis of quercetin in MeOH extracts

HPLC samples were analyzed using an Agilent HPLC. Chromatographic separation was performed with a reverse-phase INNO C_{18} (4.6×250 mm, 5 μm) column. The following gradient elution of 0.5% acetic acid in water (solvent A) and acetonitrile (solvent B) was performed: 90:10 to 50:50 for 50 min. The flow rate and injection volume were 1 mL/min and 10 μL, respectively. The UV detector was set at 270 nm.

Calibration curve

The working solutions used to create the calibration curves were prepared by diluting the standard stock solution to desired concentrations. The calibration curves were used to determine the quercetin content in the samples. Linearity was assessed based on the correlation coefficient (r^2).

Results and Discussion

Since the inhibition of AR, a key enzyme of the polyol pathway, prevents and sometimes reverts diabetic complications, this pathway has been widely studied [5,6]. Diabetes is a metabolic disorder characterized by chronic hyperglycemia, and impaired fat, lipid, as well as carbohydrate metabolism. These attributes lead to various complications such as blindness, renal failure, and nerve damage, contributing to the overall morbidity of the disease. To date, numerous AR inhibitors have been identified, including synthetic and naturally occurring compounds.

In this study, the inhibitory activity of the MeOH extracts of noxious and exotic plants on crude rat lens AR was evaluated. Among the extracts tested, *Aster pilosus* showed the most potent ARI effects with similar IC_{50} value of 0.11 μg/mL (Table 1). Recent studies have shown that the *Morus* spp. contains many bioactive compounds, including flavonoids, alkaloids, and anthocyanins [7]. Further research to characterize the bioactive components of these plants will provide insights into their inhibitory activity against AR.

Quercetin is a flavonoid found ubiquitously in nature and is known to exhibit strong anti-oxidant properties, as well as a wide

Table 1 Half maximal inhibitory concentrations (IC_{50}) of the extracts from the noxious and exotic plants against aldose reductase

Samples	Concentration (g/mL)	AR inhibition[a] (%)	IC_{50}[b] (g/mL)
Ambrosia artemisiifolia var. *elatior*	10	68.35	0.16
	1	59.86	
	0.1	47.02	
*Ambrosia trifida**	10	73.45	3.44
	1	16.37	
	0.1	5.75	
Arisaema amurense var. *serratum*	10	41.74	-
Arisaema ringens	10	39.45	-
Arisaema robustum	10	46.1	-
Aster pilosus	10	70.87	0.11
	1	69.26	
	0.1	45.87	
Caltha palustris var. *membranacea*	10	42.66	-
Cirsium japonicum var. *maackii**	10	52.21	12.24
	1	13.71	
	0.1	1.76	
Convallaria keiskei	10	60.78	1.89
	1	42.2	
	0.1	37.16	
*Lactuca serriola**	10	64.15	4.81
	1	20.35	
	0.1	6.63	
Nerium indicum	10	67.43	0.80
	1	44.27	
	0.1	41.97	
Pulsatilla koreana	10	28.21	-
Rumex acetocella	10	81.42	0.24
	1	56.88	
	0.1	45.18	
Scopolia japonica	10	77.06	0.17
	1	63.99	
	0.1	45.18	
*Sicyos angulatus**	10	18.14	-
Veratrum patulum	10	66.28	1.19
	1	54.13	
	0.1	24.77	
TMG[c]	10	99.62	0.17
	1	67.31	
	0.1	45.38	

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

range of biological activities, including anti-inflammatory, anti-diabetic, and anti-cancer effects [8-11]. The anti-diabetic properties of quercetin have been widely reported in the literature, amongst several studies have reported quercetin as an AR inhibitor [12,13]. Being a flavonoid, it has been shown to be present in many plant extracts [14,15]. Hence, the samples presenting high ARI

Table 2 Quercetin contents of the noxious and exotic plant species analyzed using high-performance liquid chromatography (HPLC)

Plant species	Quercetin content (mg/g)
Ambrosia artemisiifolia var. *elatior*	0.15±0.00
Aster pilosus	1.36±0.02
Caltha palustris var. *membranacea*	0.03±0.00
Convallaria keiskei	0.05±0.00
Lactuca serriola	0.36±0.02
Nerium indicum	0.17±0.00
Pulsatilla koreana	0.02±0.00
Rumex acetocella	2.28±0.02
Veratrum patulum	0.07±0.01

activities were further analyzed for quercetin. Content analysis using HPLC-UV was performed in order to assess whether quercetin is the AR inhibitor with the highest content among these plants and consequently the compound responsible for the apparent inhibition of AR. However, as indicated by the chromatograms, quercetin is not the dominant compound in the tested plants in terms of content. Calibration curve of quercetin is $Y = 2513.3X - 74.45$ ($r^2 = 0.9999$). Results showed that quercetin was mainly detected in *Rumex acetocella* with a content of 2.28 mg/g extract. Content quantification showed that quercetin levels among all the selected plants were very low and some were detected (Table 2, Fig. 2). This suggests that the ARI may not be due to quercetin, and that some other AR inhibitor may be present in these plants. There are recent reports on flavonoids and AR [16-20].

Conservation of the Korean native plants with positive ARI may prove to be beneficial in the long run because of their potential to be economically valuable as sources of bioactive compounds. More studies need to be conducted on the toxic plants in order to identify the phytochemicals that contribute to their bioactivity. *Aster pilosus* and other species belonging to the family Compositae are known to contain a variety of flavonoids [21]. *Ambrosia artemisiifolia* was also previously studied for its triterpenoid and caffeic acid derivatives [22]. Lastly, *Nerium indicum* has been proven to contain compounds with potential larvicidal properties against mosquito larvae [23]. In this case, quercetin was not a major compound for AR inhibition in the sample clearly. Further study is need for the investigation of other flavonoids related to AR inhibition next time.

Pinpointing the phytochemical constituents of Korean noxious and exotic plant species with potential bioactivity can help in their conservation. Moreover, figuring out possible pharmacological uses for these noxious and exotic plants can help in curbing their populations while taking full advantage of their economic value.

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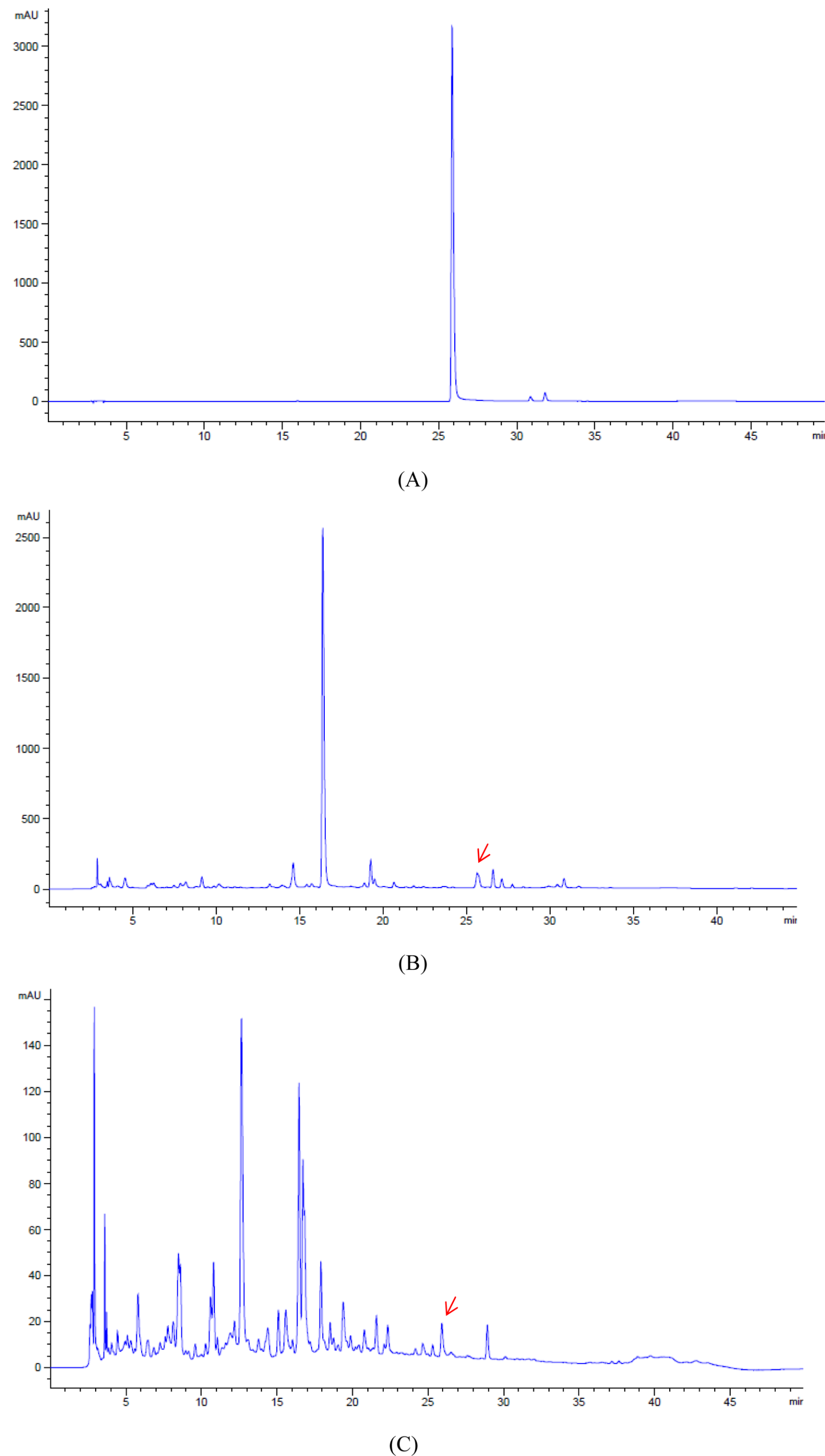

Fig. 2 HPLC chromatograms of the quercetin standard (A) and the plant species that showed quercetin peaks-*Rumex acetocella* (B), *Lactuca serriola* (C), *Nerium indicum* (D), *Ambrosia artemisiifolia* var. *elatior* (E), *Veratrum patulum* (F), *Convallaria keiskei* (G), *Caltha palustris* var. *membranacea* (H), and *Pulsatilla koreana* (I)

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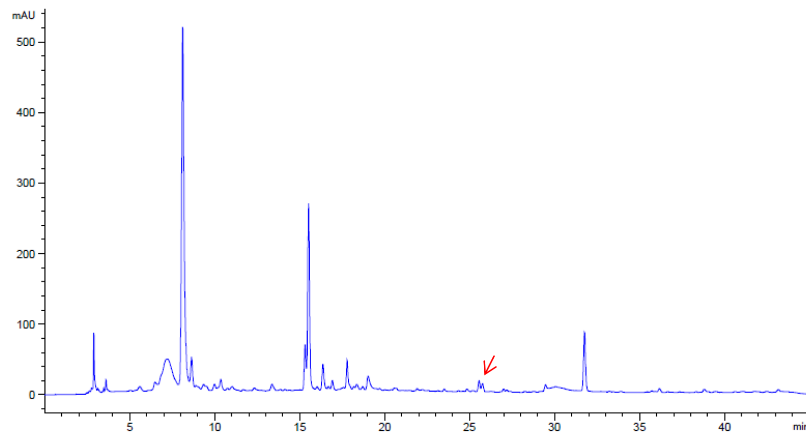

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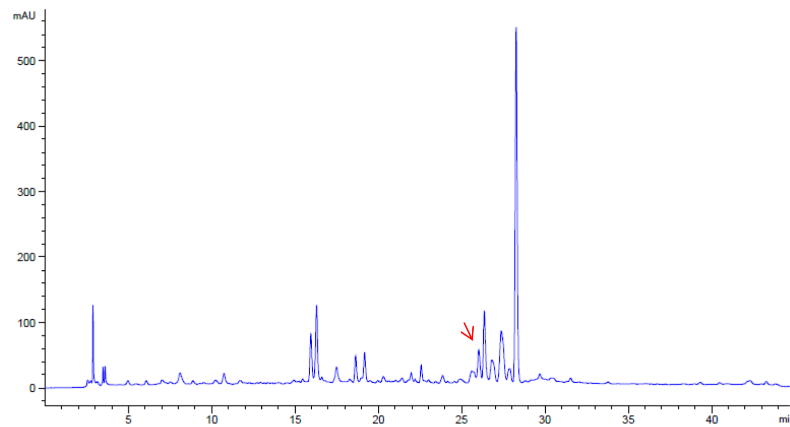

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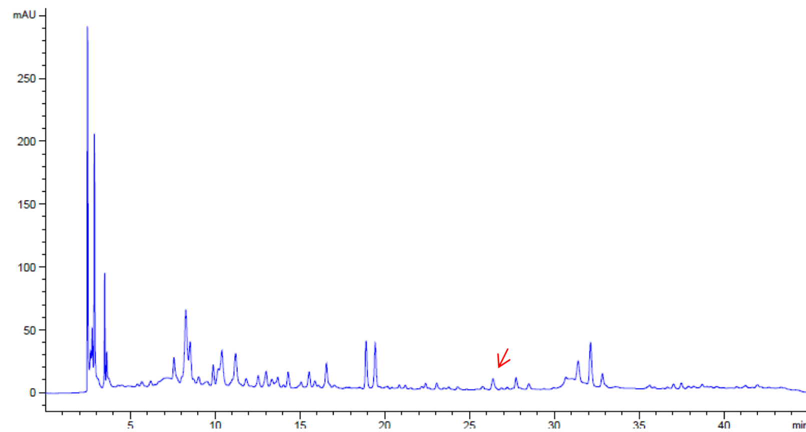

(F)

Fig. 2 Continued.

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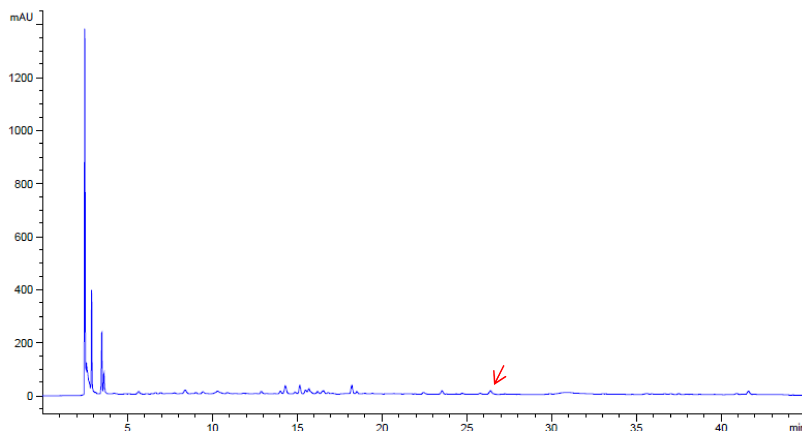

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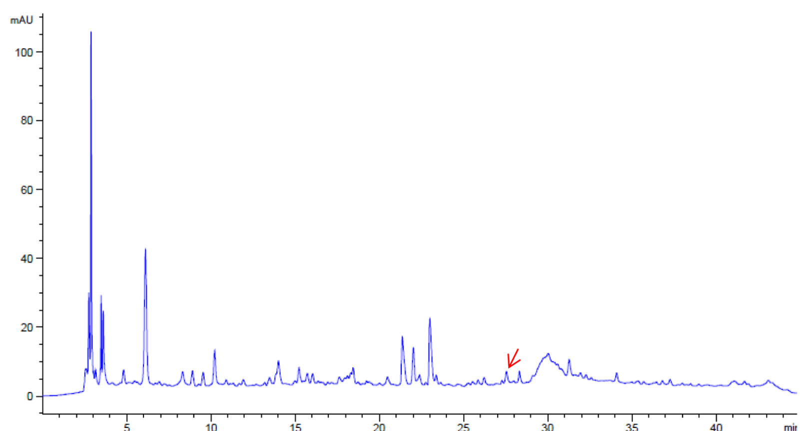

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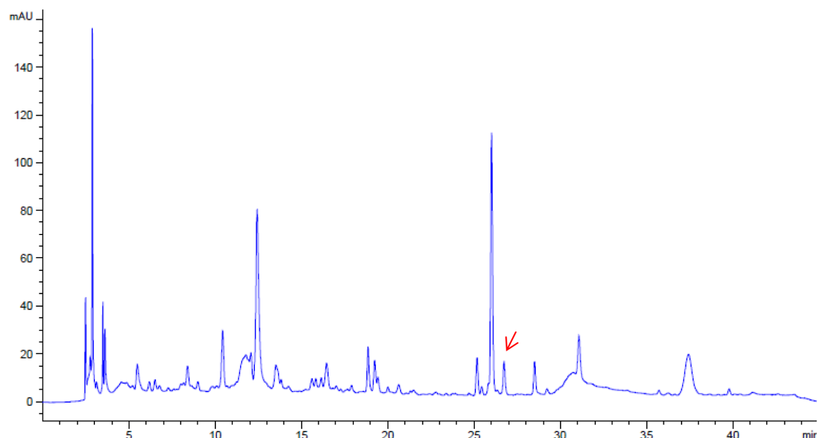

(I)

Fig. 2 Continued.

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