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Full Length Research Paper

Antiglycation Activity of *Otostegia persica* (Burm.) Boiss

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Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin and kidneys. Increased glycation of proteins and accumulation of advanced glycation endproducts (AGEPs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGEP formation are also accompanied by the formation of free radicals via autoxidation of glucose and glycated proteins. Compounds with combined antiglycation and antioxidant properties may offer therapeutic potential. Since the antioxidant activity of different extracts and fractions of aerial parts of *Otostegia persica* has been evaluated and this plant is used as an antidiabetic agent in Iranian traditional medicine, we evaluated the antiglycation activity of this species. Here, we report the isolation of known compound 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone for the antiglycation properties of this plant.

Key words: Antiglycation, *Otostegia persica*, 3',7-dihydroxy-4',6,8-trimethoxy-flavone.

INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by chronic hyperglycemia which results for a deficiency of or resistance to insulin. Diabetes affects 1-2% of the population and there are around 100 million worldwide. This figure is expected to double over the next 10-15 years. Individuals affected by diabetes are prone to complications such as retinopathy, cataract, neuropathy, atherosclerosis, nephropathy, embryopathy and wounds (Muhammed Saeed and Nessar, 2006).

Non-enzymatic glycosylation (glycation) between reducing sugar and free amino group of proteins, also known as Millard reaction, leads to the formation of glycated protein termed Amadori product. Further rearrangement, oxidation and reduction of the Amadori products result in the formation of several advanced glycation end products (AGEs) such as pentosidine, carboxymethyllysine, crossline and pyralline. Some of these products can react with a free amino group nearby and form crosslinking between proteins (Ulrich and Cerami, 2001). The crosslinked protein, example, crosslinked collagen are postulated to confer pathological conditions found in patients with diabetes and aging, such as arterial stiffness and decreased myocardial compliance, resulting from the loss of collagen elasticity (Singh et al., 2001; Aronson, 2003). Thus, agents that inhibit the formation of AGEs are purported to have therapeutic potentials in patients with diabetes and age-related disease.

The oxidation process is believed to play an important role in AGEs formation. Further oxidation of Amadori

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Abbreviations: DMSO, Dimethyl sulfoxide; **BSA,** bovine serum albumin; **TCA,** trichloroacetic acid; **AGEs,** advanced glycation endproducts.

product leads to the formation of intermediate carbonyl compounds that can react with the nearby lysine or arginine residues to form protein crosslink and AGEs. The reactive carbonyl compounds may also be generated from the metal ion-catalyzed autooxidation of glucose (Rahbar and Figarola, 2003; Voziyan et al., 2003).

Therefore, agents with antioxidative or metal-chelating property may retard the process of AGEs formation by preventing further oxidation of Amadori product and metal-catalyzed glucose oxidation (Jedsadayanmata, 2005). In this regard, several natural compounds known to possess antioxidative property, such as flavonoid-rich extracts, have been shown to prevent AGEs formation.

Otostegia persica (*Lamiaceae*) locally called "Golder", is widely distributed in south and southeastern of Iran. It is traditionally used for the treatment of malaria, fever and diabetes. The antioxidant activity of the different extracts and fractions of aerial parts of *O. persica* has been evaluated (Yassa et al., 2005) and since this plant is traditionally used in patients with diabetes, we investigated the antiglycation activity of *O. persic*a. Here, we report 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone as the active compound for antiglycation property of this plant.

MATERIALS AND METHODS

Chemicals and general experimental procedure

BSA (Bovine serum albumin) was purchased from the Research Organics Cleveland USA, while other chemicals (glucose anhydrous, trichloroacetic acid (TCA), sodium azide (NaN_3), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (Na_2HPO4), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO4) and sodium hydroxide (NaOH) were purchased from Sigma Aldrich.

Sodium phosphate buffer (pH 7.4) was prepared by mixing Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM), phosphate buffer saline (PBS) (pH 10) was prepared by mixing NaCl (137 mM) + Na_2HPO_4 (8.1 mM) + KCl (2.68 mM) + KH_2PO_4 (1.47 mM) + pH 10 was adjusted with NaOH (0.25 mM), while BSA (10 mg/ml) and glucose anhydrous (50 mg/ml) solutions were prepared in sodium phosphate buffer.

The FT IR spectra were recorded on a vector 22 instrument. The ^{1}H-NMR was recorded on a Bruker AMX 500 NMR (Avance) instruments using the UNIX data solvent. ^{1}H-^{13}C HMBC and HMQC were recorded at 500 MHz (proton) and 125 MHz (carbon), respectively. EI MS spectra were recorded on a Finnigan MAT 312. FAB mass measurements were performed on Jeol JMS HX 110 mass spectrometer using glycerol as the matrix. HREI MS was carried out on Jeol JMS 600 mass spectrometer. Column chromatography was carried out on silica gel (M and N), 70 - 230 mesh.

Plant material

The plant, *O. persica* (*Lamiaceae*) was collected from Bandar Abbas, Hormozgan province, Iran, in May 2008 and identified by Mr. M. Kamalinezhad at the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

A voucher specimen (No. 1719) has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Extraction

The air dried flowering aerial parts of *O. persica* (8 kg) was exhaustively extracted by maceration with EtOH 80% (3 × 80 lit).

The extract was evaporated to yield the residue (1112 g) which was partitioned between water (72 g), petroleum ether (188 g), CH_2Cl_2 (380 g), EtOAc (276 g) and n-butanol (132 g). The CH_2Cl_2 fraction had significant antiglycation, so we subjected it to silica gel chromatography using petroleum ether with a gradient of CH_2Cl_2 up to 100% and followed by methanol. Six fractions were collected. As fraction No. 5 had antiglycation activity, we loaded it on silica gel and eluted with petroleum ether-EtOAc in increasing order of polarity to provide two fractions with petroleum ether/EtOAc 76:24 and 81:19.

Since the second fraction was active, it was rechromatographed over silica gel and eluted with petroleum ether/EtOAc (58:42 and 52:48) to purify compound 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone (128 mg) which exhibited a very good antiglycation activity.

In vitro glycation assay

60 µl of sample was prepared by dissolving in DMSO and sample mixture (20 µl BSA + 20 µl of glucose anhydrous + 20 µl test sample). Glycated control contain 20 µl BSA + 20 µl glucose + 20 µl sodium phosphate buffer, while blank control contains 20 µl BSA and 40 µl sodium phosphate buffer. After incubation in 96 well plates at 37°C for 7 days, samples are taken out and cooled at room temperature. After incubation, 60 µl 100% TCA was added in each well and centrifuged (15000 rpm) for 4 min at 4°C. After agitation and centrifugation at 14000 rpm for 4 min, the supernatant containing glucose, inhibitor and interfering substance was removed and pellet contains AGE-BSA were dissolved in PBS. Assessment of fluorescence spectrum (ex. 370 nm) and change in fluorescence intensity (ex. 370 nm to 440 nm) based on AGEs were monitored by using Spectrofluorimeter RF-1500 (Shimadzu, Japan). % inhibition was calculated through the following formula. Rutin is used as the standard inhibitor. The comparison of fluorescence intensity at 370 nm Excitation and Emission at 440 nm is obtained by using Spectrofluorimeter.

The results are reported in % inhibition = 100 - [(OD (test) / OD (blank)] x 100

RESULTS AND DISCUSSION

The active compound was isolated as pale yellow needles and identified as 3´,7-dihydroxy-4´,6,8-trimethoxy-flavone by X-ray Crystallography (Figure 1). Despite the structure elucidation by X-ray Crystallography, we report the 1H-NMR and 13C-NMR data of this compound (Tables 1 and 2). 3´,7-dihydroxy-4´,6,8-trimethoxy-flavone has exhibited a good antiglycation activity. It was observed that this compound at 3 mM concentration has shown 65% inhibition while the standard inhibitor, Rutin showed 83% inhibition.

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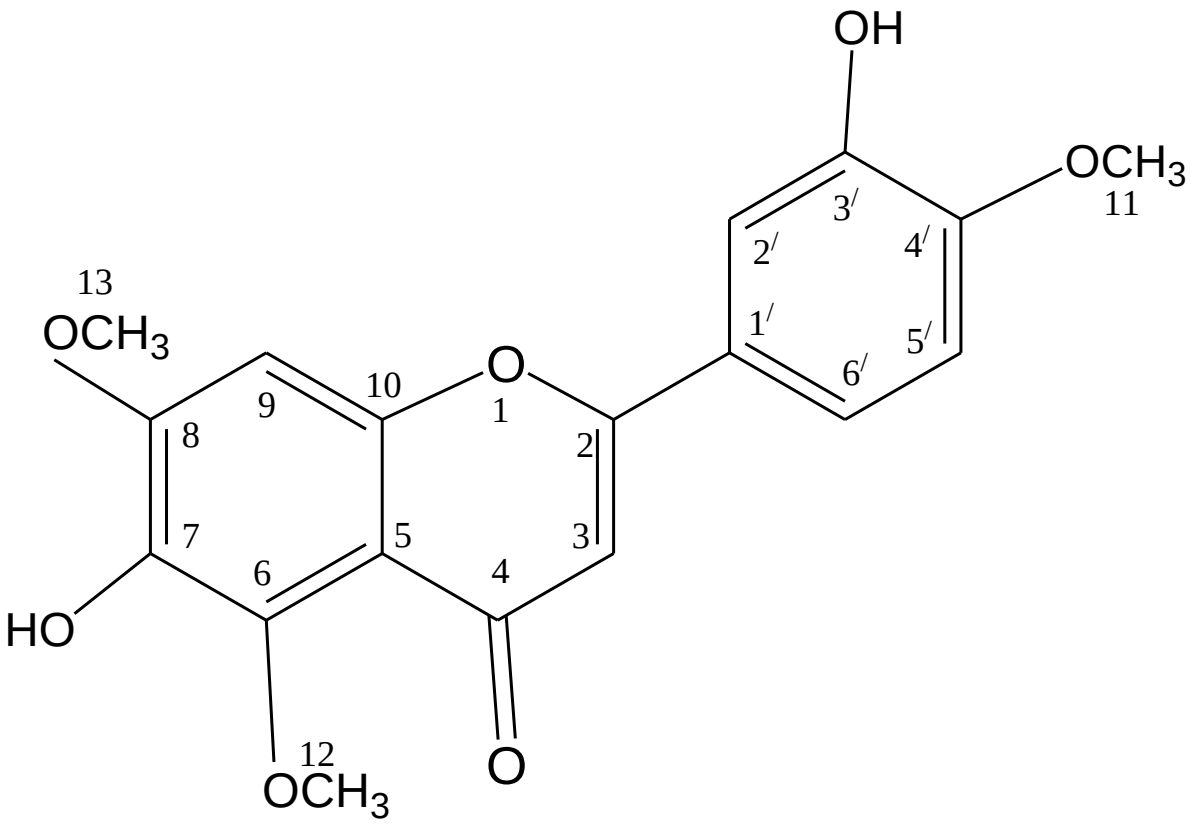

Figure 1. X-ray crystallography structure of 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone compound.

Table 1. 1H-NMR (CDCl3) data of 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone. δ in ppm, J in Hz.

Position	δ ^{1}H-NMR
C (2)	-
H–C (3)	6.54 (1H, *s*)
C (4)	-
C (5)	-
C (6)	-
C (7)	-
C (8)	-
H–C (9)	6.51 (1H, *s*)
C (10)	-
C (1´)	-
H–C (2´)	7.43 (1H, *d,* J=2.1)
C (3´)	-
C (4´)	-
H–C (5´)	6.91 (1H, *d,* J = 8.4)
H–C (6´)	7.39 (1H, *dd,* J = 8.4, 2.1)
MeO (11)	3.95 (3H, *s*)
MeO (12)	3.90 (3H, *s*)
MeO (13)	3.94 (3H, *s*)

Table 2. ^{13}C-NMR (CDCl3) data of 3´, 7-dihydroxy-4´,6,8-trimethoxy-flavone. δ in ppm.

Carbon No.	δ^{13}C-NMR
C (2)	163.8
C (3)	104.5
C (4)	182.6
C (5)	153.2
C (6)	132.7
C (7)	153.0
C (8)	158.7
C (9)	90.6
C (10)	106.2
C (1´)	124.5
C (2´)	112.3
C (3´)	146.1
C (4´)	149.6
C (5´)	110.7
C (6´)	119.0
MeO (11)	56.3
MeO (12)	60.8
MeO (13)	56.1

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