Contents lists available at ScienceDirect

IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60178-4

Inhibition of advanced glycation endproducts formation by Korean thistle, *Cirsium maackii*

Hyun Ah Jung[1], Jin Ju Park[2], Byung Sun Min[3], Hee Jin Jung[2], Md. Nurul Islam[2], Jae Sue Choi[2*]

[1]*Department of Food Science and Human Nutrition, Chonbuk National University, Jeonju 561-756, Republic of Korea*
[2]*Department of Food and Life Science, Pukyong National University, Busan 608-737, Republic of Korea*
[3]*College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Republic of Korea*

ARTICLE INFO

Article history:
Received 26 October 2014
Received in revised form 10 November 2014
Accepted 22 December 2014
Available online 20 January 2015

Keywords:
Flavonoids
Thistle
Advanced glycation endproducts
Diabetic complications
Cirsium maackii

ABSTRACT

Objective: To evaluate inhibitory potential of seven Korean thistles against the advanced glycation endproducts (AGE) formation as well as to identify responsible compounds from the most active species. **Methods:** We used an *in vitro* AGE inhibition assay to evaluate the anti-diabetic complication potential of the methanol extracts of the selected Korean thistles. **Results:** Among the seven Korean thistles, the leaves of *Cirsium maackii* (*C. maackii*) exhibited the most significant inhibitory activity against AGE formation. By means of bioassay-directed fractionation, a lignan, chlorogenic acid and 14 flavonoids were isolated from the active ethyl acetate soluble fraction of a methanol extract from *C. maackii* leaves. Luteolin and its 5-*O*-glucoside have been previously isolated; however, a lignan and 13 known compounds were isolated for the first time from *C. maackii* leaves in this study. Most of the isolated compounds exhibited inhibitory activities against potential AGE formation. Among them, cernuoside was shown to be the most potent AGE inhibitor with an IC_{50} value of 21.21 μ mol/L. Most importantly, two major flavonoids, luteolin and its 5-*O*-glucoside, also significantly inhibited AGE formation, with IC_{50} values of 36.33 and 37.47 μ mol/L, respectively. Structure activity relationship revealed that the presence of free 3′ and 4′ dihydroxyl group in flavonoids skeleton played an important role in AGE inhibition. **Conclusions:** These results indicate that *C. maackii* and *C. maackii*-derived flavonoids might be explored further to develop therapeutic agents for the prevention of diabetic complications due to their significant inhibitory activity against AGE formation.

1. Introduction

Persistent hyperglycemia induces the formation of reactive oxygen species (ROS) and advanced glycation endproducts (AGE), elevates the activity of aldose reductase, and activates protein kinase C isoforms, all of which play a key role in the pathogenesis of many long-term diabetic complications such as retinopathy, cataractogenesis, nephropathy and neuropathy[1]. In particular, increased formation of AGE due to prolonged hyperglycemia and carbonyl stress in diabetes and their subsequent accumulation in various tissues has been implicated in the pathogenesis of many diseases, including atherosclerosis, cardiac dysfunction, and vascular inflammation[2,3]. AGE are a chemically heterogeneous group of compounds formed in the Maillard reaction or browning reaction, when reducing sugars react non-enzymatically with amine residues, predominantly lysine and arginine, on proteins, lipids, and nucleic acids, ultimately leading to their chemical modification[4]. Recently, AGE formation has drawn considerable attention due to its involvement in the development of vascular complications during atherogenesis. During AGE generation, highly reactive oxygen/nitrogen species and dicarbonyl compounds are produced as intermediates that may attack numerous biomolecules. AGE also contribute to tissue injury by altering extracellular matrix structures through the formation of protein cross-links and modifying short-lived intracellular proteins such as metabolic enzymes and mitochondrial protein complexes[5]. These observations led to the development of numerous AGE inhibitors with diverse

*Corresponding author: Jae Sue Choi, Department of Food and Life Science, Pukyong National University, Busan 608-737, Republic of Korea.
Tel: 82-51-629-5845
Fax: 82-51-629-5842
E-mail: choijs@pknu.ac.kr.

Foundation project: This work was supported by the Pukyong National University Research Fund in 2011 (C-D-2011-0766).

chemical structures for use as possible therapeutic agents. Recently, there has been a growing interest in alternative therapies, especially in the therapeutic use of plant-derived natural products for the management of diabetes due to lower toxicity and fewer side effects than synthetic drugs.

Thistles, all members of the Compositae family, are perennial plants, 0.5-2.0 m in height, possessing lance-shaped, spiny-toothed leaves and white to purple flowers. The thistles grow abundantly in Korea, Japan, and China[6,7]. Thus far, 250 species of thistles have been identified worldwide, and about 10 of these species have been found in Korea[8]. Because thistle has been used in traditional folk medicine as a diuretic, antiphlogistic, hemostatic, and detoxifying agent[9,10], most thistles have been surveyed by phytochemists and pharmacologists. Especially, milk thistle is one of the most popular herbal remedies used by patients with liver disease[11]. A great deal of research has been conducted regarding the flavonoids harbored by thistles, and a variety of activities are exhibited by these species[12-15].

Previously, we reported that there is a correlation between antioxidant activity and the HPLC profiles of several Korean thistles[16], and the rat lens aldose reductase inhibitory activity of *C. maackii* and its major active constituents[17]. As a part of our ongoing search for anti-diabetic agents derived from natural sources, we investigated the AGE formation inhibitory activities of seven Korean thistles including *Cirsium chanroenicum* (*C. chanroenicum*), *Cirsium lineare* (*C. lineare*), *Cirsium maackii* (*C. maackii*), *Cirsium nipponicum* (*C. nipponicum*), *Cirsium pendulum* (*C. pendulum*), *Cirsium setidens* (*C. setidens*), and *Carduus crispus* (*C. crispus*) and identified compounds responsible for AGE inhibitory activities from the most active species.

2. Materials and methods

2.1. General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were measured using a JEOL JNM ECP-400 spectrometer (JEOL, Japan) at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Column chromatography was performed using silica (Si) gel 60 (70-230 mesh, Merck, Darmstadt, Germany) and Sephadex LH-20 (20-100 μm, Sigma, St. Louis, MO, USA). All TLC analyses were conducted on precoated Merck Kieselgel 60 F254 plates (20 cm×20 cm, 0.25 mm, Merck) using 50% H_2SO_4 as a spray reagent.

2.2. Plant extracts

Methanolic extracts of *C. lineare* collected in September 2002, *C. chanroenicum* in September 2003, *C. setidens* in September 2001, *C. nipponicum* in August 2001, *C. pendulum* in July 2003, and *C. crispus* in May 2005, were purchased from the Korean Plant Extract Bank under the Korea Research Institute of Bioscience and Biotechnology, Korea in February 2006. Whole *C. maackii* plants were collected in Ulsan, Republic of Korea, in August 2007. The plants were authenticated by a specialist in Cirsium taxonomy, Dr. Y. Kadota at the Department of Botany, National Museum of Nature and Science in Tsukuba, Japan. Each whole plant was separated into flowers, leaves, roots, and stem and dried in the shade for a week. A whole plant was registered and deposited as a voucher specimen at the herbarium of the National Museum of Nature and Science in Tsukuba, Japan as well as at the Department of Food Science and Nutrition, Pukyong National University (Professor J.S. Choi).

2.3. Chemicals and reagents

Bovine serum albumin (BSA), aminoguanidine hydrochloride, D-(-)-fructose, D-(+)-glucose, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium azide was purchased from Junsei Chemical Co. (Tokyo, Japan). All solvents used were purchased from Merck, Duksan Pure Chemical Co., unless stated otherwise.

2.4. Extraction, fractionation and isolation

The powder from leaves of *C. maackii* (1.28 kg) was refluxed with methanol (MeOH) for 3 h (4 L×5). The total filtrate was then concentrated and dried in vacuo at 40 ℃ in order to render the MeOH extract (316.3 g). This extract was suspended in distilled water and successively partitioned with methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) to yield the CH_2Cl_2 (68.8 g), EtOAc (80.6 g), and *n*-BuOH (30.7 g) fractions, respectively, as well as an aqueous residue (125.8 g). The EtOAc fraction (80.0 g) was first chromatographed over a silica gel column (8 cm×80 cm) using a mixed solvent of CH_2Cl_2 and MeOH (CH_2Cl_2:MeOH = 20:1 to 1:1, v:v, gradient) to afford 16 subfractions (F01-F16). Compounds apigenin (22 mg), luteolin (10.5 g) and quercetin (28 mg) were separately purified from EF01 (0.8 g), EF03 (13.9 g), and EF04 (0.5 g), respectively, with a solvent mixture of *n*-hexane and EtOAc (*n*-hexane:EtOAc = 8:1. v:v). Silica gel column chromatography of combined EF05 (2.38 g) and EF06 (2.43 g) using identical solvent conditions led to the isolation of apigenin 7-*O*-glucuronide methyl ester (48 mg), cernusoside (56 mg) and apigenin 7-*O*-glucuronide (27 mg). Compounds luteolin 5-*O*-glucoside (18.0 g), luteolin 7-*O*-glucoside (35 mg), tracheloside (16 mg), diosmin (230 mg), naringenin 5-*O*-glucoside (25 mg), eriodictyol 5-*O*-glucoside (15 mg), and luteolin 4′-*O*-glucoside (23 mg) were purified from EF08 (28.2 g), EF09 (0.5 g), and combined EF10 (1.5 g), EF11 (1.3 g) and EF12 (1.2 g) on Sephadex LH-20 columns (100% MeOH), respectively. Repeated chromatography of EF14 (1.1 g) over a silica gel column with CH_2Cl_2:MeOH:H_2O (26:14:4, v:v) afforded quercetin 3-*O*-glucoside (130 mg). Fraction EF13 (3.0 g) was recrystallized from 100% MeOH, yielding chlorogenic acid (350 mg). The combined EF14 and EF15 (7.4 g) was then chromatographed over a silica gel column with CH_2Cl_2:MeOH:H_2O (26:14:4, v:v) to give rutin (450 mg). The isolated compounds were identified and characterized by different spectroscopic methods, including ^{1}H- and ^{13}C-NMR as well as by comparisons with published spectral data and TLC analyses.

2.5. Inhibition of AGE formation

Inhibition of AGE formation was determined according to the modified method of Vinson and Howard[18]. To prepare the AGE reaction solution, 10 mg/mL BSA in 50 mmol/L sodium phosphate buffer (pH 7.4), with 0.2 g/L sodium azide to prevent bacterial growth, was added to 0.2 mol/L fructose and 0.2 mol/L glucose. The reaction mixture (950 μL) was then mixed with various concentrations of the samples (50 μL, final concentration: 0.20-200.00 μg/mL for the extracts and compounds; and 0.10-100.00 μg/mL for the fractions) dissolved in 10% (v/v) DMSO. After incubation at 37 ℃ for 7 d, the fluorescence intensity of the reaction products was determined using a spectrofluorometric detector (FLx800 microplate fluorescence reader, Bio-Tek Instruments, Inc., Winooski, VT, USA), with respective excitation and emission wavelengths at 350 and 450 nm. The percent inhibition of AGE formation was determined from a graphical plot of the data and is expressed as the mean±SEM (triplicate experiments). Aminoguanidine, a nucleophilic hydrazine compound, was used as a reference compound in the AGE assay.

2.6. Statistical analysis

Statistical significance was analyzed using one-way ANOVA and Student's *t*-test (Systat In., Evanston, IL., USA), with $P<0.01$ considered to be statistically significant. All results are expressed as mean±SEM from three experiments.

3. Results

3.1. AGE formation inhibitory activities of the selected Korean thistles

The inhibitory activities of the MeOH extracts of the tested Korean thistles on AGE formation were investigated. The MeOH extracts from the roots, whole plants, stem, and leaves of the same *C. maackii* exerted good inhibitory activities against AGE formation, with IC_{50} values of (129.31±5.29), (114.23±7.04), (62.74±1.41), and (28.20±0.84) μg/mL, respectively, which are comparable to the IC_{50} value of (149.04±11.27) μg/mL for the positive control, aminoguanidine (128.08 μg/mL). The inhibitory activity increased in the order of leaves > stem > whole plants > roots of the same *C. maackii* plant. In contrast, the MeOH extracts from the *C. maackii* flower, from the whole *C. crispus*, *C. nipponicum*, *C. pendulum*, *C. lineare*, and *C. setidens* plants, and both the aerial and underground parts of *C. chanroenicum* did not show any inhibitory activity at concentrations up to 200.00 μg/mL.

3.2. AGE formation inhibitory activities of different solvent soluble fractions of C. maackii

Because the MeOH extract of *C. maackii* had marked AGE inhibitory activity, we partitioned the extract with several solvents and further assessed the AGE inhibitory activity of the fractions at concentrations ranging from 1.00 to 100.00 μg/mL. The EtOAc and *n*-BuOH fractions among the several solvent-soluble fractions obtained from the MeOH extract showed significant AGE inhibitory activities with IC_{50} values of (2.78±0.04) and (87.84±4.91) μg/mL, respectively, compared to aminoguanidine with the IC_{50} value of (128.08±6.89) μg/mL. However, the CH_2Cl_2 and H_2O fractions did not show any activity at the test concentrations.

3.3. AGE formation inhibitory activities of the isolated compounds from C. maackii

Since the EtOAc fraction was found as the most active fraction, it was selected for chromatography in order to isolate active compounds. Repeated chromatography of the EtOAc fraction yielded 16 compounds, including a lignan, chlorogenic acid, and 14 flavonoids. The AGE formation inhibitory activities of the compounds isolated from *C. maackii* are summarized in the Table 1. As shown in the Table 1, most of the isolated compounds exhibited promising AGE formation inhibitory activities. Among them, cernuoside was shown to be the most potent AGE inhibitor followed by luteolin 7-*O*-glucoside and quercetin with their respective IC_{50} values of (21.21±0.25), (24.22±0.29) and (24.35±0.95) μmol/L, compared to the positive control aminoguanidine. In addition, chlorogenic acid, luteolin and its glycosides, quercetin 3-*O*-glucoside, and rutin also showed strong inhibitory activities against AGE formation. Interestingly, although apigenin showed weak inhibitory activity, its two glycosides, apigenin 7-*O*-glucuronide methyl ester and apigenin 7-*O*-glucuronide, did not show inhibitory activity at the concentration tested.

Table 1
Inhibition of AGE formation by compounds isolated from EtOAc fraction of *C. maackii* leaves.

Compounds	IC_{50} (μg/mL) [a]	IC_{50} (μM) [b]
Apigenin (1)	152.52±6.95	564.89±25.72
Luteolin (2)	10.40±0.03	36.33±0.10
Quercetin (3)	7.35±0.29	24.35±0.95
Apigenin7-*O*-glucuronide methyl ester (4)	>200	-
Cernuoside (5)	9.50±0.11	21.21±0.25
Apigenin 7-*O*-glucuronide (6)	>200	-
Luteolin 5-*O*-glucoside (7)	16.80±0.13	37.47±0.29
Luteolin 7-*O*-glucoside (8)	10.85±0.13	24.22±0.29
Tracheloside (9)	>200	-
Diosmin (10)	41.20±0.56	137.21±1.87
Naringenin 5-*O*-glucoside (11)	45.25±0.99	104.26±2.28
Eriodictyol 5-*O*-glucoside (12)	28.77±0.66	63.93±1.47
Luteolin 4′-*O*-glucoside (13)	15.24±0.07	33.87±0.16
Quercetin 3-*O*-glucoside (14)	18.80±0.43	47.00±1.13
Chlorogenic acid (15)	10.36±0.01	29.28±0.04
Rutin (16)	31.99±0.74	52.44±1.22
Aminoguanidine	129.36±1.60	1 170.15±14.47

[a]Test concentrations of samples were 0.20-200.00 μg/mL dissolved in DMSO. IC_{50} is expressed as the mean±SEM from triplicate experiments.

[b]Aminoguanidine was used as a positive control.

4. Discussion

Persistent hyperglycemia induces increased formation of AGE, which has been implicated in the onset of diabetic complications. In this point of view, we investigated the AGE formation inhibitory activities of the selected Korean thistles using an *in vitro* AGE formation inhibitory assay. Interestingly, only different parts of *C. maackii*, which was previously found to be a good inhibitor of rat lens aldose reductase, exhibited potent inhibitory activities against AGE formation. The significant variability in AGE inhibitory activities between *Cirsium* species or even between parts of the same plant might result from differences in the active components between species and different parts of the same plant, leading to variation in the inhibitory effect on diabetic complications. Therefore, the present study was designed to identify the active compounds responsible for potent AGE inhibitory activity of *C. maackii* in addition to the relationship between their structure and function.

Solvent partitioning of the MeOH extract of *C. maackii* yielded four subfractions, among which EtOAc and *n*-BuOH fractions showed significant AGE inhibitory activities. However, the CH_2Cl_2 and H_2O fractions did not show any activity at the test concentrations. It is interesting to note that both EtOAc and *n*-BuOH fractions had marked AGE inhibitory activity, whereas the other fractions showed weak or no activities at the tested concentrations. Plants possess a range of nonpolar to polar phytochemicals. The comparative inhibitory activities of several fractions derived from the MeOH extract of *C. maackii* leaves can be explained by their compositional differences. Flavonoids were expected to be present in large amounts in the polar EtOAc and *n*-BuOH fractions. From a health point of view, this may be meaningful to consumers because most of the components in the EtOAc and *n*-BuOH fractions are easily dissolved in water. In the present study, from the most active EtOAc fraction of the MeOH extract of the *C. maackii* leaves, a lignan, chlorogenic acid, and 14 known flavonoids were isolated through repeated column chromatography and were subsequently characterized by ^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC, as well as comparisons with published spectral data. luteolin and its 5-*O*-glucoside had been previously isolated, however, a lignan, along with 13 known compounds were isolated from *C. maackii* leaves for the first time in this study[19-30].

Besides apigenin, luteolin, quercetin, apigenin 7-glucuronide and its methyl ester, chlorogenic acid and rutin were previously described as AGE inhibitors, cernuoside, luteolin 4′, 5 or 7-*O*-glucoside, diosmin, naringenin 5-glucoside, eriodictyol 5-glucoside, quercetin 3-glucoside tested in our study showed concentration-dependent inhibitory activity against AGE formation for the first time. Although apigenin and diosmin had weak activities at the tested concentrations, the order of AGE inhibitory activity of most active flavonoids was similar, with IC_{50} values ranging from 21.21 to 52.44 μmol/L. Structural comparisons of these flavonoids and their inhibitory activities clearly show that the ortho-dihydroxy function of the B ring in the flavonoid skeleton, as evidenced by luteolin, quercetin, and their glycosides including rutin, quercetin 3-glucoside, and luteolin 5 or 7-*O*-glucoside, plays a pivotal role in the observed inhibitory activity. When such an *O*-dihydroxyl group was absent (i.e., apigenin and its glycosides), weak inhibitory activity was observed. The mechanism by which flavonoids inhibit AGE formation has yet to be established. The basic structure of flavonoids and other structural factors is important in the inhibitory mechanism. Muthenna *et al*. suggested the following two possible mechanisms for flavonoid-induced inhibition of AGE formation: (i) it scavenges free radicals directly or (ii) it chelates the metal ions by forming complexes with them and thereby partly inhibits post-Amadori formation[31]. It was also reported that protein glycation was accompanied by oxidative reactions[32]. Yim *et al*. has also suggested that glycation of albumin leads to the increased production of ROS[33]. Jung *et al*. demonstrated that the presence of an ortho-hydroxyl group on the B-ring plays an important role in the antioxidant activity in the total ROS system[17]. Similar trends were observed in flavonoid-induced inhibitory activity against AGE formation in the present study. Considering present and previous results on ROS inhibition of flavonoids[17,34], it appears that the inhibitory mechanism of flavonoids against glycation is at least partly due to antioxidant activity. Flavonoids are of current interest in research due to the important biological and pharmacological properties attributed to their antioxidant properties[35]. Our results indicate several major flavonoids exhibit significant AGE inhibitory potential. According to previous research, two key flavonoids, luteolin and luteolin 5-*O*-glucoside inhibit RLAR activity and the generation of ROS[34]. Our previous comparative HPLC analysis of different kinds of thistles devoid of AGE inhibitory activity revealed the absence of these compounds[16], indicating luteolin derivatives may play a role in the ability of *C. maackii* to inhibit diabetic complications.

Since a study comparing the ability of thistles to inhibit diabetic complications has not yet been reported, we studied the ability of some Korean thistles to inhibit AGE formation and isolated the active components from *C. maackii*, the most promising AGE inhibitory thistle found in the study. In conclusion, these previous data, together with our present results from AGE assays, indicate that *C. maackii* and *C. maackii*-derived flavonoids could be promising therapeutic agents for preventing diabetic complications. Based on the *in vitro* results, further experiments are needed to clarify the mechanisms of *C. maackii* and its flavonoids *in vivo*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by the Pukyong National University Research Fund in 2011 (C-D-2011-0766).

References

[1] El-Kabbani O, Ruiz F, Darmanin C, Chung RPT. Aldose reductase structures: implications for mechanism and inhibition. *Cell Mol Life Sci* 2004; **61**(7-8): 750-762.

[2] Renard C, Obberghen EV. Role of diabetes in atherosclerotic pathogenesis. What have we learned from animal models? *Diabetes Metab* 2006; **32**(1): 15-29.

[3] Basta G, Schmidt AM, Caterina RD. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc Res* 2004; **63**(4): 582-592.

[4] Bucala R, Cerami A. Advanced glycosylation: chemistry, biology, and implications for diabetes and aging. *Adv Pharmacol* 1992; **23**: 1-34.

[5] Rahbar S. Novel inhibitors of glycation and AGE formation. *Cell Biochem Biophys* 2007; **48**(2-3): 147-157.

[6] Lee CB. *Flora of Korea*. Seoul: HyangMoonSa; 1979, p. 274.

[7] Ganzera M, Pocher A, Stuppner H. Differentiation of *Cirsium japonicum* and *C. setosum* by TLC and HPLC-MS. *Phytochem Anal* 2005; **16**(3): 205-209.

[8] Lee YN. *Flora of Korea*. Seoul: Kyohaksa; 2002, p. 843.

[9] Lee SJ. *Korean folk medicine*. Seoul: Seoul National University; 1966, p. 145-146.

[10]Kim JG. *Illustrated natural drugs encyclopedia*. Seoul: Namsandang; 1984, p. 37.

[11]Flora KD, Rosen HR, Benner KG. The use of naturopathic remedies for chronic liver disease. *Am J Gastroenterol* 1996; **91**: 2654-2655.

[12]Lee HB, Kwak JH, Zee OP, Yoo SJ. Flavonoids from *Cirsium rhinoceros*. *Arch Pharm Res* 1994; **17**(4): 273-277.

[13]Perez GR, Rhamirez LM, Vargas SR. Effect of *Cirsium pascuarense* on blood glucose levels of normoglycaemic and alloxan-diabetic mice. *Phytother Res* 2001; **15**(6): 552-554.

[14]Park JC, Hur JM, Park JG, Kim SC, Park JR, Choi SH, et al. Effects of methanol extract of *Cirsium japonicum* var. *ussuriense* and its principle, hispidulin-7-*O*-neohesperidoside on hepatic alcohol-metabolizing enzymes and lipid peroxidation in ethanol-treated rats. *Phytother Res* 2004; **18**(1): 19-24.

[15]Nazaruk J, Jakoniuk P. Flavonoid composition and antimicrobial activity of *Cirsium rivulare* (Jacq.) All. flowers. *J Ethnopharmacol* 2005; **102**(2): 208-212.

[16]Jeong DM, Jung HA, Choi JS. Comparative antioxidant activity and HPLC profiles of some selected Korean thistles. *Arch Pharm Res* 2008; **31**(1): 28-33.

[17]Jung HA, Kim YS, Choi JS. Quantitative HPLC analysis of two key flavonoids and inhibitory activities against aldose reductase from different parts of the Korean thistle, *Cirsium maackii*. *Food Chem Toxicol* 2009; **47**(11): 2790-2797.

[18]Vinson JA, Howard III TB. Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J Nutr Biochem* 1996; **7**(12): 659-663.

[19]Geissman TA, Harborne JB. Anthochlor pigments. X. Aureusin and cernuoside. *J Amer Chem Soc* 1955; **77**(17): 4622-4624.

[20]Lin JH, Lin YT, Huang YJ, Wen KC, Chen RM, Ueng TH, et al. Isolation and cytotoxicity of flavonoids from Daphnis Genkwae Flos. *J Food Drug Anal* 2001; **9**(1): 6-11.

[21]Marin FR, Ortuño A, Benavente GO, Del RJA. Distribution of flavones glycoside diosmin in *Hyssopus officinalis* plants: changes during growth. *Planta Med* 1998; **64**(2): 181-182.

[22]Markham KR, Ternal B, Stanley R, Geiger H, Mabry TJ. Carbon-^{13}NMR studies of flavonoids-III; naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* 1978; **34**(9): 1389-1397.

[23]Nishibe S, Hisada S, Inagaki I. Structures of tracheloside and nortracheloside from *Trachelospermum asiaticum* Nakai var. *intermedium* Nakai. *Chem Pharm Bull* 1971; **19**(4): 866-868.

[24]Piet VL, Andre DB, Milos B. Reinvestigation of the structural assignment of signals in the ^{1}H and ^{13}C NMR spectra of the flavones apigenin. *Mag Res Chem* 1986; **24**(10): 879-882.

[25]Wang M, Simon JE, Aviles IF, He K, Zheng QY, Tadmor Y. Analysis of antioxidative phenolic compounds in Artichoke (*Cynara scolymus* L.). *J Agric Food Chem* 2003; **51**(3): 601-608.

[26]Xia H, Qiu F, Zhu S, Zhang T, Qu G, Yao X. Isolation and identification of ten metabolites of breviscapine in rat urine. *Biol Pharm Bull* 2007; **30**(7): 1308-1316.

[27]Feng XZ, Xu SX, Dong M. Two novel flavonoids from *Ixeris sonchifolia*. *J Chin Pharm Sci* 2000; **9**(3): 134-135.

[28]Mughal UR, Fatima I, Malik A, Tareen RB. Loasifolin, a new flavonoid from *Eremostachys loasifolia*. *J Asian Nat Prod Res* 2010; **12**(4): 328-330.

[29]Zhang SX, Tani T, Yamaji S, Ma CM, Wang MC, Cai SQ, et al. Glycosyl flavonoids from the roots and rhizomes of *Asarum longerhizomatosum*. *J Asian Nat Prod Res* 2003; **5**(1): 25-30.

[30]Wang LB, Gao HY, Toshi M, Sun BH, Huang J, Masayuki Y, et al. Flavonones from *Helichrysi flos* syn. *Chin J Nat Med* 2009; **7**(5):357-360.

[31]Muthenna P, Akileshwari C, Saraswat M, Bhanuprakash RG. Inhibition of advanced glycation end-product formation on eye lens protein by rutin. *Brit J Nutr* 2012; **107**(7): 941-949.

[32]Wu CH, Yen GC. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *J Agric Food Chem* 2005; **53**(8): 3167-3173.

[33]Yim YB, Yim HS, Lee C, Kang SO, Chock PB. Protein glycation: creation of catalytic sites for free radical generation. *Ann N Y Acad Sci* 2001; **928**: 48-53.

[34]Jung HA, Jung MJ, Kim JY, Chung HY, Choi JS. Inhibitory activity of flavonoids from *Prunus davidiana* and other flavonoids on total ROS and hydroxyl radical generation. *Arch Pharm Res* 2003; **26**(10): 809-815.

[35]Martinez-Florez S, Gonzalez-Gallego J, Culebras JM, Tuñón MJ. Flavonoids: properties and anti-oxidizing action. *Nutr Hosp* 2002; **17**(6): 271-278.