Natural Product Sciences
12(4) : 210-213 (2006)

Flavonols from *Houttuynia cordata* with Protein Glycation and Aldose Reductase Inhibitory Activity

Dae Sik Jang[1], Jong Min Kim[1], Yun Mi Lee[1], Jeong Lim Yoo[1], Young Sook Kim[1], Joo-Hwan Kim[2], and Jin Sook Kim[1,*]

[1]*Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Daejeon 305-811, Korea*
[2]*Department of Life Science, Daejeon University, Daejeon 300-716, Korea*

Abstract – A 4,5-dioxoaporphine type alkaloid, cepharadione B (**1**), a phenolic acid, protocatechuic acid (**2**), and flavonols, quercetin (**3**), afzelin (**4**), and quercitrin (**5**), were isolated from the EtOAc-soluble extract of the whole plants of *Houttuynia cordata*. All the isolates (**1-5**) were subjected to *in vitro* bioassays to evaluate advanced glycation end products (AGEs) formation and rat lens aldose reductase (RLAR) inhibitory activity. The three flavonols **3-5** exhibited a significant inhibitory activity on AGEs formation with IC_{50} values of 66.9, 58.9, and 32.3 μM, respectively. While the two flavonol rhamnosides **4** and **5** showed a remarkable inhibitory activity against RLAR with IC_{50} values of 0.81 and 0.16 μM, respectively.
Keywords – *Houttuynia cordata*, Saururaceae, flavonols, diabetic complications, AGEs, aldose reductase

Introduction

Hyperglycemia has an important role in the pathogenesis of long-term complications and diabetic patients with poor blood glucose control are particularly at risk (Ahmed, 2005). Persistent hyperglycemia induces abnormal changes such as the formation of advanced glycation end products (AGEs) (Makita, *et al.*, 1993), the increase of sorbitol through the polyol pathway (Shinohara, *et al.*, 1998), the overactivation of protein kinase C isoforms due to the synthesis of DAG (Larkins and Dunlop, 1992). Enhanced formation and accumulation of AGEs have been implicated as a major pathogenesis process leading to diabetic complications, normal aging, atherosclerosis, and alzheimer's disease (Rahbar, *et al.*, 2000; Kalousova, *et al.*, 2004). Aldose reductase (AR), the key enzyme in the polyol pathway, also has been demonstrated to play important roles in the pathogenesis of diabetic complications and cataract formation (Beyer-Mears and Cruz, 1985). Thus, the design and discovery of inhibitors of AGEs formation or AR can offer a promising therapeutic approach for the prevention of diabetic or other pathogenic complications (Forbes, *et al.*, 2003; Yabe-Nishimura, 1998).

In our ongoing project directed toward the discovery of preventive agents for diabetic complications from herbal medicines (Jang, *et al.*, 2006), the whole plants of *Houttuynia cordata* were chosen for more detailed investigation, since the EtOAc-soluble fraction of the MeOH extract showed a significant *in vitro* inhibitory effect on formation of AGEs. *Houttuynia cordata* Thunb. (Saururaceae) is a traditional medicinal plant used in Korea, India, Japan, and China for years for the treatment of cough, leucorrhea and ureteritis so on (Shanghai Science and Technological Publisher, 1985; Ji and Zhao, 2003). Previous phytochemical investigations on *H. cordata* have resulted in the isolation of essential oils (Liu and Deng, 1979), flavonoids (Park, *et al.*, 2000), and alkaloids (Pröbstle, *et al.*, 1994; Kim, *et al.*, 2001). In the present study, an alkaloid, a phenolic acid, and three flavonols were isolated from an EtOAc-soluble extract of the whole plants of *H. cordata*, and their inhibitory effects on AGEs and RLAR were evaluated.

Experimental

Instruments and reagents – NMR experiments were conducted on a DRX-300 FT-NMR (Bruker, Germany), and the chemical shifts were referenced to the residual solvent signals. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) and then heated at 110 °C for 5 - 10 min. Silica gel (Merck 60A, 70 - 230 or 230 -

*Author for correspondence
Fax: +82-42-868-9471; E-mail: jskim@kiom.re.kr

400 mesh ASTM) and sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Plant material – The whole plants of *Houttuynia cordata* Thunb. (Saururaceae) were collected in Guomun Island, Yeosu City, Jeonnam Province, Korea (34°43'35" N, 127°38'34" E), in October, 2004 and were identified by one of the authors, Prof. J.-H. Kim. A voucher specimen (no. 20041028-003) has been deposited at the Taejon University Taejonesis (TUT), Daejeon University, Daejeon 300-716, Korea.

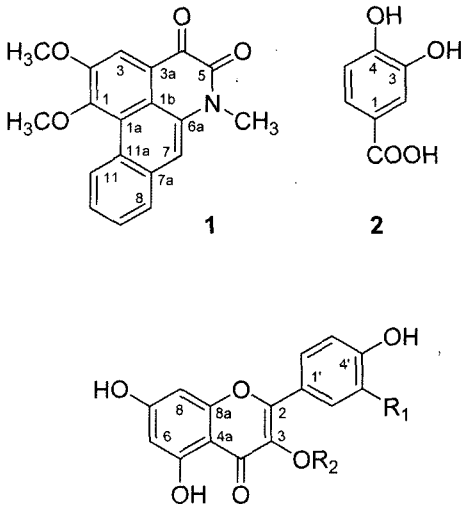
Extraction and isolation – The air dried plant material (3.3 kg) was pulverized and extracted with MeOH (3 × 20 *l*). The extracts were combined and concentrated *in vacuo* at 40 °C. The concentrated extract (440 g) was suspended in H_2O (1.5 *l*) and then partitioned with *n*-hexane (3 × 1.5 *l*) to afford a *n*-hexane-soluble fraction (123 g) on drying. Next, the aqueous layer was partitioned again with EtOAc (3 × 1.5 *l*) to give an EtOAc-soluble fraction (26.0 g) and an aqueous residue. The EtOAc-soluble fraction was subjected to repeated chromatography to afford compounds **1** (13 mg), **2** (100 mg), **3** (30 mg), **4** (140 mg), and **5** (330 mg).

Cepharadione B (1) – Orange needles; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.45 (1H, br dd, H-11), 8.15 (1H, s, H-3), 7.83 (1H, br dd, J = 9.0 and 3.6 Hz, H-8), 7.64 (2H, m, H-9 and H-10), 7.39 (1H, s, H-7), 4.09 (6H, s, OCH_3-1 and OCH_3-2), 3.78 (3H, s, N-CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 175.6 (C-4), 156.4 (C-2), 155.1 (C-1), 153.0 (C-5), 132.4 (C-6a), 131.9 (C-3a), 129.2 (C-8), 128.2 (C-11), 127.8 (C-7a/C-9), 127.1 (C-1a), 124.7 (C-10), 123.8 (C-11a), 119.7 (C-1b), 114.5 (C-7), 112.8 (C-3), 60.6 (OCH_3-1), 56.7 (OCH_3-2), 30.7 (N-CH_3).

Protocatechuic acid (2) – Colorless needles; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.33 (1H, d, J = 1.8 Hz, H-2), 7.28 (1H, dd, J = 8.1 and 1.8 Hz, H-6), 6.77 (1H, d, J = 8.1 Hz, H-5); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 167.5 (*C*OOH), 149.9 (C-4), 144.8 (C-3), 121.9 (C-1), 121.8 (C-6), 116.6 (C-2), 115.1 (C-5).

Quercetin (3) – Yellowish powder; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 12.48 (1H, br s, OH-5), 7.68 (1H, d, J = 2.1 Hz, H-2'), 7.53 (1H, dd, J = 8.0 and 2.1 Hz, H-6'), 6.88 (1H, d, J = 8.4 Hz, H-5'), 6.40 (1H, d, J = 2.1 Hz, H-8), 6.18 (1H, d, J = 2.1 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 175.3 (C-4), 165.5 (C-7), 160.3 (C-5), 155.7 (C-8a), 147.8 (C-2), 146.4 (C-4'), 144.8 (C-3'), 138.6 (C-3), 121.5 (C-1'), 115.2 (C-5'), 114.3 (C-2'), 102.6 (C-4a), 97.8 (C-6), 92.9 (C-8).

Afzelin (4) – Yellowish powder; ^{1}H-NMR (acetone-d_6, 300 MHz) δ 12.71 (1H, br s, OH-5), 7.86 (2H, d, J = 8.4 Hz, H-2'/ H-6'), 7.02 (1H, d, J = 8.4 Hz, H-3'/H-5'), 6.47 (1H, s, H-8), 6.26 (1H, s, H-6), 5.54 (1H, s, rhamnosyl H-1"), 3.26-4.23 (m, rhamnosyl H), 0.90 (3H, d, J = 5.7 Hz, rhamnosyl CH_3); ^{13}C-NMR (acetone-d_6, 75 MHz) δ 179.4 (C-4), 165.0 (C-7), 163.3 (C-5), 160.9 (C-4'), 158.5 (C-2), 158.1 (C-8a), 135.9 (C-3), 131.8 (C-2'/ C-6'), 122.6 (C-1'), 116.4 (C-3'/C-5'), 105.9 (C-4a), 102.7 (C-1"), 99.7 (C-6), 94.6 (C-8), 73.1 (C-4"), 72.6 (C-5"), 71.6 (C-3"), 71.4 (C-2"), 17.9 (C-6").



3 R_1 = OH, R_2 = H
4 R_1 = H, R_2 = rha
5 R_1 = OH, R_2 = rha

Fig. 1. Structures of compounds **1-5** from the whole plants of *Houttuynia cordata*.

Table 1. Inhibitory activity of compounds from the whole plants of *Houttuynia cordata* on advanced glycation end products (AGEs) formation and rat lens aldose reductase (RLAR) *in vitro*[a]

compound	inhibitory effects (IC_{50} value; *i*M)[a]	
	AGEs formation	RLAR
1	> 500	53.1
2	125.4	191.2
3	66.9	40.3
4	58.9	0.81
5	32.3	0.16
AG[b]	961	–
EP[b]	–	0.07
TMG[b]	–	24.7

[a]Inhibitory effect was expressed as mean ± S.D. of quadruplicate experiments. IC_{50} values were calculated from the dose inhibition curve. [b]Aminoguanidine (AG), EP (epalrestate), and TMG (3,3-tetramethyleneglutaric acid) were used as positive control.

Quercitrin (5) – Yellowish powder; ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.33 (1H, br s), 7.29 (1H, d, J = 8.1 Hz, H-6'), 6.90 (1H, br d, J = 8.4 Hz, H-5'), 6.33 (1H, s, H-8), 6.17 (1H, s, H-6), 5.34 (1H, d, J = 1.2 Hz, rhamnosyl H-1"), 3.30-4.23 (m, rhamnosyl H), 0.94 (3H, d, J = 6.0 Hz, rhamnosyl CH_3); ^{13}C-NMR (CD_3OD, 75 MHz) δ 179.6 (C-4), 167.1 (C-7), 167.0 (C-5), 163.2 (C-2), 159.3 (C-8a), 149.9 (C-4'), 146.6 (C-3'), 136.3 (C-3), 123.1 (C-1'), 122.9 (C-6'), 117.1 (C-5'), 116.5 (C-2'), 105.7 (C-4a), 103.7 (C-1"), 100.3 (C-6), 95.1 (C-8), 73.4 (C-4"), 72.2 (C-5"), 72.1 (C-3"), 72.0 (C-2"), 17.8 (C-6").

Determination of AGEs formation – According to the established method (Vinson and Howard, 1996), the reaction mixture, 10 mg/ml of bovine serum albumin (Sigma, St Louis, MO, USA) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide to prevent bacterial growth was added to 0.2 M fructose and glucose. The reaction mixture was then mixed with compounds, or aminiguanidine (Sigma, St Louis, MO, USA). After incubating at 37 °C for 14 days, the fluorescent reaction products were assayed on a spectrofluorometric detector (BIO-TEK, Synergy HT, USA; Ex: 350, Em: 450 nm).

Measurement of RLAR activity – Rat lens were removed from the eyes of 8 weeks old Sprague-Dawley rats (Dae-Han Bio Link Co., Umsung, Korea) weighing 100-150 g and homogenized in 12 volumes of a 135 mM Na, K-phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsulfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 100,000 × g for 30 min, and the supernatant fluid was used as the crude rat lens aldose reductase (RLAR). RLAR activity was assayed according to the methods described (Kim and Oh, 1999; Matsuda, *et al.*, 2002) with slight modification. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Lithium sulfate, 0.03 mM NADPH, 1 mM $_{DL}$-glyceraldehyde as a substrate, and 50 μl of enzyme fraction, with or without 25 μl of sample solution, in a total volume of 1.0 ml. The reaction was initiated by the addition of NADPH at 37 °C and stopped by the addition of 0.3 ml of 0.5 M HCl. Then, 1 ml of 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. Fluorescence was measured using a spectrofluorometric detector (Schimadzu RF-5301PC, Japan, Ex: 360, Em: 460 nm). Both AGEs and RLAR assays were performed in triplicate. The concentration of each test sample giving 50% inhibition of the activities (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity.

Results and Discussion

A 4,5-dioxoaporphine type alkaloid, a phenolic acid, and three flavonols were isolated from the EtOAc-soluble extract of the whole plants of *Houttuynia cordata*. The isolates, previously reported from the same plants, were identified as cepharadione B (**1**) (Kim, *et al.*, 2001), protocatechuic acid (**2**) (Park, *et al.*, 2000), quercetin (**3**) (Park, *et al.*, 2000), afzelin (**4**) (Park, *et al.*, 2000), and quercitrin (**5**) (Park, *et al.*, 2000) by physical and spectroscopic data measurement and by comparison with published values.

All the isolates (**1-5**) were subjected to *in vitro* bioassays to evaluate AGEs formation and RLAR inhibitory activities. The three flavonols **3-5** exhibited a significant inhibitory activity on AGEs formation with IC_{50} values of 66.9, 58.9, and 32.3 μM, respectively (IC_{50} value of a positive control aminoguanidine: 961 μM). Protocatechuic acid (**2**) also showed a remarkable inhibitory activity (IC_{50} value of 125.4 μM). In the RLAR inhibitory assay, the two flavonol rhamnosides **4** and **5**, which exhibited a strong activity on AGEs, showed an excellent inhibitory activity against RLAR with IC_{50} values of 0.81 and 0.16 μM, respectively, while compounds **1-3** showed a moderate activity. Therefore, afzelin (**4**) and quercitrin (**5**) seem worthy of additional biological tests to more fully evaluate their potential as therapeutic agents for diabetic complications and related diseases. Quercitrin (**5**) is known inhibitor of AGEs formation and RLAR, and our data are consistent with recent results on the inhibition of AGEs formation (Matsuda, *et al.*, 2003) and RLAR (Matsuda, *et al.*, 2002) by flavonoids. However, inhibitory effects of cepharadione B (**1**) on aldose reductase and protocatechuic acid (**2**) on AGEs formation have not been reported up to date.

Acknowledgment

We thank Korea Basic Science Institute (KBSI) for running NMR and MS experiments. This research was supported by a grant [L06010] from Korea Institute of Oriental Medicine and by a grant of Traditional and Biomedical Research Center [RRco4700. 2005] by ITEP.

References

Ahmed, N., Advanced glycation endproducts-role in pathology of diabetic complications, *Diabetes Res. Clin. Practice* **67**, 3-21 (2005).

Beyer-Mears, A. and Cruz, E., Reversal of diabetic cataract by sorbinil, an aldose reductase inhibitor. *Diabetes* **34**, 15-21 (1985).

Forbes, J.M., Cooper, M.E., Oldfield, M.D., and Thomas, M.C., Role of

advanced glycation end products in diabetic nephropathy. *J. Am. Soc. Nephrol.* **14**, S254-S258 (2003).

Jang, D.S., Kim, J.M., Lee, Y.M., Kim, Y.S., Kim, J.-H., and Kim, J.S., Puerariafuran, a new inhibitor of advanced glycation end products (AGEs) isolated from the roots of *Pueraria lobata*. *Chem. Pharm. Bull.* **54**, 1315-1317 (2006).

Ji, B. and Zhao, K., Clinical application of *Houttuynia cordata* injection. *Chin. Med. Pharm. J.* **2**, 14-16 (2003).

Kalousova, M., Zima, T., Tesar, V., Stipek, S., and Sulkova, S., Advanced glycation end products in clinical nephrology. *Kidney Blood Press Res.* **27**, 18-28 (2004).

Kim, S.-K., Ryu, S.Y., No, J., Choi, S.H., and Kim, Y.S., Cytotoxic alkaloids from *Houttuynia cordata*. *Arch. Pharm. Res.* **24**, 518-521 (2001).

Kim, H.-Y. and Oh, J.H., Screening of Korean forest plants for rat lens aldose reductase inhibition. *Biosci. Biotechnol. Biochem.* **63**, 184-188 (1999).

Larkins, R.G. and M.E. Dunlop., The link between hyperglycaemia and diabetic nephropathy. *Diabetologia* **35**, 499-504 (1992).

Liu, Y.L. and Deng, Z.F., Investigation of the chemical constituents of the essential oil of *Houttuynia cordata* Thunb. *Acta Botanica Sinica* **21**, 244-249 (1979).

Makita, Z., Radoff, S., Rayfield, E.J., Yang, Z.H., Skolnik, E., Delaney, V., Friedman, E.A., Cerami, A., and Vlassara, H.N., Advanced glycosylation end products in patients with diabetic nephropathy. *N. Eng. J. Med.* **325**, 836-842 (1993).

Matsuda, H., Morikawa, T., Toguchida, I., and Yoshikawa, M., Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. *Chem. Pharm. Bull.* **50**, 788-795 (2002).

Matsuda, H., Wang, T., Managi, H., and Yoshikawa, M., Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg. Med. Chem.* **11**, 5317-5323 (2003).

Park, J.-C., Hur, J.-M., Park, J.-W., Park, S.-J., Lee, J.-H., Sung, N.-J., Choi, M.-R., Song, S.-H., Kim, M.-S., and Lee, J.-W., The effects of *Houttuynia cordata* on the hepatic bromobenzene metabolizing enzyme system in rats and isolation of phenolic compounds. *Kor. J. Pharmacog.* **31**, 228-234 (2000).

Pröbstle, A., Neszmely, A., Jerkovich, G., Wagner, H., and Bauer, R., Novel pyridine and 1,4-dihydropyridine alkaloids from *Houttuynia cordata*. *Nat. Prod. Lett.* **4**, 235-240 (1994).

Rahbar, S., Yerneni, K.K., Scott, S., Gonzales, N., and Lalezari, I., Novel inhibitors of advanced glycation endproducts (Part II). *Mol. Cell Biol. Res. Commun.* **3**, 360-366 (2000).

Shanghai Science and Technological Publisher, The dictionary of Chinese Drugs Vol. 1, Shougakukan, Tokyo, 1985, p. 507.

Shinohara, R., Mano, T., Nagasaka, A., Sawai, Y., Uchimura, K., Hayashi, R., Hayakawa, N., Nagata, M., Makino, M., Kakizawa, H., Itoh, Y., Nakai, A., and Itoh, M., Effects of thyroid hormone on the sorbitol pathway in streptozotocin-induced diabetic rats. *Biochim. Biophys. Acta* **1425**, 577-586 (1998).

Yabe-Nishimura, C., Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. *Pharmacol. Rev.* **50**, 21-33 (1998).

Vinson, J.A. and Howard III, T.B., Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J. Nutr. Biochem.* **7**, 659-663 (1996).

(Accepted December 7, 2006)