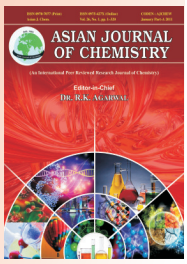
Asian Journal of Chemistry; Vol. 26, No. 15 (2014), 4582-4586

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16116



Anti-Oxidative and Anti-Inflammatory Effects of Phenolic Compounds from the Stems of *Quercus acuta* Thunberg

M.H. OH, K.H. PARK, M.H. KIM, H.H. KIM, S.R. KIM, K.J. PARK, J.H. HEO and M.W. LEE*

College of Pharmacy, Chung Ang University, Seoul 156-756, Republic of Korea

*Corresponding author: Fax: +82 2 8229778; Tel: +82 2 8205602; E-mail: mwlee@cau.ac.kr

Received: 29 July 2013; *Accepted*: 8 January 2014; *Published online*: 16 July 2014; AJC-15528

Two flavan 3-ols *i.e.*, (+)-catechin (**1**), (-)-epicatechin (**2**)], three flavanonols *i.e.*, [taxifolin (**3**), taxifolin 3-*O*-*β*-D-glucopyranoside (**4**) and taxifolin 4'-*O*-*β*-D-glucopyranoside (**5**), one proanthocyanidin *i.e.*, procyanidin B-3 (**6**) and one *i.e.*, neolignan (+)-lyoniresinol 3α-*O*-*β*-D-xylopyranoside (**7**) have been isolated from the stems of *Quercus acuta* Thunberg (QAS) by anti-oxidative activity-guided isolation. Although these compounds have been reported previously. To our best of knowledge, this is the first study to report their isolation from *Q. acuta* Thunberg. Anti-oxidative activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and superoxide dismutase (SOD)-like superoxide anion (generated by xanthine oxidase/hypoxanthine) scavenging assays. Anti-inflammatory activity was assessed by quantifying the nitric oxide in the supernatant of lipopolysaccharide (LPS)-stimulated mouse Raw 264.7 macrophage cells.

Keywords: *Quercus acuta*, Phenolic compound, DPPH, Xanthine oxidase, Nitric oxide.

INTRODUCTION

Approximately, 200 species of *Quercus grow* in the temperate regions of the northern hemisphere[1]. The 5 indigenous species of *Quercus* found in Korea include *Quercus acuta* Thunberg, *Q. gilva* Blume, *Q. glauca* Thunberg, *Q. myrsinaefolia* Blume and *Q. salicina* Blume. *Q. acuta* Thunberg is native to both Japan and Korea[2]. Phytochemical investigations of plants belonging to the Quercus genus have revealed the presence of phenolic glucosidic gallates[3,4] and tannins[5,6]. Gallic acid and protoquercitol gallates, namely, 4,5-di-*O*-galloyl (+)-protoquercitol and 3,5-di-*O*-galloyl (+)-protoquercitol, were isolated from *Q. acuta*. Evaluation of biological activity of *Q. acuta* has revealed its antibacterial effect[7]. The presence of phenolic compounds in *Q. acuta* has not been reported. In this study, we evaluated the anti-oxidative and anti-inflammatory effects of compounds from the stems of *Q. acuta*.

EXPERIMENTAL

Stationary phases for column chromatographic isolation were Sephadex LH-20 (10-25 μm; GE Healthcare Bio-Science AB, Uppsala, Sweden), MCI-gel CHP 20P (75-150 μm; Mitsubishi Chemical, Tokyo, Japan) and ODS-B gel (40-60 μm; Daiso, Osaka, Japan). ODS-B gel was used as the stationary phase in middle pressure liquid chromatography (MPLC) analysis. Thin layer chromatography (TLC) was carried out with a precoated silica gel plate (60 F_{254}; Merck, Darmstadt, Germany) with the mobile phase being chloroform, methanol and water in the ratio of 6:4:1. The spots were detected under UV radiation (254 nm) after spraying the plate with ferric chloride ($FeCl_3$) and 10 % sulfuric acid (H_2SO_4) or anisaldehyde-H_2SO_4 followed by heating.

The compounds present in *Q. acuta* were identified by several instrumental analyses. One-dimensional nuclear magnetic resonance (NMR; ^{1}H-(300 or 600 MHz) and ^{13}C-(75 or 125 MHz) NMR) and 2D-NMR (proton, proton correlation spectroscopy (HH-COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC)) were recorded with Gemini 2000 and VNS (Varian, Palo Alto, CA, USA) at the center for research facilities of Chung-Ang University. Low-resolution fast atom bombardment mass spectrum (LR-FAB-MS) was recorded using JMSAX505WA (JEOL, Tokyo, Japan) at the National Center for Inter-University Research Facilities of Seoul National University. Optical rotation was recorded with Autopol III (Rudolph Research, Hackettstown, NJ, USA) at the National Instrumentation Center for Environmental Management at Seoul National University.

The stems of *Q. acuta* (6.5 kg) were obtained from Halla Arboretum on Jeju Island (Korea) in April 2009. A voucher

specimen (NO.171) was deposited at the herbarium of College of Pharmacy, Chung-Ang University.

Extraction and isolation: The stems of *Q. acuta* (6.5 kg) were extracted several times with 80 % acetone at room temperature. Concentrating the acetone extract under vacuum yielded 430 g of the extract. *Q. acuta* constituents were isolated by solvent fractionation with hexane, ethyl acetate, butyl alcohol and water. The ethyl acetate and butyl alcohol layers (81 and 130 g, respectively) showed antioxidant activity corresponding to IC_{50} values of 18.09 ± 1.53 and 20.67 ± 2.21 μg/mL, respectively, with IC_{50} of L-ascorbic acid positive control being 4.29 ± 0.08 μg/mL (data not shown). The butyl alcohol layer applied to the Sephadex LH-20 column (25-100 μm; 2000 g; 10 × 120 cm) and eluted with water and methanol yielded 8 subfractions. Subfraction 6 was further separated on an ODS-B gel (50 μm; 400 g; 3 × 50 cm) with 20-100 % methanol in an MPLC system (5 mL/min; 280 nm) and Sephadex LH-20 (25-100 μm; 300 g; 2.5 × 40 cm; 10-100 % ethanol gradient system) to yield (+)-catechin (**1**, 370 mg) and (-)-epicatechin (**2**, 43 mg).

The ethyl acetate layer applied to the Sephadex LH-20 column (25-100 μm; 2000 g; 10 × 120 cm) and eluted with H_2O-MeOH resulted in 10 subfractions. Subfraction 6 was separated on ODS-B gels (50 μm, 300 g; 3.5 × 60 cm), (50 μm; 250 g; 3 × 50 cm) and (50 μm, 150 g; 1.5 × 45 cm) with a methanol gradient (H_2O-100, 20-100 and 30-100 %) in an MPLC system (5 mL/min; 280 nm) to obtain taxifolin 3-*O*-*β*-D-glucopyranoside (**4**, 105 mg) and taxifolin 4'-*O*-*β*-D-glucopyranoside (**5**, 310 mg). Subfraction 8 (3.75 g) was further separated on an ODS-B gel (50 μm; 300 g; 3 × 40 cm) with 20-100 % methanol gradient in an MPLC system (5 mL/min; 280 nm) and Sephadex LH-20 (25-100 μm; 200 g; 2.5 × 30 cm; 100-10 % ethanol gradient system) to obtain procyanidin B-3 (**6**, 51 mg) and taxifolin (**3**, 101 mg). Subfraction 3 (1.89 g) was separated on an ODS-B gel (50 μm; 200 g; 2.5 × 30 cm) with 10-100 % methanol gradient in an MPLC system (5 mL/min; 280 nm) and recrystallization of subfraction 3 yielded (+)-lyoniresinol 3a-*O*-*β*-D-xylopyranoside (**7**, 250 mg)

(+)-Catechin (1): White amorphous powder; EI-MS: *m/z* 290 $[M]^+$; ^{1}H NMR (300 MHz, MeOH-d_4 + D_2O): δ 2.50 (1H, dd, *J* = 8.4, 16.0 Hz, H-4_{ax}), 2.84 (1H, dd, *J* = 5.7, 16.0 Hz, H-4_{eq}), 3.97 (1H, m, H-3), 4.55 (1H, d, *J* = 7.5 Hz, H-2), 5.85 (1H, d, *J* = 2.1 Hz, H-6), 5.92 (1H, d, *J* = 2.1 Hz, H-8), 6.71 (1H, dd, *J* = 1.6, 8.1 Hz, H-6'), 6.76 (1H, d, *J* = 8.1 Hz, H-5') and 6.83 (1H, d, *J* = 1.6 Hz, H-2').

^{13}C NMR (75 MHz, MeOH-d_4 + D_2O): δ 28.5 (C-4), 68.8 (C-3), 82.9 (C-2), 95.5 (C-8), 96.3 (C-6), 100.8 (C-10), 115.3 (C-2'), 116.1 (C-5'), 120.1 (C-6'), 132.2 (C-1'), 146.3 (C-3', 4'), 157.6 (C-9), 157.8 (C-5) and 157.9 (C-7).

(-)-Epicatechin (2): White amorphous powder; EI-MS: *m/z* 290 $[M]^+$; ^{1}H NMR (300 MHz, MeOH-d_4 + D_2O):δ 2.73 (1H, dd, *J* = 2.7, 16.8 Hz, H-4_{ax}), 2.86 (1H, dd, *J* = 4.8, 16.8 Hz, H-4_{eq}), 4.17 (1H, br s, H-3), 4.81(1H, br s, H-2), 5.92 (1H, d, *J* = 2.4 Hz, H-6), 5.94 (1H, d, *J* = 2.4 Hz, H-8), 6.80 (1H, dd, *J* = 1.5, 8.1 Hz, H-6'), 6.75 (1H, d, *J* = 8.1 Hz, H-5'), 6.97 (1H, d, *J* = 1.5 Hz, H-2').

^{13}C NMR (75 MHz, MeOH-d_4 + D_2O): δ 29.3 (C-4), 67.5 (C-3), 79.9 (C-2), 96.4 (C-6, 8), 100.1 (C-10), 115.3 (C-2'), 115.9 (C-5'), 119.4 (C-6'), 132.3 (C-1'), 145.8 (C-3', 4'), 157.6 (C-9), 158.0 (C-5, 7).

Taxifolin (3): White yellow amorphous powder; EI-MS: *m/z* 304 $[M]^+$; ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O): δ 4.45 (1H, d, *J* = 11.1 Hz, H-3), 4.93 (1H, d, *J* = 11.1 Hz, H-2), 5.82 (1H, d, *J* = 2.1 Hz, H-6), 5.87 (1H, d, *J* = 2.1 Hz, H-8), 6.72 (2H, m, H-5', 6') and 6.85 (1H, d, *J* = 2.1 Hz, H-2').

^{13}C NMR (150 MHz, DMSO-d_6+ D_2O): δ 71.8 (C-3), 83.4 (C-2), 95.4 (C-8), 96.4 (C-6), 100.6 (C-10), 115.5 (C-2'), 115.6 (C-5'), 119.9 (C-6'), 128.4 (C-1'), 145.1 (C-4') 146.0 (C-3'), 162.9 (C-5, 9), 167.4 (C-7) and 197.8 (C-4).

Taxifolin 3-*O*-*β*-D-glucopyranoside (4): Brown amorphous powder; LR-FAB-MS: *m/z* 465 $[M-H]^-$; ^{1}H NMR (300 MHz, MeOH-d_4 + D_2O): δ 3.00 (1H, m, glc-5), 3.11 (1H, t, *J* = 9.0 Hz, glc-3), 3.21 (1H, m, glc-2), 3.22 (1H, t, *J* = 9.0 Hz, glc-4), 3.60 (1H, dd, *J* = 5.7, 12.0 Hz, glc-6a), 3.77 (1H, dd, *J* = 1.8, 12.0 Hz, glc-6b), 3.85 (1H, d, *J* = 7.5 Hz, glc-1), 4.92 (1H, d, *J* = 9.9 Hz, H-3), 5.22 (1H, d, *J* = 9.9 Hz, H-2), 5.89 (2H, m, H-6, 8), 6.77 (1H, d, *J* = 8.4 Hz, H-5'), 6.83 (1H, dd, *J* = 1.8. 8.4 Hz, H-6') and 6.95 (1H, d, *J* = 1.8 Hz, H-2').

^{13}C NMR (75 MHz, DMSO-d_6+ D_2O): δ 62.7 (glc-6), 71.3 (glc-4), 74.7 (glc-2), 77.2 (C-3), 77.7 (glc-5), 78.3 (glc-3), 83.6 (C-2), 96.4 (C-6), 97.2 (C-8), 102.7 (C-10, glc-1), 115.9 (C-2'), 116.2 (C-5'), 121.1 (C-6'), 129.0 (C-1'), 146.4 (C-3'), 147.4 (C-4'), 164.2 (C-9), 165.5 (C-5), 169.1 (C-7) and 196.0 (C-4).

Taxifolin 4'-*O*-*β*-D-glucopyranoside (5): Brown amorphous powder; LR- FAB-MS: *m/z* 465 $[M-H]^-$; ^{1}H NMR (300 MHz, MeOH-d_4 + D_2O): δ 3.10-3.21 (4H in total, m, glc-2, 3, 4, 5), 3.57 (1H, dd, *J* = 3.9, 11.7 Hz, glc-6a), 3.80 (1H, dd, *J* = 1.2, 11.7 Hz, glc-6b), 4.67 (1H, d, *J* = 7.8 Hz, glc-1), 4.88 (1H, d, *J* = 9.0 Hz, H-3), 5.24 (1H, d, *J* = 9.0 Hz, H-3), 5.89 (2H, s, H-6, 8), 6.75 (1H, d, *J* = 8.1 Hz, H-5'), 6.80 (1H, dd, *J* = 1.8. 8.1 Hz, H-6') and 6.96 (1H, d, *J* = 1.8 Hz, H-2').

^{13}C NMR (75 MHz, DMSO-d_6+ D_2O): δ 62.9 (glc-6), 71.5 (glc-4), 72.4 (C-3) 75.4 (glc-2), 77.7 (glc-3), 78.0 (glc-5), 83.4 (C-2), 95.4 (C-8), 97.3 (C-6), 102.4 (C-10), 104.6(glc-1), 116.0 (C-2'), 116.3 (C-5'), 121.2 (C-6'), 128.9 (C-1'), 146.0 (C-3'), 147.0 (C-4'), 164.1 (C-9), 165.6 (C-7), 169.1 (C-5) and 196.2 (C-4).

Procyanidin B-3 (6): Brown amorphous powder; LR-FAB-MS: *m/z* 577 $[M-H]^-$; ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O): δ 2.48 (1H, dd, *J* = 8.4, 16.2 Hz, H-$4t_{ax}$), 2.75 (1H, dd, *J* = 8.4, 16.2 Hz, H-$4t_{eq}$), 3.78 (1H, m, H-3t), 4.25 (1H, d, *J* = 10.2 Hz, H-4u), 4.35 (1H, t, *J* = 10.2 Hz, H-3u), 4.40 (1H, d, *J* = 8.4 Hz, H-2u), 4.53 (1H, d, *J* = 7.2 Hz, H-2t), 5.78 (1H, d, *J* = 1.2 Hz, H-6u), 5.88 (1H, d, *J* = 1.2 Hz, H-8u), 6.06 (1H, s, H-6t), 6.24 (1H, dd, *J* = 1.8, 8.4 Hz, H-6't), 6.47 (1H, dd, *J* = 2.2, 8.4 Hz, H-6'u), 6.58 (1H, d, *J* = 1.8 Hz, H-2't), 6.67 (2H, d, *J* = 8.4 Hz, H-5'u, H-5't) and 6.73 (1H, d, *J* = 2.4 Hz, H-2'u).

^{13}C NMR (150 MHz, DMSO-d_6+D_2O): δ 27.3 (C-4t), 37.1 (C-4u), 67.4 (C-3t), 72.2 (C-3u), 81.0 (C-2t), 82.5 (C-2u), 94.6 (C-8u), 95.4 (C-6u, 6t), 100.8 (C-10t), 105.7 (C-10u), 106.7 (C-8t), 114.0 (C-2't), 114.6 (C-2'u), 114.7 (C-5't), 115.0 (C-5'u), 118.4 (C-6'u), 119.1 (C-6't), 130.4 (C-1'u), 131.1 (C-1't), 144.0 (C-3'u), 144.1 (C-4'u), 144.3 (C-3't), 144.6 (C-4't), 153.4 (C-9t), 154.2 (C-9u), 154.4 (C-5t), 155.6 (C-5u, 7t) and 157.2 (C-7u).

(+)-Lyoniresinol 3α-*O*-*β*-D-xylopyranoside (7): White amorphous powder; LR- FAB-MS: *m/z* 551 $[M-H]^-$; ^{1}H NMR

(600 MHz, DMSO-d_6 + D_2O): δ 1.56 (1H, m, H-2), 1.99 (1H, m, H-3), 2.55 (1H, dd, *J* = -, 15.6 Hz, H-1_{eq}), 2.68 (1H, dd, J = 4.2, 15.6 Hz, H-1_{ax}), 3.08 (1H, dd, *J* = 7.8, 8.4 Hz, xyl-2), 3.09 (1H, dd, *J* = 10.8, 11.4 Hz, xyl-5b), 3.19 (1H, t, *J* = 9.0 Hz, xyl-3), 3.31 (3H, s, 5-OCH_3), 3.32 (1H, d, *J* = 3.6 Hz, H-3α-b), 3.35 (1H, m, xyl-4), 3.40 (1H, dd, *J* = 6.9, 10.8 Hz, H-2α-b), 3.54 (1H, dd, *J* = 3.9, 10.8 Hz, H-2α-a), 3.68 (1H, m, H-3α-a), 3.68 (3H, s, 5'-OCH_3), 3.69 (3H, s, 3'-OCH_3), 3.74 (1H, dd, *J* = 5.4, 11.4 Hz, xyl-5a), 3.82 (3H, s, 7-OCH_3), 4.18 (1H, d, *J* = 7.8 Hz, xyl-1), 4.31 (1H, d, *J* = 6.6 Hz, H-4), 6.38 (2H, s, H-2', 6') and 6.59 (1H, s, H-8).

^{13}C-NMR (150 MHz, DMSO-d_6 + D_2O): δ 34.9 (C-1), 39.7 (C-2), 41.3 (C-4) 44.9 (C-3), 56.0 (5-OCH_3), 56.4 (3', 5'-OCH_3), 59.1 (7-OCH_3), 64.0 (C-2α), 66.0 (xyl-5), 69.8 (xyl-4), 69.5 (C-3α), 73.5 (xyl-2), 76.9 (xyl-3), 104.3 (xyl-1), 106.2 (C-2', 6'), 107.0 (C-8), 125.2 (C-10), 128.8 (C-9), 133.5 (C-4'), 137.5 (C-6), 138.0 (C-1'), 146.8 (C-5), 147.2 (C-7) and 147.8 (C-3', 5').

Biological assay

DPPH radical scavenging activity: Antioxidative activity was measured by evaluating 1,1-diphenyl-2-picrylhydrzyl (DPPH) (Sigma, St. Louis, USA) scavenging activities[8]. Briefly, 20 µl of the compounds was mixed with absolute ethanol and added to 180 µL of DPPH solution (0.1 mM in absolute ethanol). After gentle mixing, the reactions were allowed to stand for 0.5 h and the optical densities were measured at 492 nm by using an enzyme linked immunosorbent assay (ELISA) reader (TECAN, Salzburg, Austria). Free radical scavenging activities were calculated as [(1-(sample optical density (OD)/control OD)) × 100] and were expressed as IC_{50} values, which were defined as the concentrations at which 50 % of DPPH free radicals were scavenged. L-ascorbic acid was used as a positive control.

Superoxide anion scavenging activity: A reaction mixture was prepared with 50 mM of phosphate buffer (pH 7.5) containing EDTA (0.05 mM), hypoxanthine (0.2 mM), 63 µL of NBT [nitroblue-tetrazolium] (1 mM) (Sigma, St. Louis, MO), 63 µL of aqueous or ethanolic extract (distilled water for the control) and 63 µL of xanthine oxidase (1.2 unit/µL) (Sigma, St. Louis, MO) in an Eppendorf tube with the final volume being 632 µL. Xanthine oxidase was added last. For each sample, a blank solution devoid of the drug was prepared. The rate of NBT reduction was determined on the basis of sequential spectrophotometric determination of absorbance at 590 nm. The solutions were freshly prepared each day and kept away from light. Results are expressed as the percentage inhibition of NBT reduction with respect to the reaction mixture without sample (buffer only). Superoxide anion scavenging activities were calculated as [(1-(sample OD-blank OD)/(control OD-blank OD)) × 100] and were expressed as IC_{50} values, which were defined as the concentrations at which 50% of NBT/superoxide anions were scavenged[9]. Allopurinol (Sigma, St. Louis, MO) was used as a positive control.

Cell culture: RAW 264.7 cells were purchased from the Korean Cell Line Bank. These cells were grown at 37 °C in a humidified atmosphere (5 % CO_2) in DMEM medium (Sigma, St. Louis, MO, USA) containing 10 % fetal bovine serum, 100 IU/mL penicillin G and 100 mg/mL streptomycin (Gibco BRL, Grand Island, NY, USA)[10]. Cell enumeration was performed with the help of a hemocytometer.

Inhibitory activity on NO production: RAW 264.7 macrophage cells were cultured in a 96-well plate and incubated for 2 h at 37 °C in a humidified atmosphere (5 % CO_2). The cells were then incubated in a medium containing 0.1 µg/mL LPS (Sigma, St. Louis, MO, USA) and the drug isolates. After incubating for an additional 24 h, the NO content was analyzed by Griess assay. The Griess reagent (0.1 % naphthylethylenediamine and 1 % sulfanilamide in 5 % phosphoric acid solution) (Sigma, St. Louis, MO, USA) was added to the supernatant obtained from drug-treated samples. L-NMMA was used as a positive control. NO content was then read at 540 nm against a standard sodium nitrite curve. Inhibitory activity on NO production was calculated as inhibition rate (%) = [1-(sample OD - blank OD)/(control OD - blank OD)] × 100 and IC_{50} values, which were defined as the concentration that could inhibit 50 % of NO production[11], was determined.

Cell cytotoxicity assay: Before performing the biological assay, cytotoxicity was measured by mitochondrial-dependent reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] (Sigma, St. Louis, MO, USA) to formazan[11]. After the cells were cultured in a 96-well plate and incubated for 2 h, they were treated with the drug isolates (12.5, 25, 50 and 100 µg/mL or µM). The cells were incubated for an additional 24 h and the medium was replaced with fresh medium contained 0.5 mg/mL MTT; the incubation was further continued for 4 h at 37 °C. The medium was then removed and the MTT-formazan complex thus formed was dissolved in 200 µL of dimethylsulfoxide (DMSO). The extent of MTT reduction to formazan within the cells was quantified by measuring the absorbance at 540 nm by using an ELISA reader (TECAN, Salzburg, Austria)[12]. Cytotoxicity was calculated as cell viability (%) = sample OD/blank OD × 100.

Statistical analysis: All data are expressed as mean ± SD. Analysis was performed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (S-N-K) test. Results were considered significantly different when *p* values were less than 0.05. Values bearing different superscripts indicate significant differences.

RESULTS AND DISCUSSION

Seven known compounds were isolated from stems of *Q. acuta* Thunberg. Structural elucidation of all the compounds was done with the help of 2-dimensional nuclear magnetic resonance (2D-NMR) and mass spectroscopy (MS) and the structure was confirmed by comparing the findings with those previously reported in the literature.

Extraction was based on solvent fractionation and was performed using hexane, ethyl acetate, butyl alcohol and water. The soluble fractions of ethyl acetate and butyl alcohol showed anti-oxidative activity with IC_{50} values of 18.09 ± 1.53 µg/mL and 20.67 ± 2.21 µg/mL, respectively; the IC_{50} of L-ascorbic acid was found to be 4.29 ± 0.08 µg/mL (data not shown). (+)-Catechin (**1**)[13-15] and (-)-epicatechin (**2**)[4,16] were isolated from the butyl alcohol soluble fraction of the acetone extract. Taxifolin (**3**)[17,18], taxifolin 3-*O*-β-D-glucopyranoside (**4**)[19,20], taxifolin 4'-*O*-β-D-glucopyranoside (**5**)[21,22], procyanidin B-3 (**6**)[23-25] and (+)-

TABLE-1
ANTI-OXIDATIVE (DPPH RADICAL AND SUPEROXIDE ANION SCAVENGING ASSAYS) AND ANTI-INFLAMMATORY (INHIBITION OF NITRIC OXIDE PRODUCTION) ACTIVITIES OF COMPOUNDS **1–7**. VALUES REPRESENT MEAN ± SD OF THREE TRIALS. VALUES SUPERSCRIPTED DIFFERENTLY WITHIN A COLUMN ARE SIGNIFICANTLY DIFFERENT ($p < 0.05$)

Compound	DPPH radical scavenging activity IC_{50} (μM)	Superoxide anion scavenging activity IC_{50} (μM)	Inhibition of NO production IC_{50} (μM)
1	37.92 ± 1.23^{d}	40.52 ± 2.15^{f}	62.01 ± 1.42^{d}
2	26.37 ± 1.72^{c}	29.91 ± 2.53^{e}	72.56 ± 1.99^{e}
3	72.48 ± 2.41^{f}	39.35 ± 4.39^{e}	20.32 ± 1.57^{a}
4	$>100^{h}$	33.75 ± 3.87^{d}	54.60 ± 4.37^{c}
5	95.22 ± 1.85^{g}	43.06 ± 1.67^{f}	43.23 ± 3.71^{c}
6	16.45 ± 0.64^{a}	5.86 ± 2.17^{b}	30.47 ± 5.26^{b}
7	45.44 ± 1.61^{e}	$>100^{g}$	15.50 ± 0.84^{a}
L-Ascorbic acid	21.47 ± 0.54^{b}	-	-
Allopurinol	-	2.53 ± 0.04^{a}	-
L-NMMA	-	-	14.32 ± 2.24^{a}

DPPH: 2, 2-diphenyl-1-picrylhydrazyl; NO: nitric oxide; L-NMMA: L-*N*-monomethylargininecitrate

lyoniresinol 3α-*O*-*β*-D-xylopyranoside (**7**)[26,27] were isolated from the ethyl acetate soluble fraction (Fig. 1).

Our results suggest that phenolic compounds (**1-7**) could play an important role in the antioxidative and antiinflammatory effects of *Q. acuta*. Although all of these compounds have been reported previously, this is the first study to report the presence of phenolic compounds in *Q. acuta*. The data from general experimental procedures, ^{1}H NMR, ^{13}C NMR and MS, of compounds **1-7** are listed in the supplementary material.

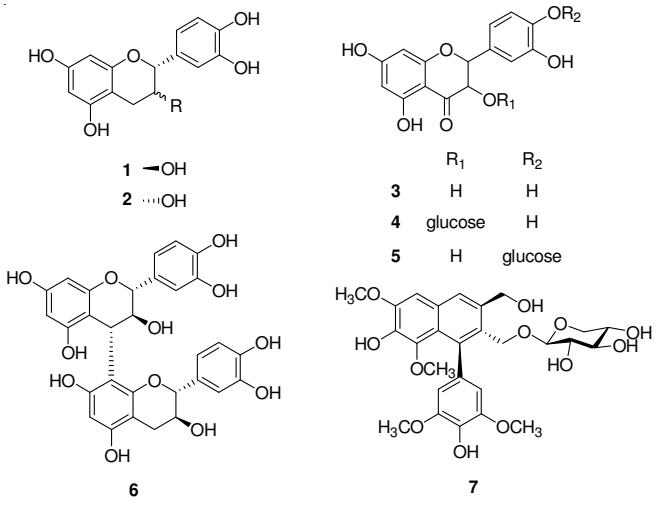


Fig. 1. Structures of compounds **1-7** isolated from *Q. acuta*

The antioxidative activities of the compounds of *Q. acuta* (**1-7**) were evaluated by DPPH free radical and superoxide dismutase (SOD)-like superoxide anion scavenging assays and expressed in terms of IC_{50} values (μM). The IC_{50} values for the DPPH free radical scavenging activities of compounds **1, 2** and **6** were 37.92 ± 1.23, 26.37 ± 1.72 and 16.45 ± 0.64 μM, respectively. All compounds except **7** exhibited potent SOD-like superoxide anion scavenging activities with IC_{50} values of 40.52 ± 2.15, 29.91 ± 2.53, 39.35 ± 4.39, 33.75 ± 3.87, 43.06 ± 1.67 and 5.86 ± 2.17 μM for compounds **1-6**, respectively (Table-1). The antioxidant activity of *Q. acuta* may be because of the *ortho*-dihydroxy-phenolic moiety, which enables the transfer of a hydrogen atom to an active free radical[28].

The antiinflammatory activity of the constituents of *Q. acuta* (**1-7**) was evaluated by the extent of inhibition of NO production in LPS-induced RAW 264.7 cells and was expressed in terms of IC_{50} values (μM). IC_{50} values for NO inhibition for compounds **3**, **6** and **7** were 20.32 ± 1.57, 30.47 ± 5.26 and 15.50 ± 0.84 μM, respectively (Table-1). Cytotoxicity of all compounds was measured by conducting the MTT assay, but there was no evidence of cytotoxicity at any of the concentrations studied. Therefore, compounds **3**, **6** and **7** may have a role in the treatment of inflammation due to NO overproduction.

Conclusion

In this study, two flavan-3-ols (**1** and **2**), three flavanonols (**3**, **4** and **5**), one proanthocyanidin (**6**) and one neolignan (**7**) were isolated and identified from *Q. acuta*. **1**, **2** and **6** showed strong antioxidative activities. Further, compounds **3**, **6** and **7** showed strong antiinflammatory activities. Therefore, these compounds present in *Q. acuta* can greatly benefit humans because of their antioxidative and antiinflammatory effects.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0022929).

REFERENCES

1. Y.N. Lee, Flora of Korea. Seoul, Republic of Korea: Kyo-Hak Publishing (1996).
2. K.S. Ko, E.S. Jeon, Ferns, Fern-Allies and Seed-Bearing Plants of Korea, Il-Jin-Sa Publishing, Seoul, Republic of Korea (2003).
3. G. Nonaka, H. Nishimura and I. Nishioka, *Chem. Pharm. Bull. (Tokyo)*, **30**, 2061 (1982).
4. G. Nonaka and I. Nishioka, *Chem. Pharm. Bull. (Tokyo)*, **30**, 4268 (1982).
5. G. Nonaka, H. Nishimura, I. Nishioka, *J. Chem. Soc., Perkin Trans. I*, 159 (1985).
6. G. Nonaka, T. Sakai, T. Tanaka, K. Mihashi and I. Nishioka, *Chem. Pharm. Bull. (Tokyo)*, **38**, 2151 (1990).
7. M. Serit, T. Okubo, R.H. Su, N. Hagiwara, M. Kim, T. Iwagawa and T. Yamamoto, *Agric. Biol. Chem.*, **55**, 19 (1991).
8. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita and T. Yasuhara, *Chem. Pharm. Bull. (Tokyo)*, **37**, 2016 (1989).
9. I. Parejo, F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerlage, J. Burillo and C. Codina, *J. Agric. Food Chem.*, **50**, 6882 (2002).

10. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
11. B. Epe, D. Ballmaier, I. Roussyn, K. Brivida and H. Sied, *Nucleic Acids Res*., **24**, 4105 (1996).
12. S.Y. Park, S.S. Hong, X.H. Han, J.S. Ro and B.Y. Hwang, *Nat. Prod. Sci.*, **11**, 85 (2005).
13. Y. Lu and L. Yeap Foo, *Food Chem.*, **65**, 1 (1999).
14. Y. Sudjaroen, R. Haubner, G. Würtele, W.E. Hull, G. Erben, B. Spiegelhalder, S. Changbumrung, H. Bartsch and R.W. Owen, *Food Chem. Toxicol.*, **43**, 1673 (2005).
15. M. Watanabe, *J. Agric. Food Chem.*, **46**, 839 (1998).
16. D.S. Yang, W.K. Whang and I.H. Kim, *Arch. Pharm. Res*., **19**, 507 (1996).
17. R. Zerback, M. Bokel, H. Geiger and D. Hess, *Phytochemistry*, **28**, 897 (1989).
18. J.H. Lee, W.J. Jeon, E.S. Yoo, C.M. Kim and Y.S. Kwon, *Nat. Prod. Sci*., **11**, (2005).
19. Z. Shen and O. Theander, *Phytochemistry*, **24**, 155 (1985).
20. S.E. Choi, K.H. Park, B.H. Han, M.S. Jeong, S.J. Seo, D.I. Lee, S.S. Joo and M.W. Lee, *Phytother. Res*., **25**, (2011).
21. T. Fossen, A.T. Pedersen and O.M. Andersen, *Phytochemistry*, **47**, 281 (1998).
22. X. Xu, H. Xie, J. Hao, Y. Jiang and X. Wei, *J. Agric. Food Chem*., **59**, 1205 (2011).
23. M.W. Lee, T. Tanaka, G.I. Nonaka and D.R. Hahn, *Arch. Pharm. Res*., **15**, 211 (1992).
24. A. Benavides, P. Montoro, C. Bassarello, S. Piacente and C. Pizza, *J. Pharm. Biomed. Anal*., **40**, 639 (2006).
25. J. Fan, X. Ding and W. Gu, *Food Chem*., **102**, 168 (2007).
26. E. Smite, P. Hefeng and L.N. Lundgren, *Phytochemistry*, **40**, 341 (2001).
27. T. Kanchanapoom, R. Kasai and K. Yamasaki, *Phytochemistry*, **57**, 1245 (2011).
28. J. Han, X. Weng and K. Bi, *Food Chem*., **106**, 2 (2008).