Korean J. Medicinal Crop Sci. 13(2) : 85 - 90 (2005)

Constituents and their DPPH Scavenging Activities from the Leaves of *Alnus hirsuta* (Spach) Rupr.

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ABSTRACT : Phytochemical study on the EtOAc fraction from a MeOH extract of the leaves of *Alnus hirsuta* Rupr. led to the isolation of nine compounds betulin (1), betulinic acid (2), hirsutanonol (3), hirsutenone (4), quercetin (5), avicularin (6), gallic acid (7), hyperin (8), and daucosterol (9). Among them, six compounds 1, 2, 57, and 9 are report from this plant for the first time. All isolated compounds were evaluated for their antioxidant activity using DPPH radical scavenging capacity and inhibition effect on mitochondrial lipid peroxidation. Six phenolic compounds 3-8 were found to have potent antioxidant activity. Of which, compounds 3, 4 and 5 showed significant free radical scavenging activity with the IC_{50} values of 18.3 ± 2.5, 15.7 ± 3.8 and 23.5 ± 3.1 ㎛, respectively. In addition, the compounds 3-8 exhibited inhibition effect on the mitochondrial lipid peroxidation with the IC_{50} values of 88.0 ± 6.5, 12.6 ± 1.2, 8.0 ± 1.1, 58.5 ± 4.3, 173.6 ± 15.2, and 75.0 ± 6.7 ㎛, respectively.

Key words **:** *Alnus hirsuta* Rupr., antioxidant activity, 1,1diphenyl2picrylhydrazyl (DPPH), mitochondrial, lipid peroxidation

INTRODUCTION

Alnus hirsuta Rupr., the species belongs to the family Betulaceae, geographically distributes in Korea, Japan, Northeast China, and Russia. It is a broad leaved deciduous tree, growing in damp places. The bark of this species had been used in Chinese and Korean traditional medicine as remedies for fever, hemorrhage, diarrhea, and alcoholism (Lee *et al.*, 1996). Previous studies on the chemical constituents of *A. hirsuta* have led to the isolation of various natural products, such as tannins (Lee *et al.*, 1992, 1998), flavonoids (Terazawa *et al.*, 1984, 1999), diarylheptanoids (Terazawa *et al.*, 1973, 1984; Jeong *et al.*, 2000), and triterpenoids (Uvarova *et al.*, 1972). It has also been reported to exhibit a variety of bioactivities, such as antioxidant activity (Lee *et al.*, 2000b, d), antiinflammation (Lee *et al.*, 2000a), cytotoxicity and antitumor (Bae *et al.*, 1997; Chang *et al.*, 1995; Joo *et al.*, 2002a, b). However, there have been few reports on constituents of the leaf, such as diarylheptanoids, flavonoids and tanins (Lee *et al.*, 1992; Bae *et al.*, 1997, Lee *et al.*, 1999, 2000c).

In the course of studies on antioxidant compounds from natural source, we found that the EtOAc fraction of the leaf of *A. hirsuta* possessed a strong scavenging activity against 1,1-diphenyl-2-pirylhydrazyl free radical (DPPH). We thus carried out the study on active principles of this plant. This paper reports the isolation, structural identification and the antioxidant activity of nine compounds isolated from the EtOAc fraction of the leaves of this plant.

MATERIALS AND METHODS

Plant materials

The leaves of *Alnus hirsuta* (Spach) Rupr. were collected at Yangu, Kangwon, Korea in Aug. 2002 and the plant was identified by one of the authors, Prof. KiHwan Bae. The voucher specimen (CNU-0304) has been deposited at the herbarium of Chungnam National University, Daejeon, Korea.

Instruments and reagents

1,1Diphenyl2picrarylhydraryl (DPPH), thiobarbituric acid (TBA), Lascorbic acid, caffeic acid, butylated hydroxyl toluene (BHT), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co., USA. Tricloroacetic acid (TCA), potassium sulphate (K_2HPO_4), potassium chloride (KCl), sodium chloride (NaCl), and ferrous sulphate ($FeSO_4$) were obtained from Daejung Chemical, Co., Ltd, Korea.

Melting points were measured by a Kofler microhotstage. Optical rotations were measured with a JASCO DIP-370 Digital polarimete (Japan). UV spectra were recorded on a Beckman DU-650 (USA). IR spectra were determined on a JASCO

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Received March 22, 2005 / Accepted March 31, 2005

IR Report100 spectrophotometer from KBr disc (Japan). ^{1}H-NMR (300 MHz, 400 MHz) and ^{13}C-NMR (75 MHz, 100 MHz) were recorded on Bruker DRX 300 and JEOL 400 spectrometers. Column chromatography were carried out using silica gel (Kesesl gel 60, 0.040~0.063 ㎜, Merck, Germany), Sephadex LH20 (Pharmacia), and Diaion (Mitsubishi Chemical, Japan). Thin layer chromatography (TLC) was done on precoated Silica gel 60 F_{254} (0.25 ㎜, Merck, Germany) and RP18 F_{254} plates (0.25 ㎜, Merck, Germany).

Extraction and isolation

The dry leaves of *Alnus hirsuta* (5.0 ㎏) were extracted with MeOH (30 ℓ × 3 times) under reflux for 5 h. The methanol extraction was combined and concentrated to obtain residue (780 g). The residue was suspended in H_2O and successively partitioned with *n*-hexane, EtOAc and BuOH, and then each layer was concentrated to obtain hexane fraction (194 g), EtOAc fraction (176 g), and BuOH fraction (161 g), respectively. The EtOAc fraction was chromatographed on a silica gel column eluting with a gradient of CH_2Cl_2-MeOH to afford six subfractions (Fr. B16). Fr. B1 (3.0 g) was subjected to a silica gel column with isocratic solvent mixture *n*-hexane-$CHCl_3$ (50:1 30:1) to afford compound 1 (26 ㎎). Fr. B2 (30 g) was subjected to a silica gel column eluting with $CHCl_3$MeOH (20:1) to give 6 fractions (Fr. B2a-2f). Fr. B2f (5 g) was further chromatographed on a silica gel column eluted with $CHCl_3$-MeOH (10:1) to obtain compound 3 (25 ㎎) and 4 (3.0 ㎎). Fr. B3 (12 g) was subjected to a silica gel column eluted with $CHCl_3$-MeOH (70:1) to give compound 2 (19.7 ㎎). Fr. B5 (22.6 g) was subjected to a silica gel column eluted with $CHCl_3$-MeOH (10:1) to give 5 fractions (Fr. B5a-5e). Fr. B5c (9 g) was subjected to a Sephadex LH20 column eluted with MeOH to yield compound 5 (21.0 ㎎), and the residue (3 g) from Fr. B5c was further choromatographed on a Sephadex LH20 column eluted with MeOH-H_2O (3:1) to afford compound 6 (66.8 ㎎), compound 7 (24.5 ㎎), and compound 9 (50.0 ㎎). Fr. B6 (20 g) was subjected to a Dianion column, eluting with water and methanol to give 6 fractions (Fr. B6a-6f). Fr. B6a (2 g) yielded compound 8 (66.0 ㎎) by a silica gel column chromatography eluted with $CHCl_3$-MeOH-H_2O (65:20:2).

Compound 1 - white amorphorous powder; mp 256~257°C; IR ν_{max} (KBr) ㎝$^{-1}$: 3350 (OH), 1630; ^{1}HNMR ($CDCl_3$, 300 MHz) : 4.70 and 4.59 (each 1H, d, J = 2.1 Hz, H-29), 3.80 and 3.35 (each 1H, d, J = 10.8 Hz, H-28), 3.20 (1H, dd, J = 5.4, 10.8 Hz, H-3), 2.40 (1H, m, H-19), 1.73 (3H, s, H-30), 1.04, 1.00, 0.99, 0.84 and 0.78 (each 3H, s, H-27, 26, 23, 25, 24); ^{13}C-NMR ($CDCl_3$, 75 MHz): 39.1 (C-1), 27.8 (C-2), 79.4 (C-3), 39.3 (C-4), 55.7 (C-5), 18.7 (C-6), 34.6 (C-7), 41.3 (C-8), 50.8 (C-9), 37.6 (C-10), 21.2 (C-11), 25.6 (C-12), 37.7 (C-13), 43.1 (C-14), 27.5 (C-15), 29.6 (C-16), 48.2 (C-17), 49.2 (C-18), 48.2 (C-19), 150.9 (C-20), 30.2 (C-21), 34.4 (C-22), 28.4 (C-23), 15.7 (C-24), 16.5 (C-25), 16.4 (C-26), 15.2 (C-27), 61.0 (C-28), 110.1 (C-29), 19.5 (C-30).

Compound 2 - white amorphous powder; mp 295~297°C; IR ν_{max} (KBr) ㎝$^{-1}$: 3420 (OH), 3080, 1695 (C = O), 1645, 1280, 1048, 1035; ^{1}H-NMR (CD_3OD, 300 MHz): 4.73 and 4.61 (each 1H, d, J = 2.1 Hz, H-29), 3.14 (1H, dd, J = 5.4, 10.8 Hz, H-3), 1.71 (3H, H-30), 1.02. 0.98, 0.97, 0.88 and 0.77 (each 3H, s, H-23, 24, 25, 26, 27); ^{13}C-NMR (CD_3OD, 75 MHz): 38.9 (C-1), 27.0 (C-2), 78.7 (C-3), 39.1 (C-4), 55.9 (C-5), 18.4 (C-6), 34.4 (C-7), 40.9 (C-8), 51.0 (C-9), 38.9 (C-10), 21.1 (C-11), 25.9 (C-12), 38.7 (C-13), 42.6 (C-14), 30.7 (C-15), 32.3 (C-16), 56.5 (C-17), 47.1 (C-18), 49.4 (C-19), 151.0 (C-20), 29.8 (C-21), 37.1 (C-22), 27.6 (C-23), 15.1 (C-24), 15.6 (C-25), 15.7 (C-26), 14.1 (C-27), 179.0 (C-28), 109.1 (C-29), 18.5 (C-30).

Compound 3 - brown oil; $[\alpha]_D$: -15.5° (*c* 1.0, acetone, 20°C); IR ν_{max} (KBr) ㎝$^{-1}$: 3390 (OH), 1690 (C = O), 1605, 1520 (C = C); ^{1}H-NMR (CD_3OD, 300 MHz): 6.68 (1H, d, J = 2.2 Hz, H-2'), 6.66 (1H, d, J = 2.1 Hz, H-2") , 6.64 (1H, d, J = 2.1 Hz, H-5'), 6.63 (1H, d, J = 2.1 Hz, H-5"), 6.52 (1H, t, J = 2.1 Hz, H-6'), 6.49 (1H, t, J = 2.1 Hz, H-6"), 4.02 (1H, tt, J = 5.1 Hz, H5), 2.46~2.65 (8H, m, H1, 2, 4, 7), 1.64~1.71 (2H, m, H-6); ^{13}C-NMR (CD_3OD, 75 MHz): 29.0 (C-1), 45.4 (C-2), 211.0 (C-3), 50.3 (C-4), 67.3 (C-5), 39.4 (C-6), 31.2 (C-7), 133.1 (C-1'), 115.3 (C-2'), 145.1 (C-3'), 143.2 (C-4'), 115.5 (C-5'), 119.5 (C-6'), 134.0 (C-1"), 115.4 (C-2"), 145.2 (C-3"), 143.5 (C-4"), 115.5 (C-5"), 119.6 (C-6").

Compound 4 - brown amorphous powder; $[\alpha]_D$: 25.2° (*c* 1.3, acetone, 20°C); UV: λ_{max} (MeOH) ㎚: 284; IR ν_{max} (KBr) ㎝$^{-1}$: 3365 (OH), 1650, 1606 and 1520, 1519 (C = C); ^{1}H-NMR (CD_3OD, 300 MHz): 6.90 (1H, td, J = 6.3, 15.9 Hz, H-5), 6.68 (1H, d, J = 8.1 Hz, H-5'), 6.67 (1H, d, J = 8.1 Hz, H-5"), 6.63 (1H, d, J = 2.1 Hz, H-2'), 6.62 (1H, J = 2.1 Hz, H-2"), 6.51 (1H, dd, J = 2.1, 8.1 Hz, H-6'), 6.50 (1H, dd, J = 2.1, 8.1 Hz, H-6"), 6.08 (1H, td, J = 1.2, 15.9 Hz, H-4), 2.472.82 (8H, m, H-1, 2, 6, 7); ^{13}C-NMR (CD_3OD, 75 MHz): 29.9 (C-1), 41.7 (C-2), 201.9 (C-3), 130.6 (C-4), 148.2 (C-5), 33.8 (C-6), 34.6 (C-7), 132.8 (C-1'), 115.3 (C-2' and C-2"), 145.2 (C-3' and C-3"), 143.5 (C-4' and C-4"), 115.5 (C-5' and C-5"), 119.7 (C-6' and C-6"), 133.0 (C-1").

Compound 5 - yellow amorphous powder; mp 313~315°C; UV: λ_{max} (MeOH) ㎚: 257, 371; IR ν_{max} (KBr) ㎝$^{-1}$: 3400 (OH), 1662 (C = O), 1616, 1520 (C = C), 1321, 1172 (CO); ^{1}H-NMR (DMSOd_6, 300 MHz): 7.67 (1H, d, J = 2.1 Hz, H-2'), 7.54 (1H, dd, J = 2.1, 8.4 Hz, H-6'), 6.88 (1H, d, J = 8.4 Hz, H-5'), 6.40 (1H, d, J = 1.8 Hz, H-8), 6.18 (1H, d, J = 1.8

Hz, H-6); ^{13}C-NMR (DMSOd_6, 75 MHz): 147.6 (C-2), 136.6 (C-3), 176.7 (C-4) 161.6 (C-5), 99.0 (C-6), 164.8 (C-7), 94.2 (C-8), 157.0 (C-9), 103.8 (C-10), 122.8 (C-1'), 116.4 (C-2'), 145.9 (C-3'), 148.6 (C-4'), 115.9 (C-5'), 120.8 (C-6').

Compound 6 - yellow amorphous powder; mp 216~217°C; IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 1650 (C = O), 1606, 1558, 1507 (C = C); ^{1}H-NMR (CD_3OD, 400 MHz): 7.47 (1H, d, J = 2.0 Hz, H-2'), 7.44 (1H, dd, J = 2.0, 8.0 Hz, H-6'), 6.85 (1H, d, J = 8.0 Hz, H-5'), 6.34 (1H, d, J = 2.0 Hz, H-8), 6.15 (1H, d, J = 2.0 Hz, H-6), 5.42 (1H, br s, H-1"), 4.28 (2H, d, J = 2.4 Hz, H-5"), 3.123.87 (m, sugar-H); ^{13}CNMR (CD_3OD, 100 MHz): 159.2 (C-2), 134.8 (C-3), 179.8 (C-4), 162.9 (C-5), 99.8 (C-6), 165.9 (C-7), 94.7 (C-8), 158.4 (C-9), 105.6 (C-10), 122.9 (C-1'), 116.4 (C-2'), 146.2 (C-3'), 149.7 (C-4'), 116.8 (C-5'), 123.0 (C-6'), 109.5 (C-1"), 83.3 (C-2"), 78.7 (C-3"), 88.0 (C-4"), 62.6 (C-5").

Compound 7 - white amorphous powder; mp 245~247°C; IR ν_{max} (KBr) cm^{-1}: 3470 (OH), 1650 (C = O), 1620, 1510 (C = C). ^{1}H-NMR (CD_3OD, 300 MHz): 7.07 (2H, s, H-2, 6); ^{13}C-NMR (CD_3OD, 100 MHz): 121.0 (C-1), 109.3 (C-2, 6), 145.4 (C-3, 5), 138.6 (C-4), 169.4 (COOH).

Compound 8 - yellow powder; mp 234~236°C; IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 1650 (C = O), 1606, 1558, 1507 (C = C), 1070 (CO); ^{1}H-NMR (CD_3OD, 300 MHz): 7.68 (1H, dd, J = 2.1, 8.4 Hz, H-6'), 7.54 (1H, d, J = 2.1 Hz, H-2'), 6.84 (1H, d, J = 8.4 Hz, H-5'), 6.42 (1H, s, H-8), 6.21 (1H, s, H-6), 5.39 (1H, d, J = 7.8 Hz, H-1"), 3.173.67 (8H, m, sugar-H); ^{13}C-NMR (CD_3OD, 75 MHz): 157.1 (C-2), 134.4 (C-3), 178.3 (C-4), 161.8 (C-5), 102.7 (C-6), 165.0 (C-7), 94.4 (C-8), 157.2 (C-9), 104.8 (C-10), 121.7 (C-1'), 116.1 (C-2'), 145.7 (C-3'), 149.3 (C-4'), 116.8 (C-5'), 122.9 (C-6'), 99.5 (C-1"), 72.1 (C-2"), 74.1 (C-3"), 68.8 (C-4"), 76.7 (C-5"), 61.0 (C-6").

Compound 9 - white needle; mp 280~282°C; IR ν_{max} (KBr) cm^{-1}: 3388 (OH), 1464, 1368, 1074, 1024; ^{1}H-NMR (DMSO-d_6, 300 MHz): 5.34 (1H, t, J = 4.5 Hz, H-6), 4.21 (1H, d, J = 7.8 Hz, H-1'), 3.083.66, (5 H, m, glu-H), 0.96 (3H, s, H-19), 0.91 (3H, d, J = 6.3 Hz, H-21), 0.82 (3H, d, J = 6.3 Hz, H-26), 0.81 (3H, d, J = 6.0 Hz, H-27), 0.79 (3H, d, J = 6.6 Hz, H-29), 0.66 (3H, s, H-18); ^{13}CNMR (DMSO-d_6, 75 MHz): 37.7 (C-1), 30.1 (C-2), 77.8 (C-3), 39.2 (C-4), 141.3 (C-5), 122.1 (C-6), 32.3 (C-7), 32.2 (C-8), 50.5 (C-9), 37.1 (C-10), 21.5 (C-11), 39.6 (C-12), 42.7 (C-13), 57.1 (C-14), 24.7 (C-15), 28.7 (C-16), 56.3 (C-17), 12.5 (C-18), 19.8 (C-19), 36.3 (C-20), 19.5 (C-21), 34.2 (C-22), 26.3 (C-23), 46.0 (C-24), 29.6 (C-25), 20.6 (C-26), 20.0 (C-27), 23.5 (C-28), 12.7 (C-29), 101.7 (C-1'), 74.3 (C-2'), 77.6 (C-3'), 71.0 (C-4'), 77.7 (C-5'), 62.0 (C-6').

DPPH radical scavenging activity

The DPPH radical scavenging effect was measured by a previously described method (Na *et al.*, 2003). Briefly, 5 μℓ of the sample, which was dissolved in DMSO, was added to 195 μℓ of 150 μm DPPH solution in MeOH in 96 well plates. The solution was rapidly mixed and incubated at room temperature for 30 min. Then the absorbance was measured at 517 nm using a microplate reader. The free radical scavenging capacity was expressed as follow:

DPPH scavenging activity (%) = [(Ac As)/Ac]×100

Where Ac was the absorbance of the control, As was the absorbance of the sample. Each sample was assayed at five concentrations and four wells were used for each concentration. All samples were carried out in triplicate. The antioxidant activity of test compounds was expressed as IC_{50}, which was defined as the concentrations of which required to reduce 50% of absorbance. Caffeic acid and BHT were used as a positive controls.

Mitochondrial Preparation and lipid peroxidation

Mitochondria were prepared according to the method of Ham and Liebler (Yen & Hsieh, 1998). Liver of Sprague-Dawley rats weighing 200~220 g were removed quickly and washed with icecold 0.9% sodium chloride. Mitochondria were minced and then homogenized in 9 volumes of icecold 5 mM K_2HPO_4-HCl buffer (pH 7.4) containing 0.25 M sucrose and 0.1 mM EDTA. The homogenate was filtered through several layers of cheesecloth and centrifuged at 600 rpm for 10 min, and the supernatant was centrifuged at 9,000 rpm for 15 min at 4°C The resulting pellet was washed three times with homogenization buffer, and the final pellet was resuspended in 0.15 M KCl in 20 mM K_2HPO_4HCl buffer (pH 7.4). The protein concentration in mitochondria was determined by BCA method using bovine serum albumin (Brenner & Harris, 1995). Oxidation of rat liver mitochondria was carried out by the reported method (Yen & Hsieh, 1998). In brief, a reaction mixture containing 390 μℓ of 20 mM K_2HPO_4HCl buffer (pH 7.4), 50 μℓ of mitochondria, 10 μℓ of test compounds in MeOH, 25 μℓ of 2.5 mM $FeSO_4 \cdot 7H_2O$, and 25 μℓ of 2.5 mM ascorbic acid. The reaction mixtures were incubated at 37°C. After 1 h, 250 μℓ of 20% TCA was added to stop oxidation. Then the mixture was added 250 μℓ of 1% TBA and heated at 100°C for 15 min. After being centrifuged at 5,000 rpm for 10 min, the absorbance was measured at 532 nm against blank containing with replacing extract, $FeSO_4$ and ascorbic acid by similar volume of potassium phosphate buffer. Lipid peroxidation inhibitory was calculated as percentage of inhibitory effect by the equation below:

Inhibition (%) = [(Ac As)/(Ac Ab)]×100

Where Ac was the absorbance of the control, As was the

absorbance of the sample and Ab was the absorbance of the blank. Caffeic acid and BHT were used as positive controls.

RESULTS AND DISCUSSION

The MeOH extract (780 g) of the leaf of this plant was suspended in H_2O and successively partitioned with *n*hexane, EtOAc and BuOH to give hexane fraction (194 g), EtOAc fraction (176 g), BuOH fraction (161 g), and water fraction (249 g), respectively. Bioassay followed revealed that the activity of the MeOH extract was mainly located in EtOAc fraction. Repeated column chromatography of this fraction led to the isolation of nine compounds (1-9).

Compound 1 was obtained as a white amorphous powder. It showed positive result in Liebermann-Burchard reaction. The IR spectrum revealed the existence of hydroxyl absorption band (3350 cm^{-1}). In addition, the ^{1}H-NMR and ^{13}C-NMR spectra suggested that 1 was a triterpenoid. In the ^{1}H-NMR, two signals at 4.70 and 4.59 (each 1H, d, J = 2.1 Hz) and the peak at 1.73 (3H, s) corresponded to three peaks at 150.9, 109.1 and 19.5, respectively, in ^{13}C-NMR suggested an isopropylene group. A set of protons at 3.80 and 3.35 (each 1H, d, J = 11.0 Hz) were assumed to be oxygenated group from whose carbon appeared at 61.0 in ^{13}C-NMR. One more oxygenated carbon at 79.4 together with double of doublets at 3.18 (1H, dd, J = 10.8, 5.4 Hz) in ^{1}H-NMR indicated a 3hydroxyl functionality. Six methyl groups, which appeared at 1.70, 1.04, 1.00, 0.99, 0.84 and 0.78 (each 3H, s) in the ^{1}H-NMR, were detected at 19.5, 16.4, 15.2, 28.4, 16.5, and 15.7 in ^{13}C-NMR, respectively. Furthermore, the ^{13}CNMR spectra exhibited 30 carbon signals in total. All above evidence together with those published in the literature (Kim *et al.*, 2002) led to the structure of 1 to be 3β, 28-dihydroxylup-20 (29)ene (betulin).

Compound 2 was also obtained as a white amorphous powder. It also showed positive result in Liebermann-Burchard reaction. The IR and NMR spectra patterns of 2 were very similar to those of 1, suggesting that they have the same skeleton. The main difference was the presence of the peak appeared at 179.0 (C-28) instead of the peak at 61.0 of 1 in the ^{13}C-NMR spectrum. Therefore, the structure of 2 was identified as betulinic acid by comparison of these data with those published in the literature (Kim *et al.*, 2002).

Compound 3 and compound 4 were obtained as brown oil and amorphous powder, respectively. Both were phenolic compounds suggested by absorption of hydroxyl group (OH) in IR spectrum and its colorization by $FeCl_3$. On the basis of the spectroscopic data (UV, IR, ^{1}H-NMR and ^{13}C-NMR), compounds 3 and 4 were assigned as hirsutanonol (Lee *et al.*, 2000c) and hirsutenone (Jeong *et al.*, 2000). These compounds

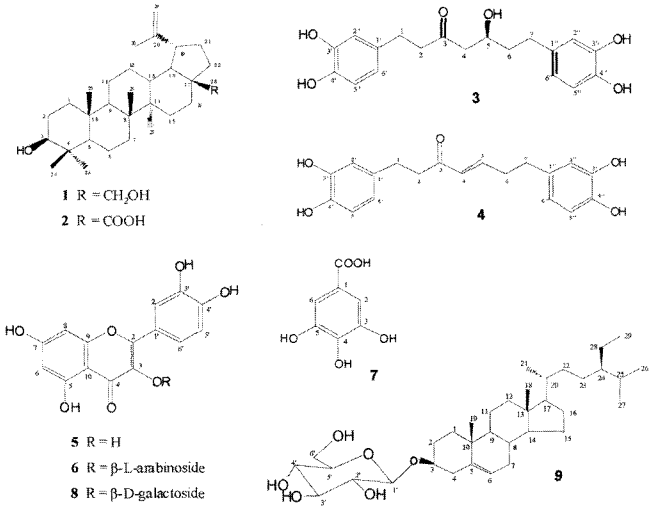

Fig. 1. Chemical structures of compounds (1-9) isolated from leaves of *Alnus hirsuta*

had been isolated from this plant previously (Terazawa *et al.*, 1973).

Compound 5 was isolated as a yellow amorphous powder. A positive reaction with $FeCl_3$ and a broad peak at 3,400 cm^{-1} in IR spectra indicated that 5 was a phenolic compound. The UV maximum absorption peaks at 257 and 371 nm suggested a flavone skeleton. Analysis of the ^{1}H-NMR and ^{13}C-NMR spectra in good agreement with those of quercetin reported in literature (Kim *et al.*, 1995).

UV, IR, ^{1}H-NMR and ^{13}C-NMR spectra patterns of compound 6 were closely similar to those of 5 except for the signals corresponding to a sugar. On the basis of the chemical shifts, multiplicity and coupling constants, the sugar was identified as L-arabinose. After comparison with authentic data (Lu & Foo, 1997), 6 was identified as avicularin (quercetin 3-*O*-α-*L*-arabinofuranoside).

The spectral data of compound 8 was closely similar to those of 6, suggesting they have a same backbone. By the comparison with reported data (Lu & Foo, 1997), 8 was identified as hyperin (quercetin 3-*O*-β-*D*-galactoside). By means of similar method, compounds 7 and 9 were identified as gallic acid and daucosterol, respectively.

Since the EtOAc fraction of the leaf of *A. hirsuta* showed a strong free radical scavenging activity, we carried out to study on phytochemical composition of this active fraction. Nine compounds were isolated from the leaves of *A. hirsuta* and their chemical structures were elucidated (Fig. 1). Among them, six compounds 1, 2, 5, 6, 7 and 9 were isolated from this plant for the first time.

All the isolated compounds were evaluated for their antioxidant activity using DPPH radical scavenging. As shown in

Table 1. Antioxidant activities of isolated compounds

compounds	Antioxidant activities	IC_{50}[1] (㎛)
	DPPH scavenging	Lipid peroxidation
1	-	-
2	-	-
3	18.3 ± 2.5	88.0 ± 6.5
4	15.7 ± 3.8	12.6 ± 1.2
5	23.5 ± 3.1	8.0 ± 1.1
6	53.2 ± 2.8	58.5 ± 4.3
7	34.4 ± 4.3	173.6 ± 5.2
8	54.7 ± 5.9	75.0 ±6.9
9	-	-
Caffeic acid[2]	39.0 ± 3.1	146.2 ± 11.0
BHT[2]	110.7 ± 6.7	1.6 ± 0.3

[1]The $IC0_{50}$ values were calculated from the regression lines using five different concentrations. The values are ± SD of three independent experiments; [2]The compounds used positive control -:Inactive

Table 1, three compounds 1, 2 and 9 were inactive. In contrast, 3 and 4 exhibited a significant inhibition in a concentrationdependent manner with IC_{50} values of 18.3 ± 2.5 and 15.7 ± 3.8 ㎛, respectively. Compounds 5, 6, 7 and 8 showed moderate activity with IC_{50} values of 23.5 ± 3.1, 53.2 ± 2.8, 34.4 ± 4.3, and 54.7 ± 5.9 ㎛, respectively. The antioxidative activities of these compounds were comparable to caffeic acid (IC_{50}= 39.4 ± 3.1 ㎛), which was used as a positive control.

The result of inhibitory effect on lipid peroxidantion of these compounds is also presented in Table 1. Of these, compounds 4 and 5 showed the most significant activity in anti-lipid peroxidation assay with IC_{50} values of 12.6 ± 1.2 and 8.0 ± 1.1 ㎛, respectively. Compounds 3, 6 and 8 were less active than 4 and 5. Gallic acid showed a weaker activity (IC_{50} = 173.6 ± 15.2 ㎛) than caffeic acid (IC_{50} = 146.0 ± 11.0 ㎛). However, all of them were much less active than BHT (IC_{50} = 1.6 ± 0.3 ㎛), which was used as a positive control. The result suggests that the methanol extract of *A. hirsuta* and its EtOAc fraction might be useful for the treatment of oxidative damages.

ACKNOWLEDGEMENTS

This research was supported by a grant of the Korea Health 21 R & D Project, Ministry of Health & Welfare, Republic of Korea (01PJ2PG601NA010002). We are grateful to Korea Basic Science Institute (KBSI) for supplying the NMR spectra.

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