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The Chemical Constituents and their Antioxidant Activity of the Stem of *Rhododendron mucronulatum*

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Abstract– From the *n*-BuOH soluble fraction of the 70% aqueous acetone extract of *Rhododendron mucronulatum* stem, twelve compounds were isolated. On the basis of spectral data, they were identified as scopoletin (**1**), (+)-taxifolin (**2**), quercetin (**3**), (−)-catechin (**4**), (+)-epicatechin (**5**), scopolin (**6**), lyoniside (**7**), ssioriside (**8**), fraxin (**9**), (+)-lyoniresinol-3α-*O*-β-D-glucopyranoside (**10**), (+)-taxifolin-3-*O*-α-L-arabinopyranoside (**11**), and astragalin (**12**), respectively. All isolated compounds were tested antioxidant activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Compounds **2** and **3** showed the potent antioxidant activity, and compounds **5**, **8**, and **11** showed moderate activity.
Keywords – *Rhododendron mucronulatum*, Ericaceae, flavonoids, lignans, coumarins, antioxidant activity

Introduction

Rhododendron mucronulatum (Ericaceae) is widely distributed in Korea (Lee, 1985), and its leaf, flower, and stem have been used as an anti hypertensive agent (Nanjing University of TCM, 1999; Lim, 1988). Meanwhile, some researchers have reported the chemical constituents of the flower of *R. mucronulatum* (Chung *et. al.*, 1996a, 1996b).

In the course of screening to evaluate antioxidant constituents from medicinal plants, we found that 70% aqueous acetone extract of the stem of *R. mucronulatum* showed a strong radical scavenging activity. This paper deals with structure elucidation of these compounds and their antioxidant activity using the DPPH radical scavenging method.

Material and Methods

General procedure – Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. NMR (Varian Gemini 200 and Bruker DPX 400) spectra (^{1}H-NMR taken at 200 and 400 MHz, and ^{13}C-NMR spectra taken at 50 and 100 MHz, respectively) were recorded in deuterated solvents using TMS as the internal standard. The MS spectra were measured using an Autospec Micromass, UV spectra using a JASCO V-530 UV/Vis spectrophotometer, and IR spectra in a KBr disk using a Bio-Rad FTS-7. TLC work was carried out using plates coated with silica gel 60 F_{254} (Merck). All solvents were routinely distilled prior to use. Silica gel and ODS column chromatography were performed on Merck silica gel 60 (70-230 mesh) and YMC gel (150 μm), respectively.

Plant materials – The stem of *Rhododendron mucronulatum* was collected at Yanggu, Kangwon in May, 2004, and identified taxonomically with respect to morphology. A voucher specimen (KNUP-S-04-02) was deposited at the College of Pharmacy, Kangwon National University.

Extraction and isolation – The air-dried stem (2.7 kg) was ground and extracted three times with 70% aqueous acetone at room temperature for 7 days each time. The resultant extracts were combined and removed acetone under reduced pressure. This acetone extract was partitioned successively with equal volume of *n*-hexane, $CHCl_3$ and *n*-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vacuo to yield the residues of n-hexane fraction (fr.) (13 g), $CHCl_3$ fr., (25 g), and *n*-BuOH fr. (78 g). The *n*-BuOH soluble fraction (50 g) was column chromatographed on a silica gel (300 g, ψ 15×50 cm) using isocratic elution with $CHCl_3$: MeOH : Water (40 : 10 : 1), in order to divide the

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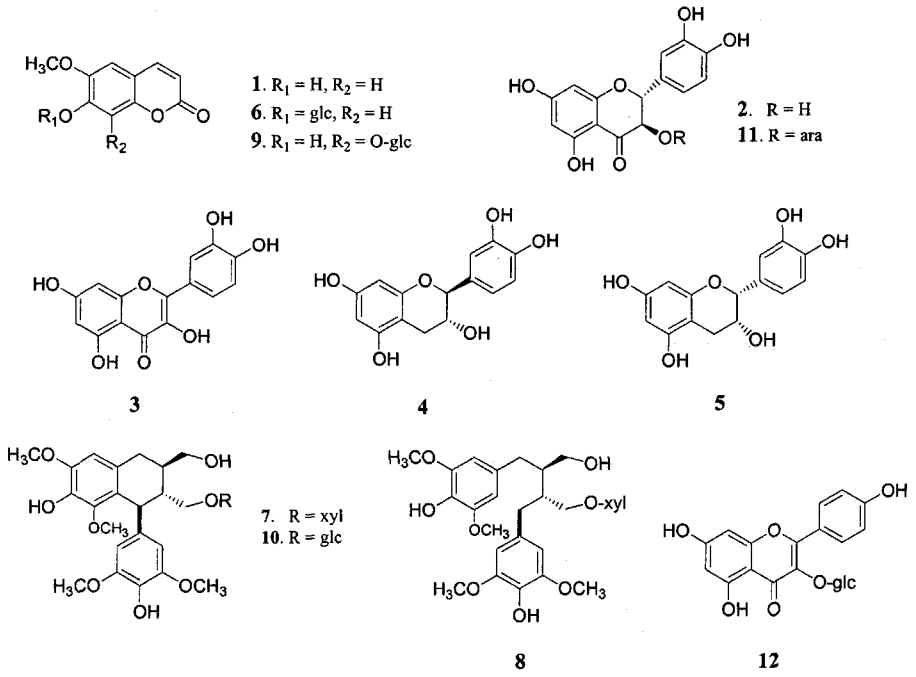

Fig. 1. Structures of **1-12**.

fraction into four sub-fractions (Fr. 1-Fr. 4). Fr. 2 (18.5 g) was re-chromatographed on silica gel column (250 g, ψ 5×50 cm) by elution with $CHCl_3$: MeOH (9 : 1) to give compounds **1** (250.2 mg) and **2** (130.1 mg). Fr. 2-1 (10.7 g) was re-chromatographed on ODS column (100 g, 150 μm, ψ 5×50 cm) by elution with MeOH : H_2O (40 : 60) to give compounds **3** (27.0 mg), **4** (69.3 mg), **5** (88.2 mg), **6** (85.2 mg), **7** (661.7 mg), **8** (794.7 mg), **9** (36.3 mg), and **10** (875.1 mg). Fr. 3 was re-chromatographed on ODS column (150 g, ψ 5×50 cm) by elution with MeOH : H_2O (30 : 70) to give compounds **11** (937.0 mg) and **12** (73.1 mg).

Scopoletin (1) – White needles; mp 203~204°C; UV (MeOH) λ_{max} 224, 252, 298, 341 nm; IR ν_{max} (KBr) 3419(OH), 1724 (C = O), 1653, 1597 (C = C), 1207, 1176 (C-O) cm^{-1}; ^{1}H-NMR (Acetone-d_6, 200 MHz) δ 7.94 (1H, d, J = 9.8 Hz, H-4), 7.29 (1H, s, H-5), 6.89 (1H, s, H-8), 6.27 (1H, d, J = 9.8 Hz, H-3), 3.99 (3H, s, -OCH_3); ^{13}C-NMR (Acetone-d_6, 50 MHz) δ 160.65 (C-2), 151.63 (C-7), 150.29 (C-9), 145.55 (C-6), 144.04 (C-4), 112.13 (C-3), 110.97 (C-10), 109.47 (C-5), 103.03 (C-8), 55.87 (-OCH_3); EI-MS *m/z* 192 [M^+].

(+)-Taxifolin (2) – White powder; mp 221~222°C; $[\alpha]_D^{19}$ 15.6° (MeOH, c 0.28); UV (MeOH) λ_{max} 242(s), 277 nm; UV (MeOH+NaOH) λ_{max} 250(s), 286 nm; UV (MeOH+NaOAc) λ_{max} 277 nm; UV (MeOH+$AlCl_3$) λ_{max} 277 nm; UV (MeOH+$AlCl_3$+ HCl) λ_{max} 277 nm; IR ν_{max} (KBr) 3409 (OH), 1610 (C = O), 1473 (C = C), 1261, 1070 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 11.97 (1H, s, 5-OH), 6.95~6.82 (3H, br. d, H-2', H-5' and H-6'), 5.98 (1H, br. s, H-8), 5.93 (1H, br. s, H-6), 5.05 (1H, d, J = 10.8 Hz, H-2), 4.57 (1H, d, J = 10.8 Hz, H-3), ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 197.23 (C-4), 166.98 (C-7), 163.39 (C-5), 162.63 (C-9), 145.82 (C-4'), 144.98 (C-3'), 128.10 (C-1'), 119.47 (C-6'), 115.38 (C-5'), 115.16 (C-2'), 100.46 (C-10), 96.06 (C-6), 95.04 (C-8), 83.07 (C-2), 71.57 (C-3); EI-MS *m/z* 304 [M^+].

Quercetin (3) – Yellow powder; mp 300°C <; UV (MeOH) λ_{max} 256, 271(s), 297, 372 nm; UV (MeOH +NaOH) λ_{max} 274, 328, 415 nm; UV (MeOH+NaOAc) λ_{max} 273, 325, 382 nm; UV (MeOH+NaOAc+H_3BO_3) λ_{max} 260, 387 nm; UV (MeOH+$AlCl_3$) λ_{max} 271, 439 nm; UV (MeOH+$AlCl_3$+HCl) λ_{max} 267, 304, 356, 429 nm; IR ν_{max} (KBr) 3298 (OH), 1655 (C = O), 1602, 1567, 1458 (C = C), 1222, 1165 (C-O) cm^{-1}; ^{1}H-NMR (MeOH-d_4, 200 MHz) δ 7.98 (1H, d, J = 2.1 Hz, H-2'), 7.86 (1H, dd, J = 2.1, 8.2 Hz, H-6'), 7.08 (1H, d, J = 8.2 Hz,, H-5'), 6.67 (1H, d, J = 2.2 Hz, H-8), 6.34 (1H, d, J = 2.2 Hz, H-6); EI-MS *m/z* 302 [M^+].

(–)-Catechin (4) – Brown powder (EtOH); mp 175~176°C; $[\alpha]_D^{19}$ −18.8° (c 0.545, MeOH); UV (MeOH) λ_{max} 281, 372 nm; UV (MeOH+NaOH) λ_{max} 288, 428 nm; UV (MeOH+NaOAc) λ_{max} 280, 430 nm; UV (MeOH+NaOAc+ H_3BO_3) λ_{max} 287, 374 nm; UV (MeOH+$AlCl_3$) λ_{max} 269, 303, 357, 424 nm; UV (MeOH+$AlCl_3$+ HCl) λ_{max} 280, 374 nm; IR ν_{max} (KBr) 3321(OH), 1455(C = C), 1257, 1080(C-O) cm^{-1}; ^{1}H-NMR (MeOH-d_4, 200 MHz) δ 6.75

(3H, m, H-2', H-5' and H-6'), 5.93 (1H, d, J = 2.4 Hz, H-8), 5.85 (1H, d, J = 2.4 Hz H-6), 4.56 (1H, d, J = 7.4 Hz H-2), 3.98 (1H, m, H-3), 2.85 (1H, dd, J = 5.2, 16.2 Hz, H-4a), 2.50 (1H, dd, J = 8.2, 16.2 Hz, H-4b); ^{13}C-NMR (MeOH-d_4, 50 MHz) δ 158.29 (C-9), 158.04 (C-5), 157.36 (C-7), 146.69 (C-3' and C-4'), 132.64 (C-1'), 120.47 (C-6'), 116.50 (C-5'), 115.67 (C-2'), 101.21 (C-10), 96.69 (C-6), 95.91 (C-8), 83.26 (C-2), 69.22 (C-3), 28.90 (C-4); EI-MS *m/z* 290 [M$^+$].

(+)-Epicatechin (5) – Brown powder; mp 235~237°C; $[\alpha]_D^{19}$ 3.1° (c 0.35, MeOH); UV (MeOH) λ_{max} 280, 378 nm; UV (MeOH+NaOH) λ_{max} 288, 431 nm; UV (MeOH +NaOAc) λ_{max} 280, 432 nm; UV (MeOH+NaOAc+ H_3BO_3) λ_{max} 286, 380 nm; UV (MeOH+$AlCl_3$) λ_{max} 287, 418 nm; UV (MeOH+$AlCl_3$+ HCl) λ_{max} 280, 385 nm; IR ν_{max} (KBr) 3320(OH), 1456 (C = C), 1258, 1081 (C-O) cm^{-1}; ^{1}H-NMR (MeOH-d_4, 200 MHz) δ 6.98 (1H, br. s, H-2'), 6.75 (2H, m, H-5' and H-6'), 5.95 (1H, d, J = 2.2 Hz, H-8), 5.92 (1H, d, J = 2.2 Hz, H-6), 4.80 (1H, s, H-2), 4.16 (1H, br. s, H-3), 2.87 (1H, dd, J = 4.6, 16.8 Hz, H-4a), 2.73 (1H, dd, J = 2.8, 16.8 Hz, H-4b); ^{13}C-NMR (MeOH-d_4, 50 MHz) δ 158.46 (C-5), 158.09 (C-3), 157.82 (C-9), 146.42 (C-3' and C-4'), 132.71 (C-1'), 119.86 (C-6'), 116.36 (C-5'), 115.74 (C-2'), 100.52 (C-10), 96.82 (C-6), 96.33 (C-8), 80.27 (C-2), 67.89 (C-3), 29.67 (C-4); EI-MS *m/z* 290 [M$^+$].

Scopolin (6) – White powder; mp 218~219°C; UV (MeOH) λ_{max} 229, 285, 334 nm; IR ν_{max} (KBr) 3465 (OH), 1705 (C = O), 1615, 1560, 1460 (C = C), 1128, 1085 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.96 (1H, d, J = 9.8 Hz, H-4), 7.29 (1H, s, H-5), 7.15 (1H, s, H-8), 6.32 (1H, d, J = 9.8 Hz, H-3), 5.09 (1H, d, J = 7.0 Hz, H-1'), 3.80 (3H, s, -OCH_3); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 160.65 (C-2), 150.01 (C-7), 149.04 (C-9), 146.11 (C-6), 144.35 (C-4), 113.41 (C-3), 112.35 (C-10), 109.77 (C-5), 103.10 (C-8), 99.69 (C-1'), 77.19 (C-5'), 76.82 (C-3'), 73.13 (C-2'), 69.67 (C-4'), 56.09 (-OCH_3); FAB-MS *m/z* 355 [M+H]$^+$.

Lyoniside (7) – White needle; mp 158~160°C; $[\alpha]_D^{19}$ 39.5° (c 0.405, MeOH); UV (MeOH) λ_{max} 241 (s), 288 nm; IR ν_{max} (KBr) 3375 (OH), 1610, 1499, 1456 (C = C), 1317, 1214, 1105 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 6.61 (1H, s, H-8), 6.41 (2H, s, H-2' and H-6'), 4.34 (1H, d, J = 5.8 Hz, H-4), 4.19 (1H, d, J = 7.0 Hz, anomeric H), 3.72 (3H, s, 5-OCH_3), 3.46 (9H, s, 7, 3', 5' -OCH_3); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 147.61 (C-3' and C-5'), 147.00 (C-5), 146.62 (C-7), 137.68 (C-1'), 137.35 (C-6), 133.38 (C-4'), 128.45 (C-9), 124.99 (C-10), 106.76 (C-2'), 106.04 (C-6'), 104.09 (xyl-1), 76.85 (xyl-3), 73.35 (xyl-2), 69.64 (xyl-4), 69.04 (C-3a), 65.81 (xyl-2), 63.76 (C-2a), 58.69 (-OCH_3), 56.13 (-OCH_3×2), 55.71 (-OCH_3), 44.62 (C-3), 40.98 (C-4), 40.78 (C-2), 32.60 (C-1); FAB-MS *m/z* 575 [M+Na]$^+$.

Ssioriside (8) – White powder; mp 100~102°C; $[\alpha]_D^{19}$ 2.2° (c 0.59, MeOH); UV (MeOH) λ_{max} 236 (s), 276 nm; IR ν_{max} (KBr) 3395 (OH), 1608, 1514, 1456 (C = C), 1327, 1213, 1107 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 6.36 (2H, s, H-2, H-6) 6.35 (2H, s, H-2', H-6'), 4.15 (1H, d, J = 7.4 Hz, anomeric H), 3.91~3.05 (overlapping, xylosyl protons), 3.83 (6H, s, -OCH_3×2), 3.81 (6H, s, -OCH_3×2), 2.62~2.49 (overlapping, H-7, H-7'), 2.12 and 1.93 (each 1H, br. s, H-8, H-8'); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 147.73 (C-3, C-5, C-3' and C-5'), 133.44 (C-4 or C-4'), 133.33 (C-4 or C-4'), 131.58 (C-1 or C-1'), 131.04 (C-1 or C-1'), 106.36 (C-2, C-6, C-2', C-6'), 103.87 (xyl-1), 76.74 (xyl-3), 73.50 (xyl-2), 69.71 (xyl-4), 68.23 (C-9), 65.82 (xyl-5), 60.44 (C-9'), 55.84 (-OCH_3× 2), 55.80 (-OCH_3×2), 42.31 (C-8'), 40.72 (C-8), 34.08 (C-7 and C-7'); FAB-MS *m/z* 577 [M+Na]$^+$.

Fraxin (9) – Yellow powder; mp 204~205°C; UV (MeOH) λ_{max} 210, 231, 293, 344 nm; IR ν_{max} (KBr) 3377 (OH), 1678 (C = O), 1591, 1491 (C = C), 1361, 1281, 1217 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 7.91 (1H, d, J = 9.4 Hz, H-4), 7.05 (1H, s, H-5), 6.23 (1H, d, J = 9.4 Hz, H-3), 4.94 (1H, d, J = 7.4 Hz, H-1"), 3.80 (3H, s, -OCH_3); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 160.37 (C-2), 145.60 (C-7), 144.92 (C-9), 144.16 (C-4), 142.80 (C-6), 131.65 (C-8), 112.02 (C-3), 109.95 (C-10), 104.93 (C-5), 103.99 (C-1'), 77.37 (C-3'), 76.26 (C-5'), 73.88 (C-2'), 69.57 (C-4'), 60.69 (C-6'), 56.07 (-O<u>C</u>H$_3$); FAB-MS *m/z* 371 [M+H]$^+$.

(+)-Lyoniresinol-3a-*O*-β-D-glucopyranoside (10) – White powder; mp 117~119°C; $[\alpha]_D^{19}$ 24.9° (c 0.825, MeOH); UV (MeOH) λ_{max} 241 (s), 278 nm; IR ν_{max} (KBr) 3375 (OH), 1610, 1499, 1456 (C = C), 1317, 1214, 1105 (C-O)) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 6.62 (1H, s, H-8), 6.42 (2H, s, H-2' and H-6'), 4.25 (1H, d, J = 7.0 Hz, anomeric H), 3.71 (3H, s, OCH_3), 3.54 (9H, s, -OCH_3×3); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 147.57 (C-3' and C-5'), 146.97 (C-5), 146.55 (C-7), 137.59 (C-1'), 137.24 (C-6), 133.33 (C-4'), 128.52 (C-9), 124.97 (C-10), 106.75 (C-2'), 105.94 (C-6'), 103.45 (glc-1), 76.94 (glc-3 and glc-5), 73.57 (glc-2), 70.13 (glc-4), 69.67 (C-3a), 63.99 (C-2a), 61.17 (glc-6), 58.91 (-OCH_3), 56.09 (-OCH_3×2), 55.69 (-OCH_3), 44.40 (C-3), 40.69 (C-4), 38.15 (C-2), 32.44 (C-1); FAB-MS *m/z* 605 [M+Na]$^+$.

(+)-Taxifolin - 3-*O*-α-L-arabinopyranoside (11) – White powder; mp 190~192°C; $[\alpha]_D^{19}$ −16.4° (c 1.44, MeOH); UV (MeOH) λ_{max} 229(s), 292 nm; UV (MeOH+NaOH) λ_{max} 246, 328 nm; UV (MeOH+NaOAc) λ_{max} 251, 329 nm; UV

(MeOH+NaOAc+H_3BO_3) λ_{max} 293 nm; UV (MeOH +$AlCl_3$) λ_{max} 295 nm; UV (MeOH+$AlCl_3$+ HCl) λ_{max} 227, 299 nm; IR ν_{max} (KBr) 3362 (OH), 1636 (C = O), 1450 (C = C), 1253, 1160 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 11.72 (1H, s, 5-OH), 6.91~6.77 (3H, m, H-2', H-5' and H-6'), 5.96 (2H, s, H-6 and H-8), 5.41 (1H, d, J = 8.4 Hz, H-2), 4.75 (1H, d, J = 8.4 Hz, H-3), 4.00 (1H, d, J = 2.4 Hz, H-1"); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 193.62 (C-4), 167.45 (C-7), 163.48 (C-5), 162.09 (C-9), 145.93 (C-4'), 145.29 (C-3'), 126.70 (C-1'), 118.82 (C-6'), 115.45 (C-3'), 114.61 (C-2'), 100.97 (C-10), 100.43 (C-1"), 96.08 (C-6), 95.20 (C-8), 81.09 (C-2), 74.92 (C-3), 71.59 (C-2"), 69.82 (C-3"), 65.14 (C-1"), 62.26 (C-5"); FAB-MS *m/z* 437 $[M+H]^+$.

Astragalin (12) – Yellow powder; mp 178°C; UV (MeOH) λ_{max} 266, 302, 350 nm; UV (MeOH+NaOH) λ_{max} 274, 327, 400 nm; UV (MeOH+NaOAc) λ_{max} 268, 308, 355 nm; UV (MeOH+NaOAc+ H_3BO_3) λ_{max} 266, 303, 350 nm; UV (MeOH+$AlCl_3$) λ_{max} 231(s), 275, 304, 350, 397 nm; UV (MeOH+$AlCl_3$+ HCl) λ_{max} 231(s), 275, 303, 347, 397 nm; IR ν_{max} (KBr) 3343 (OH), 1661 (C = O), 1598, 1438 (C = C), 1214, 1105 (C-O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 12.69 (1H, s, 5-OH), 8.12 (2H, d, J = 8.8 Hz, H-2'and H-6'), 6.96 (2H, d, J = 8.8 Hz, H-3'and H-5'), 6.50 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, d, J = 2.0 Hz, H-6), 5.54 (1H, d, J = 7.0Hz, H-1"); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ 177.52 (C-4), 164.59 (C-7), 161.30 (C-5), 160.04 (C-4'), 156.51 (C-2), 156.27 (C-9), 33.24 (C-3), 131.00 (C-2' and C-6'), 120.97 (C-1'), 115.18 (C-3' and C-5'), 103.94 (C-10), 100.88 (C-1'"), 98.84 (C-6), 93.76 (C-8), 77.54 (C-3"), 76.45 (C-5"), 74.24 (C-2"), 69.91 (C-4"), 60.84 (C-6"); FAB-MS *m/z* 449 $[M+H]^+$.

Acid hydrolysis of compounds 6-12 – Each compound (5 mg) was refluxed with 5% H_2SO_4 (5 ml) in MeOH for 1h. The reaction mixture was then concentrated under reduced pressure to remove MeOH, diluted with H_2O and fractionated by EtOAc. Each EtOAc soluble fraction was concentrated and examined by tlc. Each remaining aqueous layer was adjusted to pH 7 with $NaHCO_3$ and filtered. The filtrate was concentrated and examined by tlc.

Measurement of DPPH radical scavenging activity – The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was measured according to Hwang et al. (Hwang *et al.*, 2001). Briefly, 100 μL of 0.2 mM DPPH in methanol was added to 50 μL of each sample in methanol solution in a 96-well microtiter plate. After incubation at room temperature for 30 min, the optical density of each solution was determined at 517 nm using a microtiter plate reader (Bio Rad Laboratories Inc.). The parameter IC_{50} value represents a concentration of each compound exhibiting a 50% decrease of DPPH radicals.

Results and Discussion

The sugar moieties and aglycones of compounds **6**, **7**, **8**, **9**, **10**, **11**, and **12** were determined by acid hydrolysis and tlc. As a result, **6** gave scopoletin and D-glucose, **7** gave (+)-lyoniresinol and D-xylose, **8** gave (8S, 8'S)-4, 4'-dihydroxy-3, 5, 3', 5'-tetra- methoxy-8, 8'-butyrolignan and D-xylose, **9** gave fraxetin and D-glucose, **10** gave (+)-lyoniresinol and D-glucose, **11** gave (+)-taxifolin and L-arabinose, and **12** gave kaempferol and D-glucose, respectively.

Compounds **1**, **2**, **3**, **4**, **5**, **6**, **9**, and **12** were identified to be scopoletin (**1**) (Steck and Mazurek, 1972), (+)-taxifolin (**2**) (Mabry *et al.*, 1970; Nonaka *et al.*, 1987; Agrawal, 1989), quercetin (**3**) (Mabry *et al.*, 1970; Jin *et al.*, 2002), (−)-catechin (**4**) (Mabry *et al.*, 1970; Agrawal, 1989; Morimoto *et al.*, 1985), (+)-epicatechin (**5**) (Mabry *et al.*, 1970; Agrawal, 1989; Morimoto *et al.*, 1985), scopolin (**6**) (Steck and Mazurek, 1972; Kuroyanagi *et al.*, 1986), fraxin (**9**) (Steck and Mazurek, 1972; Kwon and Kim, 1996), (+)-taxifolin-3-*O*-α-L-arabinopyranoside (**11**) (Mabry *et al.*, 1970; Sakushima and Nishibe, 1988; Ishimaru *et al.*, 1995) and astragalin (**12**) (Mabry *et al.*, 1970; Nonaka *et al.*, 1987; Agrawal, 1989; Murakami *et al.*, 1986), respectively.

The FAB-MS spectrum of **7** exhibited pseudomolecular ion peak at *m/z* 575 $[M+Na]^+$. The IR spectrum of **7** showed significant absorption bands due to hydroxyl groups (3375 cm^{-1}) and aromatic groups (1610, 1499 cm^{-1}). The UV spectrum of **7** showed a maximum at 288 nm. The ^{1}H-NMR spectrum of **7** showed signals ascribable to four methoxyl groups, three aromatic protons, and one anomeric proton (δ 4.19, d, J = 7.0 Hz), suggesting that **7** is a monosaccharide. The ^{13}C-NMR spectrum of **7** showed carbon signals due to a pentose moiety, four methoxyl groups, and two aromatic rings. Furthermore, DEPT spectrum of **7** showed four CH_2 signals at δ 69.04, 65.81, 63.76, and 32.60. From these results **7** was presumed to be an aryl-tetralin type lignan glycoside. Acid hydrolysis of **7** gave D-xylose and (+)-lyoniresinol. The large coupling constant (J = 7.0 Hz) of **7** indicated the β-glycoside linkage for the D-xylose moiety. Based on these results and on values previously reported in the literature (Inoshiri *et al.*, 1987; Dada *et al.*, 1989; Ohashi *et al.*, 1994), **7** was identified as lyoniside.

The FAB-MS spectrum of **8** exhibited pseudomolecular ion peak at *m/z* 577 $[M+Na]^+$. The IR and UV spectra of **8** showed similar absorption patterns to those of **7**.

Table 1. DPPH radical scavenging activity of tested compounds

Tested compounds	IC_{50} (μM)[a)]
scopoletin (**1**)	>1,000
(+)-taxifolin (**2**)	49.9
quercetin (**3**)	23.0
(−)-catechin (**4**)	101.1
(+)-epicatechin (**5**)	72.2
scopolin (**6**)	>1,000
lyoniside (**7**)	111.8
ssioriside (**8**)	60.5
fraxin (**9**)	392.7
(+)-lyoniresinol-3a-*O*-β-D-glucopyranoside (**10**)	122.3
(+)-taxifolin-3-*O*-α-L-arabinopyranoside (**11**)	71.6
astragalin (**12**)	>1,000
BHA*	79.5
L-Ascorbic acid**	36.9

* and ** : positive control
a) Concentration giving a 50% decrease of DPPH radical.
The values are the means of triplicate experiments.

Furthermore, the ^{1}H-NMR spectrum of **8** was also similar to that of **7** except for signals due to protons related to two aromatic rings. From the above evidence, it has been presumed that **8** is a seco-type derivative of **7**. These results were confirmed by ^{1}H-^{1}H COSY, DEPT, HMQC, and HMBC spectra. Based on these results and on values previously reported in the literature (Yoshinari *et al.*, 1989; Shibuya *et al.*, 1992), **8** was identified as ssioriside.

The FAB-MS spectrum of **10** exhibited pseudomolecular ion peak at *m/z* 605 $[M+Na]^+$. The IR, UV, ^{1}H-NMR, and ^{13}C-NMR spectra of **10** showed almost same patterns to those of **7** except for signals due to sugar moiety. Acid hydrolysis of **10** gave D-glucose as the sugar. Based on these results and on values previously reported in the literature (Ohashi *et al.*, 1994; Min *et al.*, 2003), **10** was identified as (+)-lyoniresinol-3a-O-β-D-glucopyranoside.

In order to evaluate antioxidant activity of the isolated compounds, we determined antioxidant activity using DPPH radical scavenging activity. Among the tested compounds, (+)-taxifolin (**2**) and quercetin (**3**) exhibited potent, and (+)-epicatechin (**5**), ssioriside (**8**) and (+)-taxifolin-3-*O*-α-L-arabinoside (**11**) exhibited moderate, DPPH radical scavenging activity with an IC_{50} values of 49.9, 23.0, 72.2, 60.5 and 71.6 μM, respectively, whereas (−)-catechin (**4**), lyoniside (**7**), fraxin (**9**), (+)-lyoniresinol -3a-O-β-D-glucopyranoside (**10**) showed weak DPPH radical scavenging activity with IC_{50} values 101.0, 111.8, 392.7 and 122.3 μM, respectively (Tabel 1).

In conclusion, (+)-taxifolin (**2**), quercetin (**3**), (+)-epicatechin (**5**), ssioriside (**8**), and (+)-taxifolin-3-O-α-L-arabinoside (**11**) exhibited DPPH radical scavenging activity in the present study. scopoletin (**1**), (+)-taxifolin (**2**), (+)-epicatechin (**5**), scopolin (**6**), lyoniside (**7**), ssioriside (**8**), fraxin (**9**), (+)-lyoniresinol-3a-*O*-β-D-glucopyranoside (**10**), (+)-taxifolin-3-*O*-α-L-arabinoside (**11**), and astragalin (**12**) were for the first time isolated from this plant.

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