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Flavonoids from the Leaves of *Ailanthus altissima* Swingle and their Antioxidant Activity

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Abstract Phytochemical studies on the leaves of *Ailanthus altissima* (Simaroubaceae) have not been reported previously. Thus, the authors isolated and identified secondary metabolites from *A. altissima*. Dried and powdered leaves were extracted with 80% aqueous methanol, and the concentrated extract was successively partitioned with ethyl acetate, *n*-butanol, and water. Four flavonoids were isolated from the ethyl acetate fraction through repeated silica gel and octadecyl silica gel column chromatography. Spectroscopic data including NMR, MS, and IR allowed for identification of the chemical structures as quercetin (**1**), afzelin (**2**), quercitrin (**3**), and isoquercitrin (**4**). This is the first report of the isolation of these compounds from *A. altissima*. The four isolated flavonoids **1**–**4** as well as solvent fractions (ethyl acetate, *n*-butanol, and water), were evaluated for DPPH radical scavenging activity.

Keywords afzelin · *Ailanthus altissima* · DPPH assay · isoquercitrin · quercetin · quercitrin

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Introduction

Ailanthus altissima Swingle (Simaroubaceae), also known as tree-of-heaven, is a deciduous tree distributed mainly in China, Mongolia, Japan, and Korea (Feo et al., 2005). The diameter of a mature *A. altissima* tree is about 50 cm with stems grows up to 30 m. The upper sides of the leaves of *A. altissima* are dark green, while the under side is dark red. Flowers of *A. altissima* appear beginning from the end of June and the seeds is included in flower inside of the propeller shape. *A. altissima* has been used in Chinese traditional medicine as a bitter aromatic drug for the treatment of colds and gastric diseases (Feo et al., 2003; 2005). Some compounds isolated from the root and bark of the plant have been reported to have insecticidal (Kraus et al., 1994), antimalarial (O'Neill et al., 1986; Bray et al., 1987), antitumor (Casinovi et al., 1983), antitubercular (Rahman et al., 1997), nervous depressant (Crespi-Perellino et al., 1988), antiproliferative (Hwang et al., 2002), anti-Epstein-Barr virus (Kubota et al., 1997), alleropathy (Heisey et al., 1990; 1996), anti-inflammatory (Jin et al., 2009), and antioxidant activities (Okunade et al., 2003; Lee et al., 2005; Tamura et al., 2006; Zhang et al., 2007; Rahman et al., 2009). There have been some reports on the isolation of secondary metabolites from the cortex and roots of *A. altissima.* However, there are only a few studies that have evaluated the leaves of *A. altissima* with respect to their biological and phytochemical properties. In addition, DPPH assay performed with an alcohol extract of *A. altissima* leaves showed significant activity. Therefore, this study was initiated to identify the principal antioxidant compounds from *A. altissima* leaves.

Antioxidants are useful chemical materials for preventing oxidative deterioration of biomolecules and are widely applicable as ingredients of food supplements, cosmetics, and medicines. Many plants contain substantial amounts of antioxidants such as vitamin C, vitamin E, carotenoids, flavonoids and tannins that can be utilized to scavenge excess free radicals from the human body. Indeed, many researchers have found efficient antioxidants in plants.

This paper describes the isolation of compounds from the leaves of *A. altissima* using silica gel (SiO_2) and octadecyl silica gel (ODS) open column and flash column chromatography. For structure determination, the authors used NMR, MS, and IR spectroscopic methods. In addition, the antioxidant activity of each fraction and the isolated compounds were evaluated on the basis of their scavenging activity for stable DPPH.

Materials and Methods

Plant materials. Leaves of *Ailanthus altissima* Swingle were purchased at Daegu traditional herbal market in Daegu, Korea and identified by Prof. Dae-Keun Kim of Woosuk University, Jeonju, Korea. A voucher specimen (KHU20090515) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Reagents. Column chromatography (c.c.) was carried out using SiO_2, Kiesel gel 60 (Merck, Germany), ODS, and LiChroprep RP-18 (Merck). Flash column chromatography was carried out using SNAP Cartridge KP-Sil (Biotage, Sweden). TLC analysis was carried out using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck) and was detected using UV lamp Spectroline Model ENF-240 C/F (Spectronics Corporation, USA) and 10% H_2SO_4 solution. Deuterium solvents were purchased from Merck and Sigma-Aldrich (USA).

Instrumentation. Optical rotations were measured with a JASCO P-1010 digital polarimeter (JASCO, Japan). EI-MS and FAB-MS were recorded on a JEOL JMSAX 505-WA (JEOL, Japan) and JEOL JMS-700 (JEOL), respectively. IR spectra were obtained with a Perkin Elmer Spectrum One FT-IR spectrometer (Perkin Elmer, England). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Varian, USA). DPPH radical scavenging activity was measured with a spectrophotometer (Sunrise, USA).

Isolation of flavonoids from the EtOAc fraction. Dried leaves of *A. altissima* (5.0 kg) were extracted three times with 80% aqueous methanol (MeOH, 10 L×3) for 24 h at room temperature. The filtrates were concentrated under reduced pressure to yield a MeOH extract of 355 g. The resulting extract was then successively partitioned with water (2 L), ethyl acetate (EtOAc, 2 L×2) and *n*-butanol (*n*-BuOH, 2 L×2). The concentrated EtOAc extract (AAE, 47 g) was subjected to the Flash c.c. (SNAP Cartridge KP-sil, 100 g) and eluted with *n*-hexane-EtOAc (7:1, 1000 mL) and chloroform ($CHCl_3$)-methanol (MeOH, 10:1→5:1, each of 2000 mL) monitored by TLC to produce 17 fractions (AAE1–AAE17). AAE13 [1.9 g, elution volume/total volume (Ve/Vt) 0.706–0.824] was subjected to the Flash c.c. (SNAP Cartridge KP-Sil, 100 g) and eluted with *n*-hexane-EtOAc (5:1, 1000 mL) and $CHCl_3$-MeOH (10:1, 1300 mL) to produce nine fractions (AAE13-1–AAE13-9). AAE13-7 (620 mg, Ve/Vt 0.667–0.889) was applied to SiO_2 c.c. (3×10 cm) and eluted with *n*-hexane-$CHCl_3$-EtOAc (10:1:1, 1200 mL) to give seven fractions (AAE13-7-1–AAE13-7-7). AAE13-7-2 (250 mg, Ve/Vt 0.143–0.429) was subjected to ODS c.c. (3×4 cm) and eluted with MeOH-H_2O (7:1, 1000 mL) to give compound **1** [AAE13-7-2-11, 11 mg, Ve/Vt 0.084–0.175, TLC (60 F_{254}) R_f 0.26, *n*-hexane-$CHCl_3$-EtOAc=5:2:1]. AAE-16 (11 g, Ve/Vt 0.941–0.992) was applied to Flash c.c. (SNAP Cartridge KP-Sil, 100 g) and eluted with $CHCl_3$-MeOH (7:1→5:1, 1700 mL) to produce seven fractions (AAE16-1–AAE16-7). AAE16-3 (2.04 g, Ve/Vt 0.125–0.251) was chromatographied by the flash c.c. (SNAP Cartridge KP-Sill, 100 g; *n*-hexane-EtOAc=1:1 $CHCl_3$-MeOH=10:1→5:1, 3200 mL) to produce six fractions (AAE16-3-1–AAE16-3-6). AAE16-3-2 (300 mg, Ve/Vt 0.167–0.188) was subjected to ODS c.c. (3.5×4 cm, MeOH-H_2O=1:1→3:1, 1400 mL) to give eight fractions (AAE16-3-2-1–AAE16-3-2-8), one of which was compound **2** [AAE16-3-2-4, 28 mg, Ve/Vt 0.375–0.625, TLC (RP-18 F_{254S}) R_f 0.50, MeOH-H_2O=2:1]. AAE16-3-3 (80 mg, Ve/Vt 0.333–0.667) was subjected to ODS c.c. (3×2 cm, MeOH-H_2O=1:1, 700 mL) to give eight fractions (AAE-16-3-3-1–AAE-16-3-3-8) and afforded compound **3** [AAE16-3-3-2, 18 mg, Ve/Vt 0.112–0.298, TLC (RP-18 F_{254S}) R_f 0.60, MeOH-H_2O=2:1]. AAE16-5 (2.3 g, Ve/Vt 0.571–0.857) was subjected to Flash c.c. (SANP Cartridge KP-Sil, 100 g; *n*-hexane-EtOAc=1:1→$CHCl_3$-MeOH=10:1→5:1, 4200 mL) to produce five fractions (AAE16-5-1–AAE16-5-5). AAE16-5-3 (720 mg, Ve/Vt 0.411–0.832) was purified using ODS c.c. (3×3 cm) and eluted with MeOH-H_2O (1:1, 1500 mL) to produce seven fractions (AAE-16-5-3-1–AAE-16-5-3-7) including compound **4** [AAE16-5-3-3, 12 mg, Ve/Vt 0.251-0.496, TLC (RP-18 F_{254S}) R_f 0.62, MeOH-H_2O=2:1].

Compound **1**. yellow powder; IR ν_{max} (KBr, cm^{-1}) 3446, 1666, 1518, 1496; neg. FAB/MS *m/z* 301 [M-H]$^-$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.78 (1H, d, *J*=2.0 Hz, H-2'), 7.67 (1H, dd, *J*=8.8, 2.0 Hz, H-6'), 6.92 (1H, d, *J*=8.8 Hz, H-5'), 6.42 (1H, d, *J*=2.4 Hz, H-8), 6.22 (1H, d, *J*=2.4 Hz, H-6); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 174.6 (C-4), 165.4 (C-7), 162.3 (C-5), 158.1 (C-2), 158.0 (C-9), 148.9 (C-4'), 146.1 (C-3'), 137.1 (C-3), 124.0 (C-1'), 121.5 (C-6'), 116.1 (C-2'), 115.8 (C-5'), 104.4 (C-10), 99.1 (C-6), 94.3 (C-8).

Compound **2**. yellow crystals; $[\alpha]_D^{26}$ −104° (*c*=0.1, MeOH); IR ν_{max} (KBr, cm^{-1}) 3417, 1627, 1479; neg. FAB/MS *m/z* 431 [M-H]$^-$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.75 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.92 (2H, dd, *J*=8.8, 1.6 Hz, H-2', 6'), 6.35 (1H, br s, H-8), 6.18 (1H, br s, H-6), 5.37 (1H, d, *J*=1.6 Hz, H-1''), 0.90 (3H, d, *J*=6.0 Hz, H-6''); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 181.5 (C-4), 163.5 (C-5), 161.4 (C-4'), 158.4 (C-2, 9), 136.0 (C-3), 131.7 (C-2', 6'), 122.5 (C-1'), 116.4 (C-3', 5'), 105.9 (C-10), 103.2 (C-1''), 100.0 (C-6), 95.7 (C-8), 73.1 (C-4''), 72.0 (C-5''), 71.9 (C-3''), 71.8 (C-2''), 17.7 (C-6'').

Compound **3**. yellow powder; $[\alpha]_D^{27}$ −96° (*c*=0.1, MeOH); IR ν_{max} (KBr, cm^{-1}) 3432, 1660, 1445; neg. FAB-MS *m/z* 447 [M-H]$^-$, ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.29 (1H, d, *J*=2.0 Hz, H-2'), 7.26 (1H, dd, *J*=2.0, 8.0 Hz, H-6'), 6.87 (1H, d, *J*=8.0 Hz, H-5'), 6.29 (1H, br s, H-8), 6.13 (1H, br s, H-6), 5.32 (1H, d, *J*=1.2 Hz, H-1''), 0.92 (3H, d, *J*=6.0 Hz, H-6''); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 179.3 (C-4), 165.5 (C-7), 162.8 (C-5), 158.9 (C-2), 158.2 (C-9), 149.5 (C-4'), 146.1 (C-3'), 136.0 (C-3), 122.8 (C-1', 6'), 116.8 (C-2'), 116.2 (C-5'), 105.7 (C-10), 103.3 (C-1''), 99.7 (C-

6), 94.6 (C-8), 73.1 (C-4"), 72.0 (C-5"), 71.9 (C-3"), 71.8 (C-2"), 17.7 (C-6").

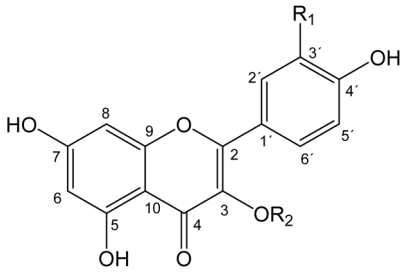
Compound **4**. yellow powder; $[\alpha]_D^{23}$ −5.2° (*c*=0.10, MeOH); IR ν_{max} (KBr, cm^{-1}) 3345, 1662, 1498; neg. FAB-MS *m/z* 463 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 7.70 (1H, d, *J*=2.4 Hz, H-2'), 7.57 (1H, dd, *J*=2.4, 7.6 Hz, H-6'), 6.85 (1H, d, *J*=7.6 Hz, H-5'), 6.36 (1H, br s, H-8), 6.17 (1H, br s, H-6), 5.24 (1H, d, *J*=7.2 Hz, H-1"); ^{13}C-NMR (100 MHz, CD_3OD, δ_C) 179.2 (C-4), 166.0 (C-7), 162.8 (C-5), 158.8 (C-2), 158.2 (C-9), 149.7 (C-4'), 145.7 (C-3'), 135.5 (C-3), 123.1 (C-1'), 122.9 (C-6'), 119.4 (C-5'), 115.9 (C-2'), 105.3 (C-10), 104.2 (C-1"), 99.9 (C-6), 94.7 (C-8), 78.3 (C-3"), 78.0 (C-5"), 75.7 (C-2"), 71.1 (C-4"), 62.5 (C-6").

Evaluation of solvent fractions and flavonol glycosides for DPPH radical scavenging activity. Antioxidant activities of the solvent fractions (EtOAc, *n*-BuOH, H_2O) and purified compounds **1**–**4** were measured on the basis of scavenging activity for the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as described previously (Masuda et al., 2001). The fractions, compounds **1**–**4**, and positive control butylated hydroxyanisole (BHA) were diluted in methanol to prepare sample solutions (100, 50, 10 μg/mL). The solvents were then added to 100 μL of a 2×10^{-4} M DPPH solution, and the mixture was shaken vigorously. After 30 min, absorbance of the methanolic DPPH tincture was measured with a spectrophotometer at 517 nm. The radical scavenging effect was calculated as follows: Radical scavenging activity (%)=(Ao–Ae)/Ao×100, where Ao is the absorbance of the negative control (DPPH plus methanol), and Ae is the absorbance of the sample (DPPH plus sample) (She et al., 2010).

Results and Discussion

When the methanol extract of the *A. altissima* leaves was developed by silica gel TLC, spots showed UV absorbance and yellow (**1**) and dark yellow (**2**, **3**, **4**) colorization after spaying with a 10% H_2SO_4 solution and heating indicated the presence of flavonoids. The methanol extract was then successively applied to EtOAc, *n*-BuOH and H_2O solvents for fractionation. Repeated SiO_2 and ODS column chromatography of the EtOAc fractions resulted in isolation of four flavonoids **1**–**4**.

Compound **1** was obtained as yellow powder and produced a molecular ion peak $[M-H]^-$ at *m/z* 301 in negative FAB/MS, thus, molecular weight of **1** was determined to be 302. The IR spectrum (KBr) showed absorbance bands consistent with hydroxyl groups (3446 cm^{-1}), conjugated ketones (1666 cm^{-1}), and double bonds (1518 cm^{-1}). In the ^{1}H-NMR spectrum, three olefin methine proton signals [(δ_H 7.78, d, *J*=2.0 Hz, H-2'), (δ_H 7.67, dd, *J*=2.0, 8.8 Hz, H-6'), (δ_H 6.92, d, *J*=8.8 Hz, H-5')] of a 1,2,4-trisubstitution benzene ring and two aromatic methine proton signals [(δ_H 6.42, d, *J*=2.4 Hz, H-8), (δ_H 6.22, d, *J*=2.4 Hz, H-6)] of a typical meta-coupled pattern due to a 1,2,3,5-tetrasubstitution benzene ring were observed. Accordingly, compound **1** was assumed to be quercetin, a well-known flavonol. A multiplicity of fifteen carbon signals observed in the ^{13}C-NMR spectrum was determined through



1: R_1 = OH, R_2 = H
2: R_1 = H, R_2 = α-L-rhm
3: R_1 = OH, R_2 = α-L-rhm
4: R_1 = OH, R_2 = β-D-glc

Fig. 1 Chemical structures of the flavonoids from the *Ailanthus altissima* leaves.

distortionless enhancement of polarization transfer (DEPT) experiments. The carbon signals in the low magnetic field region indicated the presence of conjugated ketone (δ_C 174.6, C-4), seven oxygenated olefin quaternary carbons [δ_C 165.4 (C-7), 162.3 (C-5), 158.1 (C-2), 158.0 (C-9), 148.9 (C-4'), 146.1 (C-3'), 137.1 (C-3)], two olefin quaternary carbons [δ_C 124.0 (C-1'), 104.4 (C-10)], and five olefin methines [δ_C 121.5 (C-6'), 116.1 (C-2'), 115.8 (C-5'), 99.1 (C-6), 94.3 (C-8)]. Compound **1** was identified as quercetin by comparison with published spectroscopic data (Li et al., 2009).

Compound **2**, yellow powder, showed the molecular ion peak $[M-H]^-$ at *m/z* 431 in negative FAB/MS. The IR spectrum (KBr) showed the absorbance bands consistent with a hydroxyl group (3417 cm^{-1}), conjugated ketones (1627 cm^{-1}), and double bonds (1479 cm^{-1}). ^{1}H-NMR spectrum identified four olefin methine proton signals [(δ_H 7.75, 2H, d, *J*=8.8 Hz, H-3', 5'), (δ_H 6.92, 2H, dd, *J*=8.8, 1.6 Hz, H-2', 6')] of a para-disubstitution benzene ring and two olefin methines [(δ_H 6.35, br s, H-8), (δ_H 6.18, br s, H-6)] of a typical meta-coupled pattern due to a 1,2,3,5-tetrasubstitution benzene ring. Furthermore, proton signals for one anomer proton (δ_H 5.37, d, *J*=1.6 Hz, H-1"), four oxygenated methines (δ_H 3.33–4.21, 4H, overlapped, H-2", 3", 4", 5"), and one methyl proton (δ_H 0.90, 3H, d, *J*=6.0 Hz, H-6") were also observed indicating the presence of a 6-deoxysugar. Accordingly, compound **2** was assumed to be a flavonol mono-glycoside. A total of twenty one carbon signals observed in the ^{13}C-NMR spectrum indicated compound **2** to be a flavonol 6-deoxysugar. In the low magnetic field region, signals consistent with a conjugated ketone (δ_C 181.5, C-4), six oxygenated olefin quaternary carbons [δ_C 169.5 (C-7), 163.5 (C-5), 161.4 (C-4'), 158.4 (C-2, 9), 136.0 (C-3)], two olefin quaternary carbons [δ_C 122.5 (C-1'), 105.9 (C-10)], and six olefin methane carbons [δ_C 131.7 (C-2', 6'), 116.4 (C-3', 5'), 100.0 (C-6), 95.7 (C-8)] were observed. The chemical shifts of other glycosidic carbon signals [δ_C 73.1 (C-4"), 72.0 (C-5"), 71.9 (C-3"), 71.8 (C-2"), 17.7 (C-6")] suggested the presence of an α-rhamnopyranosyl moiety. The connection between the anomer proton signal (H-1",

Table 1 DPPH radical scavenging activity of compounds **1-4**

Compound	DPPH radical scavenging activity (%)		
	10 (μg/mL)	50 (μg/mL)	100 (μg/mL)
1	20.3±1.73	92.9±0.27	93.2±0.59
2	-2.0±1.05	-1.2±1.86	-7.3±0.59
3	6.0±0.68	74.6±1.83	86.4±1.06
4	10.9±0.63	74.9±1.62	86.0±1.16
BHA	23.7±1.40	90.9±0.35	90.1±1.52

δ_H 5.37) of the rhamnopyranosyl unit and the oxygenated olefin quaternary carbon signal (C-3, δ_C 136.0) of the aglycone was verified by a cross-peak observed in the gHMBC spectrum. Thus, compound **2** was identified as kaempferol-3-*O*-*α*-L-rhamnopyranoside (afzelin), confirmed through the comparison of several physical and spectroscopic data with those of literature (Seo et al., 1978).

Compound **3**, yellow powder, showed the molecular ion peak $[M-H]^-$ at *m/z* 447 in the negative FAB/MS. The IR spectrum (KBr) showed absorbance bands consistent with a hydroxyl group (3432 cm^{-1}), conjugated ketones (1660 cm^{-1}), and double bonds (1445 cm^{-1}). Thus, Compound **3** was assumed to be a monoglycoside of quercetin (**1**) based on spectroscopic data such as MS, ^{1}H-NMR and ^{13}C-NMR. Proton signals for one anomeric proton (δ_H 5.32, d, *J*=1.2 Hz, H-1"), four oxygenated methines (δ_H 3.33~4.21, 4H, overlapped, H-2", 3", 4", 5"), and one methyl proton (δ_H 0.92, 3H, d, *J*=6.0 Hz, H-6") were also observed. The chemical shifts of glycosidic carbon signals [δ_C 73.1 (C-4"), 72.0 (C-5"), 71.9 (C-3"), 71.8 (C-2"), 17.7 (C-6")] and the coupling constant of the anomer proton (*J*=1.2 Hz) suggested the presence of an *α*-rhamnopyranosyl moiety. The connection between the anomer proton signal (H-1", δ_H 5.32) of the rhamnopyranosyl unit and the oxygenated olefin quaternary carbon signal (C-3, δ_C 136.0) of the aglycone was verified by the cross-peak observed in the gHMBC spectrum. Therefore, compound **3** was identified as quercetin-3-*O*-*α*-L-rhamnopyranoside, quercitrin (Seo et al., 1978).

Compound **4**, yellow powder, showed the molecular ion peak $[M-H]^-$ at *m/z* 463 in the negative FAB/MS. The IR spectrum (KBr) showed the absorbance bands consistent with a hydroxyl group (3345 cm^{-1}), conjugated ketones (1662 cm^{-1}), and double bonds (1498 cm^{-1}). Compound **4** was almost identical with compound **3** with the exception of a sugar moiety. Proton signals for one hemiacetal proton (δ_H 5.24, d, *J*=7.2 Hz, H-1"), ^{13}C-NMR signals of a sugar moiety [δ_C 71.1 (C-4"), 78.0 (C-5"), 78.3 (C-3"), 75.7 (C-2"), 62.5 (C-6")], and the coupling constant of the anomer proton (*J*=7.2 Hz) suggested the presence of a *β*-glucopyranosyl group. The connection between anomer proton signal (H-1", δ_H 5.24) of the glucopyranosyl unit and the oxygenated olefin quaternary carbon (C-3, δ_C 135.5) of the aglycone was verified by the cross-peak observed in the gHMBC spectrum. Compound **4** was identified as quercetin-3-*O*-*β*-D-glucopyranoside (isoquercitrin), by comparison of spectroscopic data with those of literatures (Seo et al., 1978; Lee et al., 2007).

Dried leaves of *A. altissima* were extracted with 80% aqueous methanol because the methanol extract of the leaves showed very strong DPPH radical scavenging activity in a previous screening study. The extract was separated into three fractions (EtOAc-soluble, *n*-BuOH, and H_2O-soluble fractions) by a solvent partition method. The DPPH radical scavenging activities of the solvents fractions were compared with BHA as a positive control.

BHA, exhibited a DPPH radical scavenging activity of 90.1±1.52% at 100 μg/mL, while EtOAc, *n*-BuOH, and H_2O fractions showed 97.0±0.15%, 96.5±0.08%, and 4.47±0.75%, respectively, at the same concentration.

The EtOAc and *n*-BuOH fractions exhibited higher antioxidant activities compared to the positive control BHA. As a result, the EtOAc fraction was selected for further fractionation. The EtOAc-soluble fraction was subjected to SiO_2 column chromatography with stepwise gradient elution, affording 17 fractions. The obtained fractions were assayed by the described method, and fractions 2, 3, 7, 9, 10, 12, 13, 14, 15, 16, and 17 were selected as antioxidative fractions. Finally, fractions 2, 3, 7, 13, and 16 were subjected to column chromatography using SiO_2 and ODS, affording the purified flavonoids **1-4** as major compounds.

Compounds **1**, **3**, and **4** had radical scavenging activity of 93.2±0.59%, 86.4±1.06%, and 86.0±1.16% at 100 μg/mL, respectively. Compound **1** (quercetin) showed very strong radical scavenging activity, with efficiencies similar to that of BHA. In contrast, compounds **3** (quercitrin) and **4** (isoquercitrin) had a somewhat weaker than BHA which may have been due to glycosidation at the 3-position (Burda and Oleszek, 2001). Conversely, compound **2** had an antioxidant activity of −7.3± 0.59% at 100 μg/mL. Quercetin-type flavonol glycosides **3** and **4** were stronger than kaempferol type ones because of the presence of hydroxyl groups at the B-ring C-3′ position (Bruda et al., 2001).

Antioxidant activity is tends to correlate with various bioactivities, and compounds with strong antioxidant activity have been shown to be strong effective against several diseases related to oxidative stress. In the present study, the isolated principal compounds had comparatively high DPPH radical scavenging activity, suggesting that the leaves of *A. altissima* and the isolated flavonols may be widely applicable as a source of new drugs and functional foods.

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