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Isolation and Characterization of Antioxidative Compounds from the Aerial Parts of *Angelica keiskei*

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Abstract Ethyl acetate-soluble neutral fraction of hot water extracts from the aerial parts of *Angelica keiskei* showed a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity. Six antioxidative compounds were purified and isolated by various chromatographic procedures. Based on the analyses of FAB-MS and NMR, the isolated compounds were structurally elucidated as luteolin 7-*O*-β-D-glucopyranoside (**1**), quercetin 3-*O*-β-D-galactopyranoside (**2**), quercetin 3-*O*-β-D-glucopyranoside (**3**), quercetin 3-*O*-α-D-arabinopyranoside (**4**), kaempferol 3-*O*-α-D-arabinopyranoside (**5**), and luteolin 7-*O*-rutinoside (**6**). The glycosides of flavonols and luteolin showed DPPH radical-scavenging activity. One molecule of **2**, **3**, **4**, **6**, **1**, and **5** scavenged 4.2, 4.2, 4.1, 2.5, 2.2, and 1.4 molecules of DPPH radical, respectively.

Key words: *Angelica keiskei*, DPPH radical-scavenging activity, antioxidative compound, quercetin glycoside, kaempferol glycoside, luteolin glycoside

Introduction

Antioxidants are widely used in food manufacture to increase the quality of food products by minimizing the rancidity of food components and protecting the nutritional compounds (1). In addition, they may also be associated with the scavenging of reactive oxygen species *in vivo* and therefore reduce the incidence of many subsequent diseases such as cancer, diabetes, and inflammatory disease (2-4). Moreover, it has been considered that diets containing abundant natural antioxidants, such as fruit, vegetables, and grains, are associated with the prevention of cancer and cardiovascular disease (5, 9). Natural antioxidants from dietary sources include phenolic compounds, carotenoids, as well as antioxidant vitamins and enzymes (5-8). Considerable studies have been performed on the screening, isolation, and evaluation of antioxidative activities of antioxidants in plants, and the bioavailavility and metabolism of these compounds in the human body (5-11).

Angelica keiskei Koidz (Umbelliferae) has been used as food, which is eaten as a vegetable (salad and juice) or a tea for promoting health (12). In addition, this plant has been used as a folk remedy for disease prevention such as hypertension, analeptia, neuralgia, and arteriosclerosis (12-15). In particular, it was reported that chalcones (xantho-angelol, xanthoangelol H and F, and isobavachalcone) and coumarins (laspeptin, isolaserpitin, selinidin, and pteryxin etc.) isolated from roots (16, 17) or stems (18) have anticancer activity in mouse skin and human liver carcinogenesis. Several researches on chemical constituents in the root of *A. keiskei* have been performed (16, 17, 19-21). Park *et al.* (12, 22, 23) also reported the identification of luteolin glycosides and quercetin 3-*O*-glucoside as anti-hyperlipidemic active compounds from the aerial parts of *A. keiskei*. However, not enough other chemical studies for the aerial parts of *A. keiskei* have been performed, yet.

Therefore, we carried out the isolation and identification of antioxidative compounds in the aerial parts of *A. keiskei*. This paper describes two luteolin glycosides and four flavonolglycosides isolated and structurally elucidated as antioxidative active compounds from the ethyl acetate (EtOAc)-soluble neutral fraction of hot water extracts of *A. keiskei* aerial parts and the antioxidative activities of these compounds.

Materials and Methods

Plant material The freeze-dried aerial parts of *Angelica keiskei* were obtained from Shalom Industries Co. LTD., Naju, Korea.

Chemicals 1,1-Diphenyl-2-picrylhydrazyl (DPPH), luteolin, kaempferol, and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were of reagent grade from commercial sources.

Extraction and isolation procedure The freeze-dried aerial parts of *Angelica keiskei* (2.5 kg) was extracted with boiling water (25 L) for 30 min (24). The extracted solution was adjusted to pH 8.0 by 5% $NaHCO_3$ solution, and then partitioned with ethyl acetate(EtOAc). The aqueous

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phase was also adjusted to pH 3.0 by 1 M HCl and partitioned with EtOAc. These EtOAc-soluble neutral and acidic fractions were concentrated at 38°C *in vacuo*, respectively. The EtOAc-soluble neutral fraction was chromatographed on Sephadex LH-20 (3.7×21 cm, 25-100 mesh, Pharmacia Fine Chemicals, Uppsala, Sweden) and eluted step wisely with an increasing concentration of methanol (MeOH 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%, v/v, 150 mL each) in water (25). The active fraction was subjected to HPLC under the following conditions: column, μ-Bondapak ODS column (7.8 × 300 mm, 10 μm, Milford, MA, USA); mobile phase, MeOH-H_2O (5:5 or 3.5:6.5, v/v, adjusted to pH 2.5 by trifluoroacetic acid); flow rate, 4.0 mL/min (Model 510 solvent delivery system, Waters, MA, USA); detection, UV detector (280 nm, 486 Tunable absorbance detector, Waters).

The assay for purification of antioxidative compound(s) was performed by spraying the DPPH reagent on a thin layer chromatograph (TLC, Silica gel 60 F_{254}, 0.25 mm thickness, Merck, Darmstadt, Germany). Each fraction, after column chromatography or HPLC, was spotted on the plate and developed with suitable solvents. After spraying DPPH solution, fractions visualized by decolorization of the spot were regarded as an antioxidative active.

Instrumental analyses of the isolated compounds NMR spectra were obtained from a ^{unit}INOVA 500 spectrometer (Varian, Walnut Creek, CA, USA). Fast atom bombardment-mass spectrometry (FAB-MS) was performed by mass spectrometer (Platform 2, Micromass, Menchaster, UK or JMS-HX/HX110A Tandem Mass Spectrometer, Jeol, Japan) under the following conditions: ion mode, positive; matrix reagent, 3-nitrobenzyl alcohol (3-NBA); scanning mass range, *m/z* 50~1000.

Assay of DPPH radical-scavenging Free radical-scavenging activities of the isolated compounds and their aglycones (luteolin, quercetin, and kaempferol) were determined by using DPPH radical, according to the method of Moon *et al.* (26). Briefly, an ethanol solution (500 μL) of each compound with different concentrations (10-100 μM) was added to the mixture of 10 mM Tris-HCl buffer (1 mL, pH 7.4) and 0.4 mM DPPH (500 μL) in ethanol. The solution was mixed and allowed to stand for 30 min in the dark. The free radical-scavenging activity of each compound was quantified by the decolorization of DPPH at 517 nm. The DPPH radical-scavenging activities of EtOAc-soluble neutral and acidic fractions were also determined as the percentage decreased in the absorbance shown by a blank test. Each value of 50% free radical-scavenging concentration (SC_{50}) was determined from a dose-response curve.

Results and Discussion

Isolation of antioxidative active compounds The freeze-dried aerial parts of *Angelica keiskei* (2.5 kg) was extracted with boiling water for 30 min. The hot water extract solution was solvent fractionated with EtOAc to obtain an EtOAc-soluble neutral fraction (2.4 g) and an EtOAc-soluble acidic fraction (12.5 g). Antioxidant activities of these fractions were measured using a stable radical, DPPH. Although the antioxidant activities of EtOAc-soluble neutral (SC_{50}, 18.0 μg/mL) and acidic (SC_{50}, 21.0 μg/mL) fractions were lower than that of α-tocopherol (SC_{50}, 8.0 μg/mL), their activities were comparable to that of α-tocopherol. In addition, the EtOAc-soluble neutral fraction showed more variable pattern of antioxidative active compounds than that of the EtOAc-soluble acidic fraction on the TLC after spray of DPPH EtOH solution (data not shown). Therefore, in the first place, we attempted the isolation and purification of antioxidative active compound(s) from EtOAc-soluble neutral fractions.

The EtOAc-soluble neutral fraction was subjected to Sephadex LH-20 column chromatography using a step-wise procedure, with increasing MeOH in H_2O. Relatively high DPPH radical-scavenging activities were observed at the fractions of 30, 40, 50, 60, and 70% MeOH (data not shown). Each fraction was analyzed through HPLC using a μ-Bondapak ODS column. The chromatogram pattern of 70% MeOH fraction was similar to that of 60% MeOH fraction under the same HPLC condition (mobile phase, MeOH-H_2O=5:5, v/v). In addition, the chromatogram pattern of 50% MeOH fraction was similar to that of the 40% MeOH fraction with a HPLC of the same condition (mobile phase, MeOH-H_2O=3.5:6.5, v/v). As conditions to separate antioxidative active compounds, the chromatogram patterns of 70% and 50% MeOH fractions were more purified than those of 60% and 40% MeOH fractions, respectively. Therefore, we attempted to isolate antioxidative active compounds from 70% and 50% MeOH fractions (472.0 and 221.8 mg, repectively) through semi-preparative HPLC on a μ-Bondapak ODS column. Four peaks (t_R 13.2 min, 15.9 mg, **1**; t_R 15.3 min, 15.3 mg, **2**; and **3**; t_R 18.9 min, 131.4 mg, **4**; t_R 26.7 min, 21.8 mg, **5**) were obtained from the 70% MeOH fraction. In addition, one peak (t_R 18.2 min, 22.7 mg, **6**) was isolated from the 50% MeOH fraction. The chemical structure of the isolated compounds was determined by analyses of FAB-MS and NMR.

Structural elucidation of isolated compounds

Compound 1 In FAB-MS spectrum (positive), the pseudomolecular ion peaks of **1** were detected at *m/z* 449 $[M + H]^+$ and 471 $[M + Na]^+$, indicating that the molecular weight of **1** was 448. In the ^{13}C-NMR spectrum (Table 1), **1** showed 21 carbon signals including 15 carbon signals of aglycone (δ 183.3, carbonyl carbon signal, C-4; δ 165.8~95.8, 14 sp^2 carbon signals) and 6 carbon signals of monosaccharide (δ 102.3, anomeric carbon signal; δ 79.7~62.9, 5 non-anomeric carbon signals). From the FAB-MS and ^{13}C-NMR spectroscopic data, **1** was suggested to be flavonoid monoglycoside. The ^{1}H-NMR (500 MHz, CD_3OD) spectrum (Table 2) of **1** showed evidence for aglycone of luteolin including 2 proton signals of A ring [δ 6.86 (d, J = 1.5 Hz, H-6) and 7.01 (d, J = 1.5 Hz, H-8)], 3 proton signals of B ring at δ 7.91 (d, J = 1.5 Hz, H-2'), 7.30 (d, J = 8.5 Hz, H-5') and 7.53 (dd, J = 1.5, 8.5 Hz, H-6'), and a proton signal of C ring at δ 6.94 (s, H-3). The glucose proton signals were also detected with an anomeric proton signal at δ 5.83 (H-1") and non-anomeric proton signals of 6H at δ 4.32 (H-2"), 4.30 (H-3"), 4.66 (H-4"), 4.40 (H-5"), 4.16 (H-6"a), and 4.76 (H-6"b). These results agreed with luteolin glucoside. In addition, the

Table 1. ^{13}C-NMR data for compounds 1-6 (125 MHz)

Position	1[a]	2[b]	3[b]	4[b]	5[b]	6[a,c]
2	165.8	158.9	158.9	158.9	158.6	166.0
3	104.6	135.9	135.9	135.8	135.8	104.8
4	183.3	179.9	179.9	179.6	178.3	183.3
5	163.1	163.2	163.2	163.2	163.3	163.1
6	101.1	100.0	100.0	100.0	100.0	101.3
7	164.5	166.2	166.2	166.1	166.2	164.6
8	95.8	94.2	94.8	94.8	94.9	95.7
9	158.4	158.6	158.6	158.6	158.9	158.5
10	107.1	105.8	105.8	105.8	105.7	107.2
1'	123.2	123.1	123.0	123.0	123.1	123.4
2'	115.2	116.2	116.1	117.6	132.4	115.2
3'	148.3	146.0	146.0	146.1	116.4	148.1
4'	152.4	150.1	150.1	150.1	159.9	152.4
5'	117.4	117.9	117.7	116.3	116.4	117.6
6'	120.2	123.3	123.3	123.2	132.4	120.5
1"	102.3	105.5	104.4	104.8	104.5	102.7
2"	75.3	73.3	75.9	73.0	72.9	75.2
3"	78.6	75.2	78.6	74.3	74.1	78.1
4"	71.6	70.2	71.4	69.3	69.1	72.6
5"	79.7	77.3	78.6	67.1	66.9	78.9
6"	62.9	62.1	62.7			68.2

The NMR solvents used were pyridine-d_4[a] and CD_3OD[b]. [c]The chemical shifts of rhamnose for compound **6** were assigned to δ 102.7 (C-1'''), 73.4 (C-2'''), 72.0 (C-3'''), 74.5 (C-4'''), 70.4 (C-5'''), and 19.1 (C-6''').

coupling constant (J = 7.5 Hz) of the anomeric proton signal (δ 5.83, H-1") indicated that the glucose had a β-anomeric configuration. In HMBC spectrum (data not shown), the cross peak between the anomeric proton signal at δ 5.83 (C-1") and luteolin carbon signal at δ 164.7 (C-7) confirmed that glucose was substituted for 7 position of luteolin. The HMBC spectrum of **1** showed the correspondence of the correlations of protons and carbons for luteolin glucoside. Therefore, on the basis of these assignments, the structure of **1** was luteolin 7-*O*-β-D-glucopyranoside (Fig. 1).

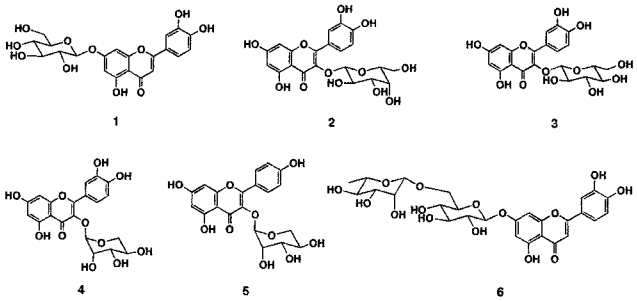

Fig. 1. The structure of antioxidative compounds 1-6 isolated from the aerial parts of *Angelica keiskei*.

Compounds 2 and 3 The molecular weight of the compound obtained from the peak of t_R 15.3 min in HPLC chromatogram of a 70% MeOH fraction was analyzed to be 464 from its FAB-MS (positive) spectrum *m/z* 465 $[M + H]^+$ and 487 $[M + Na]^+$. However, the ^{1}H- and ^{13}C-NMR spectra (Table 1 and 2) of this fraction showed the evidence for the mixture of two compounds, suggesting that the aglycone was the same but the glycosides differed. In term of the aglycone, ^{1}H-NMR spectrum of the mixture was closely related to that of **1** except for the absence of proton signal on 3 position of luteolin. Therefore, the aglycone of the two compounds is assignable to quercetin. The ^{1}H- and ^{13}C-NMR spectra of the mixture were observed with inequal intensity of two sugar proton signals at the ratio of 3:1 (the main compound, **2**; the minor compound, **3**). Comparing with NMR spectra of the literature (25) and authentic compounds, the main compound was identified as quercetin 3-*O*-β-D-galactopyranoside (**2**) and the minor compound was concluded to be quercetin 3-*O*-

Table 2. ^{1}H-NMR data for compounds 1-6 (500 MHz)

Position	δH (mult, J)					
	1[a]	2[b]	3[b]	4[b]	5[b]	6[a,c]
3	6.94 (s)	-	-	-	-	6.87 (s)
6	6.86 (d, 1.5)	6.16 (d, 2.0)	6.16 (br. d, 2.0)	6.20 (d, 2.0)	6.21 (d, 2.0)	6.82 (d, 2.0)
8	7.01 (d, 1.5)	6.37 (d, 2.0)	6.37 (d, 2.0)	6.40 (d, 2.0)	6.41 (d, 2.0)	6.97 (d, 2.0)
2'	7.91 (d, 1.5)	7.84 (d, 2.0)	7.84 (d, 2.0)	7.75(d, 1.5)	8.07 (d, 9.0)	7.99 (d, 2.0)
3'	-	-	-	-	7.89 (d, 9.0)	-
5'	7.30 (d, 8.5)	6.86 (d, 8.5)	6.86 (d, 8.5)	6.87 (d, 8.5)	7.89 (d, 9.0)	7.38 (d, 8.5)
6'	7.53 (dd, 1.5, 8.5)	7.58 (dd, 2.0, 8.5)	7.58 (dd, 2.0, 8.5)	7.58 (dd, 2.0, 8.0)	8.07 (d, 9.0)	7.56 (dd, 2.0, 8.5)
1"	5.83 (d, 7.5)	5.12 (d, 8.0)	5.26 (d, 7.5)	5.16 (d, 6.5)	5.15 (d, 6.5)	5.69 (d, 7.5)
2"	4.32 (dd, 7.5, 9.0)	4.55-3.33[d]	4.55-3.33[d]	3.89 (dd, 6.5, 8.0)	3.89 (dd, 6.5, 8.0)	4.32 (dd, 7.5, 9.0)
3"	4.30 (d, 3.5, 9.0)	4.55-3.33[d]	4.55-3.33[d]	3.63 (dd, 8.0, 3.0)	3.65 (dd, 8.0, 3.0)	4.30 (d, 3.5, 9.0)
4"	4.66 (dd, 1.5, 9.0)	4.55-3.33[d]	4.55-3.33[d]	3.81-3.84 (m)[e]	3.76-3.78 (m)[f]	4.66 (dd, 3.5, 9.0)
5"a 5"b	4.40 (dd, 9.0, 9.0)	4.55-3.33[d]	4.55-3.33[d]	3.81-3.84 (m)[e] 3.45 (m)	3.76-3.78 (m)[f] 3.40 (m)	4.40 (dd, 9.0, 9.0)
6"a 6"b	4.16 (d, 9.0) 4.76 (d, 7.0)	4.55-3.33[d]	4.55-3.33[d]	-	-	4.16 (d, 9.0) 4.76 (d, 7.0)

The NMR solvents used were [a]pyridine-d_4 and [b]CD_3OD. [c]The chemical shifts of rhamnose for **6** were assigned to δ 5.51 (br. s, H-1'''), 4.75 (s, H-2'''), 4.13 (td, 9.5, 9.5, H-3'''), 4.27 (dd, 9.5, 9.5, H-4'''), 4.34 (dd, 6.0, 9.5, H-5'''), 1.61 (d, 6.0, H-6'''). [d]The chemical shifts for glucose of **2** and **3** were shown. [e,f]The chemical shifts of arabinose for **4** and **5** were overlapped.

β-D-glucopyranoside (**3**). According to Lu and Foo (25), it was reported that these compounds had the same retention time in HPLC analysis on an ODS column. The report on the isolation of **2** and **3** by Lu and Foo was correspondent to our data. Therefore, **2** and **3** were unambiguously identified as quercetin 3-*O*-β-D-galactopyranoside and quercetin 3-*O*-β-D-glucopyranoside, respectively (Fig. 1).

Compound 4 From the FAB-MS (positive) analysis, the molecular ion peaks of **4** were revealed at *m/z* 435 $[M + H]^+$ and 457 $[M + Na]^+$, indicating that the molecular weight of **4** was 434. ^{13}C- and ^1H-NMR spectra of **4** (Table 1 and 2) were closely related to that of **3**, except for the pattern of glycosidic moiety [5 carbon signals; δ 104.8 (C-1"), 73.0 (C-2"), 74.3 (C-3"), 69.3 (C-4"), 67.1 (C-5")], suggesting that **4** was quercetin monoglycoside, which was different from the glucose of **3**. Compared with the FAB-MS and ^{13}C-NMR spectra of **3** (M.W. 464, 21 carbon signals), **4** (M.W. 434, 20 carbon signals) was assumed to be quercetin glycoside linked with pentose without the hydroxymethyl group ($-CH_2OH$) of hexose. The sugar moiety of **4** was assigned to arabinopyranose by comparison with ^{13}C- and ^1H-NMR data of the literature (27, 28). The coupling constant (J = 6.5 Hz) of the anomeric proton (δ 5.16) was derived from α-anomeric configuration. In HMBC spectrum (data not shown), the cross peak of the anomeric proton signal at δ 5.16 (H-1") with the carbon signal at δ 135.8 (C-3) confirmed that the anomeric carbon of arabinose was attached to 3 position of quercetin. Therefore, the structure of **4** could be identified as quercetin 3-*O*-α-D-arabinopyranoside (Fig. 1).

Compound 5 FAB-MS (positive) measurement of **5** showed signals related with molecular ion at *m/z* 441 $[M+Na]^+$ and 456 $[M + K]^+$, indicating that the molecular weight of **5** is 418. ^1H-NMR spectrum of **5** (Table 2) was closely related to that of **4** except for the proton signals of ABXX' system [δ 8.07 (d, J = 9.0 Hz, H-2', 6') and 7.89 (d, J = 9.0 Hz, H-3', 5')] on B ring of flavonol, suggesting that **5** was kaempferol arabinopyranoside without hydroxyl group at C-3' position of B ring of quercetin. Moreover, the cross peak between the anomeric proton signal (δ 5.15, J = 6.5 Hz) and carbon signal at δ 135.8 (C-3) in the HMBC spectrum (data not shown) indicated that arabinose was linked to 3 position of kaempferol. Therefore, the structure of **5** was concluded to be kaempferol 3-*O*-α-D-arabinopyranoside (Fig. 1).

Compound 6 FAB-MS (positive) measurement of **6** showed peaks at *m/z* 595 and 617, which were ascribable to the $[M+H]^+$ and $[M+Na]^+$ ions respectively, indicating that the molecular weight of **6** was 594. In ^{13}C- and ^1H-NMR spectra (Table 1 and 2), aglycone of **6** was closely related to that of **1**. However, the ^1H-NMR spectrum of **6** showed 15 proton signals ascribable to sugar moiety including two anomeric protons (H-1", δ 5.69, d, J = 7.5 Hz; H-1'", δ 5.51, d, br. s) and one methyl group of upfield at δ 1.61 (d, J = 6.0 Hz), indicating that **6** was luteolin diglycosides. From ^1H-NMR, TOCSY, and DQF-COSY spectra, the diglycosides of **6** were assignable to glucose [δ 5.69 (H-1"), 4.32 (H-2"), 4.30 (H-3"), 4.66 (H-4"), 4.40 (H-5"), 4.16 (H-6"a), and 4.76 (H-6"b)] and rhamnose [δ 5.51 (H-1'"), 4.75 (H-2'"), 4.13 (H-3'"), 4.27 (H-4'"), 4.34 (H-5'") and 1.61 (H-6'")] (The data of TCOSY and DQF-COSY not shown). The β-anomeric configurations of glucose and rhamnose were induced from the coupling constant of glucose (δ 5.69, d, J = 7.5 Hz, H-1") and a series of proton signals ascribable to rhamnose (Table 2). In HMBC experiment (data not shown), the presence of cross peaks from H-1" (δ 5.69) of glucose to C-7 of luteolin, and from 1'" (δ 5.51) of rhamnose to C-6" of glucose established that the anomeric carbon of glucose was bound to 7 position of aglycone and the anomeric carbon of rhamnose was linked to 6" position of glucose. Resulting from NMR and FAM-MS spectroscopic data, the structure of **6** was proved to be luteolin 7-*O*-β-L-rhamnopyranosyl (1→6)-*O*-β-D-gluco-pyranoside (luteolin 7-*O*-rutinoside) (Fig. 1).

The six antioxidative active compounds were isolated and identified as luteolin 7-*O*-β-D-glucopyranoside (**1**), quercetin 3-*O*-β-D-galactopyranoside (**2**), quercetin 3-*O*-β-D-glucopyranoside (**3**), quercetin 3-*O*-α-D-arabinopyranoside (**4**), kaempferol 3-*O*-α-D-arabinopyranoside (**5**), and luteolin 7-*O*-rutinoside (**6**) from the EtOAc-soluble neutral fraction of hot water extracts of *A. keiskei* aerial parts (Fig. 1). Park *et al.* (12, 22, 23) reported that luteolin glycosides (**1**, **6**) and quercetin 3-*O*-β-D-galactopyranoside (**2**) were identified as anti-hyperlipidemic active compounds from MeOH extracts of *A. keiskei* aerial parts. Lee *et al.* (29) also reported the result of quantitative analysis of quercetin and luteolin as major flavonoid aglycones by HPLC after acid hydrolyzing in leaves and stalks of *A. keiskei*. We reported here the entire elucidation on structure of sugar moieties bound to quercetin and luteolin. In addition, the report on the occurrence of keamferol, one of the major flavonols including quercetin, is the first time for this plant. Moreover, as far we know, isolation and identification of **3**, **4**, and **5** from the aerial parts of *A. keiskei* is also being reported for the first time.

Structure-activity relationship on DPPH radical-scavenging of isolated compounds The isolated compounds have beneficial effects, such as antimicrobial activity (30), antioxidant activity (31), anti-inflammatory activity (32), and anticancer activity (33). In particular, quercetin and luteolin are well known as flavonoids having strong antioxidative activity (34, 35). However, the structure-activity relationships of the glycosidic derivatives on antioxidative activity have not been fully characterized yet. Therefore, we determined DPPH radical-scavenging activity using the six antioxidative active compounds isolated from aerial parts of *A. keiskei* with their aglycones (luteolin, quercetin, and kaempferol) as references.

DPPH radical-scavenging activity of the compounds is shown in Table 3. The number of molecules scavenging DPPH radical per one molecule of each compound were calculated by the assumption that one molecule of α-tocopherol scavenged two molecules of DPPH radical. Free radical-scavenging activity obtained from the mole number of DPPH radicals decreased in the order quercetin (6.7 molecules) > **2** (4.2 molecules) = **3** (4.2 molecules) ≥ **4** (4.1 molecules) > luteolin (2.9 molecules) > **6** (2.5 molecules) = kaempferol (2.5 molecules) > **1** (2.2 molecules) > **5** (1.4 mole-cules). The DPPH radical-scavenging activity of quercetin was significantly higher than those of quercetin substituted with sugar (**2**, **3**, **4**) and luteolin without hydroxyl group at C-3 position. In addition, the antioxidative

Table 3. DPPH radical-scavenging activity of the isolated compounds and their aglycones

Compounds	Number of molecules of DPPH radical-scavenging[a]
α-Tocopherol	2.0
Luteolin	2.9
Quercetin	6.7
Kaempferol	2.5
Luteolin 7-*O*-β-D-glucopyranoside (**1**)	2.2
Quercetin 3-*O*-β-D-galactopyranoside (**2**)	4.2
Quercetin 3-*O*-α-D-glucopyranoside (**3**)	4.2
Quercetin 3-*O*-α-D-arabinopyranoside (**4**)	4.1
Kaempferol 3-*O*-α-D-arabinopyranoside (**5**)	1.4
Luteolin 7-*O*-rutinoside (**6**)	2.5

[a]Amount of DPPH radical trapped by 1.0 mol of α-tocopherol was assumed to be 2.0 mol; means of two replications.

ability of quercetin (6.7 molecules) having free hydroxyl group at C-3 position was considerably higher than that of luteolin (2.9 molecules) having no hydroxyl group at the C-3 position. These results indicate that the free hydroxyl group of the C-3 of flavonol is one of the important factors for the action of free radical-scavenging. Quercetin 3-*O*-glycosides (**2**, **3**, **4**) showed similar DPPH radical-scavenging activities (4.1-4.2 molecules). It is likely that the difference of sugar bound to the same position of a flavonoid does not affect antioxidant activity of the flavonoid, at least in this experimental system. However, DPPH radical-scavenging activities of these compounds (**2**, **3**, **4**) substituted with sugar at the C-3 position were stronger than those of luteolin and luteolin glycosides (**1**, **6**), which have an absence of hydroxyl group and are substituted with disaccharide (rutinose) at C-3 position, respectively. No information is available on the antioxidant effect of the ether oxygen (and/or sugar) in C-3 position of quercetin 3-*O*-glycosides. However, the ether oxygen of C-3 position may be responsible for the activation of catechol moiety of the B ring in the antioxidative mechanism. Furthermore, DPPH radical-scavenging activity of **1** (2.2 molecules) was also approximately similar to that of **6** (2.5 molecules), although they are mono- and di-saccharides, respectively. Therefore, no relation between antioxidant ability and types of sugar bound to the same position of a flavonoid is reconfirmed as in the results of comparison of the antioxidative activities between quercetin and its glycosides. Interestingly, the antioxidative ability of luteolin (2.9 molecules) was not remarkable in the comparison with those of its 7-*O*-glycosides (**1** and **6**; 2.2 and 2.5 molecules). Therefore, it is considered that the contribution to radical-scavenging of the hydroxyl group in the free form of C-7 position of luteolin is weak. Although quercetin and kaempferol were used as references, comparing the antioxidant effect of them, DPPH-trapping capacity of quercetin (6.7 molecules) was considerably higher than that of kaempferol (2.5 molecules). They are different only in the presence or absence of a hydroxyl group in C-3' position. It is already reported that the *o*-dihydroxyl structure in the B ring is essential for free radical-scavenging and metal-chelating effects in quercetin (31, 34, 36-39). Therefore, it is likely that the lowering of the radical-trapping capacity of kaempferol is caused by the lack of the hydroxyl group in C-3' position.

DPPH radical-scavenging activities for six flavonoid glycosides isolated from aerial parts of *A. keiskei* were investigated with those of their aglycones (luteolin, quercetin, kaempferol). However, we do not have sufficient results to discuss structure-activity relationships for flavonoid and its glycosides. In selection of a flavonoid for application, the characterization of the compounds is an important determinant to exert antioxidative activity. The presence of a glucoside group in flavonoid lowers its lipophilic property (40). Furthermore, considerable studies have demonstrated that the efficiency on antioxidative activity of antioxidants depends on the environment where oxidation happens. Therefore, the environment for application of flavonoid and its glycosides also have to be seriously considered.

Recently, a number of studies has been reported on biological functions and bioavailabilities of flavonoids (38, 41-47). *A. keiskei* is expected to be a useful biological source due to high level of various antioxidative active flavonoids. Investigation of bioactive compound(s) for *A. keiskei* is also underway.

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