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A Novel Benzoyl Glucoside and Phenolic Compounds from the Leaves of *Camellia japonica*

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Abstract A novel benzoyl glucoside (**4**) and 13 known phenolic compounds were isolated from the leaves of *Camellia japonica* by a guided 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The structure of **4** was determined to be 4-hydroxy-2-methoxyphenol 1-*O*-β-D-(6'-*O*-*p*-hydroxylbenzoyl)-glucopyranoside (camelliadiphenoside). The 13 known compounds were identified as (*E*)-coniferyl alcohol (**1**), (-)-epicatechin (**2**), 4-hydroxyphenol 1-*O*-β-D-(6-*O*-*p*-hydroxybenzoyl) glucopyranoside (**3**), naringenin 7-*O*-β-D-glucopyranoside (**5**), quercetin 3-*O*-β-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**6**), kaempferol 3-*O*-β-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**7**), (+)-catechin (**8**), 1,6-di-*O*-*p*-hydroxybenzoyl-β-D-glucopyranoside (**9**), phloretin 2'-*O*-β-D-glucopyranoside (**10**), quercetin 3-*O*-β-D-glucopyranoside (**11**), quercetin 3-*O*-β-D-galactopyranoside (**12**), kaempferol 3-*O*-β-D-galactopyranoside (**13**), and kaempferol 3-*O*-β-D-glucopyranoside (**14**). Their chemical structures were determined by the spectroscopic data of fast atom bondardment mass spectrometry (FABMS) and nuclear magnetic resonance (NMR). Flavonoids having the catechol moiety showed significantly higher DPPH radical scavenging activity than other isolated compounds having monohydroxy phenyl group.

Keywords: *Camellia japonica*, benzoyl glucoside, flavonoid glycoside, camelliadiphenoside, antioxidant

Introduction

Camellia japonica is an evergreen shrub of the Theaceae family, which also includes *Camellia sinensis* and *Camellia oleifera*. This plant can often be found in gardens of Korea and Japan. The seeds and young leaves of *C. japonica* have been used as an oil source and a tea material (1,2). The investigation of chemical constituents in the fruits, seeds, flowers, and leaves of *C. japonica* have been studied extensively: fruits, camelliagenins A, B, and C (3, 4); seeds, camelliasaponins A_1, A_2, B_1, B_2, C_1, and C_2 (5, 6); flowers, camellenodiol and camelledionol (7); leaves, (-)-epicatechin and (+)-catechin, camellidin II, and camelliatannins (8-10). Recently, Onodera *et al.* (11) reported the isolation of 4 flavonol glycosides including camellianoside, rutin, hyperoside, and isoquercetin from the leaves.

In the present study regarding the chemical constituents from the leaves of *C. japonica*, a novel benzoyl glucoside together with 13 known compounds were isolated via a guided 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. This paper describes the isolation and identification of the novel benzoyl glucoside (**4**) as well as 13 known phenolic compounds and their DPPH radical scavenging effects.

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Material and Methods

Plant material The leaves of *Camellia japonica* (Theaceae) were collected in January 2005 from Wando(Island) of Korea and identified by Prof. Kye-Han Lee, Laboratory of Forest Ecology, College of Agriculture and Life Science, Chonnam National University. A voucher sample has been deposited in the warm-temperate forest arboretum of Chonnam Nationnal University located in Bogildo(Island). This freeze-dried material was stored at freezer until use.

Chemicals DPPH and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol-d_4 (CD_3OD) and dimethyl sulfoxide-d_6 (DMSO-d_6) were obtained from Merck Co. (Darmstadt, Germany). Other chemicals were of reagent grade from commercial sources.

Isolation of antioxidative compounds from hot water extract The freeze-dried leaves (1.5 kg) were extracted with water (30 L) for 30 min at 90°C and filtered with a glass filter (G3). The obtained filtrate was adjusted to pH 8.0 by 5% $NaHCO_3$ and partitioned with ethyl acetate (EtOAc) to give the EtOAc-soluble neutral fraction. The aqueous layer was adjusted to pH 3.0 by 2.0 N HCl and partitioned with EtOAc to yield the EtOAc-soluble acidic fraction. A portion (5.69 g) of the EtOAc-soluble neutral fraction was fractionated by chromatography on a column (4.0×82 cm) of Sephadex LH-20 (25-100 mesh, Pharmacia Fine Chemicals, Uppsala, Sweden) eluting with H_2O/MeOH (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10, v/v, stepwise system, each 700 mL). The 3 fractions [A-5 (H_2O/MeOH, 5:5, v/v), A-6 (H_2O/MeOH, 4:6, v/v), and A-7

(H_2O/MeOH, 3:7, v/v)] were subjected to high performance liquid chromatography (HPLC) on an octadecyl silane (ODS) column (μBondapak, 19×300 mm, 10 μm, Waters, Milford, MA, USA) with a flow rate of 8.0 mL/min, a wavelength of 254 nm, and a mobile phase of H_2O/MeOH [75:25 (v/v), fraction A-5; 65:35 (v/v), fraction A-6; 60:40 (v/v), fraction A-7].

Structural analysis Nuclear magnetic resonance (NMR) spectra were obtained from ^{unit}INOVA 500 spectrometer (Varian, Walnut Creek, CA, USA) using solvents as the internal standard. Compound **4** was dissolved with DMSO-d_6 and other compounds were dissolved with CD_3OD. Chemical shifts were referenced to residual CHD_2OD at δ =3.31 ppm (^{1}H-NMR) and δ=49.15 ppm (^{13}C-NMR) and to residual DMSO-d_5 at δ=2.50 ppm (^{1}H-NMR) and δ= 39.5 ppm (^{13}C-NMR). Mass spectral data were performed using fast atom bondardment mass spectrometry (FABMS, JMS-HX100; Jeol Co., Tokyo, Japan) with matrix ingredient (3-nitrobenzyl alcohol).

Compound **1**: white powder; ^{13}C-NMR (125 MHz, CD_3OD) δ 149.2 (C-2), 147.8 (C-1), 132.2 (C-1'), 130.8 (C-4), 127.2 (C-2'), 121.1 (C-5), 116.4 (C-6), 110.7 (C-3), 64.1 (C-3'), 56.5 (-O<u>C</u>H$_3$); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.00 (1H, d, *J*=2.0 Hz, H-3), 6.84 (1H, dd, *J*=8.0, 2.0 Hz, H-5), 6.73 (1H, d, *J*=8.0 Hz, H-6), 6.50 (1H, br. d, *J*=16.0 Hz, H-1'), 6.19 (1H, td, *J*=6.0, 16.0 Hz, H-2'), 4.19 (2H, dd, *J*=6.0, 1.3 Hz, H-3'), 3.86 (3H, s, -OC<u>H</u>$_3$); FABMS (positive) *m/z* 181 $[M+H]^+$ and 203 $[M+Na]^+$.
Compound **2**: white powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 6.99 (1H, br. s, H-2'), 6.81 (1H, t, *J*=8.0 Hz, H-5'), 6.78 (1H, t, *J*=8.0 Hz, H-6'), 5.97 (1H, br. s, H-6), 5.95 (1H, br. s, H-8), 4.80 (1H, br. s, H-2), 4.17 (1H, br. s, H-3), 2.86 (1H, dd, *J*=4.5, 17.0 Hz, H-4a), 2.75 (1H, dd, *J*=2.0, 17.0 Hz, H-4b); FABMS (positive) *m/z* 291 $[M+H]^+$ and 313 $[M+Na]^+$.
Compound **3**: white powder; ^{13}C-NMR (125 MHz, CD_3OD) δ 168.1 (<u>C</u>=O), 163.8 (C-4"), 154.0 (C-4), 152.4 (C-1), 133.1 (C-2", 6"), 122.3 (C-1"), 119.7 (C-3, 5), 116.7 (C-2, 6), 116.4 (C-3", 5"), 103.8 (C-1'), 78.1 (C-3'), 75.7 (C-5'), 75.1 (C-2'), 72.2 (C-4'), 65.2 (C-6'); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.91 (2H, d, *J*=8.0 H-2", 6"), 6.94 (2H, d, *J*= 7.0 Hz, H-3, 5), 6.86 (2H, d, *J*=8.0 Hz, H-3", 5"), 6.61 (2H, d, *J*=7.0, H-1, H-2, 6), 4.73 (1H, d, *J*=7.5 Hz, H-1'), 4.67 (1H, dd, *J*=2.0, 12.0 Hz, H-6'a), 4.35 (1H, dd, *J*=8.0, 12.0, H-6'a), 3.71 (1H, ddd, *J*=2.0, 8.0, 9.5 Hz, H-5'), 3.48 (1H, dd, *J*=8.5, 8.5 Hz, H-3'), 3.45 (1H, dd, *J*=7.5, 8.5 Hz, H-2'), 3.41 (1H, dd, *J*=8.5, 9.5 Hz, H-4'); FABMS (positive) *m/z* 415 $[M+Na]^+$.
Compound **4**: white powder; $[\alpha]_D^{25}$ +15.7° (*c* 0.15, MeOH); ^{13}C- and ^{1}H-NMR data see Table 1; HR-FABMS (positive) *m/z* 445.1114 $[M+Na]^+$ (calculated for $C_{20}H_{22}O_{10}Na$, +0.4 *mmu*).
Compound **5**: yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.32 (2H, d, *J*=8.5 Hz, H-2', 6'), 6.82 (2H, d, *J*=8.5 Hz, H-3', 5'), 6.21 (1H, br. s, H-8), 6.19 (1H, br. s, H-6), 5.38 (1H, d, *J*=12.0 Hz, H-2), 4.98 (1H, d, *J*=6.5 Hz, H-1"), 3.88 (1H, d, *J*= 11.0 Hz, H-6"b), 3.69 (1H, td, *J*=8.0, 5.0, H-6"a), 3.48-3.37 (4H, m, H-2"-H-5"), 3.17 (1H, dd, *J*=13.0, 17.5 Hz, H-3b), 2.75 (1H, td, *J*=7.0 Hz, H-3a); FABMS (positive) *m/z* 435 $[M+H]^+$ and 457 $[M+Na]^+$.
Compound **6**: yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.67 (1H, br. s, H-2'), 7.63 (1H, d, *J*=8.5 Hz, H-5'), 6.87 (1H, d, *J*=8.5 Hz, H-6'), 6.36 (1H, br. s, H-8), 6.18 (1H, d, *J*=1.5 Hz, H-6), 5.07 (1H, d, *J*=7.5 Hz, H-1"), 4.52 (1H, br. s, H-1'"), 3.9-3.3 (10H, m, H-4"-H-6", H-2'"-H-5'"), 1.12 (3H, d, *J*= 6.0 Hz, H-6'"); FABMS (positive) *m/z* 633 $[M+Na]^+$.
Compound **7**: yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 8.06 (2H, d, *J*=9.0 Hz, H-2', 6'), 6.88 (2H, d, *J*=9.0 Hz, H-3', 5'), 6.32 (1H, d, *J*=1.5 Hz, H-6), 6.15 (1H, d, *J*=1.5 Hz, H-6), 5.07 (1H, d, *J*=7.5 Hz, H-1"), 4.56 (1H, br. s, H-1'"), 3.9-3.2 (10H, m, H-2"-H-6", H-2'"-H-5'"), 1.13 (3H, d, *J*=6.0 Hz, H-6'"); FABMS (positive) *m/z* 595 $[M+H]^+$ and 613 $[M+Na]^+$.
Compound **8**: white powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 6.85 (1H, br. s, H-2'), 6.77 (1H, d, *J*=8.0 Hz, H-5'), 6.72 (1H, dd, *J*= 1.5, 8.0 Hz, H-6'), 5.94 (1H, br. s, H-6), 5.87 (1H, br. s, H-8), 4.58 (1H, d, *J*=7.5 Hz, H-2), 3.99 (1H, dd, *J*=7.5, 7.5 Hz, H-3), 2.85 (1H, dd, *J*=5.5, 16.0, H-4a), 2.52 (1H, dd, *J*= 7.5, 16.0 Hz, H-4b); FABMS (positive) *m/z* 291 $[M+H]^+$ and 313 $[M+Na]^+$.
Compound **9**: dark yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) δ 168.2 (C-7"), 166.8 (C-7), 164.2 (C-4"), 163.7 (C-4), 133.5 (C-2", 6"), 133.1(C-2, 6), 122.3 (C-1), 121.6 (C-1"), 116.4 (C-3", 5"), 116.3 (C-3, 5), 96.1 (C-1'), 78.2 (C-2'), 76.6 (C-5'), 71.5 (C-3'), 74.2 (C-4'), 64.7 (C-6'); ^{1}H-NMR (500 MHz, CD_3OD) δ 7.86 (2H, d, *J* = 8.5 Hz, H-2", 6"), 7.79 (2H, d, *J*=8.5 Hz, H-2, 6), 6.73 (2H, d, *J*=8.5 Hz, H-3", 5"), 6.71 (2H, d, *J*=8.5 Hz, H-3', 5'), 5.60 (1H, d, *J*=7.5 Hz, H-1'), 4.50 (1H, dd, *J*=2.0, 12.0 Hz, H-6'a),

Table 1. NMR spectroscopic data of a novel benzoyl glucoside (4)[1)]

Position	δ_H (*int.*, *mult.*, *J* in Hz)	δ_C	HMBC correlations
1	-	138.8	
2	-	149.5	
3	6.38 (1H, d, 2.0)	100.6	C-1, C-2, C-4, C-5
4	-	152.5	
5	6.16 (1H, dd, 2.0, 8.5)	105.5	C-1, C-3, C-4
6	6.91 (1H, d, 8.5)	116.7	C-1, C-2, C-4
-OCH_3	3.68 (3H, s)	55.2	C-2
1'	4.73 (1H, d, 7.0)	100.8	C-1
2'	3.23 (1H, dd, 7.0, 9.0)	72.9	
3'	3.27 (1H, dd, 9.0, 9.0)	76.3	
4'	3.21 (1H, dd, 9.0, 9.0)	69.9	
5'	3.60 (1H, m)	73.5	
6'a 6'b	4.54 (1H, d, 10.0) 4.10 (1H, dd, 3.0, 10.0)	63.3	C-4', C-7"
1"	-	118.6	
2", 6"	7.75 (2H, d, 8.0)	131.1	C-1", C-4", C-7"
3", 5"	6.83 (2H, d, 8.0)	115.3	C-1"
4"	-	161.8	
7"	-	165.1	

[1)]Measured in DMSO-d_6 at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR.

Table 2. ^{13}C-NMR data for compounds 2, 5-8, and 11-14 (CD_3OD, 125 MHz)

Carbons	2	5	6[1)]	7[2)]	8	11	12	13	14
2	79.9	80.8	158.8	159.0	82.9	159.0	159.0	158.9	159.0
3	67.5	44.2	135.7	135.6	68.9	135.7	135.7	135.6	135.6
4	29.3	198.7	179.3	179.1	28.6	179.5	179.5	179.4	179.5
5	157.6	165.0	163.0	159.1	157.0	163.1	163.1	163.0	163.2
6	96.5	98.2	100.8	101.3	96.5	100.5	100.5	101.1	100.6
7	158.0	167.1	166.3	169.6	157.6	167.7	167.7	169.0	167.9
8	96.0	97.1	95.5	95.9	95.7	95.2	95.2	95.7	95.3
9	157.4	164.8	159.2	163.0	157.9	158.7	158.7	158.8	158.8
10	100.2	105.1	105.2	105.1	101.0	105.4	105.4	104.9	105.4
1'	132.3	131.0	123.3	123.0	132.3	123.1	123.1	122.9	123.0
2'	115.4	129.3	116.2	132.5	115.4	116.2	116.2	132.5	132.4
3'	145.9	116.5	146.0	116.3	146.3	146.1	146.1	116.3	116.2
4'	145.8	159.3	150.1	161.7	146.3	150.1	150.1	161.8	161.8
5'	116.1	101.4	117.8	116.3	116.3	117.7	117.9	116.3	116.2
6'	119.6	129.3	123.7	132.5	120.2	123.3	123.2	132.5	132.4
1"		101.4	105.1	104.8		104.6	105.7	105.4	104.4
2"		74.8	75.9	75.9		75.9	73.3	73.2	78.2
3"		78.4	78.4	77.4		78.5	75.3	75.2	75.9
4"		71.3	71.5	71.6		71.4	70.2	70.1	71.5
5"		78.0	77.4	78.4		78.3	77.3	77.3	78.6
6"		62.5	68.7	68.8		62.7	62.1	62.1	62.8

The chemical shifts of rhamnose were assigned:
[1)]Compound **6**, δ 102.6 (C-1'''), 72.4 (C-2'''), 72.2 (C-3'''), 74.1 (C-4'''), 69.9 (C-5'''), 18.0 (C-6''').
[2)]Compound **7**, δ 102.6 (C-1'''), 72.2 (C-2'''), 72.5 (C-3'''), 74.1 (C-4'''), 69.9 (C-5'''), 18.1 (C-6''').

4.31 (1H, dd, *J*=5.0, 12.0 Hz, H-6'b), 3.65 (1H, m, H-5), 3.43 (3H, H-2'-H-4'); FABMS (positive) *m/z* 421 $[M+H]^+$ and 443 $[M+Na]^+$.

Compound **10**: dark yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) δ 206.7 (C=O), 167.7 (C-4'), 166.1 (C-6'), 162.4 (C-2'), 156.5 (C-4), 134.0 (C-1), 130.5 (C-2, 6), 116.2 (C-3, 5), 106.9 (C-1'), 102.2 (C-1"), 98.5 (C-3'), 95.6 (C-5'), 78.6 (C-3" and C-5"), 74.9 (C-2"), 71.3 (C-4"), 62.6 (C-6"), 47.1 (C-α), 31.0 (C-β); ^{1}H-NMR (500 MHz, CD_3OD) δ 6.96 (2H, d, *J*=8.5 Hz, H-2, 6), 6.58 (2H, d, *J*=8.5 Hz, H-3, 5), 6.08 (1H, d, *J*=2.5 Hz, H-5'), 5.86 (1H, br. d, *J*=2.5 Hz, H-3'), 4.94 (1H, d, *J*=7.5 Hz, H-1"), 3.81 (1H, dd, *J*=1.5, 11.5 Hz, H-6"a), 3.62 (1H, dd, *J*=1.5, 11.5 Hz, H-6"b), 3.40-3.27 (4H, m, H-2"-H-5"), 3.35 (2H, t, *J*=6.5 Hz, H-α), 2.78 (2H, t, *J*=6.5 Hz, H-β); FABMS (positive) *m/z* 437 $[M+H]^+$ and 459 $[M+Na]^+$.

Compound **11**: dark yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.71 (1H, br. s, H-5'), 7.58 (1H, d, *J*=8.5 Hz, H-6'), 6.87 (1H, d, *J*=8.0 Hz, H-2'), 6.36 (1H, br. s, H-8), 6.18 (1H, br. s, H-6), 5.21 (1H, d, *J*=7.5 Hz, H-1"), 3.85-3.31 (6H, m, H-2"-H-6"); FABMS (positive) *m/z* 465 $[M+H]^+$ and 487 $[M+Na]^+$.

Compound **12**: dark yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 7.84 (1H, br. s, H-5'), 7.58 (1H, d, *J*=8.5 Hz, H-6'), 6.87 (1H, d, *J*=8.0 Hz, H-2'), 6.36 (1H, br. s, H-8), 6.18 (1H, br. s, H-6), 5.13 (1H, d, *J*=8.0 Hz, H-1"), 3.85-3.31 (6H, m, H-2"-H-6"); FABMS (positive) *m/z* 465 $[M+H]^+$ and 487 $[M+Na]^+$.

Compound **13**: yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 8.08 (2H, d, *J*=9.0 Hz, H-3', 5'), 6.88 (2H, d, *J*=9.0 Hz, H-2', 6'), 6.33 (1H, br. s, H-8), 6.15 (1H, br. s, H-6), 5.08 (1H, d, *J* =7.5 Hz, H-1"), 3.82 (1H, d, *J*=3.5 Hz, H-4"), 3.78 (1H, dd, *J* =7.5, 9.5 Hz, H-2"), 3.62 (1H, dd, *J*=6.0, 12.5 Hz, H-6"a), 3.53 (1H, dd, *J*=9.5, 3.5 Hz, H-3"), 3.52 (1H, dd, *J*=6.0, 12.5 Hz, H-6"b), 3.43 (1H, dd, *J*=6.0, 6.0 Hz, H-5"); FABMS (positive) *m/z* 449 $[M+H]^+$ and 471 $[M+Na]^+$.

Compound **14**: yellow powder; ^{13}C-NMR (125 MHz, CD_3OD) see Table 2; ^{1}H-NMR (500 MHz, CD_3OD) δ 8.05 (2H, d, *J*=9.0 Hz, H-3', 5'), 6.89 (2H, d, *J*=9.0 Hz, H-2', 6'), 6.37 (1H, d, *J*=2.0 Hz, H-8), 6.18 (1H, d, *J*=2.0 Hz, H-6), 5.22 (1H, d, *J*=7.5 Hz, H-1"), 3.69 (1H, dd, *J*=2.5, 12.5, H-6"a), 3.53 (1H, dd, *J*=2.5, 12.5 Hz, H-6"b), 3.44 (1H, dd, *J*=8.5, 8.0 Hz, H-3"), 3.43 (1H, ddd, *J*=2.5, 5.5, 12.5 Hz, H-5"), 3.42 (1H, dd, *J*=7.5, 8.5 Hz, H-2"), 3.33 (1H, dd, *J*=8.0, 8.0 Hz, H-4"); FABMS (positive) *m/z* 449 $[M+H]^+$ and 471 $[M+Na]^+$.

Assay of DPPH radical scavenging Free radical scavenging activity of the isolated compounds and α-tocopherol was determined by using DPPH radical according to the modified method of Abe *et al.* (12). Briefly, an methanol solution (200 μL) of each compound with different concentrations (10-250 μM) was added to DPPH radical ethanol solution (1,800 μL, final concentration, 100 μM). 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

solvent extracts and EtOAc-soluble neutral and acidic fractions were also determined as the percentage decrease in the absorbance shown by a blank test. Each value of 50% free radical scavenging concentration (SC_{50}) was determined from dose-response curve.

The assay for purification of antioxidative compound(s) was performed with spraying the DPPH reagent on thin layer chromatograph (TLC, Silica gel 60 F_{254}, 0.25 mm thickness, Merck) according to the modified method of Takao *et al.* (13). Each fraction purified by Sephadex LH-20 column chromatography or ODS-HPLC was spotted on the plate and developed with suitable solvents. After spraying 200 mM DPPH solution, fractions visualized as a decolorization of the spot were regarded as antioxidative active.

Results and Discussion

Antioxidative activity of solvent fractions The hot water extract of freeze-dried leaves (1.5 kg, 3.3 kg fresh wt. eq.) was solvent-fractionated with EtOAc to give the EtOAc-soluble neutral fraction (14.4 g) and EtOAc-soluble acidic fraction (16.9 g). The antioxidative activity of the EtOAc-soluble fractions was determined by a DPPH radical (final concentration, 100 μM) scavenging assay. The 50% free radical scavenging concentration (SC_{50}) of these fractions was determined from the dose-response curve. The antioxidative activity of the EtOAc-soluble neutral fraction (SC_{50}, 5.0 μg/mL) was slightly higher than that of the EtOAc-soluble acidic fraction (SC_{50}, 9.0 μg/mL). Therefore, isolation and purification of antioxidative active compound(s) from the EtOAc-soluble neutral fraction was performed.

Isolation of active compounds from the EtOAc-soluble neutral fraction The EtOAc-soluble neutral fraction (14.4 g) was fractionated by chromatography on a column of Sephadex LH-20 eluting with H_2O/MeOH (stepwise system). Each fraction was developed on TLC and then sprayed with DPPH radical EtOH solution (200 μM). All of the fractions showed DPPH free radical scavenging activity. In particular, fractions A-5 (H_2O/MeOH, 5:5, v/v, 160 mg), A-6 (H_2O/MeOH, 4:6, v/v, 270 mg), and A-7 (H_2O/MeOH, 3:7, v/v, 760 mg) exhibited more profound DPPH free radical scavenging activity than other fractions. Compound **1** (1.3 mg) was isolated from fraction A-5 (160 mg) by ODS-HPLC with 25% MeOH as a mobile phase. Compound **2** (38.2 mg), **3** (0.8 mg), **4** (1.5 mg), **5** (3.2 mg), **6** (1.3 mg), and **7** (1.3 mg) were isolated from fraction A-6 (270 mg) by ODS-HPLC with 35% MeOH as a mobile phase. Compound **8** (16.7 mg), **9** (8.2 mg), **10** (5.2 mg), mixture of **11** and **12** (1.8 mg), **13** (5.7 mg), and **14** (6.7 mg) were isolated from fraction A-7 (760 mg) by ODS-HPLC with 40% MeOH as a mobile phase. The structure of the isolated compounds was elucidated by the spectroscopic data of NMR and FABMS.

Structure determination of a novel compound The optical activity ($[\alpha]_D^{25}$) of **4** was +15.7° (*c* 0.15, MeOH). The molecular formula of **4** was determined as $C_{20}H_{22}O_{10}$ by positive ion high resolution fast atom bondardment mass spectrometry (HRFABMS) analysis (*m/z* 445.1114 $[M+Na]^+$, calculated for $C_{20}H_{22}O_{10}Na$, +0.4 *mmu*). The ^{1}H-NMR spectrum (Table 1) showed the presence of a tri-substituted aromatic ring of the signals at δ 6.38 (1H, d, *J* =2.0 Hz, H-3), 6.16 (1H, dd, *J*=2.0, 8.5 Hz, H-5), and 6.91 (1H, d, *J*=8.5 Hz, H-6), together with a methoxyl group from the signal at δ 3.68 (3H, s) and another *para*-substituted aromatic ring from the signals of the AA'BB' system at d 7.75 (2H, d, *J*=8.0 Hz, H-2", 6") and 6.83 (2H, δ, *J*=8.0 Hz, H-3", 5"). The ^{1}H-NMR spectrum (Table 1) also showed the presence of a sugar moiety from the anomeric signal at δ 4.73 (1H, d, *J*=7.0 Hz, H-1') and other signals at δ 3.21-4.54 (6H). The ^{13}C-NMR spectrum (Table 1) revealed 20 carbon signals, including a carbonyl carbon at δ 165.1 (C-7"), 12 sp^2 carbons of 2 aromatic benzene rings at δ 161.8-100.6, a methoxyl group at d 55.2, and 6 carbons of a sugar moiety at δ 100.8-63.3. These results suggest that **4** is a benzoyl glucoside with the linkage of another phenyl group. The configuration of β-D-glucopyranoside could be derived from the proton correlations at the ^{1}H-^{1}H COSY spectrum, the coupling constant value of the anomeric proton [δ 4.73 (1H, d, *J*=7.0 Hz, H-1')], and the coupling constant patterns (*J*=7.0-10.0 Hz) of other sugar protons on the ^{1}H-NMR spectrum. All of the protonated carbons were assigned according to the results of the HSQC analysis. In the HMBC spectrum (Table 1) of **4**, the aglycone moieties were assigned to be hydroxymethoxybenzene (A ring) and 4-hydroxybenzoic acid (B ring). The presence of the cross peak from the proton signal at δ 4.54 (H-6') to carbonyl carbon signals at δ 165.1 (C-7") indicated that the B ring was esterified at the C-6 position of glucose. The presence of the cross peak from the anomeric proton signal at δ 4.73 (H-1') to carbon signals at δ 138.8 (C-1) revealed that the A ring was etherified at the C-1 position of glucose.

It was already confirmed that the methoxyl group was linked with the A ring by the HMBC spectrum (Table 1). However, it remains unclear as to whether the methoxyl group positioned at C-2 or C-3 of the A ring. In the NOESY spectrum of **4**, the proton signal (δ 3.68, -OC<u>H</u>$_3$) of the methoxyl group showed a correlation with the anomeric proton signal (δ 4.73, H-1') of glucose. This result indicated that the methoxyl group may be conjugated at the C-2 position of the A ring. In order to determine the accurate structure of the A ring, **4** was methylated by diazomethane at room temperature and purified by ODS column with 70% MeOH as the mobile phase to yield the methylated **4**, and it was analyzed by the experiment of NOESY. The binding positions of the 3 methoxyl groups (δ 3.72, C-4-OC<u>H</u>$_3$ of the A ring; 3.66, C-2-OC<u>H</u>$_3$ of the A ring; 3.85, C-4"-OC<u>H</u>$_3$ of the B ring) were confirmed by the NOESY spectrum of the methylated **4**. In particular, the proton signals of the existing methoxyl group (δ 3.66, C-2-OC<u>H</u>$_3$) and the proton signal of a new methoxyl group (δ 3.72, C-4-OC<u>H</u>$_3$) showed cross peaks between the methine proton signal of H-3 (δ 6.38) at the A ring. This finding verified that the existing methoxyl group (δ 3.66, C-2-OC<u>H</u>$_3$) was linked at the C-2 position of the A ring. Therefore, the structure of **4** was elucidated as 4-hydroxy-2-methoxyphenol 1-*O*-β-D-(6'-*O*-*p*-hydroxylbenzoyl) glucopyranoside (camelliadiphenoside), a novel benzoyl glucoside (Fig. 1).

Identification of the 13 known compounds Of the 13

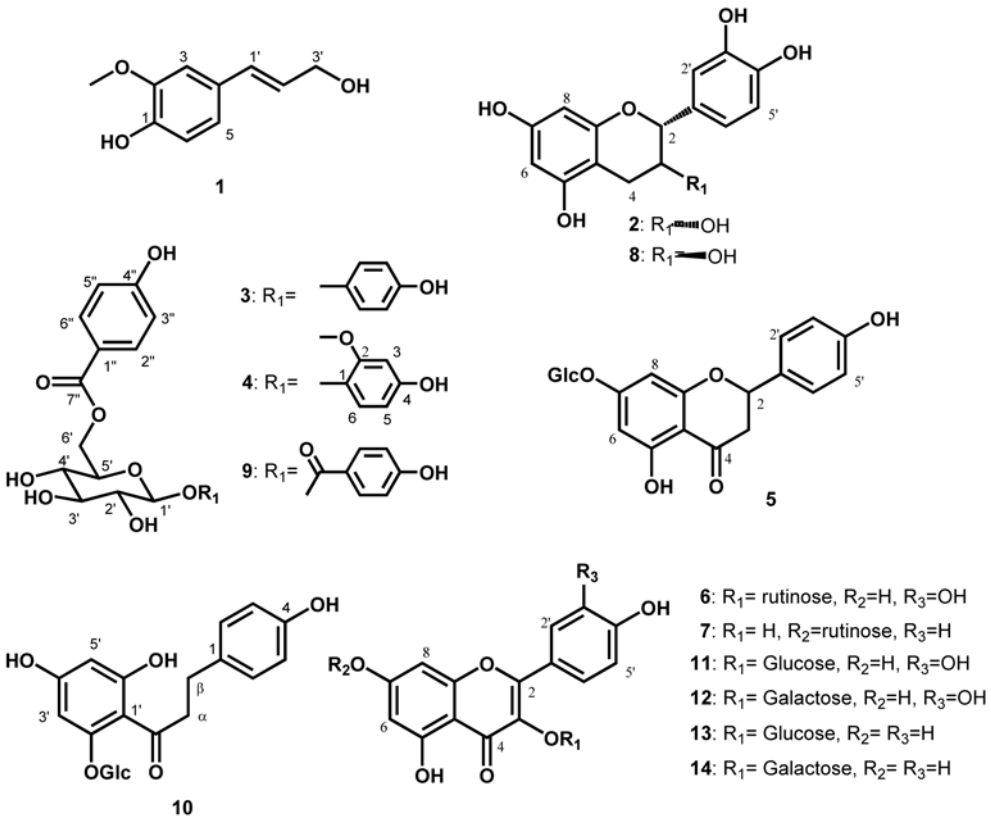

Fig. 1. Chemical structures of the compounds isolated from the leaves of *C. japonica*.

known compounds, 11 compounds were identified via comparison of the ^{1}H- and ^{13}C-NMR spectral data (Materials and Methods, Table 2) reported in relevant literature: (-)-epicatechin (**2**) from *Schotia latifolia* (14), 4-hydroxyphenol 1-*O*-β-D-(6-*O*-*p*-hydroxybenzoyl) glucopyranoside (lanceoloside A, **3**) from *Breynia officinalis* (15), naringenin 7-*O*-β-D-glucopyranoside (**5**) from *Prunus amygdalus* (16), quercetin 3-*O*-β-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (rutin, **6**) from *Prunus mume* (17), kaempferol 3-*O*-β-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (**7**) from *Camellia oleifera* (18), (+)-catechin (**8**) from *Lotus corniculatus* (19), 1,6-di-*O*-*p*-hydroxybenzoyl-β-D-glucopyranoside (**9**) from *Catalpa ovata* (20), the mixture of quercetin 3-*O*-β-D-glucopyranoside (isoquercetin, main, **11**) and quercetin 3-*O*-β-D-galactopyranoside (hyperoside, minor, **12**) from *Angelica keiskei* (21), kaempferol 3-*O*-β-D-galactopyranoside (**13**) from black tea (22), and kaempferol 3-*O*-β-D-glucopyranoside (**14**) from mulberry (23). The mixture of **11** and **12** was observed to evidence an unequal intensity of 2 sugar proton signals at a 3:1 ratio in the ^{1}H- and ^{13}C-NMR spectra of the mixture. The other 2 compounds were identified as (*E*)-coniferyl alcohol (**1**) and phloretin 2'-*O*-β-D-glucopyranoside (phloridzin, **10**) via 1D- and 2D-NMR and FABMS analyses and by comparison of their spectroscopic data as reported in the relevant literature (24,25).

Among the 14 compounds (Fig. 1) isolated from the *C. japonica* leaves, the identification of 2 catechins (**2**, **8**) and 3 quercetin glycosides (**6**, **11, 12**) has been previously reported from the leaves of the same plant (8,11). To the best of our knowledge, this study is the first to identify the other compounds (**1**, **3**, **5**, **7**, **9**, **10**, **13**, **14**) from this plant. Moreover, this study marks the first isolation of benzoyl glucosides (**3**, **4**, **9**) in Teaceaous plants.

DPPH radical scavenging activity of the isolated compounds The antioxidative activity of the 14 isolated

Table 3. DPPH radical scavenging activity of the isolated compounds from the leaves of *C. japonica*[1)]

Compound	SC_{50} (μM)
1	226
2	16
3	130
4	180
5	>250
6	20
7	>250
8	20
9	32
10	41
11	16
12	16
13	>250
14	>250
α-Tocopherol	29

[1)]α-Tocopherol was used as positive control. Each value represents mean of 2 replications.

compounds was determined by a DPPH radical scavenging assay (Table 3). DPPH radical (final concentration, 100 μM) scavenging activity decreased in the following order: **2**=**11**=**12** (SC_{50}, 16 μM)>**6**=**8** (20 μM)>**9** (32 μM)>**10** (41 μM)>**3** (130 μM)>**4** (180 μM)>**1** (226 μM)> **5**=**7**=**13** =**14** (>250 μM). Flavonoids (**2**, **6**, **8, 11**, **12**) having the catechol moiety showed significantly higher DPPH radical scavenging activity than was observed in other isolated compounds (**1**, **3**, **4**, **5**, **7**, **9, 10**) having monohydroxy phenyl group. It has already been reported that the catechol structure contributes to the antioxidant effect observed in flavonoids and phenolic compounds (26-28). Although research on the antioxidants contained in the EtOAc-soluble acidic fraction is also required, because the antioxidative activity of the EtOAc-soluble acidic fraction (SC_{50}, 9.0 μg) was comparable to that of the EtOAc-soluble neutral fraction (SC_{50}, 5.0 μg), it appears that the antioxidative activity of the EtOAc-soluble neutral fraction of *C. japonica* leaves may be attributable to flavonoids and phenolic compounds.

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