Food Sci. Biotechnol. Vol. 18, No. 3, pp. 766 ~ 770 (2009)

Food Science and Biotechnology

Phenolic Compounds from the Flower Buds of *Camellia japonica*

Jeong-Yong Cho, Hyun-Jung Ryu, Soo-Hyun Ji, Jae-Hak Moon, Kyung-Hee Jung,[1] and Keun-Hyung Park*

Department of Food Science & Technology, and Functional Food Research Center, Chonnam National University, Gwangju 500-757, Korea

[1]*Jeonnam Biotechnology Research Center, Naju, Jeonnam 520-330, Korea*

Abstracts Hot water extracts of *Camellia japonica* flower buds were found to have the higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity than the other solvent extracts. Five phenolic compounds were isolated and purified from the ethyl acetate soluble-neutral fraction of hot water extracts by Sephadex LH-20 column chromatography and octadecyl silane-high performance liquid chromatography using the guided assay of DPPH radical scavenging. Based on mass spectrometer and nuclear magnetic resonance, the isolated compounds were identified as *p*-hydroxybenzaldehyde (**1**), vanillin (**2**), dehydroxysynapyl alcohol (**3**), 7*S*,7'*S*,8*R*,8'*R*-icariol A_2 (**4**), and (-)-epicatechin (**5**). Four compounds (**1**-**4**) except for **5** were newly identified in this plant. Their DPPH radical scavenging activities as 50% scavenging concentration decreased in order to **5** (20 μM)>α-tocopherol (29 μM)>**4** (67 μM)>**3** (72 μM)>**1**=**2** (>250 μM). These results indicate that the antioxidant effect of the hot water extract of *C. japonica* flower buds may partially due to 5 isolated phenolic compounds.

Keywords: *Camellia japonica*, flower bud, phenolic compound, DPPH radical, antioxidant

Introduction

Camellia japonica (Theaceae) is cultivated in the southern coastal area of Jeolla-do(Province) and is often found in the gardens in Korea. This plant has been used in traditional folk remedies in Korea and Japan as tonic as well as to treat inflammation and stomatic problems. In addition, the flower of *C. japonica* has been used for the treatment of vomiting with blood and bleeding. Many studies have shown that *C. japonica* has anti-inflammatory (1), anti-plaque (2), antimicrobial and antioxidant (3,4), and anticancer (5) activities and that it is can also inhibit the absorption of ethanol (6). The presence of chemical constituents such as terpenes (7-9), saponins (10,11), and phenolic compounds (12,13) in the fruits, seeds, and leaves of *C. japonica* has been reported.

Recently, Lee *et al.* (4) reported that the leaves and flower buds of *C. japonica* had high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and antibacterial effects. In addition, flavonol glycosides (camellianoside, rutin, hyperoside, and isoquercetin) have been isolated as antioxidant from the leaves (14). Yashikawa *et al.* (15) reported stereo structural-elucidation of camelliosides A, B, C, and D from the flower buds of *C. japonica*. They also demonstrated that camelliosides A and B had platelet aggregation activity and exerted the gastroprotective effects against ethanol- or indomethacin-induced gastric mucosal lesions in rats. However, the studies on the chemical constituents of this plant have not yet been fully performed.

The antioxidants in the leaves and flower buds of *C. japonica* using the guided assay of DPPH radical scavenging were investigated. A novel benzyol glucoside (camelliadiphenoside) and 13 known phenolic compounds as antioxidant have been isolated from the leaves of *C. japonica* (16). This paper describes the isolation and identification of 5 phenolic compounds from the flower buds of *C. japonica* and their DPPH radical scavenging effects.

*Corresponding author: Tel: +82-62-530-2143; Fax: +82-62-530-2149
E-mail: khpark@chonnam.ac.kr
Received December 26, 2008; Revised February 3, 2009;
Accepted February 15, 2009

Materials and Methods

Chemicals 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol-d_4 (CD_3OD) was obtained from Merck (Darmstadt, Germany). All other chemicals used in this study were of reagent grade and were obtained from commercial sources.

Plant material The flower buds of *Camellia japonica* were collected in January 2005 from Wando of Korea. A voucher sample has been deposited in the warm-temperate forest arboretum of Chonnam National University located in Bogildo(Island). This freeze-dried material was stored at –40°C until use.

Extraction with various solvents The flower buds (36.7 g) of *C. japonica* were soaked with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), ethanol (EtOH), and methanol (MeOH) (each 500 mL, 2 times) for 24 hr at room temperature, respectively. The flower buds (100 g) were extracted with boiling water (1.5 L) for 15 min at 90°C. This extracted solution was filtered through a glass filter (G3) and concentrated *in vacuo* at 38°C.

Isolation of antioxidant from hot water extract The freeze-dried flower buds (1.46 kg) of *C. japonica* were extracted with boiling water (30 L) for 30 min. The obtained filtrate was adjusted to pH 8.0 by the addition of 5% $NaHCO_3$ and then partitioned with EtOAc (60 L) to give an EtOAc-soluble neutral fraction. The aqueous layer was adjusted to pH 3.0 by the addition of 2.0 N HCl and

partitioned with EtOAc (60 L) to give an EtOAc-soluble acidic fraction. The EtOAc-soluble neutral fraction (20.1 g) was fractionated to Sephadex LH-20 column (5.1×51 cm, 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, step-wise system). The active fractions were subjected to high performance liquid chromatography (HPLC) on octadecyl silane (ODS) column (mBondapak, 19×300 mm, 10 mm, Waters, Milford, MA, USA) with 8.0 mL/min of flow rate, 254 nm of wavelength, and 25 or 30% MeOH as a mobile phase.

Structural analysis Nuclear magnetic resonance (NMR) spectra were obtained with an ^{unit}INOVA 500 spectrometer (Varian, Walnut Creek, CA, USA) using solvents as the internal standard. All of the isolated compounds were dissolved with CD_3OD. Chemical shifts were referenced to residual CHD_2OD at δ=3.31 ppm in the ^{1}H-NMR spectrum and δ=49.15 ppm in the ^{13}C-NMR spectrum. Mass spectral data were performed using electron impact-mass spectrometry (EIMS, JMS-60; Jeol, Kyoto, Japan) and fast atom bombardment-mass spectrometry (FABMS, JMS-HX100; Jeol) with a matrix ingredient (3-nitrobenzyl alcohol).

Compound **1**: white powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 9.74 (1H, s, C<u>H</u>O), 7.76 (2H, d, *J*=9.0 Hz, H-2, 6), 6.99 (2H, d, *J*=9.0 Hz, H-3, 5); ^{13}C-NMR (CD_3OD, 125 MHz) δ 192.9 (<u>C</u>HO), 166.3 (C-4), 133.7 (C-3, 5), 130.0 (C-1), 117.3 (C-2, 6).
Compound **2**: white powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 9.73 (1H, s, C<u>H</u>O), 7.43 (1H, dd, *J*=7.5, 2.0 Hz, H-6), 7.43 (1H, d, *J*=2.0 Hz, H-2), 6.93 (1H, d, *J*=7.5 Hz, H-5), 3.92 (3H, s, -OC<u>H</u>$_3$); ^{13}C-NMR (CD_3OD, 125 MHz) δ 193.0 (<u>C</u>HO), 155.5 (C-4), 150.0 (C-3), 130.5 (C-1), 128.2 (C-2), 116.6 (C-5), 111.4 (C-6), 56.6 (-O<u>C</u>H$_3$).
Compound **3**: white powder; ^{1}H- (CD_3OD, 500 MHz) and ^{13}C- (CD_3OD, 125 MHz) NMR, see Table 2; EIMS *m/z* 212 (relative intensity, 66), 181 (8), 168 (100), 153 (17), 137 (14); FABMS (positive) *m/z* 213 $[M+H]^+$ and 235 $[M+Na]^+$.
Compound **4**: white powder; ^{1}H- (CD_3OD, 500 MHz) and ^{13}C- (CD_3OD, 125 MHz) NMR, see Table 2; EIMS *m/z* 436 (2), 418 (11), 400 (44), 419 (100); FABMS (positive) *m/z* 459 $[M+Na]^+$.
Compound **5**: white powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ 6.98 (1H, d, *J*=2.0 Hz, H-2'), 6.81 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.77 (1H, d, *J*=8.0 Hz, H-5'), 5.97 (1H, *J*=2.5 Hz, H-6), 5.95 (1H, *J*=2.5 Hz, H-8), 4.80 (1H, br. s, H-2), 4.17 (1H, m, H-3), 2.86 (1H, dd, *J*=5.0, 17.0 Hz, H-4a), 2.75 (1H, dd, *J*=2.5, 17.0 Hz, H-4b); ^{13}C-NMR (CD_3OD, 125 MHz) δ 79.9 (C-2), 67.5 (C-3), 29.3 (C-4), 157.6 (C-5), 96.5 (C-6), 158.0 (C-7), 96.0 (C-8), 157.4 (C-9), 100.2 (C-10), 132.3 (C-1'), 115.4 (C-2'), 145.9 (C-3'), 145.8 (C-4'), 116.1 (C-5'), 119.6 (C-6') ; FABMS (positive) *m/z* 291 $[M+H]^+$ and 313 $[M+Na]^+$.

Assay of DPPH radical scavenging The free radical scavenging activities of solvent extracts, EtOAc-soluble fractions, the isolated compounds, and α-tocopherol were evaluated by a DPPH radical according to the method described by Abe *et al*. (17), with slight modification. Briefly, a methanol solution (200 μL) containing different concentrations of each compound was added to DPPH radical ethanol solution (1,800 μL, final concentration, 100 mM). 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 observing the decolorization of DPPH at 517 nm. The DPPH radical scavenging activities of each sample were also determined as the percentage decrease when compared to in the absorbance of a blank. The 50% free radical scavenging concentration (SC_{50}) of each sample (compound) was determined using a dose-response curve.

The assay for purification of the antioxidative compound was conducted by spraying the DPPH solution on thin layer chromatograph (TLC, Silica gel 60 F_{254}, 0.25 mm thickness, Merck) using the method described by Takao *et al.* (18), with slight modification. Briefly, each fraction purified by Sephadex LH-20 column chromatography and ODS-HPLC was spotted on the TLC plate and then developed with suitable solvents. After spraying 200 μM DPPH free radical EtOH solution, fractions visualized as a decolorization of the spot were considered to have antioxidative activity.

Results and Discussion

Antioxidative activity of various solvent extracts The flower buds were extracted with *n*-hexane, $CHCl_3$, EtOAc, EtOH, MeOH, and hot water. The antioxidative activities of these extracts were evaluated by a DPPH radical. The SC_{50} value was determined based on the results of a dose-response curve. The antioxidative activities of the extracts decreased in the following order: hot water extract (SC_{50}, 28 μg/mL)>MeOH extract (30 μg/mL)>EtOH extract (32 μg/mL)>EtOAc extract (42 μg/mL)>$CHCl_3$ extract (>250 mg/mL) and *n*-hexane extract (>250 μg/mL) (Table 1). Because the hot water extract showed the higher antioxidative activity than the other solvent extracts, the antioxidative compounds in hot water extract of *C. japonica* flower buds were purified and isolated.

Antioxidative activity of the soluble fractions of the hot water extract The hot water extracted solution was partitioned with EtOAc to give EtOAc-soluble acidic (20.1 g) and EtOAc-soluble neutral (14.2 g) fractions. The antioxidative activities of these fractions and α-tocopherol as a positive control were also evaluated by a DPPH

Table 1. DPPH radical scavenging activity of various solvent extracts from the flower buds of *C. japonica*

Extraction	SC_{50} (μg/mL)[1)]
Hot water	28
Methanol	30
Ethanol	32
Ethyl acetate	42
Chloroform	>250
n-Hexane	>250
α-Tocopherol[2)]	6

[1)]Each value represents the mean of triplicates. The final concentration of the DPPH radical was 100 μM.
[2)]α-Tocopherol was used as positive control.

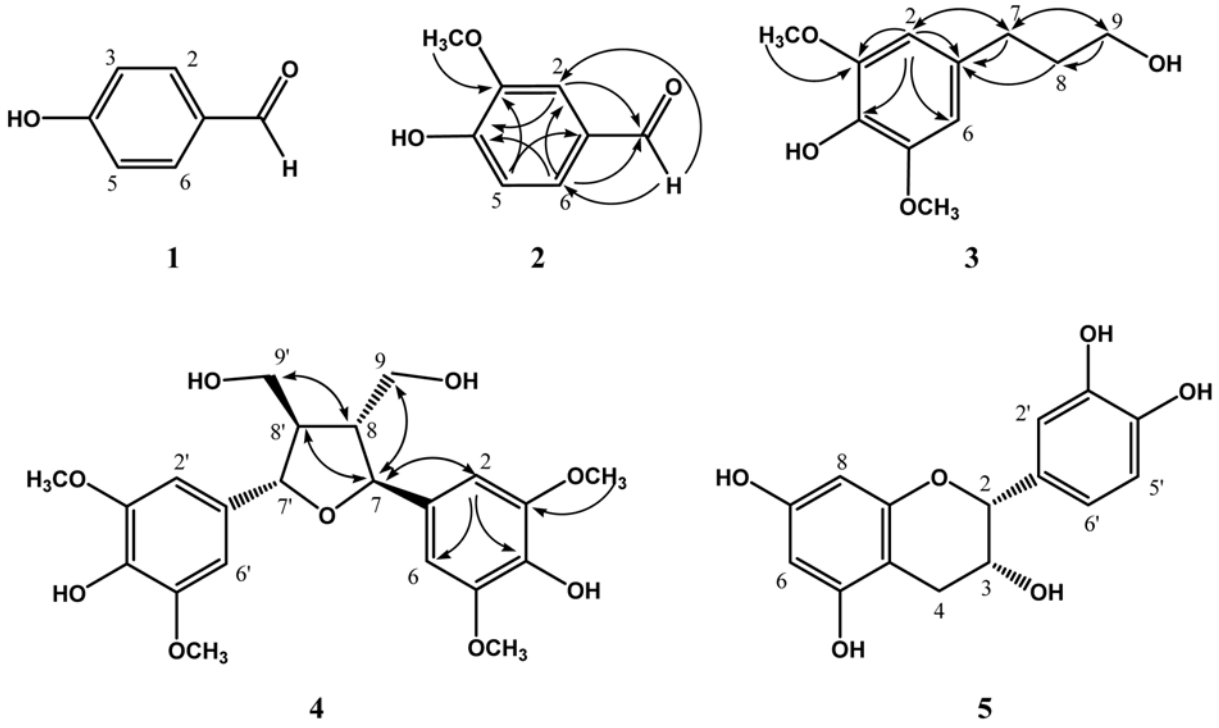

Fig. 1. Structures and HMBC correlations (arrows) of the compounds identified in the hot water extract of *C. japonica* flower buds.

radical as described above. The SC_{50} values of the EtOAc-soluble acidic fraction, EtOAc-soluble neutral fraction, and α-tocopherol were found to be 6.0, 4.0, and 6.0 μg/mL, respectively. The EtOAc-soluble neutral fraction showed the higher antioxidative activity than EtOAc-soluble neutral fraction and α-tocopherol. Therefore, isolation and purification of antioxidative compound from EtOAc-soluble neutral fraction of hot water extract was attempted.

Isolation of antioxidative compound from the EtOAc-soluble neutral fraction The EtOAc-soluble neutral fraction (20.1 g) obtained from the hot water extract of the freeze-dried flower buds was fractionated by a column of Sephadex LH-20 with elution of the mixture solvents of H_2O/MeOH. Each fraction was then developed on TLC and sprayed with DPPH free radical EtOH solution. All of the fractions showed DPPH free radical scavenging activity. In particular, activities of fractions F-1 (40% MeOH fraction, 366 mg) and F-2 (60% MeOH, 2,039 mg) were stronger DPPH free radical scavenging activity than those of the other fractions. Compound **1** (t_R 31 min, white powder, 1.1 mg), **2** (t_R 38 min, white powder, 1.3 mg), **3** (t_R 49 min, white powder, 3.3 mg), and **4** (t_R 53 min, white powder, 3.1 mg) were isolated from a portion of the fraction F-1 by ODS-HPLC with 25% MeOH as a mobile phase. Compound **5** (t_R 22 min, white powder, 50 mg) was isolated from a portion of the fraction F-2 by ODS-HPLC with 30% MeOH as a mobile phase. The 5 isolated compounds were identified based on NMR and MS spectral analyses.

Structural determination of the isolated compounds The ^{1}H-NMR spectrum of **1** revealed the presence of a *para*-substituted aromatic ring proton signals of the AA'BB' system at δ 7.76 (2H, d, *J*=9.0 Hz, H-2, 6) and 6.99 (2H, d, *J*=9.0 Hz, H-3, 5), and an aldehyde proton signal at δ 9.74 (1H, s, -C<u>H</u>O). The ^{13}C-NMR spectrum revealed 7 carbon signals including a carbonyl carbon at δ 192.9 (C-7) and 6 sp^2 carbons belonging to a benzene ring at δ 166.3-117.3. The presence of an oxygenated quaternary carbon signal (δ 166.3, C-4) suggested that the hydroxyl group was linked to the C-4 of phenylaldehyde. Compound **1** was identified as *p*-hydroxybenzaldehyde by comparison with NMR spectral data of that reported in literature (19).

The ^{1}H-NMR spectrum of **2** revealed the presence of tri-substituted benzene ring proton signals [δ 7.42 (1H, d, *J*= 2.0 Hz, H-2), 6.93 (1H, d, *J*=7.5 Hz, H-5), 7.43 (1H, dd, *J*=7.5, 2.0 Hz, H-6)], an aldehyde proton signal [δ 9.74 (1H, s, -C<u>H</u>O)], and a methoxyl proton signal [δ 3.92 (3H, s, -OC<u>H</u>$_3$)]. These data were supported by carbon signals observed in the ^{13}C-NMR spectrum. These findings suggested that **2** was a hydroxybenzaldehyde with a methoxyl group. The methoxyl group substituted in the C-3 position of 4-hydroxybenzaldehyde was assigned by an HMBC experiment (Fig. 1). Therefore, the structure of **2** was identified to be 3-methoxy-4-hydroxybenzaldehyde (vanillin) (Fig. 1).

The EIMS spectrum of **3** exhibited a molecular ion peak at *m/z* 212 (M^+, *rel. int.* 66%) together with other fragment ion peaks at *m/z* 181 ($[M\text{-}CH_2OH]^+$, 8%), 168 ($[M\text{-}(CH_2)_2OH+H]^+$, 100%), 153 ($[M\text{-}(CH_2)_3OH]^+$, 17%), and 137 ($[M\text{-}(CH_2)_3OH\text{-}OH+H]^+$, 14%). The FABMS spectrum (positive) of **3** showed the pseudomolecular ion peaks at *m/z* 213 $[M+H]^+$ and 235 $[M+Na]^+$. These results indicated that the molecular weight of **3** was 212. ^{1}H-NMR spectrum of **3** revealed the presence of an aromatic methine proton signal [δ 6.47 (2H, br. s, H-2, 6)], overlapped signal of 2 methoxyl proton [δ 3.82 {6H, s, (-OC<u>H</u>$_3$)×2}], and 3

Table 2. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data of 3 and 4 (CD_3OD)

Position	3		Position	4	
	δ_H (*int., mult., J*)	δ_C		δ_H (*int., mult., J*)	δ_C
1	-	134.3	**1, 1'**	-	134.4
2, 6	6.47 (2H, br. s)	106.8	**2, 6, 2', 6'**	6. 74 (4H, s)	105.0
3, 5	-	149.4	**3, 5, 3', 5'**	-	149.5
4	-	134.9	**4, 4'**	-	136.4
7	2.59 (2H, dd, 8.0, 8.0)	33.3	**7, 7'**	4.96 (2H, d, 8.0)	84.7
8	1.81 (2H, m)	35.8	**8, 8'**	2.32 (2H, m)	55.3
9	3.58 (2H, dd, 7.0, 7.0)	62.4	**9a, 9'a** **9b, 9'b**	3.72 (2H, dd, 3.5, 11.0) 3.63 (2H, dd, 5.0, 11.0)	61.9
-OCH_3	3.82 (6H, s)	56.9	**-OCH_3**	3.82 (12H, s)	57.0

methylene proton signals [δ 3.58 (2H, dd, *J*=7.0, 7.0 Hz, H-9), 2.59 (2H, dd, *J*=8.0, 8.0 Hz, H-7), 1.81 (2H, m, H-8)] (Table 2). The carbon signals presented in the ^{13}C-NMR spectrum were satisfied the ^{1}H-NMR data. Three methylene proton signals [δ 2.59 (H-7), 1.81 (H-8), and 3.58 (H-9)] were assigned to be propanol by their proton correlations in the ^{1}H-^{1}H COSY spectrum. Therefore, 2 partial structures of **3** are assignable to be dimethoxybenzene and propanol. In the HMBC spectrum (Fig. 1), the existence of the cross peak between the methylene proton signal at δ 2.59 (H-7) and the methine carbon signals at δ 106.8 (C-2, 6) indicated that the propanol moiety was coupled with the C-1 position of dimethoxylbenzene. Therefore, the structure of **3** was determined to be 4-hydroxy-3,5-dimethoxybenzenepropanol (dehydroxysinapyl alcohol) (Fig. 1).

The ^{1}H- and ^{13}C-NMR spectra of **4** (Table 2) were closely related to those of **3** except for propanol moiety, that is, they showed the oxygenated methine signal [δ 4.96 (2H, d, *J*=8.0, Hz, H-7, 7') in the ^{1}H-NMR spectrum and δ 84.7 (C-7, 7') in the ^{13}C-NMR spectrum] and other methine signal [δ 2.32 (2H, m, H-8, 8') in the ^{1}H-NMR spectrum and δ 55.3 (C-8, 8') in the ^{13}C-NMR spectrum]. These results suggest that **4** is lignan constituted with 2 molecules of compound **3**. The ether form of C-7 and C-7' was deduced from the oxygenated methine carbon signal (δ 84.7, C-7, 7') observed in a down-field shift when compared with the oxygenated methylene proton signal (δ 62.4, C-9, 9') (Table 2). The planar structure of **4** could be assigned to be icariol by the analyses of the 2D-NMR such as HSQC, ^{1}H-^{1}H COSY, and HMBC. The important correlations detected in the HMBC spectrum are shown in Fig. 1. The ^{1}H-NMR spectrum of **4** corresponded with that of 7*S*,7'*S*,8*R*,8'*R*-icariol A_2 that has been previously reported (20). In addition, the positive FABMS spectrum of **4** exhibited a pseudomolecular ion peak at *m/z* 459 $[M+Na]^+$, which is corresponded with the molecular weight (Mw 436) of **4**. Therefore, the structure of **4** was identified as 7*S*,7'*S*,8*R*,8'*R*-icariol A_2 (Fig. 1).

The ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **5** revealed the presence of 2 proton signals [δ 5.97 (1H, *J*=2.5 Hz, H-6), 5.95 (1H, *J*=2.5 Hz, H-8)] of A ring , 3 proton signals [δ 6.98 (1H, d, *J*=2.0 Hz, H-2'), 6.81 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), and 6.77 (1H, d, *J*=8.0 Hz, H-5')] of B ring, and 4 proton signals [δ 4.80 (1H, br. s, H-2), 4.17 (1H, m, H-3), 2.86 (1H, dd, *J*=5.0, 17.0 Hz, H-4a), and 2.75 (1H, dd, *J*=2.5, 17.0 Hz, H-4b)] of C ring. These results suggest that **5** is catechin. These findings are supported by the presence of 15 carbon signals [12 sp^2 carbon signals (δ 158.0-96.5) and 3 sp^3 carbon signals (δ 79.9-29.3)] in the ^{13}C-NMR spectrum and the detection of pseudomolecular ion peaks at *m/z* 291 $[M+H]^+$ and 313 $[M+Na]^+$ in the FAB-MS (positive) spectrum. In addition, the splitting pattern of the methine proton signal at δ 4.80 (H-2) in the ^{1}H-NMR spectrum was indicated the presence of a broad singlet, which suggests that **5** is (-)-epicatechin. Compound **5** was identified as (-)-epicatechin (Fig. 1) based on a comparison with the spectroscopic data reported in literature (21).

The 5 antioxidative compounds that were isolated from the hot water extracts of *C. japonica* flower buds were identified as *p*-hydroxybenzaldehyde (**1**), vanillin (**2**), dehydroxysynapyl alcohol (**3**), 7*S*,7'*S*,8*R*,8'*R*-icariol A_2 (**4**), and (-)-epicatechin (**5**) (Fig. 1). To the best of our knowledge, this is the first study to report the presence of 4 compounds (**1**-**4**) except for (-)-epicatechins (**5**) in *C. japonica*. *p*-Hydroxybenzaldehyde (**1**) and vanillin (**2**) are known to be the major flavor compounds in vanilla (22), and many studies have reported that vanillin (**2**) has the biological activities such as antimutagenic, anticlastogenic, and anticarcinogenic effects (22-24). This compound is also used as flavoring material in food and pharmaceutical industries. Dehydroxysynapyl alcohol (**3**) has been identified in the leaves of the sunflower (25). 7*S*,7'*S*,8*R*,8'*R*-icariol A_2 (**4**) coupled with 2 molecules of **3** was previously isolated from *Epimedium sagittatum* (26) and recently elucidated the absolute configuration with this glycoside from *Glechoma hederacea* L. (21). (-)-Epicatechin (**5**), which was identified as a constituent of the buds of *C. japonica* has previously been identified in its leaves (12). We also reported here as the constituent of this flower buds.

DPPH radical scavenging activity of the isolated compounds The antioxidative activities (Table 3) of the 5 compounds isolated here were determined by the DPPH radical scavenging method. The DPPH radical scavenging activity decreased in the following order: **5** (SC_{50}, 20 μM) >α-tocopherol (29 μM)>**4** (67 μM)>**3** (72 μM)>**1**=**2** (>250 μM). (-)-Epicatechins (**5**) showed a significantly higher DPPH radical scavenging activity than other isolated compounds and α-tocopherol. Compound **4** (7*S*,7'*S*,8*R*,8'*R*-icariol A_2), that is coupled with 2 3,5-dimethoxy-4-

Table 3. DPPH radical scavenging activities of the compounds isolated from the flower buds of *C. japonica*

Compounds	SC_{50} (μM)[1)]
p-Hydroxybenzaldehyde (**1**)	>250
Vanillin (**2**)	>250
Dehydroxysynapyl alcohol (**3**)	72
7*S*,7'*S*,8*R*,8'*R*-Icariol A_2 (**4**)	67
(-)-Epicatechin (**5**)	20
α-Tocopherol[2)]	29

[1)]Each value represents the mean of 2 replications. The final concentration of the DPPH radical was 100 μM.
[2)]α-Tocopherol was used as a positive control.

hydroxyphenyl groups, showed the higher antioxidative activity in comparison with dehydroxysynapyl alcohol (**3**) of a 3,5-dimethoxy-4-hydroxyphenyl group. Compounds **1** and **2** showed significantly lower DPPH radical scavenging activity (SC_{50}, >250 μM) than other isolated compounds. These finding indicate that the antioxidant effect of the hot water extract of flower buds may partially due to 5 isolated phenolic compounds in this study. The flowered tea that made from the flower bud of *C. japonica* has potential to become health beverages because of the high antioxidative activity of its hot water extract. Therefore, investigation of the antioxidant from the flower of *C. japonica* is being conducted.

Acknowledgments

This study was financially supported by the Regional R&D Program of Ministry of Knowledge Economy, Korea.

References

1. Akihisa T, Yasukawa K, Kimura Y, Takase S, Yamanouchi S, Tamura T. Triterpene alcohols from camellia and sasanqua oils and their anti-inflammatory effects. Chem. Pharm. Bull. 45: 2016-2023 (1997)
2. Namba T, Tsunezuka M, Takehana Y, Nunome S, Takeda K, Shu YW, Kakiuchi N, Takigi S, Hattori M. Studies on dental caries prevention by traditional Chinese medicines. IV. Screening of crude drugs for anti-plaque action and effects of *Artemisia capillaris* spikes on adherence of *Streptococcus mutans* to smooth surface and synthesis of glucan. Jpn. Soc. Pharm. 38: 263-264 (1984)
3. Hahn YS. Antimicrobial effects of *Camellia japonica* L. extract on food-borne pathogenic microorganisms. Korean J. Food Sci. Technol. 37: 113-121 (2005)
4. Lee SY, Hwang EJ, Kim GH, Choi YB, Lim CY, Kim SM. Antifungal and antioxidant activities of extracts from leaves and flowers of *Camellia japonica* L. Korean J. Med. Crop Sci. 13: 93-100 (2005)
5. Kim JH, Lee SY, Choi SI. Anti-proliferative effect of *Camellia japonica* leaves on human leukemia cell line. Korean J. Herbol. 18: 93-98 (2003)
6. Yoshikawa M, Murakami T, Yoshizumi S, Murakami N, Yamahara J, Matsuda, H. Camelliasaponins B_1, B_2, C_1, and C_2, new type inhibitors of ethanol absorption in rats from the seeds of *Camellia japonica* L. Chem. Pharm. Bull. 42: 742-744 (1996)
7. Ito S, Kodama M, Konoike M. Structure of camelliagenins. Tetrahedron Lett. 8: 591-596 (1967)
8. Itokawa H, Sawada N, Murakami T. Structures of camelliagenins A, B, and C obtained from *Camellia japonica*. Chem. Pharm. Bull. 17: 474-480 (1969)
9. Itokawa H, Nakajima H, Ikuta A, Iitaka Y. Two triterpenes from the flowers of *Camellia japonica*. Phytochemistry 20: 2539-2542 (1981)
10. Yoshikawa M, Murakami T, Yoshizumi S, Murakami N, Yamahara J, Matsuda H. Bioactive saponins and glycosides. V. Acylated polyhydroxyolean-12-ene oligoglycosides, camelliasaponins A_1, A_2, B_1, B_2, C_1, and C_2, from the seeds of *Camellia japonica* L.: Structures and inhibitory activity on alcohol absorption. Chem. Pharm. Bull. 44: 1899-1907 (1996)
11. Numata A, Kitajima A, Katsuno T, Yamamoto K, Nagahama Na, Takahashi C, Fujiki R, Nabae M. An antifeedant for the yellow butterfly larvae in *Camellia japonica*: A revised structure of camellidin II. Chem. Pharm. Bull. 35: 3948-3951 (1987)
12. Nakagawa, M, Sakamoto Y. Isolation of (-)-epicatechol and (+)-catechol from the leaf of *Camellia japonica*. Tea Indus. Technol. Res. Japan 34: 3472-3476 (1967)
13. Hatano T, Shida S, Han L, Okuda T. Tannins of theaceous plants. III. Camelliatannins A and B, two new complex tannins from *Camellia japonica* L. Chem. Pharm. Bull. 39: 876-880 (1991)
14. Onodera K, Hanashiro K, Yasumoto T. Camellianoside, a novel antioxidant glycoside from the leaves of *Camellia japonica*. Biosci. Biotech. Bioch. 70: 1995-1998 (2006)
15. Yoshikawa M, Morikawa T, Asao Y, Fujiwara E, Nakamura S, Matsuda H. Medicinal flowers. XV. The structures of noroleanane- and oleanane-type triterpene oligoglycosides with gastroprotective and platelet aggregation activities from flower buds of *Camellia japonica* L. Chem. Pharm. Bull. 55: 606-612 (2007)
16. Cho JY, Ji SH, Moon JH, Lee KH, Jung KH, Park KH. A novel benzyol glucoside and phenolic compounds from the leaves of *Camellia japonica*. Food Sci. Biotechnol. 17: 1060-1065 (2008)
17. Abe N, Nemoto A, Tsuchiya Y, Hojo H, Hirota A. Studies of the 1,1-diphenyl-2-picrylhydrazyl radical scavenging mechanism for a 2-pyrone compound. Biosci. Biotech. Bioch. 64: 306-333 (2000)
18. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotech. Bioch. 58: 1780-1783 (1994)
19. Kwon YS, Kim CM. Antioxidant constituents from the stem of *Sorghum bicolor*. Arch. Pharm. Res. 26: 535-539 (2003)
20. Yamuchi H, Kakuda R, Yaoita Y, Machida K, Kikuchi M. Two new glycosides from the whole plants of *Glechoma hederacea* L. Chem. Pharm. Bull. 55: 346-347 (2007)
21. Masika PJ, Sultana N, Afolayan AJ. Antibacterial activity of two flavonoids isolated from *Schotia latifolia*. Pharm. Biol. 42: 105-108 (2004)
22. Walton NJ, Mayer MJ, Narbad A. Vanillin. Phytochemistry 63: 505-515 (2003)
23. Maurya DK, Adhikari S, Nair CKK, Devasayayam TPA. DNA protective properties of vanillin against γ-radiation under different conditions: Possible mechanisms. Mutat. Res. 634: 69-80 (2007)
24. Duran S, Karram P. Vanillins: A novel family of DNA-PK inhibitors. Nucleic Acids Res. 31: 5501-5512 (2003)
25. Nacias FA, Lopez A, Varela RM, Torres A, Molinillo JMG. Bioactive lignans from a cultivar of *Helianthus annuus*. J. Agr. Food Chem. 52: 6443-6447 (2004)
26. Hiroyuki M, Toshio M, Akira U. Lignan and terpene glycosides from *Epimedium sagittatum*. Phytochemistry 36: 2025-2027 (1991)