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Bioactive Phenolic Constituents from the Culms of *Phyllostachys bambusoides*

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Abstract – In our search for bioactive phenolics from plants, the culms of *Phyllostachys bambusoides* has been selected for investigation of anti-cariogenic and 1,1-diphenylpicrylhydrazyl (DPPH) radical scavenging agents based on the initial screening results. Fractionation process of *n*-hexane and $CHCl_3$ extracts afforded four phenolic constituents, ferulic acid (**1**), vanillin (**2**), coniferaldehyde (**3**), and coniferyl alcohol (**4**) as guided by their DPPH free radical scavenging activities. Additionally, activity-guided fractionation of EtOAc extract with anti-cariogenic activity has resulted in the isolation of coniferaldehyde (**3**), 2,6-dimethoxy-*p*-benzoquinone (**5**), *p*-methoxy-cinnamic acid (**6**), (±)-balanophonin (**7**), and 6-methoxychromanone (**8**). The structures of **1 - 8** were determined by spectroscopic data interpretation, and also by comparison of their data with the published values. Phenolic compounds **1 - 4** exhibited similar DPPH radical scavenging activities compared with the synthetic antioxidant, butylated hydroxytoluene (BHT), and compounds **3** and **5 - 8** showed significant antibacterial activity against cariogenic oral streptococci, *Streptococcus mutans* and *S. sobrinus*.
Keywords – *Phyllostachys bambusoides*, Gramineae, phenolics, anti-cariogenic, DPPH radical scavenger

Introduction

The *Phyllostachys* species including *P. bambusoides, P. nigra* var. *henonis* and *P. pubescens* are woody perennial evergreen plants belonging to the family Gramineae, which are very popular plants in Asia. Their culms were traditionally used as medicinal materials including Bambusae Caulis in Liquamen and Bambusae Caulis in Taenis and Bambusae Concretio Salicea, which have been used as antipyretic, antitussive and antidiuretic agents (Bae, 2000). The dried mass of a secretion from the culms of *P. bambusoides* S. et Z. has been used in traditional Korean medicine, and it was reported to be useful for the clinical treatment of degenerative neuronal disorders (Lee, 1986; Ko *et al*., 1994). In addition, the culms of *Phyllostachys* were widely used as grain storage and a wrapping material for foods. Several biological activities (Nikaido *et al.,* 1984; Nishina *et al*., 1991; Cowan, 1999) and phytochemical constituents (Kweon *et al*., 2001; Tanaka *et al*., 2003; Suga *et al*., 2003) were revealed, however, little is known about its biological evaluation and chemical composition in spite of the fact that the culms of bamboos have been used extensively as a foodstuff.

In the course of screening for bioactive compounds from MeOH extract of the culms of *P. bambusoides*, phenolic compounds **1 - 8** were found to have free radical scavenging effect on DPPH radical or anti-bacterial activities against cariogenic oral streptococci, *Streptococcus mutans* and *S. sobrinus*. This paper deals with the structure elucidation and biological evaluation of the isolated phenolic compounds.

Experimental

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra, with tetramethylsilane (TMS) as the internal standard were recorded using a Varian Unity INOVA 500 spectrometer (Varian, Inc., U.S.A.) for 1D and 2D NMR experiments in MeOH-d_4 and $CDCl_3$. The chemical shifts

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(δ) were expressed in parts per million (ppm) and coupling constants (*J*) were in Hz. Mass spectra were measured on a JMS-700 (Jeol, Japan) and Varian 1200, Platform II (Varian, U.S.A.) spectrometers. IR spectra were obtained on a JASCO FT/IR-300E spectrometer (Jasco Corp., Japan) and UV spectra were recorded on a JASCO V-530 UV/Vis spectrophotometer (Jasco Corp., Japan). Optical rotation was measured on a JASCO DIP-1000 digital polarimeter.

TLC chromatographic analyses were carried out on precoated Silica gel 60 F_{254} plates, and RP-18 F_{254} plates (Merck). Visualization of the silica gel TLC was performed using 10% (v/v) H_2SO_4 followed by charring at 110 °C for 10 min. The adsorbent used for column chromatography was silica gel 60/70-230 mesh. The flash chromatography (MPLC) was performed with a Lobar™ glass prepacked column (11 mm × 300 mm), and Sephadex LH-20 (Pharmacia Biotech Co., Ltd.) was used for size exclusion chromatography. The gentamicin, gentamicin disc (10 μg), Vitamin C, BHA, and BHT were purchased from Sigma Chemical and BD Biosciences, U.S.A.

Plant material. The culms of *Phyllostachys bambusoides* were collected in Damyang, Jeonnam Province, Korea, in 2005 and were pulverized by using a grinder. A voucher specimen has been deposited in the College of Pharmacy, Chonnam National University, Korea.

Extraction and isolation. The dried bamboo powder (5 kg) was extracted with 5 L of methanol-water mixture (8 : 2) three times. After filtration, the 80% MeOH extract was combined and concentrated *in vacuo* using rotary evaporator to give a dark green residue (210 g). This 80% MeOH extract was suspended in water (1 L) and partitioned with *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and water, successively.

The *n*-hexane extract (5.2 g) was subjected to silica gel column chromatography with a gradient of $CHCl_3$-MeOH (400 : 1, 200 : 1, 50 : 1, 20 : 1, 10 : 1) mixture to give six fractions. Fraction 3 (841.1 mg) was column chromatographed on a silica gel (70 - 230 mesh, Merck), eluting with $CHCl_3$-MeOH gradient mixture to give further fractions. Subfraction 3 (202.2 mg) was subjected to MPLC ($CHCl_3$-MeOH = 200 : 1) to give three subfractions and to yield compound **1** (1.9 mg, yield 0.0009%)

The $CHCl_3$ soluble extract (7.5 g) was subjected to silica gel column chromatography with a gradient of CH_2Cl_2-MeOH (50 : 1, 20 : 1, 10 : 1) mixture to give five fractions. Fraction 1 (95 mg) was column chromatographed on Sephadex LH-20 using 100% MeOH as eluent to provide 5 fractions. Subfraction 2 was subjected to MPLC (CH_2Cl_2-MeOH = 100 : 1) to give three subfractions and compound **2** (2.3 mg, yield 0.0011%). Fraction 3 (207.2 mg) was column chromatographed on silica gel (70 - 230 mesh, Merck), eluting with CH_2Cl_2-MeOH gradient mixture to give further four fractions. Among them, subfraction 3 was separated by MPLC using CH_2Cl_2-MeOH (80 : 1) to give seven fractions and compound **3** (1.2 mg, yield 0.00057%), and subfraction 5 was subjected to MPLC (CH_2Cl_2-MeOH = 60 : 1) to yield compound **4** (2.8 mg, yield 0.0013%).

The EtOAc-soluble extract (15 g) was subjected to silica gel column chromatography with a gradient of $CHCl_3$-MeOH (100 : 1, 50 : 1, 20 : 1, 10 : 1) mixture to give nine fractions. Fraction 6 (320 mg) was chromatographed on a silica gel eluted with $CHCl_3$ to give seven fractions (a-g). Subfraction d was subjected to MPLC (*n*-hexane-CH_2Cl_2 = 2 : 8) to yield compound **3** (3.5 mg, yield 0.0016%). Fraction 7 (82 mg) was dissolved in $CHCl_3$, and 90% MeOH was added to solution and filtered. And then filtered solution was concentrated under reduced pressure using a rotary evaporator and dissolved in $CHCl_3$ to give compound **5** (20 mg, yield 0.0095%). Fraction 8 (1.34 g) was chromatographed on a silica gel, eluting with CH_2Cl_2-MeOH gradient mixture to give further fractions (A-G). Fraction B was purified further on Sephadex LH-20 using MeOH-$CHCl_3$ (3 : 2) mixture to give compound **6** (5.8 mg, yield 0.0027%), and fraction C was purified by preparative TLC using CH_2Cl_2-MeOH (20 : 1) mixture to give compound **7** (9.2 mg, yield 0.0043%) and compound **8** (1.3 mg, yield 0.0006%).

Ferulic acid (1): White powder; UV λ_{max} (MeOH): 256, 309 nm; EI-MS (*m/z*): 194; Molecular formula: $C_{10}H_{10}O_4$; IR (KBr) cm^{-1}: 1207, 1730 and 3345; ^{1}H-NMR (500 MHz in MeOH-d_4) δ 3.89 (3H, s, OCH_3), 6.30 (1H, d, *J* = 16 Hz, H-8), 6.80 (1H, d, *J* = 8 Hz, H-5), 7.06 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 7.17 (1H, d, *J* = 2.0 Hz, H-2), 7.60 (1H, d, *J* = 16 Hz, H-7). ^{13}C-NMR (125 MHz in MeOH-d_4) δ 56.6 (OCH_3), 111.9 (C-2), 116.1 (C-8), 116.6 (C-5), 124.1 (C-6), 127.9 (C-1), 147.1 (C-7), 149.5 (C-3), 150.6 (C-4), 171.1 (C-9).

Vanillin (2): Yellowish powder: UV $_{max}$ (MeOH): 229, 278 and 304 nm; EI-MS (*m/z*): 152; Molecular formula: $C_8H_8O_3$; IR (KBr) cm^{-1}: 1676 and 3205; ^{1}H-NMR (500 MHz in $CDCl_3$) δ 3.96 (3H, s, OCH_3), 6.22 (1H, brs, H-2), 7.03 (1H, d, *J* = 8.4 Hz, H-5), 7.43 (1H, dd, *J* = 8.4, 1.5 Hz, H-6), 9.83 (1H, s, H-7). ^{13}C-NMR (125 MHz in $CDCl_3$) δ 56.1 (OCH_3), 108.8 (C-2), 114.4 (C-5), 127.5 (C-6), 129.8 (C-1), 147.1 (C-3), 151.7 (C-4), 190.8 (C-7).

Coniferaldehyde (3): White amorphous solid: UV λ_{max} (MeOH): 223, 333 nm; EI-MS (*m/z*): 178, 161, 147, 107; Molecular formula: $C_{10}H_{10}O_3$; IR (KBr) cm^{-1}: 833, 1167,

1601 and 3380; ^{1}H-NMR (500 MHz in $CDCl_3$) δ 3.95 (3H, s, OCH_3), 6.60 (1H, dd, J = 15.7, 8.0 Hz, H-8), 6.97 (1H, d, J = 8.5 Hz, H-5), 7.07 (1H, dd, J = 8.5, 1.5 Hz, H-6), 7.12 (1H, d, J = 1.5Hz, H-2), 7.38 (1H, d, J = 15.5 Hz, H-7), 9.65 (1H, d, J = 8 Hz, H-9). ^{13}C-NMR (125 MHz in $CDCl_3$) δ 55.9 (OCH_3), 109.3 (C-2), 114.9 (C-5), 124.0 (C-6), 126.4 (C-8), 126.6 (C-1), 146.9 (C-3), 148.9 (C-4), 153.0 (C-7), 193.5 (C-9).

Coniferyl alcohol (4): White-pale yellow solid: UV λ_{max} (MeOH): 230, 333 nm; EI-MS (*m/z*): 180, 137, 124, 119, 91 and 77; Molecular formula: $C_{10}H_{12}O_3$; IR (KBr) cm^{-1}: 1510, 1600, 3000, 3525 and 3595; ^{1}H-NMR (500 MHz in MeOH-d_4) δ 3.85 (3H, s, OCH_3), 4.19 (2H, dd, J = 6.0, 1.1 Hz, H-9), 6.19 (1H, dt, J = 15.6, 6.0 Hz, H-8), 6.51 (1H, brd, J = 15.9 Hz, H-7), 6.74 (1H, d, J = 8.0 Hz, H-5), 6.84 (1H, dd, J = 8.0, 1.8 Hz, H-6), 6.93 (1H, d, J = 1.8 Hz, H-2). ^{13}C-NMR (125 MHz in MeOH-d_4) δ 56.1 (OCH_3), 63.4 (C-9), 109.9 (C-2), 115.1 (C-5), 120.6 (C-6), 128.0 (C-8), 130.2 (C-1), 130.5 (C-7), 147.2 (C-4), 148.5 (C-3).

2,6-Dimethoxy-*p*-benzoquinone (5): Yellow needle: EI-MS (*m/z*): 168; Molecular formula $C_8H_8O_4$; IR (KBr) cm^{-1}: 1593, and 1727; UV λ_{max} (CHC1$_3$): 286 nm; ^{1}H-NMR (500 MHz in $CDCl_3$) δ 3.82 (6H, s, OCH_3) and 5.85 (2H, s, H-3,5); ^{13}C-NMR (125 MHz in $CDCl_3$) δ 56.3 (OCH_3), 107.3 (C-3,5), 157.1 (C-2,6), 176.3 (C-4), 186.2 (C-1).

***p*-Methoxycinnamic acid (6):** White powder: EI-MS (*m/z*): 178; Molecular formula $C_{10}H_{10}O_3$; IR (KBr) cm^{-1}: 1456, 1732, 2853 and 2924; UV λ_{max} (MeOH): 217, 323 nm; ^{1}H-NMR (500 MHz in $CDCl_3$) δ 3.69 (3H, s, OCH_3), 6.38 (1H, d, J = 16 Hz, H-8), 6.81 (2H, d, J = 8.5 Hz, H-3, H-5), 7.62 (1H, d, J = 16 Hz, H-7), 7.45 (2H, d, J = 8.5 Hz, H-2, 6); ^{13}C-NMR (125 MHz in $CDCl_3$) δ 52.1 (OCH_3), 115.0 (C-8), 116.9 (C-3, 5), 127.2 (C-1), 131.2 (C-2, 6), 146.7 (C-7), 161.4 (C-4), 169.8 (COOH).

(±)-Balanophonin (7): Pale yellow oil; $[\alpha]_D$ −0.39° (c = 0.34, $CHCl_3$); EI-MS (*m/z*): 356; Molecular formula $C_{20}H_{20}O_6$; IR (KBr) cm^{-1}: 1595, 2923, 3411; UV λ_{max} (MeOH): 216, 339 nm; ^{1}H-NMR (500 MHz in MeOH-d_4) δ 3.56 (1H, brs, H-8), 3.81 (3H, s, OCH_3), 3.83 (2H, brs, H-9), 3.90 (3H, s, OCH_3), 5.60 (1H, d, J = 6.6 Hz, H-7), 6.63 (1H, dd, J = 16.5, 7.8 Hz, H-8'), 6.77 (1H, d, J = 8.1 Hz, H-5), 6.84 (1H, dd, J = 8.1, 1.8 Hz, H-6), 6.94 (1H, d, J = 1.8 Hz, H-2), 7.22 (1H, s, H-2'), 7.27 (1H, s, H-6'), 7.60 (1H, d, J = 16.5 Hz, H-7'), 9.57 (1H, d, J = 7.8 Hz, H-9'). ^{13}C-NMR (125 MHz in MeOH-d_4) δ 54.7 (C-8), 56.5 (OCH_3), 56.9 (OCH_3), 64.7 (C-9), 90.2 (C-7), 110.7 (C-5), 114.4 (C-2'), 116.4 (C-2), 119.9 (C-6), 120.1 (C-6'), 127.2 (C-8'), 129.7 (C-1'), 131.4 (C-5') 134.0 (C-1), 146.1 (C-3'), 147.9 (C-3), 149.3 (C-4), 153.1 (C-4'), 156.2 (C-7'), 196.3(C-9').

6-Methoxychromanone (8): Pale yellow powder; EI-MS (*m/z*): 178; Molecular formula: $C_{10}H_{10}O_3$. IR (KBr): 1277, 1599 and 3424 cm^{-1}; UV λ_{max} (CHC1$_3$): 257, 218, 208 nm; ^{1}H-NMR (500 MHz in $CDCl_3$) δ 3.90 (3H, s, OCH_3), 3.16 (2H, t, J = 6.5 Hz, H-3), 3.96 (2H, t, J = 6.5 Hz, H-2), 6.85 (1H, d, J = 8.5 Hz, H-8), 7.54 (1H, d, J = 2.0 Hz, H-5), 7.57 (1H, dd, J = 8.5, 2.0 Hz, H-7). ^{13}C-NMR (125 MHz in $CDCl_3$) δ 41.0 (C-3), 56.3 (OCH_3), 59.0 (C-2), 112.0 (C-5), 115.9 (C-8), 124.9 (C-7), 130.7 (C-10), 153.5 (C-9), 199.8 (C-4).

Scavenging effect on DPPH radical. The method of Blois with modification was used to measure scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Blois, 1958; Kitts *et al*., 2000). Reaction mixtures containing test samples dissolved in EtOH and 300 μM DPPH in ethanolic solution in a 96-well microtiter plate were incubated at 37 °C for 30 min. Vitamin C and BHT were used as positive control. After the reaction, absorbance was read at 517 nm by a microplate reader and mean value was obtained from triplicate, and percent inhibition was calculated. IC_{50} (inhibitory concentration) values denote the concentration of sample required to scavenge 50% of the DPPH free radical.

Antibacterial activity and MIC determination. Two bacterial strains, *S. mutans* KTCT 3065, *S. sobrinus* KTCT 3288, were used for the determination of antibacterial activity and were cultured in Brain Heart Infusion (BHI, Difco, Detroit, U.S.A.) medium under aerobic conditions at 37 °C. The screening of antibacterial activity for fractions was conducted using a disk diffusion method (Shapiro *et al*., 1994). Briefly, test samples were dissolved in DMSO to a final concentration of 50 mg/mL. 100 μL of prepared culture containing 108 CFU/ml of bacteria was spread on BHI agar. The discs of 8 mm in diameter placed on the inoculated agar were impregnated with 50 μL (2.5 mg/mL) of extract. 10 μL of DMSO was used as vehicle control and gentamicin disc was also used as positive control. The inoculated plates were incubated for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition against the test bacterial strains.

MIC values were determined for single compounds from active fractions with the highest antibacterial activity in the disc diffusion assay, using a micro-well dilution method (Shapiro *et al*., 1994). Growth inhibitory concentration of isolated compounds was tested against *Streptococcus mutans* and *S. sobrinus*, which were incubated for 24 h at 37 °C. At the end of the incubation period, optical density of the cultures was adjusted to

0.500 ± 0.050 with sterile BHI. Sterile 96-well microtiter plates were used by the respective growth medium. Four samples were used for each test concentration, and the experiments were performed in triplicate. Plates with wells containing 300 μL of BHI (100% growth controls) and 170 μL of BHI with 100 μL of substance to be tested were covered with plastic lids, then inoculated with 30 μL of bacterial culture adjusted to an optical density at 550 nm (OD_{550}). All plates were incubated at 37 ºC under appropriate atmospheric conditions and growth was estimated spectrophotometrically (630 nm) after 24 h using a microtiter plate reader. The controls included the inoculated growth medium without test compounds. Sample blanks contained uninoculated medium only.

Results and discussion

The dried powder of the culms of *P. bambusoides* was extracted with 80% aqueous MeOH, and concentrated to yield greenish brown extract. Concentrated aqueous MeOH extract was partitioned with *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH, and water. Of them, *n*-hexane and $CHCl_3$ extracts showed mild antioxidant activities against DPPH radical (IC_{50}: 273 μg/mL for *n*-hexane and 244 μg/mL for $CHCl_3$), and its EtOAc extract exhibited significant antibacterial effects against oral cariogenic *Streptococcus mutans* (10 mm of zone inhibition). Repeated column chromatography on Si gel, Sephadex LH-20, MPLC and HPLC of the *n*-hexane, $CHCl_3$, and EtOAc soluble fractions led to the isolation of eight bioactive simple phenolic compounds (**1** - **8**) (Fig. 1). Phenolics **1** -**4** were isolated as radical scavengers on DPPH radical from its *n*-hexane and $CHCl_3$ extracts, and phenolic compounds **3** and **5** - **8** were identified as anti-cariogenic agents from EtOAc layer. To determine the structures of compounds, the combined analyses with a series of 1D and 2D NMR, infrared, and mass spectra were accomplished. In addition, all physical and spectroscopic data obtained in this present study were compared with those of previously published manuscripts.

Phenolics **1**, **3** and **4** showed characteristic aromatic proton signals corresponding to a 1,3,4-trisubstituted aromatic ring which is composed of two doublets ($J = \sim 8$ Hz and ~2 Hz, respectively), doublet of doublets ($J = \sim 2$ and 8 Hz), as well as two doublets with a large J value of coupling constant ($J = \sim 16$ Hz) assignable to a *trans*-double bond. On the basis of 2D NMR spectra, the position of a methoxyl and a hydroxyl in an aromatic ring and a substituted moiety in a side chain was unambiguously elucidated. Thus, structures of these compounds were assigned as ferulic acid (**1**) (Young *et al.*, 1992), coniferaldehyde (**3**) (Carpinella *et al.*, 2005), and coniferyl alcohol (**4**) (Heravi *et al.*, 2004). Phenolic compound **6** displayed two doublets with a large coupling constant ($J = \sim 16$ Hz) assignable to a *trans*-double bond, however, two *ortho*-coupled aromatic proton doublets

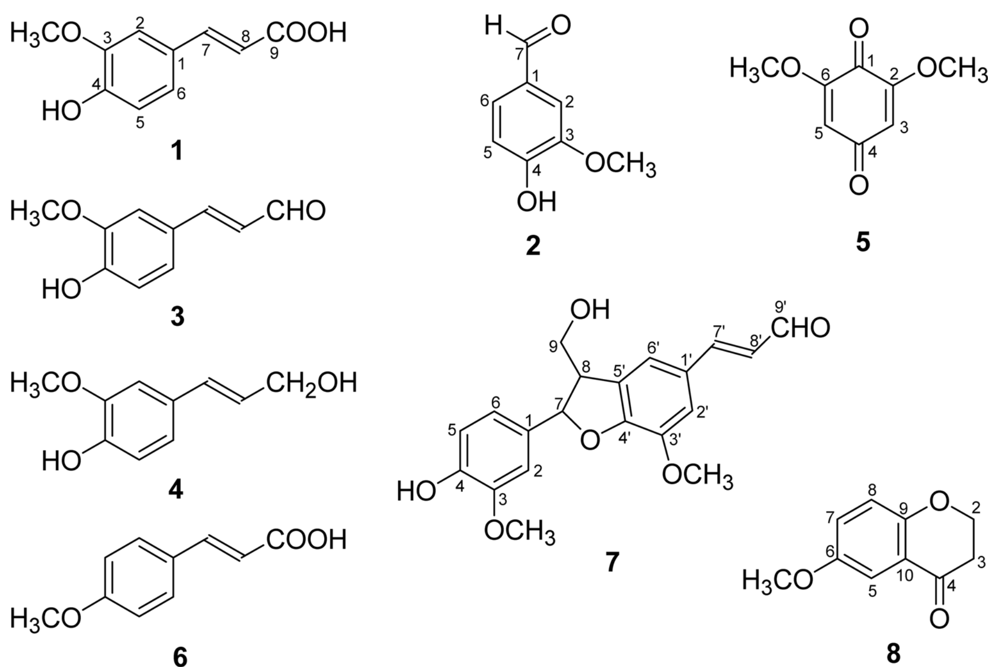

Fig. 1. Structures of phenolic compounds (**1** - **8**) from the culms of *P. bambusoides.*

were observed at δ 6.81 (2H, d, $J = 8.5$ Hz) and 7.45 (2H, d, $J = 8.5$ Hz). Therefore, compound **6** was identified as *p*-methoxycinnamic acid (Haruna *et al*., 1982).

The ^{1}H-NMR spectrum of **2** showed a singlet at δ 9.83 indicating an aldehyde group, in addition to two aromatic doublets ($J = 8.4$ and 1.5 Hz, respectively), showing a splitting pattern of a doublet of doublets, which indicated that it is a trisubstituted benzene. The ^{13}C NMR spectral data revealed the presence of a ketone carbonyl at δ 190.8, six aromatic ring carbons at δ 114.4, 127.5, 129.8, 147.1, 151.7, and a methoxyl carbon signal at δ 56.1. The structure of this compound was determined as vanillin (Harish *et al*., 2005).

Compound **5** showed a molecular ion peak at *m/z* 168, and its ^{1}H-NMR spectrum exhibited only two singlet proton signals, a methoxyl signal at δ 3.82 and an olefinic proton signal at δ 5.85. The ^{13}C-NMR spectrum showed five resonance signals, consisting of two carbonyl carbons at δ 176.3 and 186.2, and two carbons for an oxygenated olefinic bond at δ 157.1 and 107.3, and a methoxyl at δ 56.3. On the basis of this evidence and of a comparison of the published data (Nishina *et al*., 1991), the structure of **5** was determined to be 2,6-dimethoxy-*p*-benzoquinone.

Phenolic compound **7** exhibited two singlet methoxyl signals at δ 3.81 and 3.90, a trisubstituted aromatic ring at δ 6.77, 6.84, and 6.94, one pair of meta-coupled benzene signals at δ 7.22 and 7.27. In addition, dihydrobenzofuran type signals at δ 5.60 and 3.83, a hydroxymethylene at δ 64.7, two *trans*-olefinic proton signals at δ 6.63 and 7.60 with J value of 16.5 Hz, and an aldehyde group at δ 9.57 were also observed in the ^{1}H-NMR spectrum. Based on these spectral data, compound **7** was assumed to be a hgnan, and its unambiguous structure was established to be a neolignan, (±)-balanophonin by 2D NMR experiments and comparison with structural information of previously published data (Haruna *et al*., 1982; Yeun *et al*., 1998).

Compound **8** exhibited signals for three aromatic protons at δ 6.86, 7.57, and 7.54 corresponding to a typical trisubstituted aromatic ring. Two methylenes connected to ether oxygen atom showed as two triplets at δ 3.16 (2H, t, $J = 6.5$ Hz) and 3.93 (2H, t, $J = 6.5$ Hz). The ^{13}C-NMR spectral data indicated the presence of a ketone carbonyl at δ 199.8, six aromatic ring carbons at δ 112.0, 115.9, 124.9, 130.7, 149.2, 153.5, and two methylene carbons connected to an oxygen atom at δ 41.8 and 59.0. The position of a methoxyl and a ketone were confirmed by long-range correlations with the ring protons in the HMBC spectrum. By the comparison of its spectral data with those of literature values (DeWald *et al.,* 1990), the structure of **8** was identified as 6-methoxy-chromanone.

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

Compounds	IC_{50} (μg/mL)
ferulic acid (**1**)	149
vanillin (**2**)	135
coniferaldehyde (**3**)	120
coniferyl alcohol (**4**)	146
vitamin C	90
BHT	126

Table 2. Antibacterial activity of compounds **3**, **5** - **8** isolated from the culms of *P. bambusoides*[a,b]

Compounds	Antibacterial activity MIC value (μg/mL)	
	S. mutans	*S. sobrinus*
gentamicin	< 50	< 50
coniferaldehyde (**3**)	300	567
2,6-dimethoxy-*p*-benzoquinone (**5**)	110	166
p-methoxycinnamic acid (**6**)	352	470
(±)-balanophonin (**7**)	182	490
6-methoxychromanone (**8**)	127	58

[a]MIC was determined by twofold serial broth dilution method.
[b]Vehicle control (0.1% DMSO) showed no MIC.

Biological activities of the isolated compounds **1** - **8** were summarized in the Tables **1** and **2**. As shown in Table **1**, phenolic compounds **1** - **4** isolated from *n*-hexane and $CHCl_3$ extracts of *P. bambusoides* culms showed weaker radical scavenging activities on DPPH than vitamin C, however, similar antioxidant activities to that of BHT, a commercially available synthetic antioxidant. Coniferaldehyde (**3**) showed the strongest radical scavenging activity with an IC_{50} value of 120 μg/mL. The five antibacterial compounds, **3** and **5** - **8** identified from EtOAc extract exhibited inhibitory activity against the gram-positive cariogenic oral streptococci, *S. mutans* and *S. sobrinus*, frequently associated with human periodontitis, with MIC values ranging from 58 to 567 μg/mL. Antibacterial activities of all the compounds were weaker than the positive control, gentamicin. Of the isolated compounds, 2,6-dimethoxy-*p*-benzoquinone **(5)** demonstrated the strongest activity against the bacterial strain, *S. mutans* with an MIC value of 110 μg/mL. In contrast, 6-methoxychromanone (**8)** exhibited the strongest activity against *S. sobrinus* with an MIC value of 58 μg/mL.

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