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Original Research Article

Chemical Constituents and Antioxidant Properties of *Phyllostachys prominens* Gramineae (W Y Xiong) Leaf Extracts

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Abstract

Purpose: *To isolate and identify chemical components of Phyllostachys prominens (Poaceae) leaf extracts, and measure their antioxidant activities.*
Methods: *Ethanol extracts of P. prominens leaves were subjected to different chromatographic methods: macroporous resin column chromatography, Sephadex LH-20 column chromatography, and semi-preparative, reversed-phase (RP) high performance liquid chromatography (HPLC). Plant extract components were identified by ultraviolet spectroscopy (UV), mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR). DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to measure the radical scavenging activity of the compounds .*
Results: *We isolated fourteen compounds including six flavonoids, two lignans, two phenolic glycosides, a phenolic acid, a phenylpropanoid, a monoterpene glycoside, and amarusine from the leaves of Phyllostachys prominens. The DPPH assay showed that eleven compounds (compounds 1, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 14) exhibited radical scavenging activity. (The half maximal inhibitory concentration ranged from 33.52 to 100.58 µg/mL). The half maximal inhibitory concentration (IC_{50}) values of compounds 1, 4, 6 and 7 were 33.52, 40.61, 47.10, and 35.84 µg/mL respectively, while the IC_{50} of the positive control, butylated hydroxytoluene, was 46.32 µg/mL.*
Conclusion: *Fourteen compounds have been successfully all isolated from Phyllostachys prominens for the first time. Eleven of the compounds have radical scavenging activity.*

Keywords: *Phyllostachys prominens, Phytochemicals, Flavonoids, Lignans, Antioxidant activity*

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INTRODUCTION

Bamboo is a perennial plant of the Gramineae family that grows in China, Korea, Japan, and other parts of Southeast Asia, and represents an important commodity. It is used as a building material, handicraft component, food ingredient, and component of traditional medicines. Bamboo leaves have a long medicinal utilization history in China [1]. Bamboo leaf extracts have been reported to contain flavonoids, coumarins, lignans, polysaccharides, and anthraquinones[2]. In the past few years, reports have described the beneficial effects of bamboo leaf extracts on human health, which include antioxidant, anti-aging, antibacterial, lipid regulating, and anti-tumor activities [3-7].

Phyllostachys prominens, which belongs to the tribe Bambuseae, is an important bamboo species that is widely distributed in the south of China [8]. *Phyllostachys prominens* is not only used as a building material, but also as a source of high quality edible bamboo shoots. However, huge leaves of *Phyllostachys prominens* are generally disposed of as waste; thus, how to utilize such large amounts of bamboo leaves is an urgent problem. Exploring the medical value of bamboo leaves could potentially uncover a solution to this problem. Synthetic antioxidants have many risks because of their carcinogenicity and toxicity to the liver. Therefore, the development and utilization of more effective antioxidants of natural origins is desired.

However, at present, little work has been done on the chemical composition of leaf extracts from *Phyllostachys prominens*. Therefore, in this research, we isolated and identified the chemical components of *Phyllostachys prominens* leaf extracts, and evaluated the antioxidant activity of the isolated compounds.

EXPERIMENTAL

Materials and equipment

Column chromatography for fractionation of leaf extracts was carried out using a semi-preparative reversed-phase (RP) column packed with macroporous resin (AB-8, 10 × 80 mm), Sephadex LH-20 (GE Healthcare), and semi-preparative RP high performance liquid chromatography (HPLC) (Shimadzu) with an YMC-Pack ODS-A column (250 × 10 mm, 5 μm, particles). UV spectra were determined using a Waters 2695 HPLC with a photodiode array detector (PAD). NMR spectra were scanned using a Bruker instrument operating at 300 MHz. Mass spectroscopy was performed on an Agilent 6540 high-resolution quadruple time-of-flight mass spectrometer.

Plant materials

Phyllostachys prominens leaves were collected from Hangzhou City, Zhejiang Province, China in September 2013. The plant identity was confirmed by Professor Chen Shuang-Lin from the Research Institute of Subtropical Forest, Chinese Academy of Forestry. A voucher specimen (No. 201310-00) has been deposited in the state forestry administration Key Open Laboratory, International Center for Bamboo and Rattan in Beijing, China.

Extraction and isolation of compounds from *Phyllostachys prominens* leaves

Five kilograms of dried *Phyllostachys prominens* leaves were ground to a powder and extracted three times with 95 % ethanol at room temperature. The extracts were combined and evaporated under reduced pressure at 318 K on a rotary evaporator to yield a solid residue. The residue (451.0 g) was resuspended in water, followed by successive partition with ethyl acetate (111.0 g) and n-butanol (55.0 g).

During the following fractionation steps, HPLC analysis of the fractions was performed. Briefly, 1.0 ml of each fraction was filtered through a 0.45 μm membrane filter before 10 μL was injected into the UPLC system for analysis. The mobile phase was composed of solutions A (MeOH) and B (Water) with a gradient elution, and the flow rate was 1.0 mL/min. For semi-preparative HPLC steps, the fraction was filtered through a 0.45 μm membrane filter before 100 μL was injected into the semi-preparative UPLC system. The mobile phase was composed of solutions A (MeOH) and B (Water) and the flow rate of the mobile phase was 6.0 mL/min.

The n-butanol phase was separated on a macroporous resin column through successive elution with a gradient of increasing ethanol (0, 15, 30, 50, and 100 %) yielding five fractions (E1-E5) based on HPLC analysis. Fraction E2 (8.0 g) was applied to a Sephadex LH-20 column (equilibrated with H_2O) to obtain subfractions E2-1-E2-32 based on HPLC analysis. Separation of E2-14 (146.5 mg) with RP semi-preparative HPLC (14 % MeOH in H_2O) yielded compound 1 (7.5 mg), compound 2 (20.0 mg), and compound 3 (20.0 mg). Separation of E2-20 (100.0 mg) with RP semi-preparative HPLC (20 % MeOH in H_2O) yielded compound 4 (25.0 mg) and compound 5 (19.0 mg). Separation of E2-23 (175 mg) with RP semi-preparative HPLC (30 % MeOH in H_2O) yielded compound 6 (22.0 mg), compound 7 (17.0 mg), and compound 8 (9.5 mg).

The ethyl acetate fraction was further fractionated on a silica gel column by eluting with a gradient of petroleum ether and acetone with increasing polarity to obtain eight fractions (F1-F8) based on HPLC analysis. F3 was passed over a silica gel column and eluted with a gradient of petroleum ether and acetone that yielded ten sub-fractions (F3-1-F3-10). F3-7 (230 mg) were subjected to semi-preparative RP HPLC (40 % MeOH in H_2O), which yielded compound **9** (25.0 mg), compound **10** (24.0 mg), and compound **11** (41.HPLC (44 % MeOH in

H_2O) yielded compound **12** (33.0 mg), compound **13** (28.0 mg), and compound **14** (45.0 mg).

Measurement of radical scavenging activity by DPPH assay

These 14 compounds were evaluated for radical scavenging activity using a modified DPPH assay (Sigma, St. Louis, MO, USA) [9]. Briefly, each compound was dissolved in 400 µL of DMSO and serially diluted to 200, 100, 50, 20, 10, and 5 µg/mL. The reaction mixtures consisted of 200 µL of each serial dilution and 200 µg/mL DPPH in triplicate. After 30 min of incubation in the dark, the absorbance of each reaction was read at 517 nm. The positive control was butylated hydroxytoluene (BHT). The half maximal inhibitory concentration (IC_{50}) values represent the concentration of sample at which 50 % of the DPPH was scavenged. Data were calculated as mean absorbance values.

RESULTS

Fourteen compounds were isolated from leaf extracts of Phyllostachys prominens, and were identified according to the HRMS, ^{1}H-NMR, and ^{13}C-NMR analyses. Their chemical structures are displayed in Figure 1.

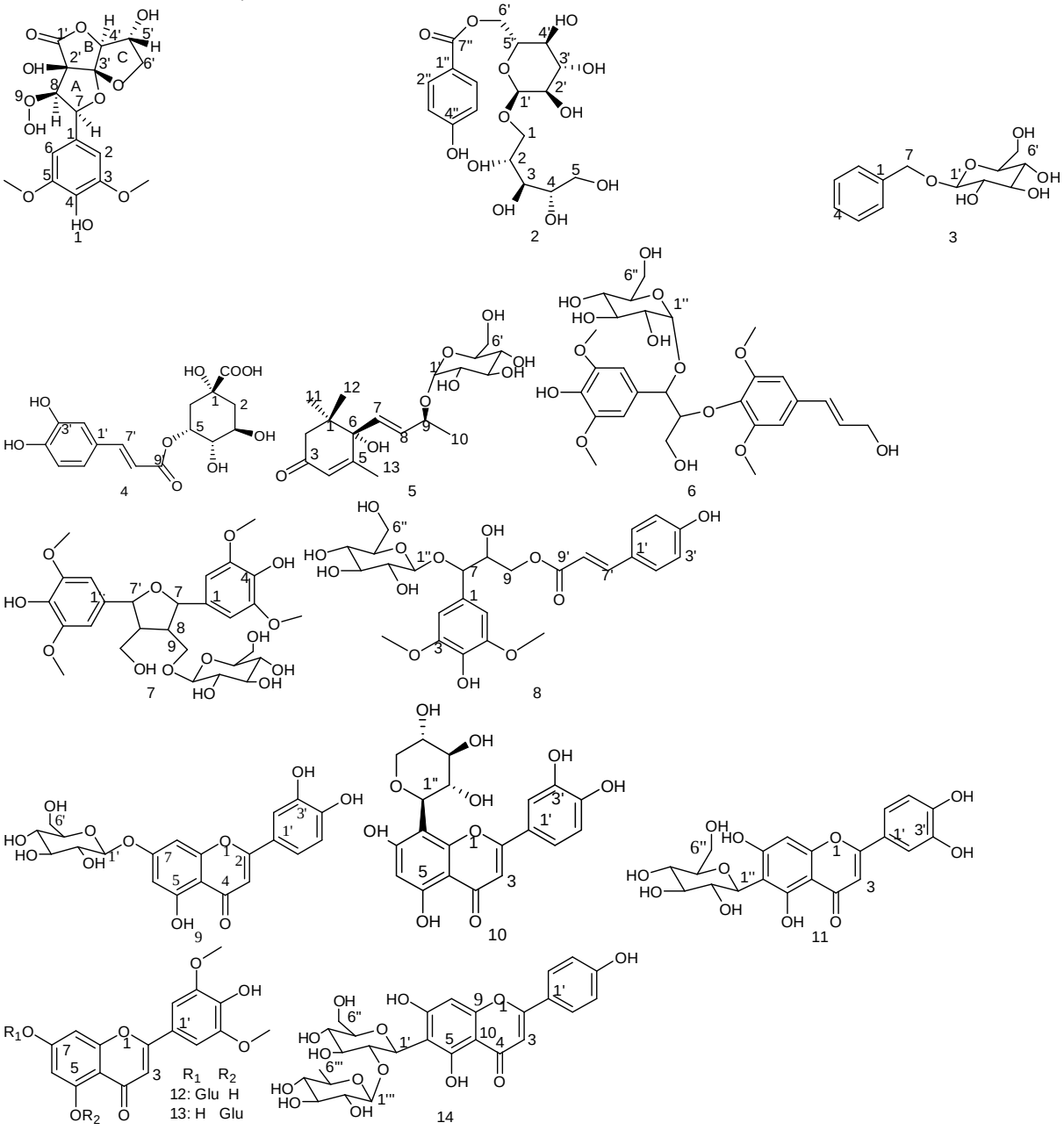

Figure 1: Structures of compounds (1-14) isolated from *Phyllostachys prominens*

Compound 1: White amorphous powder (7.5 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 8.43 (1H, s, OH-4), 6.68 (1H, s, H-2), 6.68 (1H, s, H-6), 6.41 (1H, s, OH-2′), 4.97 (1H, d, J = 10.5 Hz, H-7), 2.58 (1H, m, H-8), 3.75 (1H, m, H-9a), 3.39 (1H, m, H-9b), 4.17 (1H, s, OH-9), 3.76 (6H, s, OCH_3-3, 5), 4.97 (1H, H-4′, d, 1.5 Hz), 4.18 (1H, m, H-5′), 5.64, 5.63 (1H, OH-5′, d, 3.5 Hz), 3.99 (1H, H-6′ a, dd, J = 10.0, 5.0 Hz), 3.90 (1H, dd, J = 10.0 Hz, 5.0 Hz, H-6′ b). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 174.2 (C-1′), 148.0 (C-5, 3), 136.0 (C-4), 129.2 (C-1), 116.9 (3'), 105.3 (C-6), 89.0 (C-4′), 85.5 (C-7), 80.0 (C-2′), 74.1 (C-6′ a), 73.7 (C-5′), 56.7 (C-9a), 56.5 (OCH_3-3, 5), 55.6 (C-8). HR-EI-MS m/z: 383.0972[M-H]$^-$ (calculated for $C_{17}H_{20}O_{10}$, 384.0984). These data are in good agreement with those of amarusine A [10].

Compound 2: White amorphous powder (20.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.83 (2H, d, J = 9.0 Hz, H-2″, 6″), 6.86 (2H, d, J = 9.0 Hz, H-3″, 5″), 4.51 (1H , dd, J = 12.0, 2.0 Hz, H-6′), 4.25 (1H, d, J = 8.0Hz, H-1′), 3.97 (1H, dd, J = 10.0, 3.0 Hz, H-1), 3.74 (1H, m, H-3), 3.45 (1H, m, H-5′), 3.42 (1H, m, H-4), 3.39 (1H, m, H-2), 3.37 (1H, m, H-5), 3.21 (1H, m, H-3'), 3.22 (1H, m, H-3′), 3.04 (1H, m, H-2′). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 165.8 (C-7″), 162.5 (C-4″), 131.2 (C-6″, 2″), 120.3 (C-1″), 115.8 (C-3″, 5″), 103.8 (C-1′), 76.6 (C-3′), 74.2 (C-5′), 74.1 (C-2), 73.1 (C-4), 72.8 (C-2′), 71.9 (C-1), 71.2 (C-3), 70.5 (C-4′), 63.5 (C-5), 64.1 (C-6′). HR-EI-MS m/z: 433.1433 [M-H]$^-$ (calculated for $C_{18}H_{26}O_{12}$, 434.1424). These data are in good agreement with those of xylitol 1-O-(6'-O-p-hydroxylbenzoyl)-glucopyranoside [11].

Compound 3: White powder (20.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.42 (2H, d, J= 7.5 Hz, H-2, 6), 7.33 (2H, t, J= 7.5 Hz, H-3, 5), 7.28 (1H, d, J= 7.5 Hz, H-4), 4.93 (1H, d, J = 11.9 Hz, H-7a), 4.67 (1H, d, J= 11.9 Hz, H-7b), 4.36 (d, J= 7.7 Hz, H-1′), 3.91 (1H, dd, J= 11.9, 1.9 Hz, H-6′), 3.70 (1H, dd, J= 11.9, 5.7 Hz, H-6′), 3.25-3.33 (2H, m, H-2′, 5′). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 138.0 (C-1), 128.2 (C-3, C-5), 128.0 (C-2, C-6), 127.8 (C-4), 102.1 (C-1′), 77.7 (C-5′), 78.1 (C-3'), 74.9 (C-2′), 71.5 (C-4′), 70.5 (C-7), 61.7 (C-6′). HR-EI-MS m/z: 269.1109 [M-H]$^-$ (calculated for $C_{13}H_{18}O_6$, 270.1103). These data are in good agreement with those of benzyl-O-β-D-glucopyranoside [12].

Compound 4: Yellowish amorphous powder (25.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 1.99 (2H, m, H-2), 3.93 (1H, brs, H-3), 3.55 (1H, m, H-4), 5.09 (1H, d, J = 4.9 Hz , H-5), 1.78 (2H, m, H-6), 7.04 (1H, d, J = 1.6 Hz, H-2'), 6.77 (1H, d, J = 8.1Hz, H-5'), 6.98 (1H, dd, J = 1.6, 8.2 Hz, H-6'), 7.43 (1H, d, J = 15.9Hz, H-7'), 6.16 (1H, d, J = 15.9 Hz, H-8′). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 175.5 (C-7), 166.2 (C-9′), 148.7 (C-4′), 145.9 (C-7′), 145.2 (C-3'), 126.0 (C-1′), 121.6 (C-6'), 116.1 (C-5′), 115.2 (C-2′), 114.8 (C-3′), 71.3 (C-1, 3), 68.7 (C-4, 5), 37.7 (C-2), 36.8 (C-6). HR-EI-MS m/z: 353.0934 [M-H]$^-$ (calculated for $C_{16}H_{18}O_9$, 354.0950). These data are in good agreement with those of 5-O-caffeoylquinic acid [13].

Compound 5: White powder (19.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 5.93 (1H, d, J = 15.3 Hz, H-7), 5.74 (1H, s, H-4), 5.62 (1H, dd, J = 15.3 Hz, 6.0 Hz, H-8), 4.98 (1H, brs, 6-OH), 4.40 (1H, m, H-9), 4.06 (1H, d, J = 7.2 Hz, H-1c), 2.52 (1H, d, J =16.5Hz, H-2a), 2.03 (1H, d, J = 16.5 Hz, H-2e), 1.79 (1H, s, H-11), 1.16 (1H, d, J = 6.16 Hz, H-10), 0.90 (1H, s, H-13), 0.89 (1H, s, H-12), 2.89~5.12 (glu-H). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ:198.7 (C-3), 164.4 (C-5), 131.9 (C-8), 131.7 (C-7), 125.8 (C-4), 100.1 (C-1′), 78.1 (C-6′), 77.6 (C-3'), 73.4 (C-9), 72.3 (C-2′), 70.1 (C-4′), 61.2 (C-6'), 49.6 (C-2), 41.2 (C-1), 24.3 (C-12), 23.3 (C-13), 22.2 (C-11), 18.9 (C-10). HR-EI-MS m/z: 385.1945 [M-H]$^-$ (calculated for $C_{19}H_{30}O_8$, 386.1940). These data are in good agreement with those of (6s,9s)-drummondol-9-O-β-D-glucopyranoside [14].

Compound 6: Yellowish amorphous powder (22.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 6.75 (1H, s, H-2), 6.75 (1H, s, H-6), 5.51 (1H, s, H-7), 4.25 (1H, m, H-8), 3.61 (2H, s, H-9), 6.73 (1H, s, H-2′), 6.73 (1H, s, H-6′), 6.38 (1H, s, H-7′), 6.46 (1H, s, H-8′), 4.11 (2H, s, H-9′), 4.34 (1H, s, H-1‴), 3.08 (1H, m, H-2‴), 3.15 (1H, s, H-3‴), 3.05 (1H, s, H-4‴), 3.04 (1H, s, H-5‴), 4.11 (2H, s, H-6‴), 3.73 (6H, s, 3-OCH_3), 3.74 (6H, s, 5-OCH_3). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 153.1 (C-3′), 153.1 (C-5′), 147.7 (C-3), 147.7 (C-5), 135.9 (C-4′), 135.0 (C-4), 132.9 (C-1′), 130.6 (C-7′), 129.4 (C-1), 128.9 (C-8′), 105.7 (C-2′), 105.7 (C-6′), 104.1 (C-2), 104.1 (C-6), 102.5 (C-1‴), 84.5 (C-8), 79.1 (C-7), 77.7 (C-5‴), 76.9 (C-3‴), 74.6 (C-2‴), 70.4 (C-4‴), 61.8 (C-9′), 61.3 (C-6‴), 60.6 (C-9), 56.4 (3, 5-OCH_3). HR-EI-MSm/z: 597.2255 [M-H]$^-$ (calculated for $C_{28}H_{38}O_{14}$, 598.2261). These data are in good agreement with those of 3,5,3',5'- tetramethoxy -4-hydroxyl-(8-O-cinnamyl alcohol)-7-O-glucoside [15].

Compound 7: White powder (17.0 mg).^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 6.66 (4H, s, H-2, 6, 2', 6′), 4.91 (1H, s, H-7′), 4.99 (1H, s, H-2″), 4.85 (1H, s, H-7), 4.16 (1H, s, H-1″), 3.76 (12H, s, H-OCH_3), 3.89, 3.56 (2H, s, H-9), 3.48, 3.53 (1H, s, H-9′), 3.44, 3.46 (2H, m, H-6″), 3.11 (1H, s, H-5″), 3.08 (1H, m, H-3″), 3.05 (1H, m, H-4″), 2.32 (1H, s, H-8′), 2.12 (1H, s, H-8). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 148.3 (C-3), 148.3 (C-

3′), 135.2 (C-4), 135.0 (C-4′), 133.5 (C-1′), 133.2 (C-1), 104.4 (C-6), 104.4 (C-6′), 104.4 (C-2), 104.4 (C-2′), 103.7 (C-1″), 82.6 (C-7′), 86.2 (C-7), 77.3 (C-5″), 74.0 (C-2″), 77.2 (C-3″), 70.5 (C-4″), 69.4 (C-2), 61.52 (C-6″), 60.36 (C-9′), 55.5 (C-OCH_3), 53.7 (C-8), 50.7 (C-8′). HR-EI-MS m/z: 597.2213 $[M-H]^-$ (calculated for $C_{28}H_{38}O_{14}$, 598.2261). These data are in good agreement with those of 4, 4', 9'-trihydroxyl-3, 5, 3', 5'-tetramethoxy-7, 7'-monoepoxylignan-9-O-glucoside [15].

Compound 8: Yellowish syrup (9.5 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.58 (1H, d, J = 16.1, H-7′), 7.53 (2H, d, J= 8.5 H-2′, 6′), 6.78 (2H, d, J = 8.5, H-3′, 5′), 6.67 (2H, s, H-2, 6), 6.36 (2H, s, H-8′), 4.47 (2H, d, J = 6.3, H-7), 4.35 (1H, s, H-1″), 4.80 (dd, J = 6.3, 13.2), 4.21 (2H, s, H-9), 4.10 (1H, s, H-8), 3.73 (6H, s, 3, 5-OCH_3), 3.61, 3.41 (2H, s, H-6″), 3.14 (2H, s, H-3″), 3.04 (2H, s, H-4″), 3.03(2H, s, H-5″).^{13}C-NMR (DMSO-d_6, 300 MHz) δ:166.8(C-9′), 160.7 (C-4′), 147.9 (C-3, 5), 145.1 (C-7'), 135.2 (C-4), 133.2 (C-1') 129.7 (C-1), 116.2 (C-3′, 5′), 114.3 (C-8), 105.0 (C-2), 104.3 (C-1″), 83.6 (C-7), 77.3 (C-3″), 72.6 (C-8), 70.4 (C-4″), 65.4 (C-9), 61.4 (C-6″), 56.4 (3, 5-OCH_3). HR-EI-MS m/z: 551.1853 $[M-H]^-$ (calculated for $C_{26}H_{32}O_{13}$, 552.1842). These data are in good agreement with those of 3,5-dimethoxy-4,4'-dihydroxyl-9-O-benzylacrylicester-phenylpropano-7-O-glucopyranoside [16].

Compound 9: Yellow amorphous powder (25.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 12.98 (1H, s, 5-OH), 7.41 (2H, dd, J = 8.5, 2.5 Hz, H-2′, 6′), 6.88 (1H, d, J = 8.5 Hz, H-5′), 6 .76 (1H, d, J = 2.5 Hz, H-8), 6.76 (1H, s, H-3), 6.44 (1H, d, J = 2.5 Hz, H-6), 5.07 (1H, d, J = 6.0 Hz, H-1″), 3.15-3.70 (4H, m, Glu). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 182.3 (C-4), 164.9 (C-2), 163.0 (C-7), 161.1 (C-5), 157.3 (C-9), 150.5 (C-4′), 145.9 (C-3′), 121.6 (C-1′), 119.5 (C-6′), 116.4 (C-5′), 113.9 (C-2′), 105.7 (C-10), 103.5 (C-3), 100.3 (C-1″), 99.9 (C-6), 95.1 (C-8), 77.6 (C-5″), 76.8 (C-3″), 73.5 (C-21″), 70.5 (C-4″), 61.0 (C-6″). HR-EI-MS m/z: 447.1015[M-H]$^-$ (calculated for $C_{21}H_{20}O_{11}$, 448.1005). These data are in good agreement with those of luteolin-7-O-glucoside [17].

Compound 10: Yellow powder (24.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.48 (2H, dd, J = 8.5, 2.5 Hz, H-2′, 6′), 6.88 (1H, d, J = 8.5 Hz, H-5'), 6.76 (1H, s, H-3), 6.44 (1H, d, J = 2.5 Hz, H-6), 3.15-3.70 (4H, m, L-arabinose). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 182.4 (C-4), 164.6 (C-2), 163.2 (C-7), 161.0 (C-5), 156.0 (C-9), 150.2 (C-4′), 145.9 (C-3′), 121.9 (C-1′), 120.7 (C-6′), 116.6 (C-5′), 114.1 (C-2′), 104.9 (C-10), 104.3 (C-3), 102.6 (C-6), 99.0 (C-8), 75.2 (C-1″), 75.1 (C-3″), 71.3 (C-5″), 69.3 (C-4″), 68.4 (C-2″). HR-EI-MS m/z: 417.0887 $[M-H]^-$ (calculated for $C_{20}H_{18}O_{10}$, 418.0899). These data are in good agreement with those of luteolin -8-C-α-L-arabinose [17].

Compound 11: Yellowish powder (41.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 13.15 (1H, brs, 5-OH), 7.44 (1H, dd, J = 2.5, 9.0 Hz, H-6′), 7.38 (1H, d, J = 2.5 Hz, H-2′), 6.90 (1H, d, J = 9.0 Hz, H-5′), 6.64 (1H, s, H-3), 4.58 (1 H, d, J =10.0 Hz, H-1″). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 181.4 (C- 4), 163.4 (C-7), 163.4 (C-2), 160.6 (C-5), 156.3 (C-9), 150.4 (C-4′), 146.0 (C-3′), 121.6 (C-1′), 118.8 (C-6′), 116.0 (C-5′), 112.9 (C-2′), 108.9 (C-6), 102.8 (C-10), 102.4 (C-3), 93.7 (C-8), 81.4 (C-5″), 79.0 (C-1″), 73.2 (C-2″), 70.5 (C-3″), 70.2 (C-4″), 61.3 (C-6″). HR-EI-MS m/z: 447.1050[M-H]$^-$ (calculated for $C_{21}H_{20}O_{10}$, 448.1056). These data are in good agreement with those of isoorientin [18].

Compound 12: Yellow powder (33.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.48(2H, dd, J = 8.5, 2.5 Hz, H-2′, 6′), 7.02 (1H, s, H-3), 6.91 (1H, d, J = 2.5 Hz, H-6), 6.46 (1H, d, J = 2.0 Hz, H-8), 3.89 (6H, s, 3′, 5′-OCH_3), 5.01 (1H, d, J = 8.0 Hz, H-1″). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 182.3 (C-4), 164.4 (C-2), 163.2 (C-7), 161.3 (C-5), 157.1 (C-9), 148.4 (C-3′, 5′) 140.3 (C-4′), 120.4 (C-1′), 105.6 (C-10), 104.7 (C-2′, 6′), 104.0 (C-3), 99.8 (C-6), 95.6 (C-8), 100.4 (C-1″), 77.5 (C-5″), 76.7(C-3″), 73.4 (C-2″), 69.9 (C-4″), 60.6 (C-6″). HR-EI-MS m/z: 491.1451[M-H]$^-$ (calculated for $C_{23}H_{24}O_{12}$, 492.1268). These data are in good agreement with those of tricin-7-O-β-D-glucoside [18].

Compound 13: Yellow powder (28.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.48(2H, dd, J = 8.5, 2.5 Hz, H-2′, 6′), 7.02 (1H, s, H-3), 6.91 (1H, d, J = 2.5 Hz, H-6), 6.46 (1H, d, J = 2.0 Hz, H-8), 3.89 (6H, s, 3′, 5′-OCH3), 5.01 (1H, d, J = 8.0 Hz, H-1''). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 177.5 (C-4), 163.0 (C-2), 161.5 (C-7), 159.0 (C-5), 158.8 (C-9), 148.6 (C-3′, 5′), 139.9 (C-4′), 120.9 (C-1′), 108.7 (C-10), 106.8 (C-3), 104.9 (C-6), 104.8 (C-2′, 6′), 104.6 (C-1″), 98.9 (C-8), 77.9 (C-5″), 76.0 (C-3″), 74.0 (C-2″), 70.1 (C-4″), 61.3 (C-6″), 56.8 (3′, 5′-OCH_3). HR-EI-MS m/z: 491.1451 $[M-H]^-$ (calculated for $C_{23}H_{24}O_{12}$, 492.1268). These data are in good agreement with those of tricin-5-O-β-D-glucoside [17].

Compound 14: Yellow powder (45.0 mg). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 7.82 (2H, d, J = 6.0 Hz, H-2′, 6′), 6.66 (1H, s, H-3), 6.40 (1H, s, H-8), 6.82 (2H, d, J = 6.0 Hz, H-3′, 5′), 4.97 (2H, d, J = 6.0 Hz, H-1″), 4.27 (1H, d, J = 3.0 Hz, H-1‴). ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 182.5 (C-4), 163.5 (C-2), 156.7 (C-4′), 161.6 (C-5), 160.5

(C-9), 128.9 (C-2', 6′), 121.6 (C-1′), 116.6 (C-3′, 5′), 105.2 (C-10), 109.3 (C-6), 100.9 (C-1‴), 81.9 (C-5″), 80.6 (C-3″), 76.3 (C-2″), 75.3 (C-2‴), 72.1 (C-4‴), 71.9 (C-1″), 71.1 (C-4″), 70.6 (C-3‴), 68.7 (C-5‴), 62.2 (C-6″), 18.0 (C-6‴). HR-EI-MS m/z: 577.1632 $[M-H]^-$ (calculated for $C_{27}H_{30}O_{14}$, 578.1635). These data are in good agreement with those of isovitexin-2″-xylopyranoside [18].

Compounds **1-14** were tested for antioxidant activity using the DPPH method. The results are shown in Table 1. Compounds **1**, **4**, **6**, **7**, **9**, **10**, **11**, **12**, **13**, and **14** exhibited the ability to scavenge radicals with IC_{50} of 33.52 µg/mL, 40.61 µg/mL, 47.10 µg/mL, 35.84 µg/ml, 67.89 µg/mL, 56.24 µg/mL, 100.58 µg/mL, 78.11 µg/mL, 83.06 µg/mL, and 88.25 µg/mL, respectively. BHT was used as the positive control; the IC_{50} of BHT was 46.32 µg/mL. By comparison, compounds **2**, **3**, **5**, and **8** had no radical scavenging capacity.

Table 1: Antioxidant activity of compounds 1 - 14

Test sample	Antioxidant (IC_{50}, µg/mL)
1	33.52±1.17
2	-
3	-
4	40.61± 2.39
5	-
6	47.10±1.95
7	35.84± 0.97
8	-
9	67.89± 2.77
10	56.24± 2.13
11	100.58 ± 5.84
12	78.11± 4.13
13	83.06± 3.25
14	88.25± 2.28
BHT	46.32±1.61

BHT as positive control; "-"= Below detection limit

DISCUSSION

In the present study, 14 compounds, including six flavonoids, two lignans, two phenolic glycosides, a phenolic acid, a phenylpropanoid, a monoterpene glycoside, and amarusine, were isolated from the leaves of *Phyllostachys prominens*. To our knowledge, these were the first compounds isolated from leaf extracts of *Phyllostachys prominens* that subsequently were shown to have anti-oxidant activities.

Bamboo leaves are rich in flavonoids [3],which were considered to have many functions, such as removing active oxygen, preventing hemal sclerosis, improving nutrition for tissue, antiaging and preventing aging dementia[6]. In our study, we also six flavonoids exhibting antioxidant activity. In addition to flavonoids, we isolated two lignans from the bamboo leaf extracts. Lignans, which are a type of phytoestrogen, have a variety of biological activities including antioxidant activity [19]. In the future research on the chemical constituents of bamboo leaves, more attention should be paid on lignans.

The DPPH assay results showed that, in addition to flavonoids, the two lignans and phenolic acid, which are important compounds in bamboo leaves, also had antioxidant activity. These findings suggest that the antioxidant activity of the bamboo leaf extracts could be attributed to several different compounds.

CONCLUSION

Chemical utilization of *Phyllostachys prominens* leaves may be a way to solve the problem of excess quantities of bamboo leaves that are disposed of as waste. Importantly, bamboo leaves have been reported to contain compounds with anti-oxidant properties. To our knowledge, we were the first to isolate fourteen different (**1-14**) compounds from the leaves of Phyllostachys prominens. Moreover, 11 compounds (1, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 14) showed measurable radical scavenging activity with IC_{50}s ranging from 33 to 100 µg/mL. Compounds 1, 4, 6, 7, 8, 9, 10, 11, 12, 13 and 14 showed radical scavenging activity. Compound 1, 4 and 7 each had lower IC_{50} values than the positive control. These findings suggest that Phyllostachys prominens leaves have potential applications in medicine.

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REFERENCES

1. *Hu XM. Zhonghuabencao Pharmacopoeia, Shanghai Science and Technology Press, Shanghai, China; 1999; pp 336-337.*
2. *Shibata M, Yamatake Y, Sakamoto M, Kanamori M, Takagi K. Phamacological studies on bamboo grass (1). Acute toxicity and anti-inflammatory and antiulcerogenic activities of water-soluble fraction (Folin) extracted from Sasaalbomarginata Makino et Shibata. Nippon Yakurigaku Zasshi 1975; 71(5): 481-490.*
3. *He YJ, Yue YD. A Review of the effective component and applications of extracts from bamboo Leaves. Biomass ChemEng 2008; 42: 31-37.*
4. *Perry G, Raina AK, Nonomura A, Wataya T, Sayre LM, Smith MA. How important is oxidative damage? Lessons*

from Alzheimer's disease. Free Radical BiolMed 2000; 28(5): 831-834.

5. *Lee MJ, Moon GS. Antioxidative effects of Korean bamboo trees, Wang-dae, Som-dae, Maengjong-juk, Jolit-dae and O-juk.Korean J Food SciTechnol 2003; 35: 1227-1231.*
6. *Lu BY, Wu X, Tie X, Zhang Y, Zhang Y. Toxicology and safety of anti-oxidant of bamboo leaves. Part I: acute and sub-chronic toxicity studies on antioxidant of bamboo leaves. Food Chem Toxicol 2005; 43(5): 783-792.*
7. *Lin YL, Collier AC, Liu WY, Berry MJ, Panee J. The inhibitory effect of bamboo extract on the development of 7,12-Dimethylbenz[a] anthracene (DMBA)-induced breast cancer. Phytother Res 2008; 22(11): 1440-1445.*
8. *Yi PT, Shi JY, Ma LS. Icono-graphia bambusoidearumsinicarum. Science Press, Beijing, China, 2008; pp342.*
9. *Park EJ, Jhon DY. The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. LWT - Food SciTechnol 2010; 43(4): 655-659.*
10. *Sun J, Zhang PC, Wei Q, Xun H, Tang F, Yue YD, Li L, Guo XF, Zhang Rong. Amarusine A, Anew dioxaspiro [4.4] nonane derivative with a butyrolactone ring from Pleioblastus amarus. Tetrahedron Lett 2014; 55: 4529-4531.*
11. *Sun J, Tang F, Yue YD, Xun H, Guo XF. Two new compounds from the cry leaves of Pleioblastus amarus (Keng) keng f. J Asian Nat Prod Res 2014; 16(9): 930-935.*
12. *Simona DM, Carmen F, Franco Z, Maria I. Phenolic glycosides from Cucumismelo var. in odorus seeds. Phytochem Lett 2009; 2(3): 130-133.*
13. *Jung HA, Park JC, Chung HY, Kim J, Choi JS. Antioxidant flavonoids and chlorogenic acid from the leaves of Eriobotrya japonica. Arch Pharmacal Res 1999; 22(2): 213-218.*
14. *Ìhsan Ç, Ayse KU, Piergiorgio AL, Peter R. (6S)-hydroxy-3-oxo-α-ionolglucosides from Capparis spinosa fruits. Phytochemistry2002; 59: 451-457.*
15. *Tan XR, Jakupovic J, Jia JZ. Aromatic constituents' from Vladimiria souliei. Planta Med 1990; 56(5): 475-477.*
16. *Nasim S, Lee NH. New phenyl propanoids from Sasaquel paertensis Nakai with tyrosinase Inhibition activities. Bull. Korean Chem. Soc 2009; 30(8): 1729-1732.*
17. *Lu YR, Foo FL. Flavonoid and phenolic glycosides from Salvia officinalis. Phytochemistry 2000; 55(3): 263-267.*
18. *Park HS, Lim JH, Kim HJ, Choi HJ, Lee IS. Antioxidant flavone glycosides from the leaves of Sasa borealis. Arch. Pharmacal Res 2007; 30(2): 160-166.*
19. *Kitts DD, Yuan YV, Wijewickreme AN, Thompson LU. Antioxidant activity of the flaxseed lignan secoisolarici resinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. Mol Cell Biochem. 1999; 202(1): 91-100.*