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Phenolic Antioxidants from the Leaves of *Camellia pachyandra* Hu.

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Camellia pachyandra Hu. is a species in *Camellia* sect. Heterogenea (Theaceae), whose leaves have been used for making tea and consumed by the local people living in Yunnan province, China. This is the first investigation of the chemical constituents in the leaves of *C. pachyandra*, from which 22 phenolic compounds including nine hydrolyzable tannins (**1**−**9**), 11 flavonol glycosides (**10**−**20**), and two simple phenolics (**21**, **22**) were isolated. It was noted that the leaves of the title plant contained no caffeine and no catechin, whereas hydrolyzable tannins were found to be the major constituents, of which the content of ellagitannin **5** reached to 3.7%. All the isolates were evaluated for their antioxidant activities by DPPH radical scavenging and tyrosinase inhibitory assays. Though the secondary metabolites without caffeine and catechins are different from the commonly consumed tea plants, the results suggested that the leaves of *C. pachyandra*, rich in hydrolyzable tannins as potent antioxidants, could be developed as an ideal resource for a natural beverage without caffeine.

KEYWORDS: *Camellia pachyandra* Hu; *Camellia* sect. Heterogenea; hydrolyzable tannins; flavonol glycosides; antioxidants

INTRODUCTION

According to Min's system (*1*), the genus *Camellia* includes two subgenera, 14 sections and 119 species, and most of them are distributed in the southwest of China. Among them, the leaves of two cultivated species *Camellia sinensis* (L.) O. Kuntze var. *sinensis* and *C. sinensis* var. *assamica* (Masters) Kitamura, belonging to the *Camellia* sect. Thea (Theaceae), are traditionally used for producing tea, one of the most popular beverages consumed in the world. In addition, some other species from the genus *Camellia* have also been used for making tea and consumed widely by the local people in China.

Camellia pachyandra Hu, a shrub or tree about 5−12 m tall, is one of the members in *Camellia* sect. Heterogenea (Theaceae). Its leaves are glabrous, abaxially pale green and thinly leathery. The year-old branchlets are grayish brown, while the current year branchlets are green to yellowish green. The buds are purple. It is an endemic species distributed in the south of Yunnan province at an altitude of 1400−1900 m (*2*). The leaves of *C. pachyandra* have been used for making green tea or Pu-er tea by the local people and called "Purple-bud" tea.

To date, most of the studies on the chemical constituents of tea and its raw materials have focused on two cultivated tea plants (*3*−*5*) and flavan-3-ols, e.g. catechins were proved to be the bioactive principles of green tea due to their obvious antioxidative properties (*6*−*8*). However, there are no reports on the chemical constituents of *C. pachyandra*. As a part of our continuing research on tea and its original and related plants (*3*, *9*, *10*), the chemical constituents in the leaves of *C. pachyandra* were studied, which led to the identification of 22 phenolic constituents. Moreover, the isolated compounds were tested by DPPH radical scavenging and tyrosinase inhibitory assays in order to evaluate the antioxidant activities of this special tea.

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MATERIALS AND METHODS

General. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. ^{1}H, ^{13}C, and 2D-NMR (including ^{1}H−^{1}H COSY, HSQC, and HMBC) spectra were recorded in acetone-d_6 or DMSO-d_6 with Bruker AM-400 and DRX-500 spectrometers operating at 400 and 500 MHz for ^{1}H, and 100 and 125 MHz for ^{13}C, respectively. Coupling constants were expressed in hertz, and chemical shifts were given on a ppm scale with tetramethylsilane as internal standard. FAB-MS (negative ion mode) were recorded on a VG Auto Spec-300 spectrometer with glycerol as the matrix. DPPH radical scavenging and tyrosinase inhibitory assays were performed on an Emax precision microplate reader. Column chromatography was done on Sephadex LH-20, 25−100 μm (Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP20P, 75−100 μm (Mitsubishi Chemical Co., Ltd.), and Toyopearl HW-40F (Tosoh Co., Ltd.), Diaion HP20SS (Mitsubishi Chemical Co., Ltd.) and silica gel (Qingdao Haiyang Chemical Co.). Thin-layer chromatography (TLC) was performed on precoated silica gel H plates, 0.2−0.25 mm thick (Qingdao Haiyang Chemical Co.), with benzene/ethyl formate/formic acid (3:6:1 or 2:7:1, v/v/v) and trichloromethane/methanol/water (7:3:0.5 or 8:2:0.2, v/v/v), and spots were detected by spraying with 2% ethanolic $FeCl_3$ or anisaldehyde−H_2SO_4 reagent followed by heating.

Kojic acid, tyrosinase mushroom, L-tyrosine, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemicals (Steinheim, Germany); ascorbic acid was obtained from Xinxing Chemical Industrial Reagent Institute (Shanghai, China).

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HPLC analysis was carried out on a Waters 2695 separation module combined with the accessory of a Waters 2996 column chromatophotodiode array detector, using a computer-controlled system with the millennium[32] software, along with a Zorbax SB-C_{18} (250 × 4.6 mm, 5 μm) reversed phase column (Agilent, USA). Water was purified in a Milli-Q apparatus (Millipore, USA). Acetonitrile (chromatographic grade) and phosphoric acid (reagent grade) were purchased from Merck (Darmstadt, FR, Germany).

Plant Materials. The leaves of *Camellia pachyandra* Hu. were plucked from the tea garden of Tea Research Institute, Yunnan Academy of Agricultural Science, Xishuangbanna, Yunnan, China, in 2007, and identified by Ms. Mei Xu.

Extraction and Isolation. The dried leaves (900 g) of *Camellia pachyandra* Hu. were soaked with 60% aqueous (aq) acetone under room temperature for three times (2 L × 3, each time one week). Then the filtrates were combined and concentrated to 500 mL under reduced pressure, and then partitioned successively with petroleum ether (5 × 500 mL) and ethyl acetate (5 × 500 mL), to yield ethyl acetate-soluble (fraction E, light brown residue, 20 g) and water-soluble (fraction W, pale yellow residue, 120 g) fractions.

Part of fraction W (70 g) was subjected to a Diaion HP20SS column chromatography (CC), eluting with H_2O−MeOH (1:0−0:1), to get 4 subfractions (fr. W-1−W-4). Fr. W-1 (20 g) was subjected to MCI-gel CHP20P (0−40% aq MeOH), Toyopearl HW-40F (0−30% aq MeOH) and silica gel ($CHCl_3$−MeOH−H_2O, 7: 3: 0.5) CC to give compounds **3** (0.83 g), **5** (10.0 g), **9** (25 mg) and **18** (135 mg). Fr. W-2 (7.0 g) was chromatographed on Sephadex LH-20 (20−40% aq MeOH) and MCI-gel CHP20P (0−20% aq MeOH) to give compounds **1** (132 mg) and **2** (165 mg). Fr. W-3 (16 g) was chromatographed over MCI-gel CHP20P (0−60% aq MeOH) to yield compounds **4** (47 mg), **6** (117 mg), **19** (36 mg) and **20** (28 mg).

Fraction E was passed through a Sephadex LH-20 (7 × 45 cm, i.d.) column, eluting with a gradient-elution system from H_2O to MeOH, and finally 50% (v/v) aq Me_2CO, to afford 5 subfractions (fr. E-1−E-5). Fr. E-1 (6.0 g, eluted with 0−20% aq MeOH) was then subjected to MCI-gel CHP20P CC, eluted with 20% aq MeOH to yield compound **5** (2.2 g). Fr. E-2 (2.0 g) was chromatographed over MCI-gel CHP20P (0−30% aq MeOH) and Toyopearl HW-40F (0−20% aq MeOH) to yield compounds **7** (120 mg) and **8** (260 mg). Fr. E-3 (3.0 g) was subjected to MCI-gel CHP20P (10% aq MeOH) and Sephadex LH-20 (20% aq MeOH) CC to give compound **22** (35 mg). Fr. E-4 (5.0 g) was passed through Sephadex LH-20 (50−80% aq MeOH), MCI-gel CHP20P (30−60% aq MeOH) and silica gel [$CHCl_3$−MeOH−H_2O (7:3:0.5)] CC to yield compounds **10** (35 mg), **11** (42 mg), **12** (46 mg), **13** (61 mg), **14** (108 mg), **15** (23 mg), **16** (36 mg), **17** (9 mg), and **21** (0.55 g).

Casuariin (1) (*11*, *12*). **1** was obtained as a brownish yellow amorphous powder, $[\alpha]_D^{26}$ + 49.7 (*c* 0.1, H_2O + acetone); FAB-MS (negative ion mode) *m*/*z* 783 $[M - H]^-$; ^{1}H NMR data see Okuda et al. (*12*); ^{13}C NMR (100 MHz, acetone-d_6: D_2O) δ 66.8 (glc C-1), 76.7 (glc C-2), 70.4 (glc C-3), 76.6 (glc C-4), 68.0 (glc C-5), 67.8 (glc C-6), 114.7, 115.8, 115.9, 116.1 (HHDP C-1), 119.6, 124.8, 126.8, 127.0 (HHDP C-2), 104.9, 106.8, 108.3, 116.8 (HHDP C-3), 144.9, 145.0, 145.5, 145.9 (HHDP C-4), 134.7, 135.5, 136.4, 138.6 (HHDP C-5), 143.3, 143.5, 144.1, 144.2 (HHDP C-6), 165.6, 168.6, 169.6, 170.2 (HHDP C-7).

5-Desgalloylstachyurin (2) (*11*, *13*). **2** was obtained as a brownish yellow amorphous powder, $[\alpha]_D^{26}$ +7.0 (*c* 0.2, H_2O + acetone); FAB-MS (negative ion mode) *m*/*z* 783 $[M - H]^-$; ^{1}H NMR data see Lee et al. (*13*); ^{13}C NMR (100 MHz, acetone-d_6: D_2O) δ 64.4 (glc-1), 81.0 (glc C-2), 72.5 (glc C-3), 75.8 (glc C-4), 68.1 (glc C-5), 67.7 (glc C-6), 114.8, 115.7, 116.0, 116.1 (HHDP C-1), 121.9, 124.9, 126.7, 127.2 (HHDP C-2), 105.2, 106.9, 108.4, 118.4 (HHDP C-3), 144.9, 145.0, 145.5, 145.6 (HHDP C-4), 134.8, 135.5, 136.4, 138.3 (HHDP C-5), 143.3, 143.9, 144.1, 144.2 (HHDP C-6), 166.4, 168.6, 169.6, 169.9 (HHDP C-7).

2,3-*O*-(*S*)-Hexahydroxydiphenoyl-β-D-glucopyranose (3) (*14*). **3** was obtained as a brownish yellow amorphous powder, $[\alpha]_D^{26}$ +29.4 (*c* 0.2, H_2O + acetone); FAB-MS (negative ion mode) *m*/*z* 481 $[M - H]^-$; ^{1}H NMR (500 MHz, Me_2CO-d_6: D_2O) δ 6.65, 6.64, 6.57, 6.56 (2H in total, HHDP-H), α-glc 5.33 (0.5H, d, *J* = 3.2 Hz, H-1), 4.85 (0.5H, dd, *J* = 3.4, 9.6 Hz, H-2), 5.28 (0.5H, t, *J* = 3.4 Hz, H-3), 3.76 (0.5H, m, H-4), 3.84 (0.5H, m, H-5), 3.91 (0.5H, m, H-6a), 3.50 (0.5H, m, H-6b), β-glc 4.91 (0.5H, d, *J* = 7.0 Hz, H-1), 4.65 (0.5H, t, *J* = 8.8 Hz, H-2), 4.95 (0.5H, t, *J* = 9.4 Hz, H-3), 3.71 (0.5H, m, H-4), 3.87 (0.5H, m, H-5), 3.79 (0.5H, m, H-6a), 3.49 (0.5H, m, H-6b); ^{13}C NMR (125 MHz, Me_2CO-d_6: D_2O) δ α-glc 90.8 (C-1), 72.5 (C-2), 77.3 (C-3), 67.6 (C-4), 77.8 (C-5), 63.3 (C-6), 114.2, 114.4 (HHDP C-1), 126.1, 126.4 (HHDP C-2), 106.9, 107.2 (HHDP C-3), 144.7, 144.8 (HHDP C-4), 135.8, 136.0 (HHDP C-5), 144.0, 144.1 (HHDP C-6), 169.3, 170.0 (HHDP C-7), β-glc 93.9 (C-1), 75.1 (C-2), 77.4 (C-3), 67.8 (C-4), 80.1 (C-5), 63.4 (C-6), 114.3, 114.5 (HHDP C-1), 126.1, 126.6 (HHDP C-2), 107.1, 107.2 (HHDP C-3), 144.7, 144.8 (HHDP C-4), 135.9, 136.0 (HHDP C-5), 144.1, 144.2 (HHDP C-6), 169.5, 169.9 (HHDP C-7).

Pedunculagin (5) (*15*). **5** was obtained as a brownish yellow powder, $[\alpha]_D^{26}$ +78.8 (*c* 0.5, H_2O + acetone); FAB-MS (negative ion mode) *m*/*z* 783 $[M - H]^-$; ^{1}H NMR (500 MHz, Me_2CO-d_6: D_2O) δ α-glc 6.30, 6.49, 6.58, 6.62 [2H in total, HHDP-H], 5.44 (0.5H, brs, H-1), 5.22 (0.5H, m, H-2), 5.03 (0.5H, t, *J* = 2.1 Hz, H-3), 5.04 (0.5H, m, H-4), 4.57 (0.5H, t, *J* = 8.7 Hz, H-5), 3.75 (0.5H, dd, *J* = 1.8, 12.9 Hz, H-6a), 5.21 (0.5H, m, H-6b), β-glc 6.31, 6.54, 6.59, 6.64 [2H in total, HHDP-H], 5.20 (0.5H, m, H-1), 4.81 (0.5H, t, *J* = 11.2 Hz, H-2), 5.17 (0.5H, t, *J* = 9.1 Hz, H-3), 5.03 (0.5H, m, H-4), 4.16 (0.5H, t, *J* = 10.0 Hz, H-5), 3.82 (0.5H, dd, *J* = 7.8, 12.8 Hz, H-6a), 5.25 (0.5H, d, *J* = 12.9 Hz, H-6b); ^{13}C NMR (125 MHz, Me_2CO-d_6: D_2O) δ α-glc 90.7 (C-1), 69.5 (C-2), 76.9 (C-3), 66.8 (C-4), 75.0 (C-5), 63.9 (C-6), 113.7, 113.9, 115.0, 115.1 (HHDP C-1), 124.8, 125.1, 125.2, 125.3 (HHDP C-2), 107.2 (×2), 107.7, 108.3 (HHDP C-3), 145.1 (×4) (HHDP C-4), 135.9, 136.0 (×2), 136.3 (HHDP C-5), 144.1 (×2), 144.2 (×2) (HHDP C-6), 169.6, 170.0, 170.5, 169.3 (HHDP C-7), β-glc 93.9 (C-1), 69.8 (C-2), 77.4 (C-3),7 1.6 (C-4), 75.4 (C-5), 63.8 (C-6), 113.6, 113.8, 115.0, 115.1 (HHDP C-1), 124.7, 125.1, 125.2, 125.2 (HHDP C-2), 107.2 (×2), 107.5, 107.7 (HHDP C-3), 144.9 (×2), 145.1 (×2) (HHDP C-4), 135.9, 136.0 (×2), 136.2 (HHDP C-5), 143.9 (×2), 144.1 (×2) (HHDP C-6), 169.7, 169.9, 170.4, 169.4 (HHDP C-7).

DPPH Radical-Scavenging Activity. The DPPH assay was performed as described in our previous paper (*16*), and ascorbic acid was used as positive control. Reaction mixtures containing an ethanolic solution of 200 μM DPPH (100 μL) and 2-fold serial dilutions of sample (dissolved in 100 μL of ethanol, with amounts of sample ranging from 2 to 1000 μg/mL) were placed in a 96 well microplate and incubated at 37 °C for 30 min. After incubation, the absorbance was read at 517 nm and the scavenging activity was determined by following equation: % scavenging activity = $[A_{control} - A_{sample}]/A_{control} \times 100$. The SC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals. The data presented are means ± SD of three determinations

Tyrosinase Inhibitory Assay. The tyrosinase inhibitory activity was measured as previously described, using L-dopa as the substrate (*9*). Kojic acid was used as positive control. Mushroom tyrosinase aqueous solution (40 μL, 25 IU/mL), phosphate buffer (pH 6.8) (120 μL) (A) or phosphate buffer (pH 6.8) (80 μL) along with testing samples (40 μL) (B) were mixed. A revised value of 40 μL of sample added to 120 μL of phosphate buffer (pH 6.8) (C) was made to eliminate the absorbance brought with the colored samples. The mixture was preincubated at 37 °C for 10 min, and then 40 μL of 10 μM/mL L-dopa was added. The reaction mixture was then further incubated at 37 °C for 15 min. The amount of dopachrome was measured at 475 nm in a microplate reader. The inhibiting activity was determined by the following equation: % inhibition rate = [A − (B − C)]/A. The IC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to inhibit 50% of tyrosinase activity. The data presented are means ± SD of three determinations.

RESULTS AND DISCUSSION

Identification of Compounds 1−22. The 60% aqueous acetone extract of dried leaves of *Camellia pachyandra* Hu. was suspended into H_2O and then partitioned successively with petroleum ether and EtOAc. The EtOAc and H_2O fractions were separately subjected to repeated column chromatography over Diaion HP-20SS, Sephadex LH-20, MCI-gel CHP20P, and Toyopearl HW-40F to give 22 phenolic compounds. The known compounds were identified as nine hydrolyzable tannins {casuariin (**1**) (*11*, *12*), 5-desgalloylstachyurin (**2**) (*11*, *13*), 2,3-*O*-(*S*)-hexahydroxy- diphenoyl (HHDP)-β-D-glucopyranose (**3**) (*14*), gemin D (**4**) (*17*),

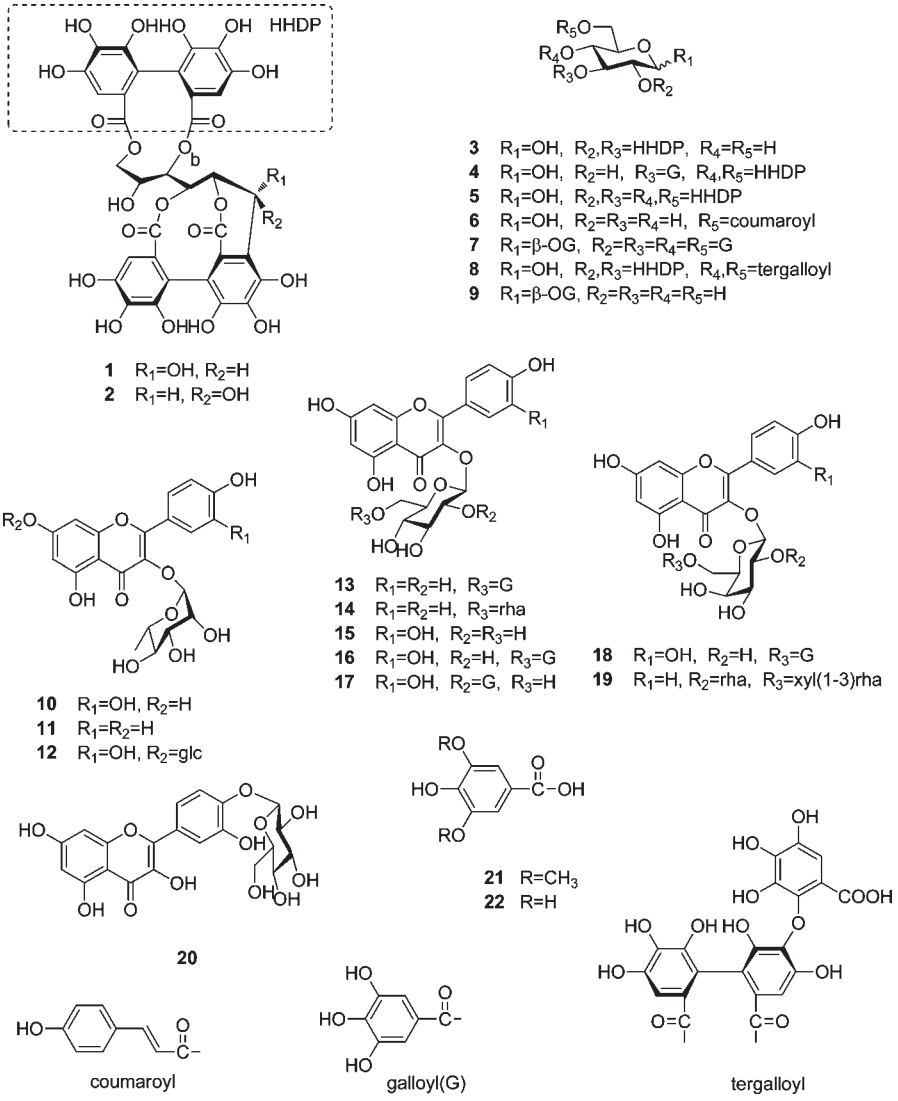

Figure 1. Compounds **1**−**22** isolated from *C. pachyandra*.

pedunculagin (**5**) (*15*), 1-*O*-coumaroyl-*β*-D-glucopyranose (**6**) (*18*), 1,2,3,4,6-*O*-pentagalloyl-*β*-D-glucopyranose (**7**) (*19*), alnusnins B [2,3-*O*-(*S*)-HHDP-4,6-*O*-(*S*)-tergalloyl-*β*-D-glucopyranose] (**8**) (*20*), 1-*O*-galloyl-*β*-D-glucopyranose (**9**) (*9*)}, 11 flavonol glycosides {[quercetin-3-*O*-α-L-rhamnopyranoside (**10**) (*21*), kaempferol-3-*O*-α-L-rhamnopyranoside (**11**) (*21*), 7-*O*-*β*-D-glucopyranosylquercetin-3-*O*-α-L-rhamnopyranoside (**12**) (*22*), kaempferol-3-*O*-(6-*O*-galloyl)-*β*-D-glucopyranoside (**13**) (*21*), kaempferol-3-*O*-rutinoside (**14**) (*3*), quercetin-3-*O*-*β*-D-glucopyranoside (**15**) (*21*), quercetin-3-*O*-(6-*O*-galloyl)-*β*-D-glucopyranoside (**16**) (*21*), and quercetin-3-*O*-(2-*O*-galloyl)-glucopyranoside (**17**) (*23*), quercetin-3-*O*-(6-*O*-galloyl)-*β*-D-galactopyranoside (**18**) (*21*), kaempferol-3-*O*-{[6-*O*-(3-*O*-*β*-D-xylopyranosyl)-α-L-rhamnopyranosyl][2-*O*-α-rhamnopyranosyl]}-*β*-D-galactopyranoside (**19**) (*24*), quercetin-4′- *O*-*β*-D-glucopyranoside (**20**) (*22*)}, and two simple phenolics [syringic acid (**21**) (*25*) and gallic acid (**22**) (*3*)], by comparison with authentic samples and of their spectroscopic and physical data with previously reported values (**Figure 1**). Part of the spectroscopic data for known compounds **1**−**3** and **5** were first reported in detail, based on 2D NMR experiments. Of the isolated compounds, only gallotannin **9**, flavonol glycosides **14** and **15**, and gallic acid (**22**) were reported from the cultivated tea leaves, while the *C*-glycosidic tannin **1** and ellagitannins **4** and **5** were first isolated from the leaves of *Camellia oleifera* (*26*). The flavonol glycoside gallates **13** and **16**−**18** were isolated from the genus *Camellia* for the first time.

HPLC Analysis. The leaves of *Camellia pachyandra* were analyzed by the HPLC method, and the chromatogram is shown in **Figure 2**. Pedunculagin (**5**), a hydrolyzable tannin existing as tautomers, was found to be the major component, whose content reached 3.7% as dry weight. It is well-known that caffeine and catechins are characteristic bioactive principles in the two cultivated tea plants (*3*, *9*, *10*). However, the co-HPLC analysis with authentic samples suggested that the leaves of *C. pachyandra* contained no caffeine and flavan-3-ols. In Min's taxonomical system (*1*), the plants in sect. Heterogenea are "distant relatives" to the species in sect. Thea. The present study shows that the secondary metabolites in *Camellia* sect. Heterogenea are different from the species in sect. Thea. This could be a supportive evidence for Min's theory.

Antioxidative Assays. The antioxidant activities of compounds **1**−**22** were evaluated by DPPH radical scavenging and mushroom tyrosinase inhibitory assays (**Table 1**).

All the compounds with the galloyl or HHDP groups (**1**−**5**, **7**−**9**, **13**, **16**−**18**, **22**) exhibited stronger DPPH radical scavenging activity than the other types of compounds. It is in accordance with the previously reported data (*27*) and showed that more

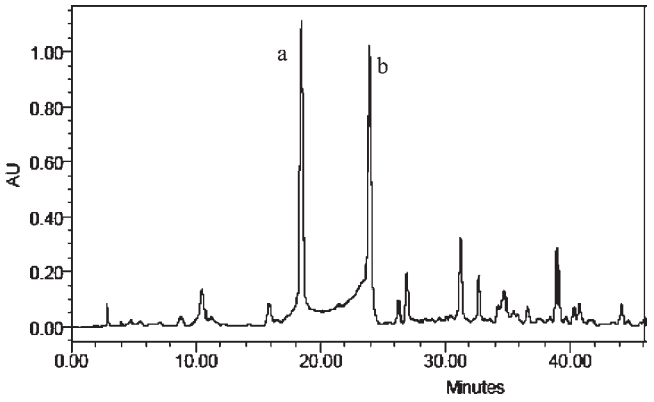

Figure 2. HPLC chromatogram of 70% aq MeOH extract of the leaves of *Camellia pachyandra* (peaks a and b: pedunculagin (**5**) in α and β forms).

Table 1. The DPPH Radical Scavenging (SC_{50}) and Tyrosinase Inhibitory (IC_{50}) Activities of Compounds **1**−**22** from the Leaves of *C. pachyandra*

compounds	yield (%)	SC_{50} [a,b]	IC_{50} [b,c]
1	0.023	13.6 ± 0.1	453 ± 6
2	0.069	12.8 ± 0.2	467 ± 8
3	0.003	24.5 ± 0.3	928 ± 12
4	0.013	16.7 ± 0.2	835 ± 16
5	1.356	13.8 ± 0.1	678 ± 9
6	0.005	53.8 ± 0.6	936 ± 24
7	0.013	6.3 ± 0.2	234 ± 3
8	0.029	11.5 ± 0.1	556 ± 5
9	0.015	29.3 ± 0.2	−[d]
10	0.003	53.3 ± 0.7	914 ± 16
11	0.004	−	−
12	0.002	69.1 ± 0.8	−
13	0.005	21.8 ± 0.2	873 ± 15
14	0.007	125.5 ± 1.6	1083 ± 14
15	0.061	32.2 ± 0.3	753 ± 24
16	0.005	22.5 ± 0.7	611 ± 15
17	0.007	18.9 ± 0.3	725 ± 9
18	0.004	22.1 ± 0.4	768 ± 11
19	0.004	−	−
20	0.012	119.5 ± 1.0	−
21	0.001	99.5 ± 5	−
22	0.005	13.5 ± 1.7	261 ± 5
ascorbic acid		33.2 ± 1.6	
kojic acid			281 ± 2

[a] SC_{50}: DPPH radical scavenging activity (concentration in μM necessary for 50% reduction of DPPH radical). [b] Values represent means ± SD ($n = 3$). [c] IC_{50}: tyrosinase inhibitory activity (concentration in μM necessary for 50% inhibition of tyrosinase activity). [d] −: no activity at a concentration of 1.0 mg/mL.

pyrogallol groups attached to the molecules led to a stronger radical scavenging activity. All the flavonol glycosides (**10**−**20**) displayed moderate DPPH radical scavenging activity since the *O*-glycosylation at the C-3 position in the C-ring of the flavonols could be a hindrance in the free radical reactions (*28*).

In the tyrosinase inhibitory assay, most of the tested compounds showed moderate activities. Nevertheless, gallotannin **7** and gallic acid (**22**) displayed stronger activity than that of the positive control, kojic acid. Compared to the ellagitannins with a glucopyranosyl moiety, the *C*-glycosidic ellagitannins (**1**, **2**) with an open-chain form for glucosyl moiety displayed more inhibitory potency on tyrosinase. It suggested that the conformation of the tannins may also influence their antioxidant activities.

In the present study, 22 phenolic compounds including nine hydrolyzable tannins (**1**−**9**), 11 flavonol glycosides (**10**−**20**), and two simple phenolics (**21**, **22**) were isolated for the first time from the leaves of *C. pachyandra*. Among them, compounds **1** and **2** were characterized as an open-chain *C*-glycosidic moiety in the structures, rarely found to exist in plants. These compounds were reported to have insulin-like (*29*) and vasorelaxant and hypotensive (*30*) effects. The ellagitannin pedunculagin (**5**), reported with various bioactivities, e.g., inhibitory activity on human DNA topoisomerase II(*31*), fatty acid synthase (*32*), and reverse transcriptase in mouse leukemia virus-infected cells (*33*), was found to be the major constituent in *C. pachyandra* by co-HPLC analysis. The flavonol glycoside gallates (**13**, **16**−**18**), reported to be antimicrobial compounds (*34*), as an inhibitor in inflammatory disorders (*35*) and of HIV-2 integrase (*36*), were isolated from the genus *Camellia* for the first time. Most of the isolates showed certain antioxidant activities by DPPH radical scavenging and tyrosinase inhibitory assays. Though the secondary metabolites without caffeine and catechins are different from the commonly consumed tea plants, the results suggested that the leaves of *C. pachyandra*, rich in hydrolyzable tannins as potent antioxidants, could be developed as an ideal resource for a natural beverage without caffeine.

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Supporting Information Available: Spectroscopic and physical data of compounds **1**−**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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