JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic Antioxidants from Green Tea Produced from *Camellia crassicolumna* Var. *multiplex*

QING LIU,[†,‡] YING-JUN ZHANG,*[,†] CHONG-REN YANG,[†] AND MEI XU[§]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China; Graduate School of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China; and Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai 666200, People's Republic of China

Camellia crassicolumna var. *multiplex* (Chang et Tan) Ming belonging to *Camellia* sect. *Thea* (Theaceae), is endemic to the southeastern area of Yunnan province, China, where the leaves have been commonly used for making tea and beverages consumed widely. HPLC analysis showed that there was no caffeine or theophylline contained in the leaves; however, thin layer chromatography (TLC) analysis suggested the abundant existence of phenolic compounds. Further detailed chemical investigation of the green tea produced from the leaves of the plant led to the identification of 18 phenolic compounds, including four flavan-3-ols (**1**−**4**), six flavonol glycosides (**5**−**10**), three hydrolyzable tannins (**11**−**13**), two chlorogenic acid derivatives (**14**, **15**), and three simple phenolic compounds (**16**−**18**). The isolated compounds were evaluated for their antioxidant activities by 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and tyrosinase inhibitory assays. Most of them exhibited significant DPPH radical scavenging activities, whereas flavan-3-ols and hydrolyzable tannins showed stronger inhibitory activities on tyrosinase. The results suggest that *C. crassicolumna* could be an ideal plant resource for a noncaffeine beverage.

KEYWORDS: Tea; *Camellia crassicolumna*; phenolic antioxidants; DPPH; tyrosinase

INTRODUCTION

Tea is one of the most popular beverages consumed in the world due to its rich content of polyphenols, which are reported to have various bioactivities, such as antioxidative, antimicrobial, antitumor, and antimutagenesis (*1*−*3*).

According to Min's system (*4*), there are 12 species and 6 varieties in *Camellia* sect. *Thea* (Theaceae), in which the commonly cultivated tea plants, *Camellia sinensis* L. and *C. sinensis* var. *assamica* (Masters) Kitamura, are included. *Camellia crassicolumna* Chang, as one member of *Camellia* sect. *Thea*, is mainly distributed in the evergreen broad-leaved forests at an altitude of 100−2540 m above sea level in southeastern Yunnan province, China (*5*). The leaves, which were reported to contain little caffeine (<0.19%) but some theophylline (*6*), are often used to make a tea that is widely consumed by the local people. Our preliminary study showed that the leaves of *C. crassicolumna* var. *multiplex* collected from Menghai, Yunnan province of China, contained no caffeine or theophylline by high-performance liquid chromatographic (HPLC), but significant amounts of phenolic compounds by thin-layer chromatographic (TLC) analysis. To evaluate this tea plant, further detailed chemical investigation on green tea produced from leaves of this plant was carried out. Antioxidant activities were tested by 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and tyrosinase inhibitory assays.

MATERIALS AND METHODS

General Procedure. ^{1}H, ^{13}C, and 2D NMR spectra were recorded in acetone-d_6 with Bruker AM-400 and DRX-500 spectrometers operating at 400 and 500 MHz for ^{1}H and at 100 and 125 MHz for ^{13}C, respectively. Coupling constants are expressed in hertz, and chemical shifts are given on a parts per million scale with tetramethylsilane as internal standard. The MS data were recorded on a VG Auto Spec-3000 spectrometer with glycerol as the matrix, and LC-MS data were recorded on an API Qstar Pulsar LC/TOF spectrometer. DPPH radical scavenging and tyrosinase inhibitory assays were performed on an Emax precision microplate reader. HPLC analysis was performed on a Shimadzu LC-10AD (Japan) equipped with an SPD-M10AVP diode array detector and a 250 × 4.6 mm, i.d., 5 μm, Zorbax SB-C_{18} column (Agilent, Palo Alto, CA). Column chromatography (CC) was done on 25−100 μm Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd. Uppsala, Sweden), 75−100 μm MCI-gel CHP20P (Mitsubishi Chemical Co., Ltd. Tokyo, Japan), and 37−70 μm Toyopearl HW-40F (Tosoh Co., Ltd. Tokyo, Japan). Thin-layer chromatography (TLC) was carried out on precoated 0.2−0.25 mm thick slica gel H plates (Qingdao Haiyang Chemical Co., Qingdao, China), with benzene/ethyl

* Corresponding author (telephone +86-871-5223235; fax +86-871-5150124; e-mail zhangyj@mail.kib.ac.cn).
† Kunming Institute of Botany.
‡ Graduate School of Chinese Academy of Sciences.
§ Tea Research Institute, Yunnan Academy of Agricultural Sciences.

10.1021/jf802974m CCC: $40.75
Published on Web 01/07/2009

formate/formic acid (3:6:1 or 2:7:1, v/v/v), and spots were detected by spraying with 2% ethanolic $FeCl_3$ or anisaldehyde/H_2SO_4 reagent followed by heating.

Chemicals and Reagents. DPPH radical, tyrosinase, L-dopa, and kojic acid were purchased from Sigma-Aldrich Chemicals (Steinheim, Germany), and ascorbic acid was obtained from Xinxing Chemical Industrial Reagent Institute (Shanghai, China). Acetonitrile (chromatographic grade) and phosphoric acid (reagent grade) were purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA). Authentic samples used for HPLC analysis were isolated from *C. crassicolumna* var. *multiplex* in the present study or from other tea plants of *Camellia* Sect. *Thea* by our group previously. Theophylline was purchased from Shanghai Shunbo Bioengineering Co. (Shanghai, China).

Plant Materials. The green tea used in this study was produced from the leaves of *C. crassicolumna* var. *multiplex*, collected from the tea plant garden of the Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai.

Extraction and Isolation. Green tea (860 g) produced from the leaves of *C. crassicolumna* var. *multiplex* was extracted three times with 60% aqueous acetone at room temperature (each 7 days × 3 L). After removal of the organic solvent under reduced pressure, the aqueous fraction was extracted with $CHCl_3$ and EtOAc successively, to give a $CHCl_3$ fraction (20 g) and an EtOAc fraction (90 g). TLC detection demonstrated that phenolic compounds were contained in the EtOAc and H_2O fractions, on which further investigation was focused.

The EtOAc fraction (90 g) was subjected to CC over Sephadex LH-20 and eluted with ethanol to afford five fractions (1−5). Further repeated CC on MCI-gel CHP20P, Sephadex LH-20, and Toyopearl HW-40F, eluted with MeOH/H_2O (0:1−1:0) gave **17** (5 mg), **5** (55 mg), **6** (6 mg), **7** (100 mg), **8** (63 mg), and **9** (12 mg) from fraction 1 (2.0 g); **3** (16 mg) and **12** (40 mg) from fraction 2 (8.7 g); **2** (1.1 g), **4** (5.6 g), and **16** (200 mg) from fraction 3 (15.3 g); **1** (9 mg) and **13** (16 mg) from fraction 4 (0.3 g); and **10** (10 mg) from fraction 5 (1.7 g), respectively.

The H_2O fraction (24 g) was chromatographed repeatedly over MCI-gel CHP20P and Sephadex LH-20 and eluted with MeOH/H_2O (0:1−1:0) to give **11** (13 mg), **14** (19 mg), **15** (64 mg), and **18** (4 mg).

HPLC Analysis. Two and a half grams of green tea produced from the leaves of *C. crassicolumna* var. *multiplex* in a volumetric flask was saturated with 70% aqueous methanol (100 mL) for 12 h at room temperature, during which ultrasonication for 15 min was carried out twice. Authentic samples were prepared as methanolic solutions (0.5 mg/mL), respectively. All sample solutions were filterd through a 0.45 μm (Millipore) filter before injection into the HPLC.

The optimal mobile phase for analysis was a binary gradient elution system consisting of solvent A (water containing 0.34% phosphoric acid) and solvent B (acetonitrile). The gradient program (I) used was from 4 to 40% B in 45 min. Another gradient program (II) from 5 to 15% of B in 45 min was used for the analysis of caffeine. The flow rate was 1 mL/min, and the column temperature was set at 30 °C. The injection volume was 10 μL. The UV detection wavelength was monitored at 280 nm. The peaks were confirmed by the UV absorptions and retention times of authentic samples, which were 27.97 (**1**), 27.59 (**2**), 21.11 (**3**), 21.95 (**4**), 30.08 (**6**), 33.45 (**7**), 30.29 (**8**), 26.14 (**9**), 18.21 (**11**), 12.32 (**12**), 26.62 (**13**), 14.17 (**15**), 7.89 (**16**), 8.54 (theogallin, peak a), 18.77 (caffeine), and 14.21 (theophylline), respectively, under gradient program I.

Quantification of the Main Constitutes. Gradient amounts of 1−20 μL of theogallin and catechin analogues **2**−**4** solutions (0.5 mg/mL) were injected to examine the linear relations. Then calibration curves were made accordingly to yield the following regression equations and ranges for quantificative analysis: $y = 2E + 06x + 1E + 06$, $r = 0.9983$, 0.53−7.76 μg (theogallin); $y = 2E + 06x + 252277$, $r = 0.9995$, 0.55−7.63 μg (**2**); $y = 657134x + 62236$, $r = 0.9997$, 0.42−8.48 μg (**3**); $y = 1E + 06x + 209659$, $r = 0.9994$, 1.02−10.20 μg (**4**).

LC-MS Analysis. LC-MS analysis was carried out to identify the two big unknown peaks d and e in **Figure 2A**. The tea extracts were analyzed on an API Qstar Pulsar LC/TOF spectrometer equipped with a 250 × 4.6 mm, i.d., 5 μm, Zorbax SB-C_{18} column (Agilent). The mobile phases were solvent C (water containing 0.5% acetic acid) and solvent D (methanol). The gradient was 0−20 min, 5−20% C; 20−30 min, 20−60% C; 30−31 min, 60−100% C; and 31−40 min, 100% C, at a flow rate of 1 mL/min. The column temperature was set at 30 °C, and the effluent was monitored at 280 nm.

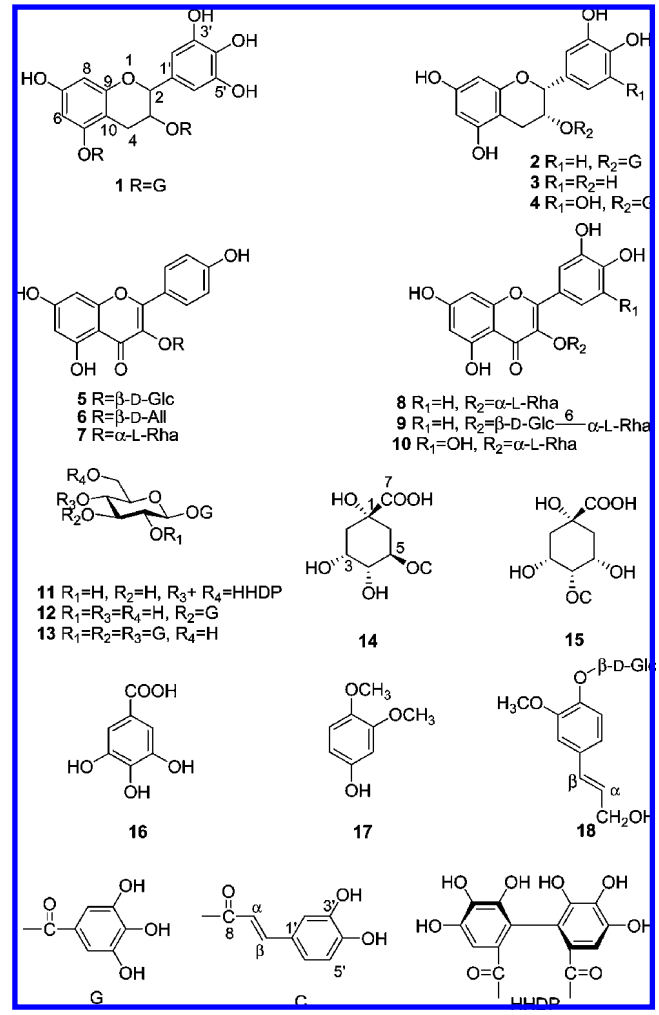

Figure 1. Compounds **1**−**18** from *Camellia crassicolumna* var. *multiplex*.

DPPH Radical Scavenging Assay. The DPPH assay was performed as described previously (*7*). A MeOH solution of DPPH (100 μM, 100 μL) was added to the MeOH solution of samples at series of concentrations (1−1000 μg/mL, 100 μL). After storage at room temperature for 15 min, the absorption values of the reaction mixtures were read at 517 nm, and DPPH radical scavenging activity was calculated according to the following equation: percentage of DPPH reduction (%) = 100 × [$A_{blank} - A_{sample}$]/A_{blank} (ascorbic acid was used as positive control). Then, a linear plot of percentage of DPPH reduction and sample concentration was made (correlation coefficient R^2 = 0.99−1). The antioxidant activity was evaluated by SC_{50} values (the concentration of sample required to scavenge 50% of DPPH radicals), which were obtained through extrapolation from the linear plot. In this assay, each sample was evaluated in triplicate.

Inhibitory Activity on Tyrosinase. A tyrosinase inhibition assay was performed as reported previously (*8*, *9*) with slight modification. First, 80 μL of L-dopa (2.5 mM) solution and 80 μL of sample solution (2 mM) were mixed, and then 80 μL of tyrosinase solution (25 IU/mL) was added. After incubation at room temperature for 20 min, the mixture was monitored at 475 nm to obtain absorption values. The inhibiting activity was determined according to the following equation: % inhibition rate = 100 × ($A_{blank} - A_{sample}$)/A_{blank} [phosphoric acid buffer solution (pH 6.8) was used as blank]. Second, samples possessing stronger activity, which had an inhibitory rate of >50%, were further evaluated to obtain their IC_{50} values. In this case, a series concentration (1−1000 μg/mL) of samples was prepared, and the inhibiting activity

was determined according to the equation % inhibition rate = 100 × [(*A* − *B*) − (*C* − *D*)]/(*A* − *B*), where *A* = absorption values at 475 nm without test samples, *B* = absorption values at 475 nm without test samples and enzyme, *C* = absorption values at 475 nm with test samples, and *D* = absorption values at 475 nm with test samples but without enzyme. Inhibitory activity on tyrosinase was evaluated by IC_{50} values obtained through extrapolation from standard curve. All measurements were carried out in triplicate. All of the solutions were prepared with phosphoric acid buffer solution (pH 6.8). Kojic acid was used as positive control.

RESULTS AND DISCUSSION

The 60% aqueous acetone extract of *C. crassicolumna* var. *multiplex* was partitioned successively with $CHCl_3$ and EtOAc. Then, the EtOAc and H_2O fractions were separately subjected to repeated column chromatography over Sephadex LH-20, MCI-gel CHP20P, and Toyopearl HW-40F to afford 18 known phenolic compounds. These were determined to be four flavan-3-ols [(±)-gallocatechin-3,5-di-*O*-gallate (**1**) (*10*), (−)-epicatechin-3-*O*- gallate (**2**) (*11*), (−)-epicatechin (**3**) (*12*), and (−)-epigallocatechin-3-*O*-gallate (**4**) (*12*)], six flavonol glycosides [kaempferol-3-*O*-*β*-D-glucopyranoside (astragalin) (**5**) (*13*), kaempferol-3-*O*-*β*-D-allopyranoside (asiaticalin) (**6**) (*14*), kaempferol-3-*O*-α-L-rhamnopyranoside (afzelin) (**7**) (*11*), quercetin-3-*O*-α-L-rhamnopyranoside (quercetrin) (**8**) (*15*), rutin (**9**) (*16*), and myricitrin (**10**) (*17*)], three hydrolyzable tannins [strictinin (**11**) (*18*), 1,3-di-*O*-galloyl-*β*-D-glucopyranose (**12**) (*18*), and 1,2,3,4-tetra-*O*-galloyl-*β*-D-glucopyranose (**13**) (*19*)], two chlorogenic acid derivatives [chlorogenic acid (**14**) (*12*) and 3α,5α-dihydroxy-4α-*O*-caffeoylquinic acid (**15**) (*12*)], and three simple phenolic compounds [gallic acid (**16**), 3,4-dimethoxyphenol (**17**), and coniferin (**18**) (*12*)], respectively, by comparison with authentic samples and of their spectroscopic and physical data with previously reported values (**Figure 1**). This is the first time that all of the compounds were isolated from this plant, and compounds **6**, **8**, **10**, **12**, **13**, and **17** were reported from tea and tea plants.

The chemical constituents in green tea produced from the leaves of *C. crassicolumna* var. *multiplex* were analyzed by HPLC method, and the chromatogram was shown in **Figure 2**. With co-HPLC analysis (gradient program I) with authentic samples, 14 phenolic compounds, **1**−**4**, **6**−**9**, **11**−**13**, **15**, **16**, and theogallin (peak a), were identified (**Figure 2A**). The retention times of caffeine and theophylline under gradient program I were 18.77 and 14.21 min, respectively, suggesting that there was no theophylline contained in this tea plant. To confirm the existence of caffeine, another analytical gradient program (II) was applied, and the chromatogram is shown in **Figure 2B**. With this HPLC analytical condition, the retention time of caffeine (27.47 min) was similar to those of peaks b and c (27.05 and 27.81 min, respectively). However, the ultraviolet absorptions of peaks b and c were different from that of caffeine (**Figure 2B**). The result demonstrated that there was no caffeine contained in the leaves of *C. crassicolumna* var. *multiplex* either. Moreover, two main unknown peaks, d and e, were observed at 11.75 and 22.57 min in **Figure 2A**. These were further supposed to be epimers of theogallin and EGCG due to their quasi molecular ion peaks at *m*/*z* 343 $[M - H]^-$ (peak d) and 457 $[M - H]^-$ (peak e) and fragment ion peaks at *m*/*z* 191 $[M - 153 (galloyl)]^-$ (peak d), 305 $[M - 153 (galloyl)]^-$ (peak e), and 169 for galloyl fragment, respectively, by LC-MS analysis. However, further evidence is necessary to confirm the structures.

The contents of theogallin and catechin analogues **2**−**4**, which are the main components in *C. sinensis* var. *assamica*, a widely

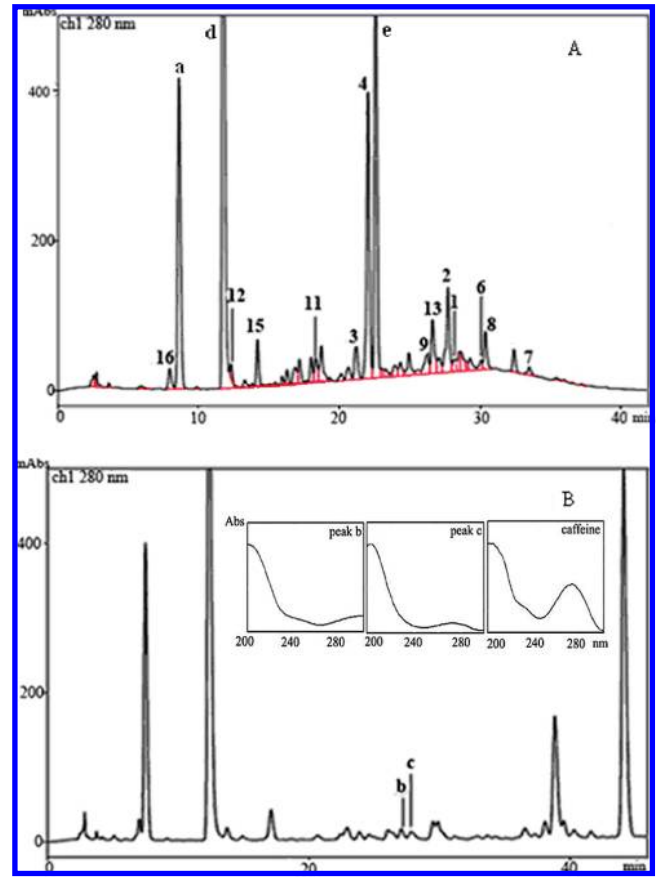

Figure 2. (**A**) HPLC chromatogram of *Camellia crassicolumna* var. *multiplex* (**A**) with program I. The number of each identified peak is consistent with that of the compounds as shown in **Figure 1**; peak a is theogallin. (**B**) HPLC chromatogram with program II and ultraviolet absorption spectra of peaks b and c and caffeine.

cultivated and consumed tea plant, were quantitatively analyzed as 0.99, 0.31, 0.43, and 1.97%, respectively, in the green tea produced from *C. crassicolumna* var. *multiplex*. Both tea plants contain similar amounts of (−)-epicatechin-3-*O*-gallate (**2**). However, compared with *C. sinensis* var. *assamica* (*20*), the contents of (−)-epicatechin (**3**) and (−)-epigallocatechin-3-*O*-gallate (**4**) were much lower, whereas the content of theogallin was higher in *C. carssicolumna* var. *multiplex*. Moreover, neither caffeine nor theophylline was detected in the leaves of *C. crassicolumna* var. *multiplex*, which is inconsistent with previous results (*6*, *21*). According to Min's system (*4*), about 47 reported species and 3 varieties in Sect. *Thea* and Sect. *Glaberrima* of genus *Camellia* were merged into sect. *Thea* as 12 species and 6 varieties. The present study suggested that there are significant differences for the secondary metabolites of *C. crassicolumna* collected from different sources. Further systematic research on all species of *Camellia* sect. *Thea* is necessary to clarify their relationships and components.

The antioxidant activity of **1**−**17** was evaluated by DPPH radical scavenging and mushroom tyrosinase inhibitory assays, and the results are shown in**Table 1**.

Compounds **1**−**4** exhibited stronger DPPH radical scavenging activity than that of ascorbic acid, and their activity order was **1** > **4** > **2** > **3**. This result is in accord with a previous paper (*22*) and showed that the more galloyl and B-ring hydroxyl groups attached to the molecule, the stronger the antioxidative activity. The presence of at least an *o*-dihydroxy group in the B-ring and a hydroxy or a galloyl group at the C-3 position of the C-ring is essential for the radical scavenging activities of

Table 1. Antioxidant and Tyrosinase Inhibitory Activity of Compounds **1**−**17** from *Camellia crassicolumna* Var. *multiplex*

sample	DPPH[a] SC_{50}(μM)[c]	tyrosinase[b] IC_{50} (μM)[c]
(−)-epigallocatechin-3,5-digallate (**1**)	8.9 ± 0.4	20.4 ± 0.3
(−)-epicatechin-3-*O*-gallate (**2**)	13.3 ± 1.2	212 ± 8.9
(−)-epicatechin (**3**)	24.8 ± 0.4	−
(−)-epigallocatechin-3-*O*-gallate (**4**)	11.1 ± 0.1	203 ± 13
astragalin (**5**)	405 ± 4	−
asiaticalin (**6**)	744 ± 33	−
afzelin (**7**)	1039 ± 49	−
quercetrin (**8**)	31.0 ± 0.5	−
rutin (**9**)	32.6 ± 0.9	−
myricitrin (**10**)	18.3 ± 0.6	396 ± 19
strictinin (**11**)	10.0 ± 0.6	143 ± 11
1,3-di-*O*-galloyl-β-D-glucopyranose (**12**)	13.2 ± 0.8	247 ± 10
1,2,3,4-tetra-*O*-galloyl-β-D-glucopyranose (**13**)	10.1 ± 0.4	55.3 ± 4.8
chlorogenic acid (**14**)	57.8 ± 3.0	−
3α,5α-dihydroxy-4α-caffeoylquinic acid (**15**)	67.9 ± 2.9	−
gallic acid (**16**)	15.4 ± 1.2	−
3,4-dimethoxyphenol (**17**)	196 ± 11	−
ascorbic acid (positive control)	42.8 ± 0.6	
kojic acid (positive control)		94.2 ± 5.2

[a] SC_{50} = concentration in μM required to scavenge 50% of DPPH radical. [b] IC_{50} = concentration in μM required for 50% reduction of tyrosinase activity. −, the inhibitory rate on tyrosinase is <50% at a concentration of 2 mM. [c] Values represent means ± SD (n = 3).

flavan-3-ols, and the galloyl group could considerably strengthen the activity (*23*). Flavan-3-ols **1**−**4** are major antioxidants in various plants, and more of their uses, such as natural antioxidant in oils and fats against lipid oxidation and supplement for animal feeds, have been reported (*24*).

Flavonol glycosides **5**−**10** displayed DPPH radical scavenging activity in the order **10** > **8** > **9** > ascorbic acid > **5** > **6** > **7**. The antioxidative activity of flavonoids was related to the structure of the B-ring and C-ring, when the A-ring was the same. An increase of *o*-hydroxy structures in the B-ring would enhance the activity, whereas O-glycosylation at the C-3 position in the C-ring would decrease the activity (*25*). Furthermore, the larger the substituent at the C-3 position, the weaker will be the activity (**8** > **9**), probably as the result of stereospecific hindrance.

Hydrolyzable tannins **11**−**13** exhibited stronger radical scavenging activity than ascorbic acid and flavan-3-ols (**Table 1**), due to their possessing one or more galloyl moieties. Their activity was in the order **11** > **13** > **12**. This demonstrated that an increase of galloyl and/or catechol groups enhanced the activity of the tannins significantly (*15*, *23*). Besides, gallotannins also showed the potential for HIV therapy (*26*).

Gallic acid (**16**), a well-known and widely used natural antioxidant, also exhibited stronger antioxidative activity than ascorbic acid. When the hydroxyl group was substituted by a methoxy group (compound **17**), the antioxidative activity decreased.

The inhibitory activities on tyrosinase of the tested compounds were in the order gallotannins > flavan-3-ols > flavonol glycosides. Of these, compounds **1** and **13** displayed stronger activity than kojic acid. The tyrosinase inhibitory activity of gallotannins became stronger with the increase of the number of galloyl moiety in molecule (**13** > **12**). This trend was also applicable to flavan-3-ols (**1** > **4** > **2** > **3**). It was shown that the galloyl group was indispensable to the activity. Moreover, the number of hydroxy groups in the B-ring influenced the tyrosinase inhibitory activity of flavan-3-ols to some extent, and the increase of *o*-hydroxy groups also strengthened the activity (**4** > **2**).

Tyrosinase, also known as polyphenol oxidase (PPO), is the key enzyme in the undesirable browning of fruits and vegetables (*27*). It is also involved in the initial step in melanin synthesis (*28*). Thus, tyrosinase inhibitors are used as skin-whitening agents in the cosmetic industry and enzymatic browning inhibitors in the food industry. Now, there is a constant search for better tyrosinase inhibitors from natural sources as they are largely free of any harmful side effects (*29*).

In the present study, 18 phenolic compounds, including 4 flavan-3-ols (**1**-**4**), 6 flavonol glycosides (**5**−**10**), 3 hydrolyzable tannins (**11**−**13**), 2 chlorogenic acid derivatives (**14**, **15**), and 3 simple phenolic compounds (**16**−**18**) were isolated for the first time from the leaves of *C. crassicolumna* var. *multiplex*. HPLC analysis showed that the contents of catechin anologues **3** and **4** were much lower, whereas theogallin was higher in *C. crassicolumna* var. *multiplex*, compared to those in *C. sinensis* var. *assamica*. In addition, neither caffeine nor theophylline was detected in the leaves of *C. crassicolumna* var. *multiplex*. Most of the isolated compounds exhibited significant DPPH radical scavenging activities, whereas flavan-3-ols and hydrolyzable tannins showed stronger inhibitory activities on tyrosinase. The above results will promote the reasonable usage of this tea plant, and *C. crassicolumna* var. *multiplex* could be an ideal plant resource for a noncaffeine beverage.

ACKNOWLEDGMENT

We are grateful to the staff of the analytical group at State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring all of the spectral data.

Supporting Information Available: Physical and spectroscopic data of compounds **1**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Khan, N.; Mukhtar, H. Tea polyphenols for health promotion. *Life Sci.* **2007**, *81*, 519–533.
(2) Khan, N.; Mukhtar, H. Multitargeted therapy of cancer by green tea polyphenols. *Cancer Lett.* **2008**, *269*, 269–280.
(3) Kuroda, Y.; Hara, Y. Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutat. Res.* **1999**, *436*, 69–97.
(4) Min, T. L. A revision of *Camellia* sect. *Thea*. *Acta Bot. Yunnan* **1992**, *14*, 115–132.
(5) Luo, J. F.; Long, C. L.; Zhou, Y. L. Ecological environment and introduction experiments of three wild tea species from Yunnan. *J. Bot. Res. Wuhan* **2001**, *19*, 39–46.
(6) Min, T. L.; Li, B. J.; Wang, C.; Long, C. L.; Zhang, W. J.; Yang, S. X.; Li, X. D.; Xu, X. K. A health tea containing abundant theophylline and its production method Chinese Patent CN 96112804.6.
(7) Yoshida, T.; Mori, K.; Hatano, T.; Okumura, T.; Uehara, I.; Komagoe, K. Studies on inhibition mechanism of autoxidation by tannins and flavonoids V. radical-scavenging effects of tannins and related polyphenols on 1,1′-diphenyl-2-picrylhydrzyl radical. *Chem. Pharm. Bull.* **1989**, *37*, 1919–1921.
(8) Masamoto, Y.; Ando, H.; Murata, Y.; Shimoishi, Y.; Tada, M.; Takahata, K. Mushroom tyrosinase inhibitory activity of esculetin isolated from seeds of *Euphorbia lathyris* L. *Biosci., Biotechnol., Biochem* **2003**, *67*, 631–634.
(9) Karioti, A.; Protopappa, A.; Megoulas, N.; Skaltsa, H. Identification of tyrosinase inhibitors from *Marrubium velutinum* and *Marrubium cylleneum*. *Bioorg. Med. Chem.* **2007**, *15*, 2708–2714.
(10) Kumar, N. S.; Rajapaksha, M. Separation of catechin constituents from five tea cultivars using high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1083*, 223–228.

(11) Barakat, H. H.; El-Mousallamy, A. M. D.; Souleman, A. M. A.; Awadalla, S. Flavonoids of *Ochradenus baccatus*. *Phytochemistry* **1991**, *30*, 3777–3779.
(12) Zhou, Z. H.; Yang, C. R. Chemical constituents of crude green tea, the material of Pu-er tea in Yunnan. *Acta Bot. Yunnan* **2000**, *22*, 343–350.
(13) Li, J. B.; Ding, Y. Studies on the chemical constituents from *Myristica yunnanensis*. *Zhongguo Zhongyao Zazhi* **2001**, *26*, 479–481.
(14) Toru, O.; Keiko, H.; Yukio, H.; Goichi, K.; Tsunematsu, T. The constituents of *Osmunda* spp. II.: a new flavonol glycoside of *Osmunda asiatica*. *Chem. Pharm. Bull.* **1978**, *26*, 3071–3074.
(15) Wang, K. J.; Yang, C. R.; Zhang, Y. J. Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toona sinensis*). *Food Chem.* **2007**, *101*, 365–371.
(16) Wenkert, E.; Gottlieb, H. E. Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds. *Phytochemistry* **1977**, *16*, 1811–1816.
(17) Addae-Mensah, I.; Achenbach, H. Terpenoids and flavonoids of *Bridelia ferruginea*. *Phytochemistry* **1985**, *24*, 1817–1819.
(18) Jiang, Z. H.; Hirose, Y.; Iwata, H.; Sakamoto, S.; Tanaka, T.; Kouno, I. Caffeoyl, coumaroyl, galloyl, and hexahydroxydiphenoyl glucoses from *Balanophora japonica*. *Chem. Pharm. Bull.* **2001**, *49*, 887–892.
(19) Nishizawa, K.; Nakata, I.; Kishida, A.; Ayer, W. A.; Browne, L. M. Some biologically active tannins of *Nuphar variegatum*. *Phytochemistry* **1990**, *29*, 2491–2494.
(20) Gao, D. F.; Zhang, Y. J.; Yang, C. R.; Chen, K. K.; Jiang, H. J. Phenolic antioxidants from green tea produced from *Camellia taliensis*. *J. Agric. Food Chem.* **2008**, *56*, 7517–7521.
(21) Chen, J.; Wang, P. S.; Xia, Y. M.; Xu, M.; Pei, S. J. Genetic diversity and differentiation of *Camellia sinensis* L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. *Genet. Resour. Crop Evol.* **2005**, *52*, 41–52.
(22) Villano, D.; Fernandez-Pachon, M. S.; Moya, M. L.; Troncoso, A. M.; Garcıa-Parrilla, M. C. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta* **2007**, *71*, 230–235.
(23) Cai, Y. Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. Structure−radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci.* **2006**, *78*, 2872–2888.
(24) Yilmaz, Y. Novel uses of catechins in foods. *Trends Food Sci. Technol.* **2006**, *17*, 64–71.
(25) Tsimogiannis, D. I.; Oreopoulou, V. The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3′,4′-hydroxy substituted members. *Innovative Food Sci. Emerging Technol.* **2006**, *7*, 140–146.
(26) Notka, F.; Meier, G. R.; Wagner, R. Inhibition of wild-type human immunodeficiency virus and reverse ranscriptase inhibitor-resistant variants by *Phyllanthus amarus*. *Antiviral Res.* **2003**, *58*, 175–186.
(27) Martinez, M. V.; Whitaker, J. R. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol.* **1995**, *6*, 195–200.
(28) Rangkadilok, N.; Sitthimonchai, S.; Worasuttayangkurn, L.; Mahidol, C.; Ruchirawat, M.; Satayavivad, J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem. Toxicol.* **2007**, *45*, 328–336.
(29) Seo, S. Y.; Sharma, V. K.; Sharma, N. Mushroom tyrosinase: recent prospects. *J. Agric. Food Chem.* **2003**, *51*, 2837–2853.

Received for review September 24, 2008. Revised manuscript received November 13, 2008. Accepted November 18, 2008. This work was supported by the State Key Laboratory of Phytochemistry and Plant Resources in West China, the Yunnan Provincial Forestry Department, and the "West Light" Program of the Chinese Academy of Sciences.

JF802974M