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Chemical Constituents and Anticancer Activity of Yellow Camellias against MDA-MB-231 Human Breast Cancer Cells

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ABSTRACT: Yellow camellia, with its golden yellow flowers, is rare in the world. Most studies of yellow camellia have focused on its ornamental properties; however, there are fewer published studies on its medical values. The purpose of this study was to define the chemical constituents and the biological potential of the water extract of leaves in six species of yellow camellia. The data showed that *Camellia murauchii* had significantly higher total catechins and total polyphenol content than others; *Camellia euphlebia* had the highest total amino acids and γ-aminobutyric acid. The results indicated that *Camellia tunghinensis* exhibited the highest free radical scavenging capacity and showed potent anticancer activities. *Camellia nitidissima* had stronger inhibitory effect than other species on fatty acid synthesis. In addition to catechins, 3-*p*-coumaroylquinic acid, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-glucoside were detected in *C. tunghinensis* using liquid chromatography−tandem mass spectrometry. Taken together, yellow camellias possess biological activity and are worthy of continued study.

KEYWORDS: *yellow camellia, catechins, free radical scavenging, anticancer effects, breast cancer*

INTRODUCTION

Camellia is a genus of flowering plants in the family Theaceae. These plants are native to eastern and southern Asia. Although camellias are acknowledged worldwide as garden plants, the range of flower colors is almost exclusively limited to red, pink, and white. The yellow petals of the flowers are rarely found in the world.[1] Until recently, 42 species and 5 variants of the yellow-flowering camellias have been found. *Camellia nitidissima* is the most well-known species of yellow camellia, and the leaves of *C. nitidissima* has been used to make tea.[2]

Over the years, several studies have demonstrated that the extract of yellow camellia leaves contains components with health beneficial effects, including flavonoids, saponins, polyphenols, amino acids, and trace elements.[3] The antioxidant properties of tea have been attributed to the polyphenolic compounds.[4] Catechins having antioxidant and anticarcinogenic activities are the main polyphenols in green tea. Song et al. recently reported the antioxidant activity and polyphenolic constituents of yellow camellia leaves extracted with acetone/ethanol/water.[5] Theanine (γ-glutamylethylamide or 5-*N*-ethylglutamine), a unique amino acid exclusively found in tea, can increase the level of the inhibitory neurotransmitter.[6] The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA). Theanine has been shown to have neuroprotective effects. In addition, theanine is the major component of sweet taste in green tea.[7]

Several studies have reported the chemical compositions of yellow camellia, but few studies have discussed their biological activities. In the present paper, we tested six species of yellow camellia. Our objectives are (1) to measure the content of caffeine, catechins, and amino acids, (2) to assess the antioxidant activity and total polyphenol content, (3) to determine whether yellow camellia can induce apoptosis in MDA-MB-231 cells, and (4) to investigate the effect of yellow camellia on fatty acid synthesis.

MATERIALS AND METHODS

Chemicals and Reagents. (−)-Epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (+)-catechin (C), (−)-gallocatechin (GC), (−)-gallocatechin gallate (GCG), (−)-catechin gallate (CG), sodium carbonate, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu's phenol reagent, gallic acid, ninhydrin, glycine, 3-(4,5-dimethylth-

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iazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Oil Red-O, antibodies for β-actin, standard amino acids, GABA, and dimethylaminoazobenzene sulfonyl chloride (dabsyl chloride) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and sodium dodecyl sulfate (SDS) were purchased from Merck (Darmstadt, Germany). Antibodies for poly(ADP-ribose) polymerase (PARP), B-cell lymphoma 2 (Bcl-2), and fatty acid synthase (FASN) were purchased from Cell Signaling Technology (Beverly, MA, USA).

Samples. Six species of fresh leaves used in this study (*Camellia murauchii*, *Camellia impressinervis*, *Camellia euphlebia*, *Camellia tunghinensis*, *Camellia nitidissima* var. *microcarpa*, and *C. nitidissima*) were provided by the Tea Flower Manor (Ping Xi, Taipei, Taiwan). Fresh leaves (1 g) of each species were placed in boiling distilled water (100 mL) for 30 min, and the infusion was filtered through a 0.45 μm polyvinylidene difluoride (PVDF) filter (Millipore, Bedford, MA, USA). The filtrate was analyzed with an HPLC system as described below. The filtrate was dried under reduced pressure using a rotavapor to afford powdered crude extract and kept in a refrigerator at −20 °C until use. In the following experiments, the extract was dissolved in DMSO (100 mg/mL) and diluted to desired concentrations.

For amino acid determination, each species of fresh leaves (1 g) was placed in 50 mL of hot distilled water (80−90 °C) for 30 min. The extract was cooled to room temperature and filtered through a 0.45 μm PVDF filter. The solution was adjusted to pH 3 by the addition of 100 μL of 1 M HCl and then partitioned with ethyl acetate twice to get the ethyl acetate soluble fraction. The water layer was adjusted to pH 9 by the addition of 2 mL of 1 M $NaHCO_3$ and then partitioned with ethyl acetate once to get the water-soluble fraction.

Reverse-Phase HPLC Analysis of Caffeine and Catechins. The compositions of caffeine and catechins in yellow camellia leaves were analyzed by reverse-phase HPLC using a Shimadzu Class-VP system controller (Shimadzu Scientific Instruments, Columbia, MD, USA). The Shimadzu SPD-M20AVP UV−vis photodiode array (PDA) detector was used to detect the constituents at 280 nm, and all peaks were plotted and integrated by the Shimadzu Class-VP 10.1 Chromatography Workstation system data module. The HPLC set consisted of a 250 × 4.6 mm i.d., 7 μm, TSK ODS-100S packed column (Tosoh, Tokyo, Japan). The extract of yellow camellia leaves was filtered through a 0.45 μm filter disk and then injected into the column. The concentrations of caffeine and tea polyphenol working solutions were 100 μg/mL. Each authentic standard compound (500 ng) (caffeine and catechins) was injected. Briefly, the mobile phase consists of a mix of methanol/water/formic acid (19.5:82.5:0.3) at a flow rate of 1 mL/min. Identification of caffeine or individual catechins was based on comparison of the retention times of unknown peaks to those of reference authentic standards. The amounts of each constituent in the yellow camellia leaves extract were estimated by the integrated datum provided by the Waters data module.

Amino Acid Dabsylation and HPLC Quantitation. The method for the determination of amino acids was conducted as described by Syu et al.[8] The 22-compound calibration mixture used for this study was prepared by adding GABA and theanine to a commercial 20-amino acid calibration mixture and diluted with the dabsylation buffer to achieve a final concentration of 1 mg/mL. The amino acid solution (1 mg/mL) was adjusted to pH 9 by adding 1 M NaOH. The solution was added to 1 mL of dabsyl chloride (1 mg/mL, in acetone) and incubated at 67 °C for 10 min. The mixture was adjusted to pH 9 by the addition of 1 M $NaHCO_3$. The reaction was stopped by cooling water, and then the sample was filtered through a 0.45 μm PVDF filter. Amino acids were analyzed by a HPLC gradient system using a Waters 600E system controller and a Jasco UV−visible 975 detector setting the wavelength at 425 nm. The dabsyl derivatives of amino acids were separated on a C18-MS column (250 × 4.6 mm i.d.; 5 μm particle size; Merck) packed by Nacalai Tesque (Kyoto, Japan). The optimized mobile phase consisted of CH_3CN/0.045 M CH_3COONa (30:70) (solvent A) and CH_3CN/0.045 M CH_3COONa (75:25) (solvent B). The gradient started with 15 min at 100% A; 5 min at 85% A/15% B; a 15 min hold at 85% A/15% B; 15 min at 75% A/25% B; 15 min at 70% A/30% B; a 10 min hold at 70%A/30% B; and 15 min at 100% A. The elution was performed at a flow rate of 0.9 mL/min using a gradient system, and the absorbance was monitored at 425 nm.

Determination of Total Polyphenol Content. Total polyphenol content was conducted as described by Isabelile et al.[9] The assay conditions were as follows: a 20 μL sample (10 mg/mL) was added to 80 μL of a 7.5% sodium carbonate solution in 96-well plates. After 3 min, 100 μL of 0.2 N Folin−Ciocalteu's phenol reagent was added to the mixture and subsequently incubated at room temperature for 30 min. The resulting absorbance of the mixture was measured at 765 nm using a microplate reader. The total polyphenol content was calculated on the basis of a standard curve using gallic acid standard solutions (5−160 ppm). Results were expressed in milligrams of gallic acid equivalent (GAE) per gram of dried extract. Experiments were carried out in triplicate.

Determination of Total Amino Acid Content. The total amino acid content was determined using the ninhydrin method. The assay conditions were as follows: a 100 μL sample (25 mg/mL) was added to a 1.5 mL eppendorf tube and mixed with 1 mL of 3.5% (w/v) ninhydrin solution. The mixtures were incubated in a heat block at 90 °C for 15 min. The total amino acid content was calculated on the basis of a standard curve determined using glycine standard solutions (37.5−375 μg/mL). Results were expressed in milligrams of glycine equivalent (GE) per gram of dried extract. Experiments were carried out in triplicate.

Determination of Antioxidant Activity by the DPPH Free Radical Scavenging Assay. The DPPH free radical scavenging assay was analyzed using the method of Dietz et al.[10] The assay conditions were as follows: a 20 μL sample (25 mg/mL) was mixed with 80 μL of a 100 mM Tris-HCl buffer at pH 7.4 and 100 μL of a 200 μM DPPH ethanol solution in 96-well plates. The mixtures were incubated at room temperature for 20 min in darkness, and then the absorbance was measured at 517 nm using a microplate reader. The percentage of DPPH removal effect was calculated by measuring the absorbance of the sample and applying the following equation: % of inhibition = $[1 - (A_s/A_0)] \times 100$, where A_s is the absorbance of sample and A_0 is the absorbance of the DPPH solution. The DPPH free radical scavenging assay was calculated on the basis of a standard curve using ascorbic acid solutions (5−100 μg/mL). Experiments were carried out in triplicate.

Cell Culture. MDA-MB-231 human breast cancer cells were obtained from Bioresource Collection and Research Center (Hsin-Chu, Taiwan) and maintained in Dulbecco's modified Eagle's medium/nutrient mixture F12 (DMEM/F12) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) and 1% penicillin−streptomycin (Invitrogen) at 37 °C under 5% CO_2. Cells were enzymatically passaged at ∼80% confluence using 0.25% trypsin−EDTA (Invitrogen).

Cell Viability. Cell viability on MDA-MB-231 cells was measured by MTT assay. The cells were dispensed in 24-well plates at a density of 2×10^4 cells/well. After overnight incubation, they were treated with various concentrations of samples at 37 °C for 48 h. Two hours before the end of incubation, 40 μL of MTT solution (2 mg/mL) was added to each well. The formazan crystals formed were dissolved in DMSO. Results were measured using a microplate reader at 590 nm. Experiments were carried out in triplicate.

Western Blot Analysis. Cells treated with various crude extracts were lysed at the time indicated. Equal amounts of cell lysates were electrophoresed on sodium dodecyl sulfate−polyacrylamide gels. The proteins were transferred to the PVDF membrane and incubated with primary antibodies recognizing FASN, PARP, Bcl-2, and β-actin. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 60 min and developed using enhanced chemiluminescence (ECL; Millipore, Billerica, MA, USA).

Oil Red-O Staining. The lipid deposits on MDA-MB-231 cells were visualized by Oil Red-O staining. The cells were dispensed in 6-well plates at a density of 1.5×10^5 cells/well. After overnight incubation, they were treated with various extracts (400 μg/mL) at 37 °C for 48 h. Cells were fixed with 10% formaldehyde for 1 h and washed twice with PBS, followed by 50% isopropanol. Then cells were added with the Oil Red-O staining working buffer (stock solution, 3

Table 1. Levels of Caffeine and Catechins in Yellow Camellia Leaves[a]

	C. murauchii	*C. impressinervis*	*C. euphlebia*	*C. tunghinensis*	*C. nitidissima* var. *microcarpa*	*C. nitidissima*
caffeine	18.02 ± 0.03	27.42 ± 0.56	24.32 ± 2.23	25.69 ± 0.35	18.62 ± 0.86	33.83 ± 5.99
EGCG	353.29 ± 4.17	9.48 ± 0.05	125.12 ± 7.57	16.49 ± 0.85	14.32 ± 3.53	15.23 ± 1.86
EGC	11.96 ± 0.48	25.79 ± 0.62	28.22 ± 3.84	41.90 ± 0.11	64.02 ± 4.49	78.84 ± 9.79
C	83.40 ± 1.01	17.97 ± 0.28	52.31 ± 7.35	ND	10.52 ± 0.48	ND
EC	26.00 ± 0.12	10.49 ± 1.16	36.41 ± 2.74	ND	41.06 ± 14.07	12.00 ± 1.93
ECG	404.47 ± 2.65	195.35 ± 0.31	23.76 ± 1.09	211.94 ± 4.28	91.37 ± 2.24	140.57 ± 60.30
GC	ND[b]	ND	ND	ND	ND	ND
GCG	39.54 ± 0.46	27.36 ± 0.42	108.86 ± 10.58	68.73 ± 14.54	26.85 ± 3.43	51.69 ± 14.84
CG	152.86 ± 0.14	59.06 ± 0.41	114.24 ± 4.79	130.48 ± 7.96	49.42 ± 3.85	64.56 ± 27.05
total catechins	1071.52	345.50	488.92	469.54	297.56	362.89

[a]Values (μg/g) = mean ± SD (n = 3). [b]ND, not detectable.

Table 2. Quantification of Amino Acid Contents in Yellow Camellia Leaves[a]

	C. murauchii	*C. impressinervis*	*C. euphlebia*	*C. tunghinensis*	*C. nitidissima* var. *microcarpa*	*C. nitidissima*
GABA	315.16 ± 113.74	270.41 ± 2.48	409.62 ± 127.02	ND[b]	257.45 ± 11.74	290.59 ± 13.54
theanine	6.88 ± 5.28	ND	ND	ND	6.97 ± 0.73	ND
cystine[c]	394.21 ± 36.48	150.73 ± 35.57	108.81 ± 6.10	612.29 ± 119.30	373.29 ± 39.95	1238.48 ± 325.03
Glu[d]	804.17 ± 32.37	596.14 ± 40.51	996.98 ± 11.33	5219.09 ± 942.35	1194.78 ± 18.69	4230.31 ± 1130.50
Gln[e]	39.17 ± 5.69	36.99 ± 1.86	81.81 ± 5.40	ND	126.73 ± 9.06	ND
Arg[f]	2995.38 ± 31.33	2521.08 ± 172.44	3139.11 ± 25.98	5685.21 ± 904.94	5320.01 ± 44.45	14364.10 ± 787.19
Ser	83.33 ± 15.98	84.63 ± 12.17	130.00 ± 12.07	1103.53 ± 212.77	ND	ND
Thr	634.91 ± 2.31	26.24 ± 3.86	648.38 ± 0.38	186.55 ± 50.24	956.27 ± 3.26	1177.88 ± 92.90
Gly	121.94 ± 1.33	81.54 ± 4.67	184.34 ± 8.09	75.18 ± 5.87	166.82 ± 2.62	61.19 ± 18.81
Lys	130.12 ± 5.55	107.80 ± 1.90	13.40 ± 17.17	ND	137.43 ± 4.89	143.58 ± 9.42
Ala	11.92 ± 2.71	35.37 ± 13.02	60.03 ± 31.21	ND	36.86 ± 3.11	332.34 ± 14.58
Pro	192.22 ± 0.28	42.20 ± 4.04	624.45 ± 178.14	ND	239.53 ± 21.70	ND
Tyr	1434.93 ± 23.32	802.59 ± 1.25	3949.19 ± 307.94	448.28 ± 92.96	3637.49 ± 119.01	202.41 ± 78.42
Met	274.29 ± 4.82	64.70 ± 5.26	1188.67 ± 441.83	ND	902.14 ± 80.97	659.02 ± 436.83
Val	148.76 ± 4.21	55.54 ± 17.26	74.47 ± 20.47	ND	345.24 ± 123.27	100.34 ± 18.38
Ile	207.24 ± 0.53	697.15 ± 18.58	2425.01 ± 258.66	ND	770.40 ± 14.88	659.02 ± 364.46
Trp	415.18 ± 20.77	294.43 ± 27.24	28.36 ± 0.98	ND	902.97 ± 5.58	1153.93 ± 74.77
Leu	82.09 ± 2.34	291.64 ± 34.04	103.60 ± 6.22	451.72 ± 37.68	2839.38 ± 104.01	4525.12 ± 239.90

[a]Values (μg/mL) = mean ± SD (n = 3). [b]ND, not detectable. [c]Peak = Phe (major) + cystine. [d]Peak = Glu (major) + Cys + Asn. [e]Peak = Gln (major) + His + OH-Pro. [f]Peak = Arg (major) + buffer solution + Asp.

mg/mL in isopropanol; working solution, 60% Oil Red-O stock solution) for 30 min and washed with PBS and 70% ethanol. Finally, cells were added with 200 μL of isopropanol to dissolve the Oil Red-O and determine the absorbance using the microplate reader at 510 nm. Experiments were carried out in triplicate.

LC-MS/MS. The extract was analyzed on an Accela liquid chromatography system coupled to a Thermo Scientific LTQ Orbitrap XL mass spectrometer (Thermo Finnigan Corp., San Jose, CA, USA). Separations were performed using a 250 × 4.6 mm i.d., 5 μm, Waters Xbridge C18 column from Waters Corp. (Milford, MA, USA). The mobile phase consisted of (A) water containing 0.5% acetic acid and (B) acetonitrile. The program for gradient elution started at 95% solvent A and decreased linearly to 70% solvent A in 100 min. In all experiments, the column was kept at room temperature, the flow rate was 0.6 mL/min, and the injection volume was 5 μL. The UV absorbance detection wavelength was set at 280 nm. The mass spectra were obtained at a mass-to-charge ratio (m/z) scan range from 150 to 2000. Sample was analyzed in negative mode. The following MS parameters were used for the analysis: spray voltage, 3.0 kV; capillary temperature, 300 °C; sheath gas flow rate, 65 arbitrary units; auxiliary gas flow rate, 15 arbitrary units. The relative collision energy was 20–30% in collision-induced dissociation (CID) mode except for the instrumental parameters used in the full-scan mode.

Statistics. All values are expressed as the mean ± standard deviation (SD). Each value is the mean of at least three separate experiments in each group. All data were analyzed with a paired t test. An asterisk indicates that the value is significantly different from that of the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

RESULTS AND DISCUSSION

Analysis of Caffeine and Catechins in Yellow Camellia Leaves. According to previous studies, the major components in tea leaves are caffeine and catechins.[11] There are eight tea catechins, including EGCG, EGC, ECG, EC, C, GC, GCG, and CG. We used our previously established isocratic HPLC method[12] to identify the content of caffeine and catechins in yellow camellia leaves. As shown in Table 1, the caffeine content of samples varied from 33.83 μg/g (*C. nitidissima*) to 18.02 μg/g (*C. murauchii*), and total catechins varied from 1071.52 μg/g (*C. murauchii*) to 297.56 μg/g (*C. nitidissima* var. *microcarpa*). These results showed that *C. murauchii* possessed the highest level of total catechins, followed by *C. euphlebia*, *C. tunghinensis*, *C. nitidissima*, *C. impressinervis*, *C. murauchii*, and *C. nitidissima* var. *microcarpa*. However, GC was not detectable in six species of yellow camellia leaves. Interestingly, similarly to green tea (*C. sinensis*), *C. murauchii* possessed higher EGCG and ECG levels. As summarized in Table 1, *C. murauchii* contained less caffeine but more catechins among six species.

Quantification of Amino Acid Contents in Yellow Camellia Leaves. To examine the composition of amino acids

Table 3. Bioactivities and Total Compound Content of Yellow Camellia Leaves

	C. murauchii	*C. impressinervis*	*C. euphlebia*	*C. tunghinensis*	*C. nitidissima* var. *microcarpa*	*C. nitidissima*
TPC (mg GAE/g)[s]	10.30 ± 0.43	9.70 ± 0.91	9.70 ± 1.06	5.87 ± 0.15	8.61 ± 0.71	8.36 ± 0.43
TAA (mg GE/g)[b]	6.11 ± 0.51	6.46 ± 24.92	11.52 ± 0.52	3.92 ± 0.37	8.27 ± 0.26	6.05 ± 0.50
DPPH (removal effect, %)[c]	42.71 ± 0.62	64.03 ± 2.46	74.61 ± 2.48	87.86 ± 2.22	67.91 ± 1.05	65.86 ± 3.55

[s]The total polyphenol content (TPC) is expressed as mg of gallic acid equivalent (GAE)/g of dried extract. [b]The total amino acids (TAA) are expressed as mg of glycine equivalent (GE)/g of dried extract. [c]The DPPH free radical scavenging assay is expressed as percentage (%) of inhibition of the DPPH radical.

in yellow camellia leaves, HPLC was performed to analyze the amino acid content. The quantitative data of amino acids are shown in Table 2. The GABA content of yellow camellia leaves varied from 409.62 μg/mL (*C. euphlebia*) to 257.45 μg/mL (*C. nitidissima* var. *microcarpa*), but it was not detected in *C. tunghinensis*. Theanine was detected only in *C. nitidissima* var. *microcarpa* (6.97 μg/mL) and *C. murauchii* (6.88 μg/mL) and not in other species of yellow camellia leaves. GABA is an important inhibitory neurotransmitter with its major activity in the mammalian central nervous system and is known to exhibit antihypertensive effects.[6] In our study, *C. euphlebia* possessed the highest content of GABA among the six species. Theanine is a unique free amino acid found exclusively in tea plants. It is a main component responsible for giving tea its sweet and umami taste and possesses some pharmacological activities. Theanine is usually used as an index for determining the characteristics and quality of tea. In our study, *C. nitidissima* var. *microcarpa* possessed the highest content of theanine among the six species.

Total Polyphenol and Amino Acid Content and Bioactivities of Yellow Camellia Leaves. To determine the total polyphenol content of yellow camellia leaves, the Folin−Ciocalteu assay was used to assess the total polyphenol using gallic acid as a standard. The total polyphenol content varied from 10.30 mg GAE/g (*C. murauchii*) to 5.87 mg GAE/g (*C. tunghinensis*) (Table 3). To determine the total amino acid of yellow camellia leaves, the ninhydrin colorimetric method was used with glycine as a standard. As depicted in Table 3, the content of total amino acids of yellow camellia leaves varied from 11.52 mg GE/g (*C. euphlebia*) to 3.92 mg GE/g (*C. tunghinensis*). To characterize the bioactivity, we next examined the antioxidant activity of yellow camellia leaves by DPPH free radical scavenging assay. Ascorbic acid was used as a standard for DPPH free radical scavenging assay. As shown in Table 3, the DPPH free radical scavenging activities were found to range from 87.86% (*C. tunghinensis*) to 42.71% (*C. murauchii*). Our results demonstrated that *C. tunghinensis* possessed the highest free radical scavenging assay among all six species.

Cytotoxicity of Yellow Camellia Leaves. The cytotoxic effect of yellow camellia leaves was first evaluated in MDA-MB-231 human breast cancer cells. Following 48 h of treatment with 100−800 μg/mL of yellow camellia leaf extracts, cell viability was measured by MTT assay. As shown in Figure 1, the extracts of yellow camellia leaves could reduce cell viability in MDA-MB-231 cells. The inhibitory rate of cell viability at 800 μg/mL ranged from ∼80% in *C. tunghinensis* to ∼25% in *C. impressinervis* and *C. nitidissima* var. *microcarpa*. The cytotoxic effect of yellow camellia leaves against MDA-MB-231 cells at 800 μg/mL was in the order *C. tunghinensis* > *C. murauchii* > *C. euphlebia* > *C. nitidissima* > *C. nitidissima* var. *microcarpa* > *C. impressinervis*.

Induction of Apoptosis by Yellow Camellia Leaves. PARP cleavage is often associated with apoptosis and has served as a hallmark of apoptosis. As shown in Figure 2, treatment with 400 μg/mL of yellow camellia leaves represented the ratio of the cleaved to proform PARP, and the relative level in control group was set at 1.00. Among six samples, *C. tunghinensis* was the best inducer in PARP cleavage. In addition, the proto-oncogene Bcl-2 is an important suppressor of apoptosis. To investigate the expression of Bcl-2 protein, Western blotting was performed to detect Bcl-2. As shown in Figure 2, treatment with 400 μg/mL yellow camellia leaves caused Bcl-2 expression to decrease significantly in *C. impressinervis*, *C. tunghinensis*, *C. nitidissima* var. *microcarpa*, and *C. nitidissima*.

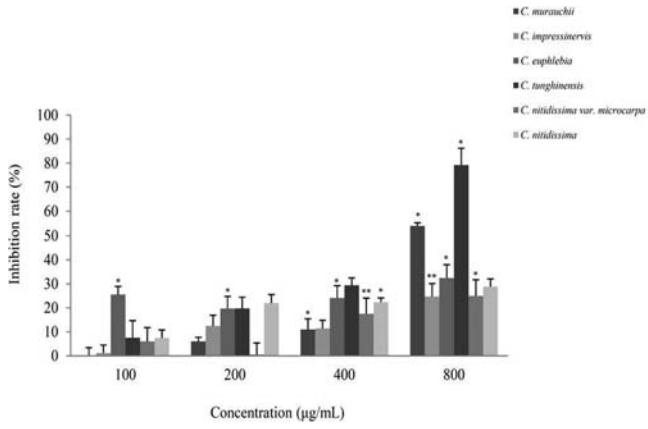

Figure 1. Proliferation inhibitory effect of different yellow camellia species on MDA-MB-231 cells. After treatment with different concentrations of yellow camellia leaves for 48 h, the effect on cell growth was examined by MTT assay. The percentage of viable cells was compared with that of the vehicle control and expressed as the mean of at least three times. Each bar represents the mean ± SD. *, $p < 0.05$ compared with the untreated group; **, $p < 0.01$ compared with the untreated group.

Inhibition of Fatty Acid Synthesis. FASN has been shown to play an important role in lipogenesis and cancer progression.[13] To elucidate the effect of yellow camellia leaves on fatty acid biosynthesis, Western blot analysis and Oil Red-O staining were performed. Our result showed that treatment with 400 μg/mL of yellow camellia leaves, *C. nitidissima*, had the best inhibitory effect on FASN (Figure 3A). Lipid accumulation was assessed by Oil Red-O staining. As shown in Figure 3B, *C. nitidissima* was shown to have a better inhibitory effect than the others. Blockade of FASN expression attenuates tumor cell growth and proliferation. FASN inhibition may be related to the end-product starvation, disturbance of membrane function, inhibition of DNA replication, p53-regulated nongenotoxic metabolic stress, toxic accumulation of malonyl-coenzyme A, and inhibition of antiapoptotic proteins.

Profile of Constituents in Yellow Camellia Leaves. The bioactivity of tea has been attributed to catechin compounds;

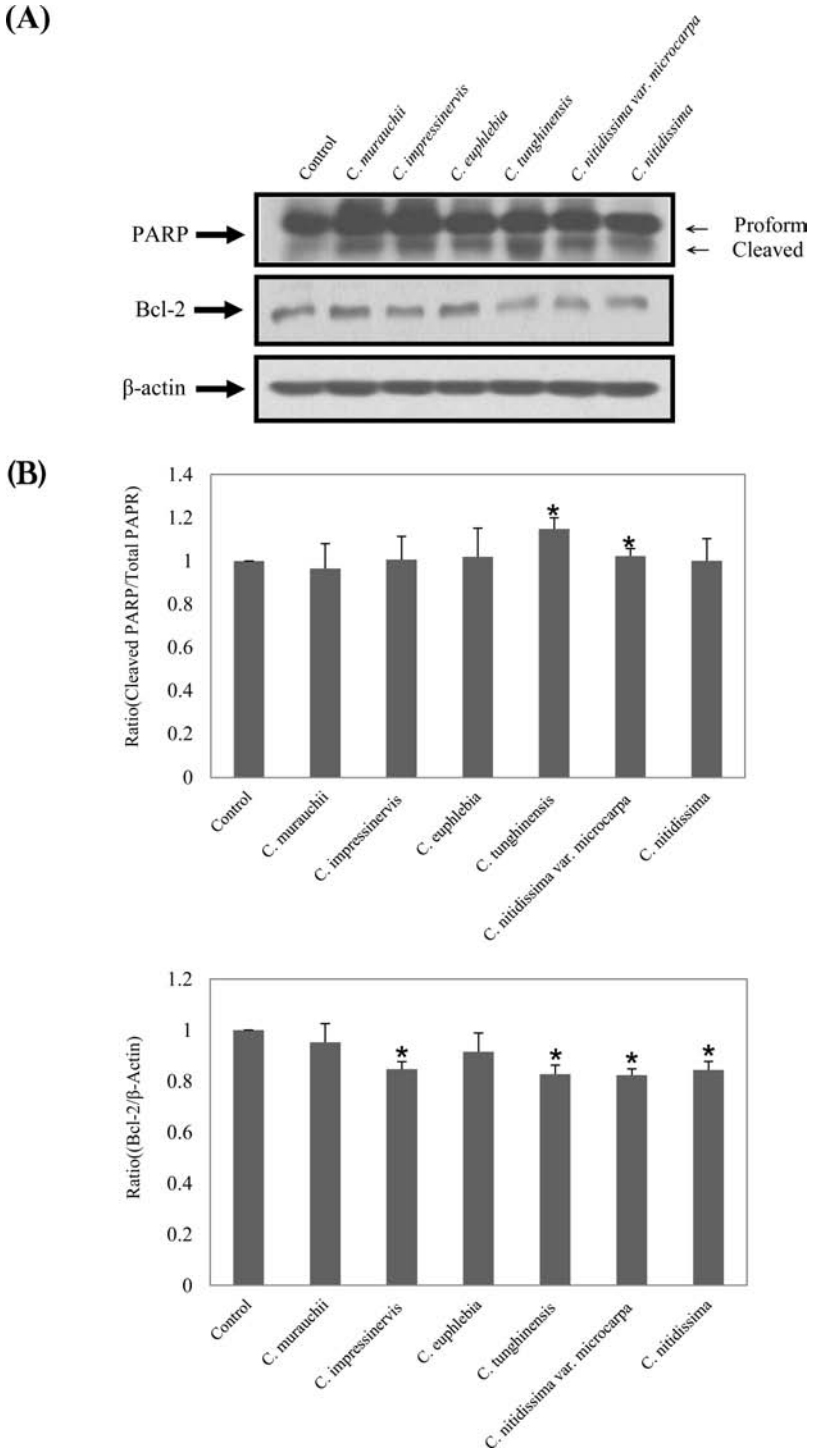

Figure 2. Effect of yellow camellia leaves on the cleaved PARP and Bcl-2 in MDA-MB-231 cells. (A) Cells were treated with 400 μg/mL of yellow camellia leaves for 48 h, and levels of PARP cleavage and Bcl-2 were analyzed by Western blot. β-Actin was used as the loading control. (B) Western blot data shown are representative of those obtained in at least three separate experiments. Relative expression levels were determined by calculating the fold change in yellow camellia-treated cells versus untreated cells. Each bar represents the mean ± SD. *, $p < 0.05$ compared with the untreated group.

however, *C. tunghinensis* does not contain high catechin content. It is likely that bioactive molecules other than catechins existed in *C. tunghinensis* to lead to its high cytotoxicity. On the basis of these findings, we next examined the chemical constituents of *C. tunghinensis*. To investigate the chemical constituents of *C. tunghinensis*, LC-MS/MS analysis was performed. The chemical constituents were identified by comparing retention times and mass fragmentation patterns with a previous study.[14] The peaks were determined as 3-*p*-coumaroylquinic acid, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-glucoside in *C. tunghinensis*. You et al. indicated that quercetin-3-*O*-glucoside showed antiproliferative effect in cancer cell lines.[15]

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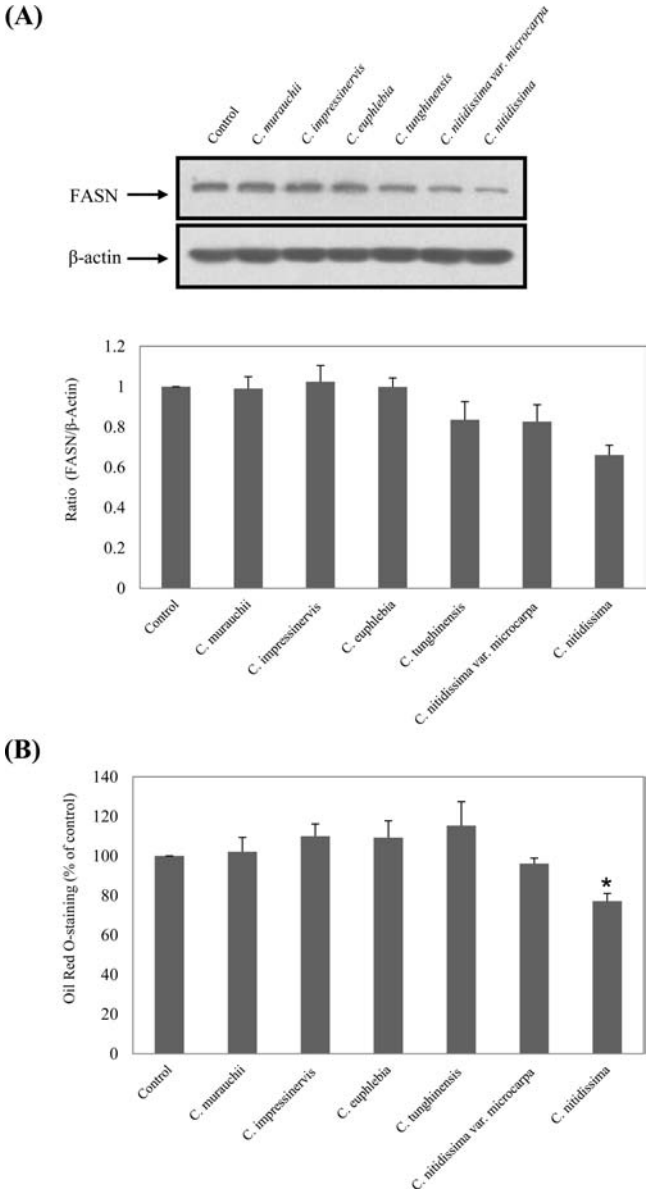

Figure 3. Effect of yellow camellia leaves on lipogenesis in MDA-MB-231 cells. (A) Cells were treated with 400 μg/mL of yellow camellia leaves for 48 h, and FASN expression was analyzed by Western blot. β-Actin was used as the loading control. Western blot data shown are representative of those obtained in at least three separate experiments. Relative expression levels were determined by calculating the fold change in yellow camellia-treated cells versus untreated cells. Each bar represents the mean ± SD. (B) To measure the total lipids, cells were treated with 400 μg/mL yellow camellia leaves for 48 h. The total lipid content was measured by Oil Red-O staining. Data shown are representative of those obtained in at least three separate experiments. Relative expression levels were determined by calculating the fold change in yellow camellia-treated cells versus untreated cells. Each bar represents the mean ± SD. *, $p < 0.05$ compared with the untreated group.

In this study, we tested the constituents and bioactivity in ellow camellia leaves. These results indicate that *C. murauchii* has the highest level of total catechins and total polyphenol content and lowest level of caffeine. The high amounts of GABA and total amino acids are present in *C. euphlebia*. *C. nitidissima* possesses stronger fatty acid synthesis inhibition than the other species. *C. tunghinensis* possesses highly potent cytotoxic activity and cleaved PARP production in MDA-MB-231 cells. The chemical constituents of *C. tunghinensis* are characterized as 3-*p*-coumaroylquinic acid, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-glucoside by LC-MS/MS analysis.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

GABA, γ-aminobutyric acid; LC-MS/MS, liquid chromatography−tandem mass spectrometry; HPLC, high-performance liquid chromatography; EGCG, (−)-epigallocatechin 3-gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; C, (+)-catechin; GC, (−)-gallocatechin; GCG, (−)-gallocatechin; CG, (−)-catechin gallate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PARP, poly(ADP-ribose) polymerase; Bcl-2, B-cell lymphoma 2; FASN, fatty acid synthase; PVDF, polyvinylidene difluoride; GAE, gallic acid equivalent; GE, glycine equivalent; DMEM/F12, Dulbecco's modified Eagle's medium/nutrient mixture F12; FBS, fetal bovine serum; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; ECL, enhanced chemiluminescence

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