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Determination of Tea Polyphenols and Caffeine in Tea Flowers (*Camellia sinensis*) and Their Hydroxyl Radical Scavenging and Nitric Oxide Suppressing Effects

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The native occurrence of tea polyphenols, namely, (−)-epicatechin, (+)-catechin, (−)-epigallocatechin 3-gallate, (−)-epicatechin, and (−)-epicatechin 3-gallate, and caffeine in tea flowers was assessed by an isocratic HPLC procedure. The levels of total catechins and caffeine were determined in tea flowers collected from 10 different species of *Camellia sinensis*. The results showed the levels of total catechin ranged from 10 to 38 mg/g, whereas the level of caffeine ranged from 3 to 8 mg/g. Levels of catechins and caffeine in tea leaves and various teas were also determined and ranged from 2 to 126 mg/g and from 23 to 49 mg/g, respectively. Both tea flower and tea leaf extracts exert their strong hydroxyl radical scavenging effects in the Fenton reaction system and nitric oxide suppressing effects in LPS-induced RAW 264.7 cells. Most tea flowers contain less caffeine, but comparable amounts of total catechins, compared to tea leaves and teas. The present study demonstrates that both tea flowers and tea leaves contain appreciable amounts of catechins and caffeine. It is likely that tea flowers might be useful for making alternative tea beverages.

KEYWORDS: Tea flowers; tea leaves; catechins; tea polyphenols; caffeine; hydroxyl radical scavenging; nitric oxide suppression

INTRODUCTION

Tea is one of the most widely consumed beverages in the world. During the past decade numerous in vitro and in vivo studies have suggested the possible protective effects of tea and tea polyphenols in cancer and neurodegenerative and cardiovascular disease development (*1*−*3*). The molecular mechanisms of cancer chemoprevention by tea and tea polyphenols in animals and man have been intensively investigated (*2*, *4*−*11*). It has been demonstrated that the extracellular signals for cell proliferation have been suppressed by tea polyphenols through down-regulation of EGF-receptor signaling (*2*, *4*, *5*). The prooxidant enzymes nitric oxide synthase and xanthine oxidase have been shown to be strongly inhibited by tea polyphenols (*6*−*8*). Suppression of lipopolysaccharide-induced NFκB activity by tea polyphenols through down-regulation of IKK activity in macrophages has been demonstrated (*9*). Furthermore, induction of apoptosis by oolong tea polyphenol theasinensin A through cytochrome *c* release and activation of caspases-9 and -3 in human U-937 cells has been observed (*10*). It is proposed that cancer chemoprevention by tea polyphenols may occur through modulation of signal transduction pathways (*2*, *3*, *11*).

Although dried tea has been exported from China for at least five centuries, it was not until the early 19th century that its cultivation spread to other parts of Asia. An apparently wild tea with larger leaves was discovered in Assam, and eventually all Indian plantations were planted with this "Assam tea" (*Camellia sinensis* var. assamica). It is now thought that the species originated somewhere in southern China. It is also possible that Assam tea is of hybrid origin, with *Camellia irrawadiensis* one possible parent.

There are several kinds of flower teas in the commercial markets, namely, jasmine, cinnamon, rose, lotus, and daisy (*chrysanthemum*) flower teas. Jasmine flower tea (a green tea) is a very popular tea and widely consumed in China, especially in northern China. The aroma and fragrance of jasmine flower are mild and lasting. The flowered teas are made from tea leaves (*Camellia sinensis*) processed with different species of flowers. However, a commercial drinking beverage from tea leaves with tea flowers has never been made. It is important to determine whether tea flowers are suitable for making a consumable beverage. To answer this question, we have collected tea flowers from tea plants and prepared their extracts with hot water. The flavor of the extracts is similar to that of daisy flower tea, and a pleasant bitter taste is persistent in the mouth after drinking. We have performed a series of experiments to analyze the chemical composition of tea flowers. We now report the high-performance liquid chromatographic (HPLC) determination of

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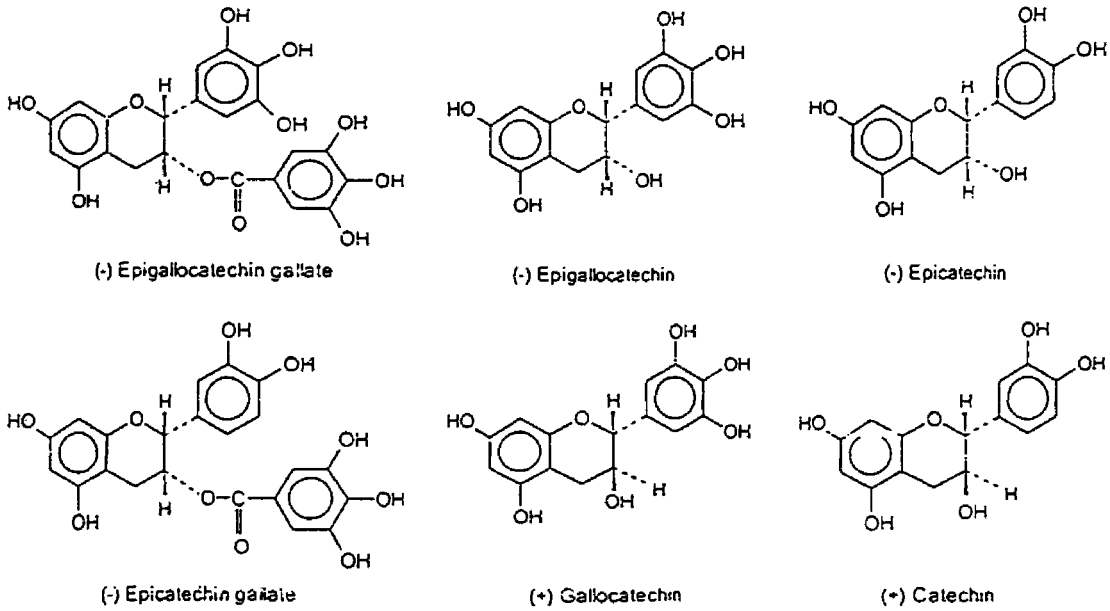

Figure 1. Chemical structures of tea catechins.

tea polyphenols and caffeine in tea flowers as well as the hydroxyl radical scavenging and nitric oxide suppressing effects of the tea flower extracts.

Numerous HPLC methods for the determination of tea polyphenols and methylxanthines have been published (*12*−*14*). In most studies the tea polyphenols and tea methylxanthines are determined separately by HPLC (*14*) or other nonchromatographic methods. Because both tea polyphenols (catechins) and tea methylxanthines (caffeine, theophylline, and theobromine) have been demonstrated to exert their significant healthy effects in humans, we have developed a reliable and rapid HPLC method for the simultaneous determination of the levels of these two constituents in a given tea sample (*12*, *13*). In this study, we have adapted these procedures with slight modifications and developed a simple and precise isocratic HPLC method for the determination of tea polyphenols and caffeine in various tea samples.

MATERIALS AND METHODS

Chemicals and Reagents. (−)-Epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin 3-gallate (ECG), (−)-gallocatechin 3-gallate (GCG) (chemical structures of these tea catechins are depicted in **Figure 1**), caffeine, lipopolysaccharide (LPS, *Escherichia coli* 0127:E8), sulfanilamide, dithiothreitol, and naphthylethylenediamine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Agarose, ethidium bromide, dimethyl sulfoxide (DMSO), hydrogen peroxide, and ferrous sulfate were purchased from E. Merck Co. (Darmstadt, Germany). The plasmid pcDNA 3, 1-E expression vector was purchased from the Invitrogen Life Technologies, Carlsbad, CA.

Collection of Tea Flowers. Fresh tea flowers from 10 species were plucked from the Taitung Tea Experiment Substation (Taitung, Taiwan). The collected tea flowers were dried at 70 °C overnight in an electric oven with a rotating fan to keep the heat evenly distributed. The weight of the tea flowers was checked from time to time until a constant weight was reached.

In this study, 10 species of tea flowers were analyzed by HPLC for their tea polyphenols and caffeine. Three species, namely, Wuu-Yi, Huang-Gan, and Dayeh-Oolong, are local varieties, whereas another seven species, TTES 1−6 and 12, are hybrid varieties established by the Taiwan Tea Experiment Station (TTES) (*15*).

Preparation of Extracts from Tea Leaves and Tea Flowers. Each of the dry tea leaves or tea flowers (1 g) was steeped in boiling distilled water (100 mL) for 30 min or in 75% ethanol (100 mL) at 60 °C for 30 min. The infusion was filtered with a 0.45 μm PVDF filter disk (Millipore, Bedford, MA). The filtrate was analyzed with the HPLC system as described below.

The filtrate was dried under reduced pressure by rotavapor to give a powdered crude extract and kept in a refrigerator at −20 °C until use. In most experiments, the crude extract was dissolved in DMSO (50 mg/mL) and diluted to desired concentrations.

Reverse-Phase HPLC Analysis of Tea Polyphenols and Caffeine. The compositions of tea polyphenols (catechins) and caffeine in different samples (from tea flowers or tea leaves) were determined by HPLC analysis using a Waters 600E system controller. The HPLC method used a 250 × 4.6 mm i.d., 5 μm Cosmosil 5C18-MS packed column (Nacalei Tesque, Inc., Kyoto, Japan). The tea or tea flower extract was filtered through a 0.45 μm filter disk and then was injected onto the column. The concentrations of caffeine and tea polyphenol working solutions were 100 μg/mL. Five hundred nanograms of each authentic standard compound [caffeine, (−)-epigallocatechin 3-gallate, (−)-epigallocatechin, catechin, (−)-epicatechin, (−)-epicatechin 3-gallate, and (−)-gallocatechin 3-gallate] was injected. The mobile phase was methanol/doubly distilled water/formic acid (19.5:82.5:0.3, v/v/v) degassed by sonication (Branson 5200), with isocratic elution at a flow rate of 1.0 mL/min. A Waters 484 tunable absorbance detector was used to detect tea constituents at 280 nm, and all peaks were plotted and integrated by a Waters 745 data module. Identification of caffeine or individual tea polyphenols was based on the comparison of the retention times of unknown peaks to those of authentic reference standards. The amount of each constituent in the tea leaf or tea flower extract was estimated by the integrated datum provided by the Waters data module.

Protection of Supercoiled DNA from Strand Breakage by Fenton Reaction. pcDNA-3 superhelix form plasmid DNA (200 ng) was incubated with 0.35% H_2O_2 and 50 μM ferrous sulfate in the presence or absence various concentrations of crude extract of tea or tea flower at 37 °C for 30 min. DNA relaxation to an open circular form was induced by the hydroxyl radicals generated by the Fenton reaction (H_2O_2 and Fe^{2+}). DNA was separated on 1% agarose gel and stained with ethidium bromide (*16*). The percentage of supercoiled forms of DNA among total DNA was calculated using a densitometer (IS-100 Digital Imaging System) and expressed as the ratio of supercoiled forms plasmid DNA to total plasmid DNA.

Cell Culture. RAW 264.7 cells, which were derived from murine macrophages, were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). RAW 264.7 cells were cultured in DMED (without phenol red) supplemented with 10% endotoxin-free,

Table 1. Polyphenol Composition of Various Fresh Tea Flowers in Water or 75% Ethanol Extract[a]

	mg/g of flower													
	caffeine		EGCG		EGC		C		EC		ECG		total catechins	
	water	ethanol	water	ethanol	water	ethanol	water	ethanol	water	ethanol	water	ethanol	water	ethanol
TTES 1	3.48 ± 0.14	7.04 ± 0.24	3.89 ± 0.43	10.08 ± 0.58	2.54 ± 0.44	11.93 ± 1.28	0.47 ± 0.08	ND[b]	1.00 ± 0.06	1.22 ± 0.15	2.96 ± 0.30	5.68 ± 0.67	10.87	28.91
TTES 2	5.71 ± 0.57	8.32 ± 0.27	6.73 ± 0.23	9.41 ± 1.20	9.82 ± 3.16	19.57 ± 3.01	1.15 ± 0.29	0.95 ± 0.06	2.98 ± 0.09	2.00 ± 0.42	5.08 ± 0.54	6.26 ± 0.66	25.76	38.19
TTES 3	4.33 ± 0.35	6.73 ± 0.35	5.51 ± 0.43	7.29 ± 0.96	12.98 ± 3.95	16.60 ± 12.9	0.12 ± 0.01	ND	0.70 ± 0.01	0.38 ± 0.02	3.30 ± 0.39	4.00 ± 0.40	22.62	28.27
TTES 4	5.20 ± 0.23	6.31 ± 0.12	5.93 ± 0.18	6.50 ± 0.85	8.40 ± 2.24	10.50 ± 1.71	0.63 ± 0.18	ND	1.46 ± 0.13	0.68 ± 0.05	3.64 ± 0.19	3.94 ± 0.08	20.06	21.62
TTES 5	6.16 ± 0.20	7.35 ± 0.12	6.45 ± 0.14	5.61 ± 0.47	12.41 ± 0.80	7.61 ± 1.42	3.13 ± 0.45	0.30 ± 0.03	4.06 ± 0.07	2.42 ± 0.18	5.38 ± 0.26	4.50 ± 0.08	31.43	20.45
TTES 6	6.35 ± 0.28	6.75 ± 0.20	6.83 ± 0.76	5.23 ± 0.61	16.53 ± 4.86	9.11 ± 3.34	1.08 ± 0.20	0.28 ± 0.09	3.22 ± 0.16	1.83 ± 0.52	5.27 ± 0.20	4.24 ± 0.34	32.93	20.69
TTES 12	5.56 ± 0.35	7.11 ± 0.15	4.77 ± 0.43	6.95 ± 1.45	8.34 ± 3.35	12.47 ± 0.87	0.55 ± 0.19	2.03 ± 0.36	1.22 ± 0.04	0.99 ± 0.38	3.86 ± 0.32	4.06 ± 0.33	18.74	26.49
Wuu-Yi	5.85 ± 0.45	6.83 ± 0.24	7.08 ± 0.46	7.01 ± 1.02	9.78 ± 6.21	17.01 ± 2.64	1.12 ± 0.17	0.64 ± 0.21	2.21 ± 0.21	1.58 ± 0.10	7.11 ± 0.78	6.61 ± 0.41	27.29	32.86
Huang-Gan	5.12 ± 0.16	6.95 ± 0.36	5.20 ± 0.34	7.40 ± 1.56	12.48 ± 2.01	13.70 ± 4.96	1.66 ± 0.46	1.30 ± 0.63	2.41 ± 0.07	2.00 ± 0.33	4.55 ± 0.15	4.65 ± 0.18	26.30	29.04
Da-Yeh oolong	5.50 ± 0.13	6.82 ± 0.39	7.42 ± 0.18	7.73 ± 0.24	16.22 ± 3.61	12.65 ± 6.21	1.38 ± 0.43	0.60 ± 0.41	3.28 ± 0.16	2.09 ± 0.21	5.67 ± 0.21	5.04 ± 0.49	33.98	28.12

[a] Each of the tea leaves (1 g) was extracted by 100 mL of boiling water or 75% ethanol at 60 °C for 30 min. Each value represents the mean ± SE of five individual determinations. [b] ND, not detectable.

heat-inactivated fetal calf serum (GIBCO, Grand Island, NY). When the cells reached a density of (2−3) × 10^6 cells/mL, they were activated by incubation in medium containing *E. coli* 0127:E8 LPS (50 ngmL). Various test crude extracts dissolved in DMSO were added together with lipopolysaccharide at a final concentration of 50 μg/mL or indicated concentration.

HL-60 (human promyelocytic leukemia) cells obtained from ATCC were grown in 90% RPMI 1640 and 10% fetal bovine serum (Gibco, BRL) supplemented with 2 mM glutamine. The medium was normally changed to phenol red-free RPMI 1640 before tea or tea flower crude extract treatment.

Nitrite Determination. The nitrite concentration in the cultured RAW264.7 cells medium was measured as an indicator of NO production, according to the Griess reaction (*17*). One hundred microliters of each supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water). Absorbance of the mixture at 550 nm was determined with a Dynatech MR-7000 enzyme-linked immunosorbant assay plate reader (Dynatech Labs, Chantilly, VA).

RESULTS

HPLC Analysis of Authentic Standard Tea Polyphenols and Caffeine. A mixture of authentic standard tea catechins including (−)-epigallocatechin, catechin, (−)-epigallocatechin 3-gallate, (−)-epicatechin, (−)-gallocatechin 3-gallate, and (−)-epicatechin 3-gallate as well as caffeine was analyzed by isocratic HPLC. The structures of six catechins are shown in **Figure 1**. The amount of each tea constituent was 0.5 μg except that of (−)-epigallocatechin (2 μg). A representative HPLC chromatogram is illustrated in **Figure 2A**. Complete baseline separation of these catechins and caffeine was achieved by this HPLC procedure in 80 min. It has been demonstrated that (−)-gallocatechin 3-gallate is an artifact produced during the chemical handling of the catechin mixture. Therefore, (−)-gallocatechin 3-gallate is absent from most freshly and naturally extracted tea samples.

HPLC Analysis of Tea Polyphenols and Caffeine in Tea Flowers. The levels of tea polyphenols and caffeine in tea flowers were analyzed from 10 different tea varieties, and the data are summarized in **Table 1**.The chemical compositions of tea polyphenols and caffeine in tea flowers were analyzed by the HPLC procedure described. The tea flowers (1 g) were extracted by boiling water (100 mL) or 75% ethanol (100 mL) at 60 °C for 30 min. The HPLC chromatograms for these two extracts are very similar, as illustrated in parts B and C, respectively, of **Figure 2**. Furthermore, the HPLC chromatogram

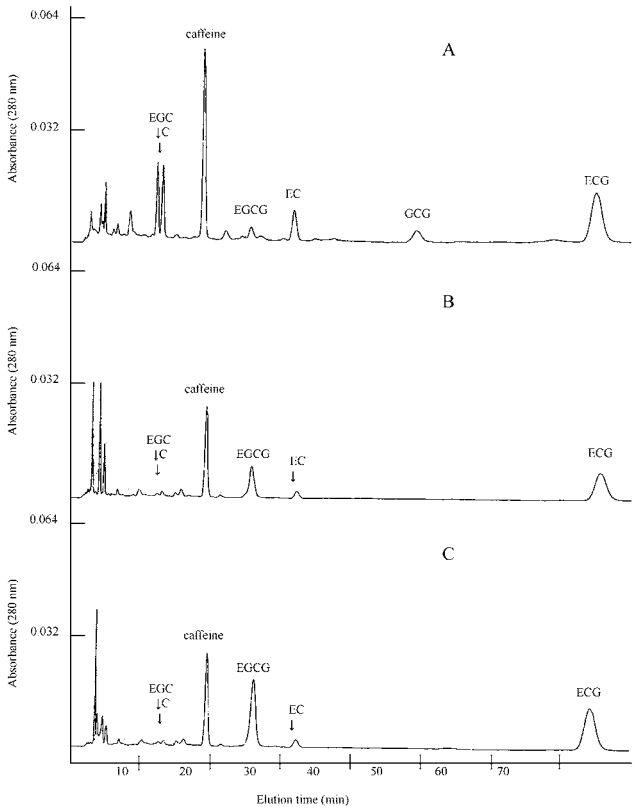

Figure 2. Isocratic HPLC separation of tea catechins and caffeine: (A) mixture of authentic standard compounds, 2 μg of EGC, and 0.5 μg each of other compounds; (B) water extract of tea flower (Wuu-Yi species); (C) 75% ethanol extract of tea flower (Wuu-Yi species). Abbreviations of tea catechins are given under Materials and Methods.

of tea flower extract is very similar to that of tea leaf extracts (data not shown). Qualitatively, the compositions of tea polyphenols and caffeine in tea flowers are similar to that of tea leaves, but quantitatively, tea flowers contain far less caffeine and (−)-epigallocatechin 3-gallate and more (−)-epicatechin 3-gallate in some cases (**Tables 1** and **2**).

Levels of Tea Polyphenols and Caffeine in Tea Flowers. For comparison purposes, the levels of tea polyphenols and caffeine in nine kinds of tea leaves were also analyzed and are summarized in **Table 2**. Among the tea flower extracts, the water extracts gave lower levels of caffeine and (−)-epigallocatechin 3-gallate as compared with 75% ethanol extracts (**Table 1**). Levels of (−)-epigallocatechin, catechin, (−)-epicatechin,

Table 2. Polyphenol Composition in 75% Ethanol Extract of Various Tea Leaves

		mg/g of tea						
		caffeine	EGCG	EGC	C	EC	ECG	total catecins
fresh tea leaves	TTES 8	26.81 ± 2.04	21.19 ± 2.42	36.21 ± 10.4	0.72 ± 0.18	1.11 ± 0.22	5.36 ± 0.61	64.59
	TTES 12	23.40 ± 1.21	4.33 ± 0.48	17.58 ± 4.17	1.77 ± 0.41	0.29 ± 0.09	1.51 ± 0.17	25.48
	wild type	41.36 ± 0.56	14.20 ± 3.41	ND[b]	ND	0.27 ± 0.09	5.54 ± 0.43	20.01
	av	**30.52**	**13.24**	**17.93**	**0.83**	**0.56**	**4.14**	**36.72**
green tea	TTES 12	26.74 ± 1.70	30.12 ± 5.36	83.19 ± 15.1	1.51 ± 0.27	4.21 ± 0.84	4.46 ± 0.62	123.49
	wild type	47.50 ± 1.45	59.03 ± 5.00	44.13 ± 10.8	0.87 ± 0.28	4.72 ± 0.77	20.00 ± 2.61	128.75
	av	**37.12**	**44.58**	**63.66**	**1.19**	**4.47**	**12.23**	**126.13**
oolong tea	TTES 12	35.26 ± 0.86	27.75 ± 2.35	39.87 ± 6.89	0.68 ± 0.10	3.64 ± 0.25	5.94 ± 0.62	77.88
	wild type	49.14 ± 1.20	65.15 ± 4.45	31.20 ± 7.70	0.55 ± 0.14	4.23 ± 0.45	19.30 ± 1.53	120.43
	av	**42.2**	**46.45**	**35.54**	**0.62**	**3.94**	**12.62**	**99.16**
black tea	TTES 8	42.74 ± 0.55	5.01 ± 0.67	ND	0.41 ± 0.00	0.64 ± 0.04	3.02 ± 0.21	9.08
	wild type	49.79 ± 4.11	1.31 ± 1.24	ND	ND	0.16 ± 0.08	1.51 ± 0.37	2.98
	av	**46.27**	**3.16**	**ND**	**0.21**	**0.40**	**2.27**	**6.03**

[a] Each of the tea leaves (1 g) was extracted by 100 mL of 75% ethanol at 60 °C for 30 min. Each value represents the mean ± SE of five individual determinations. [b] ND, not detectable.

and (−)-epicatechin 3-gallate in tea flowers were quite comparable in both extracts.

The total tea polyphenols (catechins) in tea flowers varied greatly from species to species. The total catechins in tea flower water extracts were found to range from 33 mg/g in TTES 6 to 11 mg/g in TTES 1. Meanwhile, the total catechins in tea flower 75% ethanol extracts were found to range from 39 mg/g in TTES 2 to 21 mg/g in TTES 5 (**Table 1**). The total catechins in fresh tea leaves and different manufactured teas (green, oolong, and black teas) as estimated from their 75% ethanol extracts are shown in **Table 2**. Three tea varieties, namely, TTES 8 (large leaves), TTES 12 (small leaves), and a wild local species (large leaves), were used as fresh starting tea leaves for making green, oolong, and black teas. It appeared that green tea provided more total catechins in TTES 12 (123 mg/g) or wild species (129 mg/g). Oolong tea also provided high levels of total catechins in TTES 12 or wild species (120 mg/g), whereas black tea contained very low levels of total catechins in TTES 8 (∼9 mg/g) or wild species (∼2 mg/g). It is interesting to note that the freshly plucked tea leaves contained a moderate amount of total catechins in TTES 12 (25 mg/g) or wild species (20 mg/g). The levels of total catechins in freshly plucked tea flowers (11−39 mg/g; as indicated in **Table 1**) are comparable with that in freshly plucked tea leaves (20−25 mg/g) as indicated in **Table 2**.

Hydroxyl Radical Scavenging Effects of Tea Flowers. The antioxidant effects of tea flower extracts were evaluated by the Fenton reaction system. The hydroxyl radical-induced DNA damage was significantly inhibited by the presence of tea flower extracts as shown in **Figure 3A**,**B**. Treatment of pcDNA-3 plasmid with Fenton reagent (hydrogen peroxide and ferrous sulfate) relaxed the supercoiled form DNA concentration- and time-dependently (data not shown). However, on cotreatment of plasmid DNA and tea flower extracts, the latter provided a protective effect on the damage of plasmid DNA in a concentration-dependent manner (**Figure 3A**). The arbitrary values coming from the densitometric analysis represent the ratio of supercoiled forms of plasmid DNA to relaxed forms plasmid DNA, and the relative level was calculated as the ratio of supercoiled/relaxed observed relative to the control group (**Figure 3B**). The potency of the hydroxyl radical scavenging effect of tea flower extract is stronger than that of vitamin E and 75% ethanol extract of fresh tea leaf extract. It appeared that the potency of tea flower extracts is lower than that of water extracts of fresh tea leaves (**Figure 3B**).

Suppressing Effects of Tea Flowers on the LPS-Induced NO Production in Macrophages. Treatment of RAW 264.7 cells with LPS for 16 h produced nitric oxide (NO) in the culture medium (**Table 3**). The production of NO in this cell culture was strongly inhibited by the presence of green, oolong, black, pu-erh, and fresh tea leaf extracts. It seemed that more inhibitory substances were found to be present in the 75% ethanol extracts (**Table 3**). It has been demonstrated that (−)-epigallocatechin 3-gallate in green tea and theaflavins in black tea suppress the NO production in the LPS-activated macrophages (*8*−*10*). An appreciable amount of this NO inhibitory substance was alsofound in the tea flower extracts. The chemical properties of this inhibitory substance in tea flowers are worthy of further investigation.

Induction of Apoptosis by Tea Extracts but Not by Tea Flower Extracts. In a preliminary experiment, the induction of apoptosis in HL-60 cells by tea and tea flower extracts was performed. The results indicated that both oolong and green tea extracts induced strong apoptosis in HL-60 cells, whereas black tea extracts induced moderate apoptosis at the same concentration. Pu-erh tea extract was inactive in this experiment. Old fresh tea leaf extracts showed strong apoptotic induction in HL-60 cells, whereas no apoptotic effect was observed in young tea leaf extracts. It is also noteworthy that the tea flower extracts (either from water extraction or from 75% ethanol extraction) showed no apoptotic effects in HL-60 cells under the same experimental conditions.

DISCUSSION

The isocratic HPLC system successfully separated the five tea catechins in the tea flower extracts (**Figure 2**). Some flavonoids, such as quercetin, quercetin glycosides, rutin, apigenin, genistein, genistin, hesperetin, hesperidin, myricetin, kaempferol, and others, could not be resolved by this system. Another gradient elution HPLC system has been developed for these flavonoids. Further studies on the flavonoid composition of tea flowers are now in progress in our laboratory.

Both green and oolong teas were manufactured from freshly plucked tea leaves. The total catechin contents of green and oolong teas were higher than that of fresh tea leaves as indicated in **Table 2**. It appears that the catechin biosynthesis has

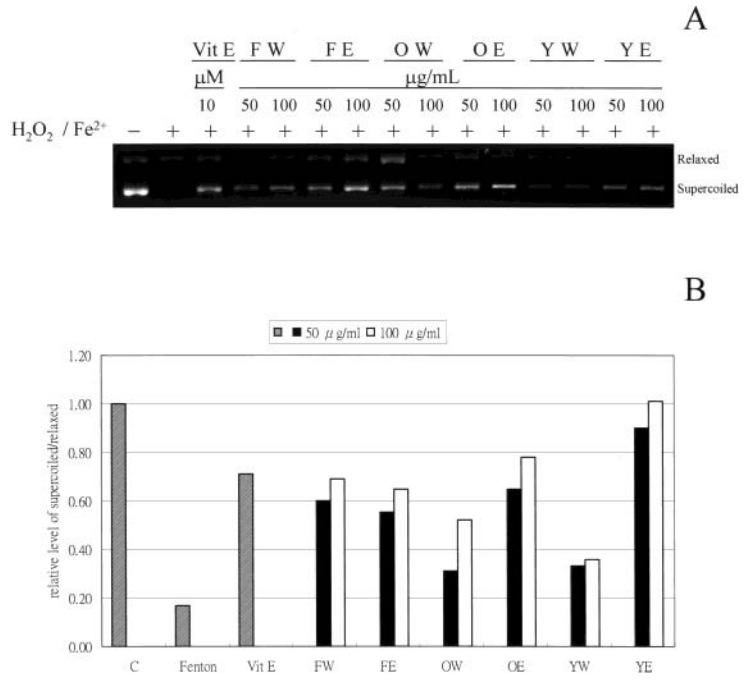

Figure 3. Hydroxyl radical scavenging effects of tea flowers and tea leaves: (A) protection of plasmid DNA damage by different tea and tea flower extracts (50 and 100 μg/mL, respectively); (B) relative level calculated as the ratio of supercoiled to relaxed forms coming from densitometric analysis. The ratio of supercoiled/relaxed observed in the control group is set at 1.00. Abbreviations: C, blank control system; Fenton, Fenton reaction mixture containing plasmid DNA as positive control; Vit E, vitamin E; FW, tea flower, water extract; FE, tea flower, 75% ethanol extract; OW, old tea leaves, water extract; OE, old tea leaves, 75% ethanol extract; YW, young tea leaves, water extract; YE, young tea leaves, 75% ethanol extract.

Table 3. Effects of Tea and Tea Flower Extracts on Suppression of Nitric Oxide Production in the Culture Medium of LPS-Stimulated Macrophage RAW 264.7 Cells

treatment	nitric oxide (nM)
control	0
LPS	43
LPS + black tea (W)	2
LPS + black tea (E)	0
LPS + oolong tea (W)	30
LPS + oolong tea (E)	4
LPS + pu-erh tea (W)	1
LPS + pu-erh tea (E)	0
LPS + green tea (W)	17
LPS + green tea (E)	0
LPS + tea flower (W)	27
LPS + tea flower (E)	8
LPS + old fresh tea leaves	3
LPS + old fresh tea leaves (E)	0
LPS + young fresh tea leaves (W)	27
LPS + young fresh tea leaves (E)	0

[a] The content of nitric oxide was determined as described under Materials and Methods. Abbreviations: W, water extract; E, 75% ethanol extract. Fresh tea leaves are tea leaves plucked freshly from the tea plants and dried immediately.

continued during the manufacturing processes, increasing the amounts of total catechins. Another explanation is that the mechanical operation in the withering process may rupture the outer membrane of the tea leaves, liberating the intracellular catechins and rendering them available for extraction.

Two solvent systems, namely, boiling water and 75% ethanol at 60 °C, were used to extract the total catechins and caffeine from the tea flowers. The results indicated that more catechins and caffeine were extracted by the 75% ethanol system.

The occurrence of tea polyphenols [five catechins, namely, (−)-epigallocatechin, catechin, (−)-epigallocatechin 3-gallate, (−)-epicatechin, and (−)-epicatechin 3-gallate] and caffeine in tea flowers (10 species of *C. sinensis*) has been demonstrated by an HPLC procedure (**Table 1**). The levels of tea catechins and caffeine in most tea flowers are lower or sometimes comparable with that of tea leaves. To our knowledge, this may be the first description of the occurrence and levels of tea catechins and caffeine in tea flowers (**Table 1**).

In the process of tea cultivation, the growth and proliferation of tea leaves are the most important, because the harvested tea leaves are used for manufacturing different kinds of teas. It has been demonstrated in some tea plants such as Chinsin Oolong and TTES 12 that blossoming of tea flowers may hamper the growth and proliferation of tea leaves; on the other hand, in some tea plants such as TTES 8 and wild species, the blossoming of tea flowers does not affect the growth and proliferation of tea leaves (*18*). Some chemicals, such as ethephon and NAA, have been employed to inhibit the blossoming of tea flower to promote the production of tea leaves (*18*).

In the present study, tea flowers were found to contain tea catechins and caffeine in quantities approaching those of tea leaves. From an economic point of view, both tea flowers and tea leaves should be treated equally. We propose that more scientific and field work should be done on the promotion of tea flowers as beneficial to health and well-accepted agricultural products.

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