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ARTICLE

Comparison of Major Phenolic Constituents and in Vitro Antioxidant Activity of Diverse Kudingcha Genotypes from *Ilex kudingcha*, *Ilex cornuta*, and *Ligustrum robustum*

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A total of seven Kudingcha genotypes from three plant species (*Ilex kudingcha*, *Ilex cornuta*, and *Ligustrum robustum*) with different geographic origins in China were investigated for their major phenolic compounds, individual and total phenolics contents, and in vitro antioxidant properties (ABTS, DPPH, FRAP, and OH assays). LC-PDA-APCI-MS analysis showed that Kudingcha genotypes from *Ilex* and *Ligustrum* had entirely different phenolic profiles. Major phenolics in Kudingcha genotypes from two *Ilex* species were mono- and dicaffeoylquinic acids, whereas those in a Kudingcha genotype from *Ligustrum* were phenylethanoid and monoterpenoid glycosides. All Kudingcha genotypes of *Ilex* exhibited significantly stronger antioxidant capacities than that of *Ligustrum*. Within six *Ilex* genotypes, great variation existed in their composition of individual phenolic compounds and their antioxidant properties. The comparative data and LC fingerprints obtained in this study may provide useful information for screening and breeding of better Kudingcha genotypes and also for their authentication and quality control.

KEYWORDS: Kudingcha; *Ilex kudingcha*; *Ilex cornuta*; *Ligustrum robustum*; functional tea; antioxidant activity; phenolic compounds; LC-APCI-MS

INTRODUCTION

Kudingcha, literally bitter spike-leaf tea, is a popular functional bitter tea beverage in China and also other countries (e.g., Singapore, Malaysia, and Vietnam) of Southeast Asia. It has been consumed as a traditional medicinal herbal tea in China for nearly 2000 years (since the Dong Han Dynasty) (*1*, *2*). Kudingcha, as an ancient functional tea, has been gaining in research attention in the past two decades because of its potential health and economic significance. Kudingcha has been recognized as a treatment for sore throat and an aid to weight loss, relief of hypertension, and for its potential cardiovascular benefits as well as possessing antioxidant, antidiabetic, hepatoprotective, neuroprotective, anti-inflammatory, and diuretic effects (*2*−*7*).

Kudingcha is a collective name in Chinese. It was earlier reported that the original plants for production of Kudingcha were from about 10 known species (*8*, *9*). Recent investigations have showed that Kudingcha could be made from leaves of many diverse plants that belong to more than 30 species from 13 genera in 12 families (*2*, *10*, *11*). Some plants for production of Kudingcha are native to Southern China, such as *Ilex kudingcha* C. J. Tseng. The most common Kudingcha categories consumed in China are from two genera in two families, i.e., the genus *Ilex* in the family Aquifoliaceae and the genus *Ligustrum* in the family Oleaceae. To some extent, Chinese Kudingcha is like "mate" or "yerba mate" (tea-like bitter beverage) prepared from the leaves and twigs of *Ilex paraguariensis* St. Hil., a plant originated from South America where it is widely consumed (*12*, *13*). Representative *Ilex* plant species for producing Chinese Kudingcha include *Ilex kudingcha* C. J. Tseng, *Ilex latifolia* Thunb., *Ilex cornuta* Lindl. et Paxt., and *Ilex pentagona* S. K. Chen, Y. X. Feng et C F. Liang; whereas representative *Ligustrum* species have *Ligustrum robustum* (Roxb.) Blume, *Ligustrum pedunculare* Rehd., and *Ligustrum purpurascens* Y. C. Yang, and *Ligustrum henryi* Hemsl. (*2*, *6*, *8*, *10*, *11*).

Because many different plant species with diverse geographic origins in China have been claimed to be used as raw materials for Chinese Kudingcha, commercial Kudingcha products in markets are easily confusable and the differences in their quality and health effects are also unclear (*6*, *14*). Misuse or confusion of certain plants as Kudingcha materials may cause safety problems. This seriously hinders further development and application of Chinese Kudingcha. So far there have been no reports on systematic comparison of chemical components and bioactivities of different Kudingcha products from various production areas in China, and their differences in main phenolic and antioxidant profiles have not been elucidated. Additionally, HPLC fingerprints for authentication and comparison of a number of Kudingcha genotypes with different origins in China are still lacking.

Although there have been a number of reports on phenolic compounds (mainly phenylethanoid and monoterpenoid glycosides) and antioxidant activity of the plants from the genus *Ligustrum* (e.g., *L. pedunculare*, *L. purpurascens,* and *L. robustum*) (*3*, *4*, *8*, *15*, *16*)

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Table 1. Typical LC-PAD-APCI-MS Data of Major Phenolic Compounds Identified in the Methanolic Crude Extracts of Kudingcha Samples from the Genus *Ilex* with Different Origins

					mass of obsd adduct ions				mass of obsd fragmented ions	
					negative		positive		positive	negative
peak no.[a]	retention time (min)	tentative names of phenolic compounds	UV/vis spectra λ_{max} (nm)	molecular mass	$[M-H]^-$	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$		
x	15.3	unknown	258	359?	358				152, 314	
1	17.1	3-*O*-caffeoylquinic acid	215, 300sh 325	354	353	355	377	393	163, 337	179, 191, 335, 707[b]
2	18.7	5-*O*-caffeoylquinic acid	218, 299sh, 329	354	353		377	393	163, 337	161, 191, 335, 707[b]
3	19.6	4-*O*-caffeoylquinic acid	213, 300sh, 328	354	353	355	377	393	163, 377	179, 191, 335, 707[b]
4	20.8	coumaroylquinic acid	312	338	337	339		377		191
5	24.3	3,5-di-*O*-caffeoylquinic acid	218, 301sh, 329	516	515	517	539	555	163, 337, 355, 499	191, 353, 497
6	25.5	rutin (quercetin 3-rutinoside)	255, 351	610	609	611		649	303, 465	301, 463
7	26.6	4,5-di-*O*-caffeoylquinic acid	214, 302sh, 329	516	515	517	539	555	163, 337, 355, 499	191, 335, 497,
8	28.2	kaempferol 3-rutinoside	265, 330	594	593	595			287, 449	285, 447

[a] The numbers of the peaks in this table coincide with the numbers of the peaks in **Figure 1** and the numbers of the phenolic compounds in **Table 2**. [b] Dimeric adduct ion.

and also several studies on phenolic components (mainly caffeoylquinic acid derivatives) and antioxidant activity of the plants from the genus *Ilex* (e.g., *I. kudingcha, I. latifolia,* and *I. paraguariensis*) (*7*, *13*, *17*, *18*), these previous investigations are mostly based on single plant species (single Kudingcha genotype) from China or other countries and by using different experimental conditions and methods. It is difficult to directly compare the results for diverse Kudingcha plant species or genotypes from previous different studies.

Major production regions of Kudingcha are located in Southern China including the provinces of Guangdong, Guangxi, Hainan, Yunan, Sichuan, Guizhou, Hunan, Hubei, Anhui, Fujian, and Zhejiang (*1*, *2*, *10*, *11*). These provinces differ greatly in their ecogeography and biodiversity and harbor rich genetic resources of the Kudingcha plants. The plant materials of Kudingcha tested in this study were collected from most of the mentioned-above provinces. The objectives of this study were to characterize and compare the main phenolic constituents of seven Kudingcha genotypes from *I. kudingcha*, *I. cornuta*, and *L. robustum* with different origins in China by employing LC-PDA-APCI-MS technique and to assess their antioxidant activity in vitro using different assay methods.

MATERIALS AND METHODS

Plant Materials and Chemicals. Dried leaf samples of six Kudingcha genotypes from two *Ilex* species (*I. kudingcha* C. J. Tseng and *I. cornuta* Lindl. et Paxt.) and one Kudingcha genotype from *Ligustrum robustum* (Roxb.) Blume, greatly differing in their geographic origins, which represent major Kudingcha production provinces in China, were collected from a well-known market for herbal tea and beverages in Wuhan, China in April, 2008. Five *I. kudingcha* genotypes were from Guangxi (Z2), Fujian (Z3), Hainan (Z6), Sichuan (Z8), and Guangdong (Z9), respectively. One *I. cornuta* genotype (Z1) and one *L. robustum* genotype (Z10) were from Hubei and Guangxi, respectively. The specimen authentication was kindly assisted by Wuhan Botanical Garden, Chinese Academy of Sciences, Hubei, China. A representative variety of green tea (*Camellia sinensis*) was used as control. All the collected samples were sealed with plastic bags and stored in a desiccator with silica gel at room temperature in the laboratory of the University of Hong Kong.

D-2-deoxyribose, EDTA, hydrogen peroxide, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6 tripyridyl-*s*-triazine (TPTZ), $FeCl_3$, potassium persulfate, authentic standard compounds of gallic acid, chlorogenic acid (5-*O*-caffeoylquinic acid), and rutin (quercetin-3-*O*-rutinoside) were purchased from Sigma/Aldrich (St. Louis, MO); Trolox (6-hydroxy-2,5,7,8-tetramethylchromane 2-carboxylic acid) was from Fluka Chemie (Buchs, Switzerland), and Folin−Ciocalteu reagent, HPLC grade organic reagents, and formic acid were from BDH (Dorset, U.K.).

Preparation of Kudingcha Extracts. Dried kudingcha were ground into fine powder using a Kenwood Multi-Mill (Kenwood, Watford, U.K.), and passed through a sieve (24 mesh). The powdered sample (2.0 g) was extracted with 50 mL of 80% methanol at room temperature (~23 °C) for 24 h in a shaker. The crude extract was filtered by a Millipore filter with a 0.2 μm nylon membrane under vacuum at room temperature. The filtrate was then stored at 4 °C within 24 h for assays of in vitro antioxidant properties. Additionally, sample preparation for LC-APCI-MS analysis was: the powdered sample (20 mg) was added in a 1.5 mL vial and extracted with 1 mL of 80% methanol at room temperature (~23 °C) for 8 h. The extract was filtered using a Millipore filter (nylon membrane, 0.2 μm i.d.) at 23 °C and then injected into LC for analysis.

LC-PDA-APCI-MS Analysis. A LC-APCI-MS-2010EV system (Shimadzu; Kyoto, Japan) consisted of an LC-10ADvp binary pump, a SIL-10Avp autosampler, a photodiode array detector (PDA), a central controller, and a single quadrupole MS detector with atmospheric pressure chemical ionization (APCI) interface. The analytical column was a VP-ODS C18 column (250 mm × 2.0 mm, 5 μm) (Nomura Chemical, Seto, Japan). LC conditions were as follows: solvent A, 0.1% formic acid, and solvent B, MeOH with 0.1% formic acid. Two gradient elution procedures were used in this study. For the analysis of Kudingcha samples from *I. kudingcha* and *I. cornuta*, a shorter gradient elution (65 min) was used: 0−5 min, 5% B; 5−14 min, 5−35% B; 14−20 min, 35−45% B; 20−30 min, 45−55% B; 30−40 min, 55−70% B; 40−50 min, 70−100% B; 50−58 min, 100% B; 58−60 min, 100−5% B; 60−65 min, 5% B. For the analysis of Kudingcha samples from *L. robustum*, a longer gradient elution (112 min) was used: 0−5 min, 5% B; 5−15 min, 5−30% B; 15−40 min, 30−40% B; 40−60 min, 40−50% B; 60−65 min, 50−55% B; 65−90 min, 55−100% B; 90−100 min, 100% B; 100−100.5 min, 100−5% B; 100.5−112 min, 5% B. For all samples, the flow rate was 0.2 mL/min, injection volume was 8 μL, and detection was at 280 nm. MS conditions: the scan range of APCI-MS was m/z 150−700 in both positive and negative ion modes. APCI temperature was 400 °C, curved desolvation line (CDL) temperature 250 °C, and heat block temperature 200 °C. The APCI probe voltage was 4.5 kV. A nebulizing gas of 2.5 L/min and a drying gas of 2.0 L/min were applied for ionization using nitrogen in both cases.

Quantitative Analysis of Individual and Total Phenolics. Individual phenolics identified in the Kudingcha extracts were quantified using the same LC-PDA system and same chromatographic conditions described as above and by establishing external standard curves of the corresponding known phenolics. The concentrations of the phenolics without standards were calculated using the calibration curves of the standards closest in their chemical structures because the same categories of phenolics usually have similar chromatographic behavior and UV/vis spectroscopic characteristics. In the present study, chlorogenic acid (5-*O*-caffeoylquinic acid) was used as an external standard to quantify monocaffeoylquinic acids, dicaffeoylquinic acids, and coumaroylquinic acid in Kudingcha extracts because these phenolic acids shared similar UV/vis spectroscopic traits (λ_{max} = 325−329 nm in **Table 1**). Thus quantification of caffeoylquinic acid derivatives was based on data of the peak area at λ_{max} = 326 nm. Rutin (quercetin 3-rutinoside) was used to estimate the

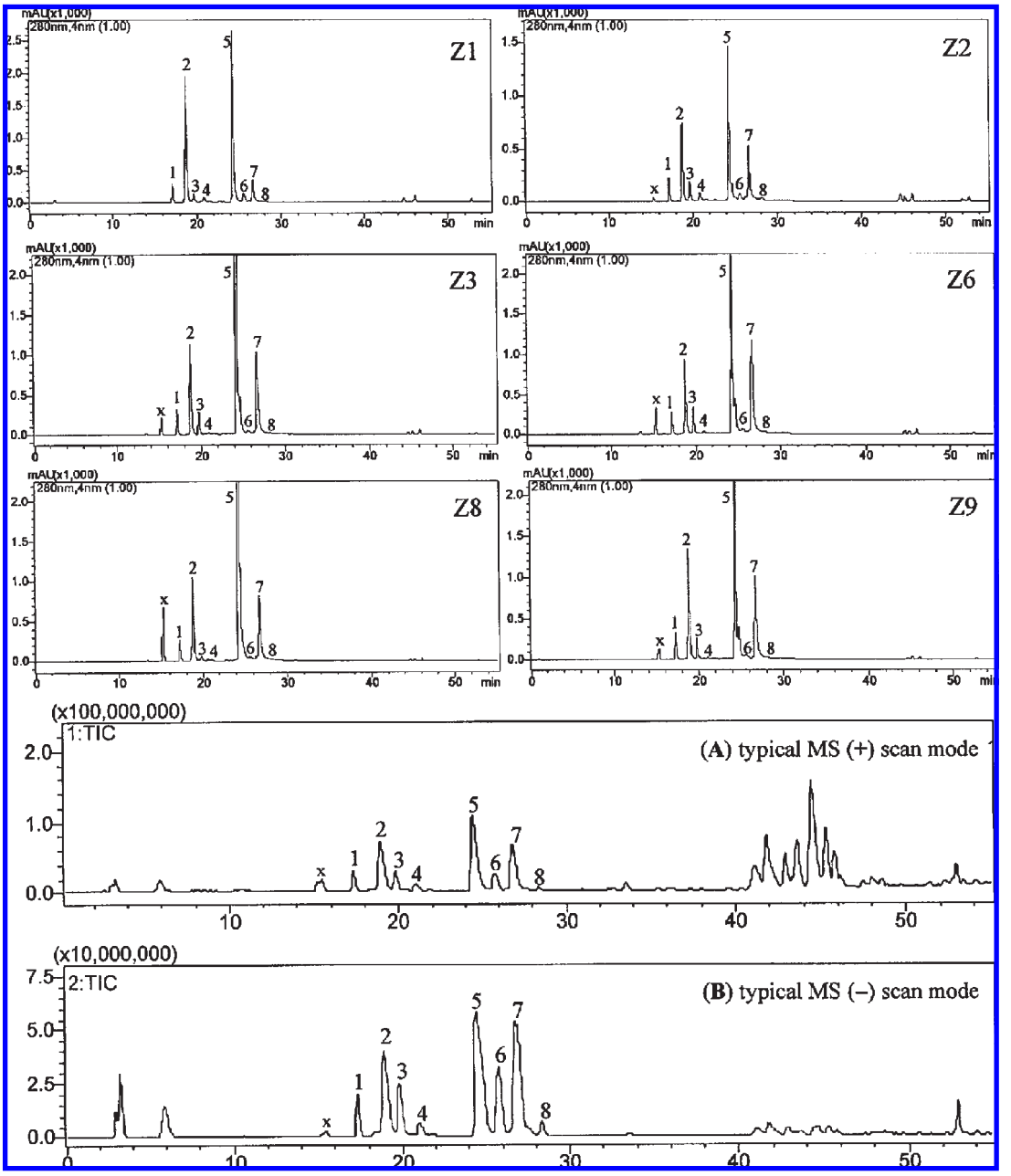


Figure 1. LC-PDA-APCI-MS chromatograms of the methanolic crude extracts from six Kudingcha genotypes: typical LC-PDA profiles of major phenolic compounds (peaks 1−8) from different genotypes in the genus *Ilex* (Z1, Z2, Z3, Z6, Z8, and Z9) and representative APCI-MS profiles in positive (**A**) and negative (**B**) full scan modes from Z2. Peak numbers in this figure correspond to the numbers of the phenolic compounds identified in **Table 1**.

Table 2. Contents of Phenolic Acids and Flavonoids of Six Kudingcha Genotypes from the Genus *Ilex* with Different Origins [a]

	compositions of individual phenolic compounds [b]									
sample code	1	2	3	4	5	6	7	8	total phenolic acid (mg CA/100 g DW) [c]	total flavonols (mg rutin /100 g DW) [d]
Z1	115.7a	979.2a	56.4a	53.6a	1286.8a	79.7a	214.5a	tr	2744.8 ± 32.2a	79.7 ± 1.75a
Z2	87.1b	334.7b	87.1b	39.3b	729.3b	49.7b	331.3b	8.4a	1649.1 ± 12.1b	58.2 ± 1.03b
Z3	133.4c	593.0c	140.8c	15.9c	2005.1c	19.4c	678.3c	tr	3693.8 ± 57.5c	19.4 ± 0.08c
Z6	83.4b	346.6b	104.2d	11.2c	1303.0a	34.9d	575.9d	4.3b	2576.0 ± 34.0d	39.2 ± 2.01d
Z8	112.4a	566.5c	36.0e	12.6c	2877.6d	20.1c	535.0e	tr	4478.3 ± 24.4e	20.1 ± 1.03c
Z9	135.4c	761.3d	124.4f	10.6c	1804.5e	41.2e	691.8c	tr	3620.4 ± 20.9c	41.2 ± 1.33d
mean	111.2	596.9	91.5	15.3	1667.7	40.8	504.5	2.1	3127.1	43.0

[a] Six Kudingcha genotype samples were from different provinces in China: **Z1** from Hubei, **Z2** from Guangxi, **Z3** from Fujian, **Z6** from Hainan, **Z8** from Sichuan, and **Z9** from Guangdong. Different letters in the same column indicate significance difference ($p < 0.05$) between mean values of various genotypes. [b] The numbers of the phenolic compounds in this table coincide with the numbers of the peaks in **Table 1** and **Figure 1**. The compounds **1**−**5** and **7** were expressed as mg chlorogenic acid (CA)/100 g DW, and the compounds **6** and **8** were expressed as mg rutin/100 g DW. tr, trace amount. [c] The sum of the contents of compounds **1**−**5** and **7**. [d] The sum of the contents of compounds **6** and **8**.

concentrations of rutin and kaempferol 3-rutinoside in Kudingcha extracts because these two flavonols possessed similar aglycone and the same sugar moiety (rutinoside). Additionally, total phenolics content (TPC) was estimated using the Folin−Ciocalteu colorimetric method described in our previous studies (*19*, *20*). Results were expressed as grams of gallic acid equivalents (GAE) per 100 g dry weight.

ABTS Assay. Antioxidant activity against $ABTS^{\bullet+}$ radicals was measured with a spectrophotometer (U-1800, Hitachi, Japan) using the improved ABTS method (*21*). Briefly, The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by the reaction of ABTS (7 mM) and potassium persulphate (2.45 mM) after incubation at room temperature in the dark for 16 h. The $ABTS^{\bullet+}$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.002 at 734 nm. $ABTS^{\bullet+}$ solution (3.9 mL; absorbance of 0.700 ± 0.002) was added to 0.1 mL of the test sample, which was properly diluted and mixed thoroughly. The reaction mixture was kept at room temperature for 6 min, and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 μM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard, and the results were expressed as mmol trolox equivalents per 100 g dry weight of plant material.

DPPH Assay. The DPPH method was performed according to Cai et al. (*21*). The measurement procedure was similar to the ABTS method described above. Briefly, the DPPH radical ($DPPH^{\bullet}$) solution (60 μM) was prepared in 80% methanol. The same samples of plant extracts diluted with 80% methanol during the ABTS assay were also used in the DPPH assay. $DPPH^{\bullet}$ solution (3.9 mL) was added to 0.1 mL of the extracts. The reaction was carried out at 23 °C in the dark for 2 h and then the absorbance of the reactive mixture was recorded using a spectrophotometer at 515 nm. A Trolox calibration curve was established. The results were expressed as mmol Trolox equivalents per 100 g dry weight of plant material.

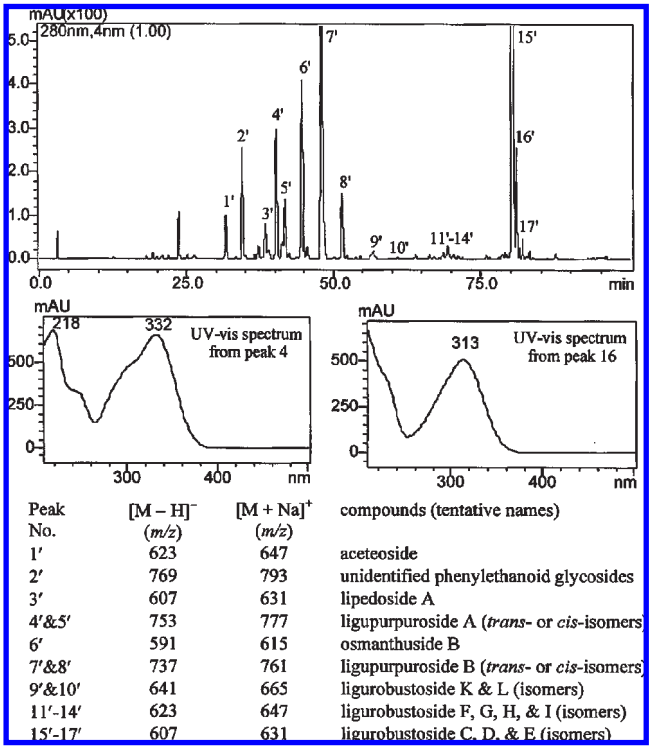
FRAP Assay. Ferric reducing antioxidant power (FRAP) assay was performed according to Surveswaran et al. (*20*). Briefly, the FRAP reagent was prepared by adding 10 volumes of acetate buffer (300 mM, pH 3.6), including 3.1 g sodium acetate and 16 mL of glacial acetic acid, 1 volume of 10 mM TPTZ prepared in 40 mM HCl, and 1 volume of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and prewarmed at 37 °C. This reagent (3 mL) was mixed with 0.1 mL diluted test samples similar to those used for the ABTS and DPPH assays. The mixture was shaken and incubated at 37 °C for 8 min, and the absorbance was read at 593 nm. A blank with only 0.1 mL methanol was used for calibration. A standard curve was made with Trolox, and the results were expressed as μmol Trolox equivalents per one gram dry weight of plant material.

Scavenging Activity against Metal Ion-Dependent Hydroxyl Radicals. The hydroxyl radical ($OH^{\bullet}$) scavenging activity was determined using the deoxyribose method described previously (*22*). Briefly, 0.5 mL of appropriately diluted plant extract sample was added to 1.0 mL of potassium phosphate buffer (20 mM, pH 7.4) including D-2-deoxyribose (2.8 mM), EDTA (104 mM), $FeCl_3$ (100 mM), ascorbic acid (100 mM), and hydrogen peroxide (1 mM). The mixtures were incubated at 37 °C for 1 h. After incubation, 1 mL of 0.05% thiobarbituric acid in 10% trichloroacetic acid was added and the mixtures were boiled at 100 °C for 15 min in a sealed tube. Deionized water was used as a blank. The absorbance at the wavelength of 532 nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity (%) was calculated using the following equation: (A_{532} of blank − A_{532} of sample) × 100/A_{532} of blank.

Statistical Analysis. All above tests were performed in triplicate. All the results were calculated as mean ± SD (standard deviation). Statistical comparisons of the mean values were performed by one-way ANOVA, followed by Duncan's multiple-range test at $p < 0.05$ or 0.01 confidence levels using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

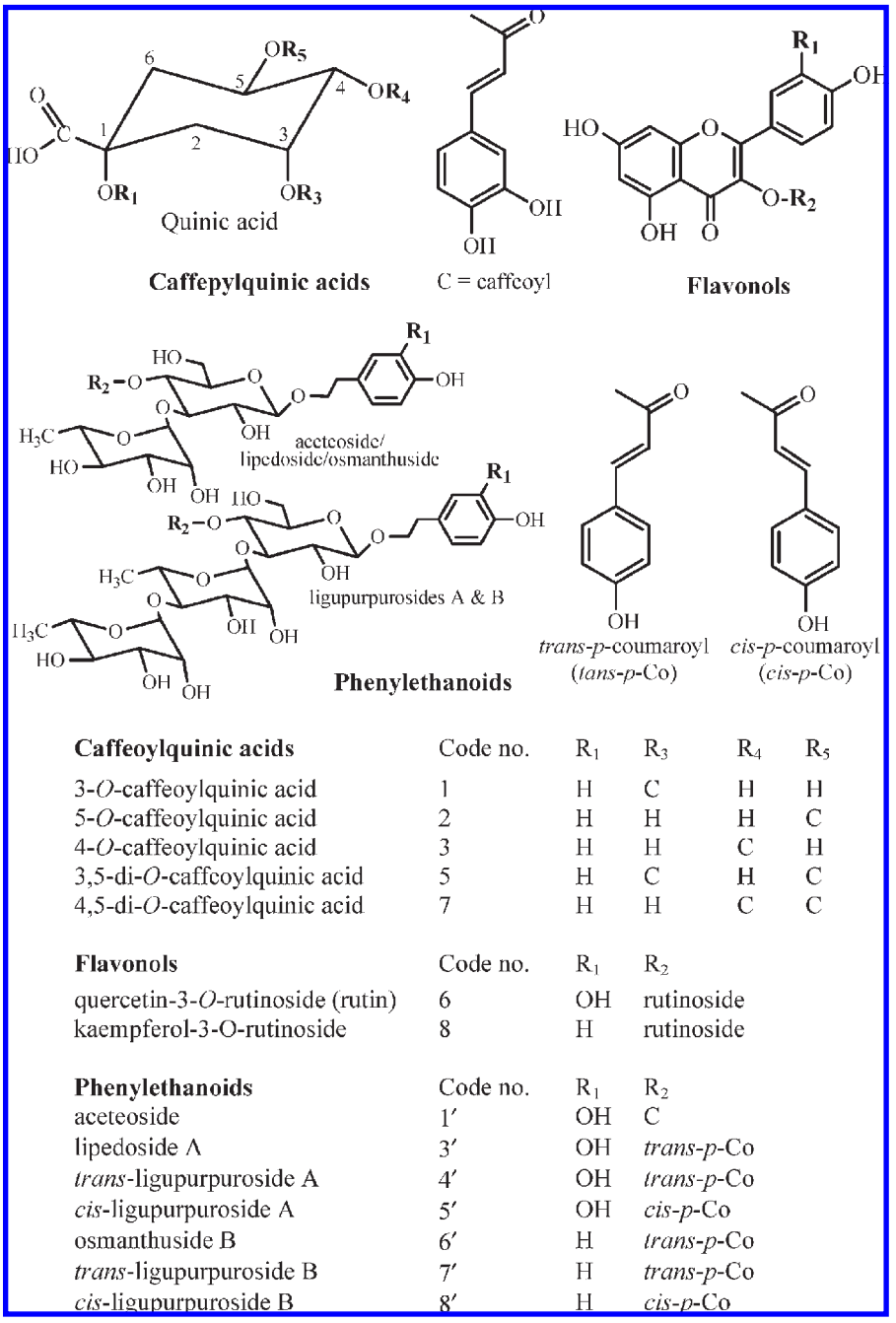
LC-PDA-APCI-MS Identification of Phenolic Compounds in Different Kudingcha Genotypes. *I. kudingcha* and *I. cornuta* belong to the same genus *Ilex* in the family Aquifoliaceae with similar taxonomic origin. The results of LC-PDA-APCI-MS analysis showed that six Kudingcha genotypes from two *Ilex* species (Z1 from *I. cornuta* and Z2, 3, 6, 8, and 9 from *I. kudingcha*) showed similar phenolic profile fingerprints (**Figure 1**) and mainly contained caffeoylquinic acid derivatives (**Table 1**). In comparison with the Kudingcha genotypes from *Ilex*, a Kudingcha genotype (Z10) from *L. robustum* in the genus *Ligustrum* of the family Oleaceae had an entirely different phenolic profile that showed various kinds of phenolic compounds (**Figure 2**). Major phenolic compounds in Z10 (*L. robustum*) were readily identified as glycosides of phenylethanoids (peaks 1−8) and monoterpenoids (peaks 9−17) with coumaroyl and/or caffeoyl moieties on the bases of LC-PDA and MS data, as shown in **Figure 2** and with reference to literature data on the Kudingcha genotypes from *L. robustum*, *L. pedunculare*, and *L. purpurascens* (*3*, *4*, *8*, *15*, *16*, *23*).



Peak No.	$[M - H]^-$ (*m/z*)	$[M + Na]^+$ (*m/z*)	compounds (tentative names)
1′	623	647	aceteoside
2′	769	793	unidentified phenylethanoid glycosides
3′	607	631	lipedoside A
4′&5′	753	777	ligupurpuroside A (*trans*- or *cis*-isomers)
6′	591	615	osmanthuside B
7′&8′	737	761	ligupurpuroside B (*trans*- or *cis*-isomers)
9′&10′	641	665	ligurobustoside K & L (isomers)
11′-14′	623	647	ligurobustoside F, G, H, & I (isomers)
15′-17′	607	631	ligurobustoside C, D, & E (isomers)

Figure 2. LC-PDA chromatogram (280 nm) with typical UV/vis spectra and MS data of major identified/unknown phenolic compounds in the methanolic crude extract of Kudingcha genotype (Z10) from *Ligustrum robustum*. Tentative identification of phenylethanoid glycosides (peaks 1−8) and monoterpenoid glycosides (peaks 9−17) with coumaroyl and/or caffeoyl moieties was based on our LC-PDA-APCI-MS data and according to previous literature data (*3*, *4*, *8*, *14*, *16*, *23*).

Previous studies on the authentication of different types of Chinese Kudingcha have been focused on the morphological features using microscopic techniques (*14*) and polymorphism analysis of different molecular markers by DNA sequencing and RAPD techniques (*10*, *11*). High performance liquid chromatography based molecular fingerprints have been proved to be a feasible and robust technique in authentication of specimens and samples, and quality control of commercial products. In this study, LC fingerprints (**Figures 1** and **2**) of different Kudingcha genotypes from two genera (*Ilex* and *Ligustrum*) were developed and compared, suggesting the significant differences in the phenolic profiles between the *Ilex* and *Ligustrum* species. Because of the big differences in the phenolic categories and polarities between the *Ilex* and *Ligustrum* species, the chromatographic conditions used for their respective phenolic separation were quite different, i.e., the gradient elution procedure (65 min) of the *Ilex* samples was significantly shorter than that (112 min) of the *Ligustrum* samples. Furthermore, within the *Ilex* species, an unknown phenolic compound (peak *x*, retention time 15.3 min,



Caffeoylquinic acids	Code no.	R_1	R_3	R_4	R_5
3-*O*-caffeoylquinic acid	1	H	C	H	H
5-*O*-caffeoylquinic acid	2	H	H	H	C
4-*O*-caffeoylquinic acid	3	H	H	C	H
3,5-di-*O*-caffeoylquinic acid	5	H	C	H	C
4,5-di-*O*-caffeoylquinic acid	7	H	H	C	C

Flavonols	Code no.	R_1	R_2
quercetin-3-*O*-rutinoside (rutin)	6	OH	rutinoside
kaempferol-3-O-rutinoside	8	H	rutinoside

Phenylethanoids	Code no.	R_1	R_2
aceteoside	1′	OH	C
lipedoside A	3′	OH	*trans*-*p*-Co
trans-ligupurpuroside A	4′	OH	*trans*-*p*-Co
cis-ligupurpuroside A	5′	OH	*cis*-*p*-Co
osmanthuside B	6′	H	*trans*-*p*-Co
trans-ligupurpuroside B	7′	H	*trans*-*p*-Co
cis-ligupurpuroside B	8′	H	*cis*-*p*-Co

Figure 3. Chemical structures of representative phenolic compounds present in the extracts of Kudingcha genotypes from the genus *Ilex* (caffeoylquinic acids and flavonols) and *Ligustrum* (phenylethanoids). Code numbers in this figure correspond to the numbers of the peaks in **Figures 1** and **2** and major phenolic compounds identified in **Table 1**.

$\lambda_{max} = 235$ nm) (**Figure 1** and **Table 1**) was detected in all five Kudingcha genotypes (Z2, 3, 6, 8 and 9) from *I. kudingcha* but not found in a genotype (Z1) from *I. cornuta*. This might provide valuable information for authentication and quality control of different Kudingcha genotypes. However, the unknown compound needs further identification with NMR or other techniques in the future.

Compared with literature on phenolic components in Kudingcha from many *Ligustrum* species (*6*, *24*), information on phenolic compounds in a number of Kudingcha genotypes from the *Ilex* species is limited. Six Kudingcha genotypes from two *Ilex* species with different origins were selected in the present study for phenolic identification by LC-PDA-APCI-MS. The retention times, UV/vis spectra, calculated molecular masses, and MS adduct ions and fragmented ions in both positive and negative modes are shown in **Table 1**. Major phenolic acids in the tested Kudingcha genotypes were identified as caffeoylquinic acid derivatives including mono and dicaffeoylquinic acids, whereas major flavonoids were identified as rutin and kaempferol 3-rutinoside (both belong to flavonols). Their chemical structures were illustrated in **Figure 3**. Peaks 1, 2, and 3 were easily identified as 3-*O*-caffeoylquinic acid (neochlorogenic acid), 5-*O*-caffeoylquinic acid (chlorogenic acid), and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), respectively, peaks 5 and 7 were assigned as 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid, and peaks 6 and 8 as rutin and kaempferol 3-*O*-rutinoside, according to the available standard samples (5-*O*-caffeoylquinic acid and rutin) and by comparison with literature data (retention

times, λ_{max}, and UV/vis spectral shapes) at the similar chromatographic conditions (*13*, *17*, *25*). The results were generally in agreement with those of a few Kudingcha genotypes from the *Ilex* species in previous different studies (*7*, *17*, *18*). However, in our LC profiles (**Figure 1**), all the major phenolic compounds were eluted within 29 min, considerably shorter than the retention times (55−95 min) reported in previous studies on Kudingcha (*7*, *17*, *18*), indicating the improved elution efficiency of the LC system in this study.

A more reliable and direct confirmation of phenolic identification came from APCI-MS that gave the expected protonated molecular ions and fragmented ions. The molecular masses of the phenolic constituents isolated in all six Kudingcha genotypes were determined from the prominent $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ ions in positive mode and from the prominent $[M-H]^-$ ions in negative mode as well as their fragmented ions (**Table 1**).

Three monocaffeoylquinic acid isomers (peaks 1, 2, and 3) had similar protonated ions and fragmented ions, i.e., $[M-H]^-$ at *m*/*z* 353, and $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ at *m*/*z* 355, 377, and 393, respectively (**Table 1**). Further fragmented ions could be more informative for structural elucidation. The fragmented ions in positive mode were observed at *m*/*z* 163 and 337. The former would correspond to a caffeoyl group (caffeic acid by the loss of one H_2O molecule). The latter would correspond to caffeoylquinic acid with the loss of one H_2O molecule. The fragmented ions in negative mode were observed at *m*/*z* 179, 191, 335, and 707. The ion at *m*/*z* 179 would represent the caffeic acid group. The ion at *m*/*z* 191 would correspond to the deprotonated quinic acid, which was also observed for all dicaffeoylquinic acids. The ion at *m*/*z* 335 would correspond to deprotonated caffeoylquinic acid with the loss of one H_2O molecule. The ion at *m*/*z* 707 could be a dimeric adduct of caffeoylquinic acid. Such a mechanism of fragmented formation was noted to occur in aromatic carboxylic acids (*26*). Most of the fragmented ions for monocaffeoylquinic acids were also observed by employing ESI-MS (*13*, *27*). APCI-MS was employed in this study to observe more abundant ions (fragmented and intact) in both positive and negative mode for structural elucidation than ESI-MS in previous study (*13*). In addition, although three monocaffeoylquinic acid isomers had similar protonated ions and fragmented ions and similar UV/vis spectra, their retention times were quite different, enabling us to clearly identify them (**Table 1**).

Two dicaffeoylquinic acid isomers (peaks 5 and 7) had similar protonated ions and fragmented ions, i.e., $[M-H]^-$ at *m*/*z* 515, and $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ at *m*/*z* 517, 539, and 555, respectively (**Table 1**). Further fragmented ions at *m*/*z* 499 in the positive mode and *m*/*z* 497 in the negative mode would correspond to a dicaffeoylquinic acid with the loss of one H_2O molecule. Further fragmented ions (*m*/*z* 163, 337, and 355 in positive mode and *m*/*z* 191 and 335 in negative mode) were identical with those observed for the monocaffeoylquinic acids as discussed above. Most of the fragmented ions for dicaffeoylquinic acids were also consistent with those observed using ESI-MS (*13*, *27*). Additionally, like the monocaffeoylquinic acid isomers, two dicaffeoylquinic acid isomers had quite different retention times to clearly distinguish them.

One minor constituent of phenolic acid (peak 4) was identified as coumaroylquinic acid (**Figure 3**), which was indicated by $[M-H]^-$ at *m*/*z* 337 in the negative mode and $[M+H]^+$ and $[M+K]^+$ at *m*/*z* 339 and 377 in the positive mode (**Table 1**). Its fragmented ion at *m*/*z* 191 in the negative mode corresponded to a deprotonated quinic acid group, and it was also observed for caffeoylquinic acids as discussed above. Furthermore, the UV/vis spectrum of coumaroylquinic acid was characteristic, with a maximum at around 321 nm. This was in agreement with that was detected in previous study (*13*).

Two flavonols (small peaks 6 and 8) were also minor phenolic constituents and identified as quercetin 3-rutinoside (rutin) and kaempferol 3-rutinoside (**Figure 3**), respectively, by APCI-MS data (**Table 1**). Rutin, the most common flavonoid in plants, was easily differentiated by its MS data (peak 6). Peak 8 produced typical protonated ions of kaempferol 3-rutinoside, such as $[M+H]^+$ ion at *m*/*z* 595 and $[M-H]^-$ ion at *m*/*z* 593. Further fragmented ions at *m*/*z* 287 (positive) and 285 (negative) would represent its aglycon kaempferol, whereas the fragmented ions at *m*/*z* 449 (positive) and 447 (negative) were indicative of kaempferol 3-rutinoside, with the loss of a dehydrated rhamnose moiety (*m*/*z* 146). The existence of rutin and kaempferol in Vietnam bitter tea was also reported (*18*). However, kaempferol 3-rutinoside identified in Chinese Kudingcha as in this study, has not been reported previously.

LC-PDA Quantification of Phenolic Compounds in Different Kudingcha Genotypes. Because plants of the *Ilex* species are major base materials for production of Chinese Kudingcha, the contents of individual phenolic compounds in all six *Ilex* genotypes were quantified and compared (**Table 2**). The dicaffeoylquinic acids (especially 3,5-di-*O*-caffeoylquinic acid, compound **5**) were the predominant compounds in the extracts of all the *Ilex* genotypes, followed by the monocaffeoylquinic acids (especially 5-*O*-caffeoylquinic acid, compound **2**). On average, the content of 3,5-di-*O*-caffeoylquinic acid was 1667.7 mg chlorogenic acid (CA)/100 g DW, significantly higher than that of 4,5-di-*O*-caffeoylquinic acid (504.5 mg/100 g). These two dicaffeoylquinic acids (compounds **5** and **7**) accounted for 67.4% of total peak area, whereas three monocaffeoylquinic acids (compounds **1**, **2**, and **3**) accounted for 26.4%. Among the monocaffeoylquinic acids in all *Ilex* genotypes, 5-*O*-caffeoylquinic acid (chlorogenic acid) had the highest content (596.9 mg/100 g). The average content of coumaroylquinic acid (compound **4**) in all *Ilex* genotypes was considerably lower (15.3 mg/100 g). Additionally, two flavonols (compounds **6** and **8**) were minor components identified in the *Ilex* samples and only accounted for 1.5% of total peak area, and the content of rutin (40.8 mg/100 g) was much higher than that of kaempferol 3-rutinoside (2.1 mg/100 g).

Significant variations in the contents of individual phenolic compounds among the six genotypes from the genus *Ilex* (Z1, 2, 3, 6, 8 and 9) with different geographic origins were observed and compared ($p < 0.05$) (**Table 2**). For example, contents of the most abundant compound 3,5-di-*O*-caffeoylquinic acid (**5**) varied to a large extent from 729.3 (Z2) to 2877.6 mg CA/100 g DW (Z8). Contents of chlorogenic acid (**2**) varied from 334.7 (Z2) to 979.2 mg/100 g (Z1). Significant variations in minor components were also detected among the six genotypes. For instance, coumaroylquinic acid (**4**) varied from 10.6 (Z9) to 53.6 mg/100 g (Z1). Rutin (**6**) varied from 19.4 (Z3) to 79.7 mg/100 g (Z1). Kaempferol 3-rutinoside (**8**) was detected only in two Kudingcha genotypes (8.4 and 4.3 mg/100 g for Z2 and Z4, respectively), whereas the other four genotypes contained trace amounts of kaempferol 3-rutinoside.

The great diversity in the phenolic compositions among six *Ilex* genotypes was also reflected in their total contents of phenolic acids and flavonols (**Table 2**). Total content of phenolic acids varied considerably from 1649.1 (Z2) to 4478.3 mg CA/100 g DW (Z8), with a mean value of 3127.1 mg/100 g. Z1 had the highest content of total flavonols (rutin and kaempferol 3-rutinoside) (79.7 mg rutin/100 g DW), whereas Z3 had the lowest (19.4 mg/100 g). The diversities in phenolic compositions among six Kudingcha genotypes from the genus *Ilex* could be attributed to the differences in their genetic, environmental, and processing conditions.

In Vitro Antioxidant Activities and Total Phenolics of Different Kudingcha Genotypes and Their Correlations. The methanolic crude extracts from seven Kudingcha genotypes from two genera (*Ilex* and *Ligustrum*) exhibited a wide range of antioxidant activities (**Table 3**). The ABTS values of six *Ilex* genotypes ranged from 16.3 to 38.5 mmol Trolox/100 g DW (i.e., Z2 and Z8,

Table 3. Total Antioxidant Capacities, Hydroxyl Radical-Scavenging Activity, and Total Contents of Phenolics in the Crude Extracts of Six Kudingcha Genotypes from the Genus *Ilex* in Comparison with One Kudingcha Genotype from the Genus *Ligustrum* and a Control (Green Tea)[a]

genus	sample code	ABTS assay (mmol Trolox/100 g DW)	DPPH assay (mmol Trolox/100 g DW)	FRAP assay (μmol Trolox/g DW)	OH• scavenging activity (%)	TPC (g gallic acid/100 g DW)
Ilex	Z1	25.1 ± 0.31a	42.4 ± 0.67a	211.1 ± 6.7a	28.1 ± 1.10a	8.9 ± 0.06a
	Z2	16.3 ± 0.19b	9.9 ± 0.12 b	98.1 ± 2.3b	24.3 ± 0.87b	6.0 ± 0.14b
	Z3	31.0 ± 0.43c	43.1 ± 0.41a	214.8 ± 3.1a	28.7 ± 1.60a	10.7 ± 0.34c
	Z6	33.1 ± 0.69d	37.0 ± 0.67c	236.0 ± 4.5c	29.7 ± 1.02a	11.0 ± 0.42c
	Z8	38.5 ± 0.51e	46.1 ± 0.52d	317.2 ± 2.2d	30.6 ± 1.91a	15.0 ± 0.13d
	Z9	30.7 ± 0.29c	44.5 ± 1.2ad	231.8 ± 1.6c	27.9 ± 1.24a	10.3 ± 0.20c
	mean	29.1	37.2	218.6	28.2	10.3
Ligustrum	Z10	7.1 ± 0.08	8.2 ± 0.24	45.6 ± 0.31	16.9 ± 2.0	7.6 ± 0.21
Camellia	green tea	47.7 ± 0.67	42.6 ± 0.81	314.7 ± 5.31	25.1 ± 1.31	14.9 ± 0.13

[a] Six Kudingcha genotypes of the genus *Ilex* were from different provinces in China: **Z1** from Hubei, **Z2** from Guangxi, **Z3** from Fujian, **Z6** from Hainan, **Z8** from Sichuan, and **Z9** from Guangdong, and one Kudingcha genotype (**Z10**) of the genus *Ligustrum* was from Guangxi. Different letters in the same column indicate significance difference ($p < 0.05$) between mean values of various genotypes.

respectively), with an average of 29.1 mmol/100 g, which was significantly lower than that of green tea (47.7 mmol/100 g) but considerably higher than that of the *Ligustrum* genotype (Z10) (7.1 mmol/100 g). The DPPH values of six *Ilex* genotypes varied significantly from 9.9 to 46.1 mmol Trolox/100 g DW (i.e., Z2 and Z8, respectively), with an average of 37.2 mmol/100 g, which was much higher than that of Z10 from *Ligustrum* (8.2 mmol/100 g). Z8 and Z9 showed slightly higher DPPH values (46.1 and 44.5 mmol/100 g) than green tea (42.6 mmol/100 g). The FRAP values also ranged widely from 98.1 to 317 μmol Trolox/g DW (i.e., Z2 and Z8, respectively), with an average of 219 μmol/100 g, which was significantly lower than that of green tea (315 μmol/100 g) but much higher than that of Z10 from *Ligustrum* (45.6 μmol/100 g). As to the OH• radical scavenging activity, Z8 showed significantly lower value (24.3%) than the other five *Ilex* genotypes (27.9−30.6%). The mean value of six *Ilex* genotypes was 28.2%, higher than that of green tea (25.1%) and much higher than that of Z10 from *Ligustrum* (16.9%). Green tea is rich in catechin/epicatechin and their derivatives (tea polyphenols), which are good dietary antioxidants (*28*, *29*). In this study, certain Kudingcha genotypes from *Ilex* possessed potent antioxidant activity similar to green tea and to some extent had slightly better antioxidant properties than green tea. This indicated that certain Kudingcha genotypes from *Ilex*, rich in caffeoylquinic acids and their derivatives, might be a functional alternative as good dietary antioxidants, especially when caffeoylquinic acids could function better compared with tea catechins in some biological and pharmaceutical situations (*28*, *30*).

Many previous studies showed that total phenolics contents of crude extracts from medicinal herbs and dietary plants could significantly contribute to their total antioxidant capacities (*19*, *20*, *29*). In this study, total phenolics contents (TPC) of all the tested Kudingcha samples were estimated using the Folin−Ciocalteu colorimetric method. The TPC values of six Kudingcha genotypes from the genus *Ilex* varied from 6.0 to 15.0 g gallic acid/100 g DW, with a mean value of 10.3 g/100 g, higher than that of Kudingcha genotype (Z10) from the genus *Ligustrum* (7.6 g/100 g) but significantly lower than that of green tea (14.9 g/100 g) (**Table 3**). We selected six Kudingcha genotypes from the genus *Ilex* to analyze their correlative relationships among total antioxidant activity parameters (ABTS, DPPH, FRAP, and OH• radical scavenging activity (OH)) and total phenolics content (TPC) as well as total phenolic acids (TPA) and total flavonols (TF). The results (**Table 4**) showed that significantly positive correlations ($p < 0.01$ or 0.05) existed between various parameters except for TF. Among the antioxidant assays (ABTS, DPPH, FRAP, and OH), they all showed high correlation coefficients, suggesting the reliability and interchangeability between these spectrophotometer-based methods in predicting antioxidant activities of the samples despite their different chemical mechanisms of action (*31*). For example, ABTS correlated well with DPPH ($r = 0.83$), FRAP (0.96), and OH (0.95). This was consistent with a previous study on only one Kudingcha genotype (*7*) and also in agreement with several other studies focusing on a large number of different medicinal and/or dietary plant materials (*19*, *20*, *29*, *32*). Total phenolic acids (TPA) positively and highly correlated with ABTS ($r = 0.86$), DPPH (0.84), FRAP (0.88), and TPC (0.89), whereas total flavonols (TF) showed no such trends with other parameters because TF accounted for very small proportion of total phenolics content (TPC) in the tested Kudingcha genotypes, as well as certain Kudingcha genotypes (e.g., Z8 and Z3) with the highest TPA values contained the lowest TF values (**Table 2**). This further suggested that the caffeoylquinic acids and their derivatives (belong to TPA) in the crude extracts of different Kudingcha genotypes were the major phenolic compounds that significantly contributed to their total phenolics contents and were also responsible for their in vitro antioxidant activities. Because ABTS assay had high correlations with other parameter assays and was also performed within a few minutes, it could be employed to better and faster estimate total antioxidant capacity of a large number of Kudingcha genotypes and might be used as an efficient parameter for quality control of Kudingcha.

Table 4. Pearson Correlation Coefficients (r) between Different Antioxidant Capacity Parameters (ABTS, DPPH, FRAP, and OH• Scavenging Assays (OH)) and Total Phenolics Contents (TPC), as well as Total Phenolic Acids (TPA) and Total Flavonols (TF) of Six Kudingcha Genotypes from the Genus *Ilex*

	ABTS	DPPH	FRAP	OH	TPC	TPA
DPPH	0.832[a]					
FRAP	0.963[b]	0.870[a]				
OH	0.962[b]	0.860[a]	0.954[b]			
TPC	0.968[b]	0.763	0.969[b]	0.926[b]		
TPA	0.861[a]	0.836[a]	0.876[a]	0.776	0.887[a]	
TF	−0.702	−0.366	−0.535	−0.548	−0.694	−0.687

[a] *$p < 0.05$; [b] **$p < 0.01$

In conclusion, this study showed that six Kudingcha genotypes (*I. kudingcha* and *I. cornuta*) from the genus *Ilex* had the similar phenolic profiles and their major phenolic compounds were identified as isomers of mono- and dicaffeoylquinic acids (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid), which accounted for 93.8% calculated by peak area, whereas their minor phenolic components were coumaroylquinic acid, rutin, and kaempferol 3-rutinoside. However, the phenolic compounds of a Kudingcha genotype (*L. robustum*) from the genus *Ligustrum* were entirely different

from those of Kudingcha genotypes from *Ilex* and were identified mainly as phenylethanoid glycosides and phenolic monoterpenoids. Moreover, Kudingcha genotypes of the genus *Ilex* showed significantly better in vitro antioxidant activities than that of the genus *Ligustrum*. Certain genotypes from *Ilex* showed similar or better antioxidant properties in comparison with green tea against some free radicals (e.g., $OH^{\bullet}$ and $DPPH^{\bullet}$). This study suggests that some Kudingcha genotypes from *Ilex* may be consumed as a functional herbal beverage with good antioxidant contribution to our diet.

Large variations in chemical compositions of individual phenolic compounds and in vitro antioxidant activity were observed among diverse Kudingcha genotypes from the genus *Ilex* from different geographic origins in China. The diversity in these phenolic compositions may be of significance for employing different Kudingcha genotypes to develop and produce special Kudingcha beverages and also may be potentially employed to breed advanced lines with improved contents of certain types of phenolic compounds. Additionally, our developed LC fingerprints of different Kudingcha genotypes may be employed for authentication and quality control of Kudingcha during production and processing.

LITERATURE CITED

(1) Chen, X. Y. History of Chinese Gao-Lu tea (Ku-Ding-Cha) and differentiation of its original plant species. *Agric. Archaeol.* **1992**, *2*, 214–219.

(2) Yan, D. H. Health functions, plant species and name evolution of Kuding tea. *Guizhou Agric. Sci.* (in Chinese with English abstract) **2007**, *35*, 114−116.

(3) Wong, I. Y. F.; He, Z. D.; Huang, Y.; Chen, Z. Y. Antioxidative activities of phenylethanoid glycosides from *Ligustrum purpurascens*. *J. Agric. Food Chem.* **2001**, *49*, 3113–3119.

(4) Chen, Z. Y.; Wong, I. Y. F.; Leung, M. W. S.; He, Z. D.; Huang, Y. Characterization of antioxidants present in bitter tea (*Ligustrum pedunculare*). *J. Agric. Food Chem.* **2002**, *50*, 7530–7535.

(5) Lau, K. M.; He, Z. D.; Dong, D.; Fung, K. P.; But, P. P. H. Antioxidative, anti-inflammatory and hepato-protective effects of *Ligustrum robustum*. *J. Ethnopharmacol.* **2002**, *83*, 63–71.

(6) Shu, R. G.; Yao, M. N.; Min, L. Research advances in chemical components and pharmacology of different origins of Kuding Tea. *J. Jiangxi Univ. TCM* (in Chinese with English abstract) **2008**, *20*, 91−94.

(7) Liu, L. X.; Sun, Y.; Laura, T.; Liang, X. F.; Ye, H.; Zeng, X. X. Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C. J. Tseng. *Food Chem.* **2009**, *112*, 35–41.

(8) He, Z. D.; Ueda, S.; Akaji, M.; Fujita, T.; Inoue, K.; Yang, C. R. Monoterpenoid and phenylethanoid glucosides from *Ligustrum pedunculare*. *Phytochemistry* **1994**, *36*, 709–716.

(9) Zhang, C. K. Investigation on the original plants and commercial products of Ku-Ding-Cha. *J. Chinese Med. Mater.* (in Chinese with English abstract) **1994**, *3*, 14−15.

(10) Zhang, F. Q.; Liu, G. M.; Zhou, P.; Xu, L. X.; Guo, A. P. A study on genetic variations, relationships and classification of several "Kudingcha" species in *Ilex* (Aquifoliaceae) based on RAPD analysis. *Acta Bot. Yunnan* (in Chinese with English abstract) **2004**, *26*, 637−644.

(11) Zheng, D. J.; Liang, Y. F.; Liu, G. M.; Yan, D. H.; Linghu, C. D.; Tian, Y. H. RADP analysis of germplasm resources of Kudingcha in Oleaceae. *Scientia Agricultura Sinica* (in Chinese with English abstract) **2008**, *41*, 4164−4172.

(12) Filip, R.; Lotito, S. B.; Ferraro, G.; Fraga, C. G. Antioxidant activity of *Ilex paraguariensis* and related species. *Nutr. Res. (N.Y.)* **2000**, *20*, 1437–1446.

(13) Bravo, L.; Goya, L.; Lecumberri, E. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Res. Int.* **2007**, *40*, 393–405.

(14) Tam, C. F.; Peng, Y.; Liang, Z. T.; He, Z. D.; Zhao, Z. Z. Application of microscopic techniques in authentication of herbal tea−Ku-Ding-Cha. *Microsc. Res. Tech.* **2006**, *69*, 927–932.

(15) Tian, J.; Zhang, H. J.; Sun, H. D.; Pan, L. T.; Yao, P.; Chen, D. Y. Monoterpenoid glycosides from *Ligustrum robustum*. *Phytochemistry* **1998**, *48*, 1013–1018.

(16) She, G. M.; Wang, D.; Zeng, S. F.; Yang, C. R; Zhang, Y. J. New phenylethanoid glycosides and sugar esters from Ku-Ding-Cha, a herbal tea produced from *Ligustrum purpurascens*. *J. Food Sci.* **2008**, *73*, 476–481.

(17) Negishi, O.; Negishi, Y.; Yamaguchi, F.; Sugahara, T. Deodorization with ku-ding-cha containing a large amount of caffeoyl quinic acid derivatives. *J. Agric. Food Chem.* **2004**, *52*, 5513–5518.

(18) Thuong, P. T.; Su, N. D.; Ngoc, T. M.; Hung, T. M.; Dang, N. H.; Thuan, N. D.; Bae, K. H.; Oh, W. K. Antioxidant activity and principles of Vietnam bitter tea *Ilex kudingcha*. *Food Chem.* **2009**, *113*, 139–145.

(19) Shan, B.; Cai, Y. Z.; Sun, M.; Corke, H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.* **2005**, *53*, 7749–7759.

(20) Surveswaran, S.; Cai, Y. Z.; Corke, H.; Sun, M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chem.* **2007**, *102*, 938–953.

(21) 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.

(22) Song, Y. C.; Huang, W. Y.; Sun, C.; Wang, F. W.; Tan, R. X. Characterization of graphislactone A as the antioxidant and free radical-scavenging substance from the culture of *Cephalosporium* sp. IFB-E001, an endophytic fungus in *Trachelospermum jasminoides*. *Biol. Pharm. Bull.* **2005**, *28*, 506–509.

(23) Tian, J.; Sun, H. D. New monoterpenoid glycosides from *Ligustrum robustum*. *Chin. J. Appl. Environ. Biol.* **1999**, *5*, 501–506.

(24) Zhou, J. N.; Ouyang, M. N. Research advancement on the chemical constituents of *Ligustrum*. *Nat. Prod. Res. Dev.* (in Chinese with English abstract) **2003**, *15*, 77−86.

(25) Schütz, K.; Kammerer, D.; Carle, R.; Schieber, A. Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC-DAD-APCI/MS^n. *J. Agric. Food Chem.* **2004**, *52*, 4090–4096.

(26) Miketova, P.; Schram, K. H.; Whitney, J.; Kearns, E. H.; Timmermann, B. N. Mass spectrometry of 3,5- and 4,5-dicaffeoylquinic acids and selected derivatives. *J. Mass Spectrom.* **1999**, *34*, 1240–1252.

(27) Schram, K.; Miketova, P.; Slanina, J.; Humpa, O.; Taborska, E. Mass spectrometry of 1,3- and 1,5-dicaffeoylquinic acids. *J. Mass Spectrom.* **2004**, *39*, 384–395.

(28) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89–143.

(29) Cai, Y. Z.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157–2184.

(30) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.

(31) Huang, D. J.; Ou, B. X.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.

(32) Li, H. B.; Wong, C. C.; Cheng, K. W.; Chen, F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT—Food Sci. Technol.* **2008**, *41*, 385–390.

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