JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Polyphenolic Compounds, Antioxidant Capacity, and Quinone Reductase Activity of an Aqueous Extract of *Ardisia compressa* in Comparison to Mate (*Ilex paraguariensis*) and Green (*Camellia sinensis*) Teas

SONIA CHANDRA AND ELVIRA GONZALEZ DE MEJIA*

Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 228 ERML, MC-051, 1201 W. Gregory Drive, Urbana, Illinois 61801

Aqueous extracts of the leaves of *Ardisia compressa* (AC) have been used in folk medicine to treat various liver disorders including liver cancer. The objective of this study was to partially characterize and determine the total polyphenol content, antioxidant capacity, and quinone reductase activity of *A. compressa* tea in comparison to mate (*Ilex paraguariensis,* MT) and green (*Camellia sinensis,* GT) teas. Total polyphenol content, antioxidant capacity, and phase II enzyme induction capacity were measured by the modified Folin-Ciocalteu, ORAC, and quinone reductase (QR) assays, respectively. The major polyphenols in AC were not catechins. HPLC retention times and standard spikes of AC indicated the presence of gallic acid, epicatechin gallate, ardisin and kaempferol. Using catechin as standard, the total polyphenol value of AC (36.8 ± 1.1 mg/mg DL) was significantly lower than GT (137.2 ± 5.8 mg equivalent of (+)-catechin/mg dried leaves, DL) and MT (82.1 ± 3.8 mg/mg DL) ($P < 0.001$). Antioxidant capacity (AC, 333; GT, 1346; MT, 1239 mmol Trolox equivalents/g DL) correlated with total polyphenol values ($r^2 = 0.86$, $P < 0.01$). AC (4.5−12.5 μg/mL) induced QR enzyme, in Hepa1c1c7 cells, up to 15%. MT and GT showed no induction at the concentrations tested (0.5−10.5 and 0.5−12.5 mg/mL, respectively). These results suggest that AC has a different mechanism of protection against cytotoxicity that is not related to its antioxidant capacity. Further studies are needed to determine such mechanisms and to explore its potential as a chemopreventive or therapeutic agent.

KEYWORDS: ***Ardisia compressa*; mate; green tea; polyphenols; quinone reductase; ORAC**

INTRODUCTION

Aqueous extracts of the leaves of *Ardisia compressa* (AC) have been used by indigenous Mexican people as a folk medicine for the treatment of liver disorders, including liver cancer. However, the corresponding scientific information is lacking. Only recently, research in our laboratory has demonstrated that aqueous extracts of AC can protect cultured rat hepatocytes from cytotoxicity, genotoxicity, and oxidative damage induced by benomyl and 1-nitropyrene even better than epigallocatechin gallate (EGCG) (*1*, *2*). Aqueous extracts of AC also showed anti-topoisomerase I and II activities, leading to the identification of ardisin (*2*).

These findings provide some scientific information that supports the potential biological action of *A. compressa* against liver disorders. However, the molecular mechanism and the active compounds are still unknown and need to be investigated.

Antioxidant capacity is a common mechanism of compounds in teas that show chemoprotective and therapeutic activity (*3*). For example, black, oolong, and green teas, through their antioxidative activity, can inhibit various mutagens in cell culture assays (*4*). It has also been reported that polyphenolic compounds extracted from green tea, especially epicatechin (EC) and epicatechin gallate (ECG), are effective antioxidants against peroxidation of rat liver microsomes induced by 2,2′-azobis-2-amidinopropane hydrochloride (*5*). Epigallocatechin gallate (EGCG) shows a potent antioxidant capacity against oxidative stress from ultraviolet A radiation (*6*). Antioxidative properties have also been found in mate tea (*Ilex paraguariensis*), an herbal tea consumed as a stimulating drink in Paraguay, Argentina, Uruguay, and southern Brazil (*7*). The aqueous extract of mate can reduce the oxidability of human plasma low-density lipoprotein (LDL) in vivo (*8*) and protect DNA oxidation and in vitro LDL lipoperoxidation (*9*).

Another proposed chemopreventive and anticarcinogenic mechanism has been related to the capacity to induce phase II enzymes during the detoxification process (*10*, *11*). Upregulation of phase II detoxification enzymes such as quinone reductase (QR) is thought to be a useful biomarker for anticarcinogenesis (*12*).

* To whom correspondence should be addressed. Tel.: +1 (217) 244−3196. Fax: +1 (217) 265−0925. E-mail: edemejia@uiuc.edu.

10.1021/jf0352632 CCC: $27.50
Published on Web 05/05/2004

An assay for measuring the induction activity on phase II enzymes has been developed using quinone reductase. The QR enzyme reduces quinones to phenolic compounds, which are further conjugated to less reactive glucuronides and sulfate ester, which are then secreted in urine (*13*). The QR assay is used as a simple screening method to detect the capacity of a compound to act as a potential anticarcinogen (*14*).

The objective of this study was to determine total polyphenol content and composition, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate and green teas using the same standardized methodology. It was also thought that the information obtained would shed new light on the mechanism of action of *A. compressa*.

MATERIALS AND METHODS

Biological Material. The Hepa1c1c7 cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD). *Ardisia compressa* leaves were collected from the Pacific coast of Mexico (State of Michoacan). Fine dried leaves of mate and green tea (Romance and Lipton brands, respectively) were obtained from the local market.

Chemicals. 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 99% caffeine anhydrous, 98% gallic acid monohydrate, 90% (−)-epicatechin, 97% flavone, and 99% *trans*-4-hydroxy-3-methoxy cinnamic acid were purchased from Aldrich (Milwaukee, WI). Biorad protein reagent was from Bio-Rad (Hercules, CA). Tween 20 and methanol (HPLC grade) were obtained from Fisher Scientific (Hanover Park, IL). Quercetin-3-β-glucoside (>90%), 97% myricetin, *p*-coumaric acid (>98% HPLC grade), syringic acid (>97%), and 99% theophylline were purchased from Fluka (Milwaukee, WI). Formic acid (>96%, ACS grade), (+)-catechin hydrate (>98%), (−)-catechin gallate, (−)-epicatechin gallate (98% HPLC grade), (−)-gallocatechin, 98% epigallocatechin, (−)-gallocatechin gallate, (−)-epigallocatechin gallate (>80%, HPLC grade), (−)-caffeic acid, quercetin dihydrate, rutin hydrate (>95%), 90% kaempferol, bergenin monohydrate, theobromine (>99%), and theaflavin (>80%) were obtained from Sigma (St. Louis, MO). All the other materials used were also obtained from Sigma (St. Louis, MO).

Preparation of Herbal Tea. The aqueous extract of AC was prepared as described previously (*2*). Fresh leaves of *A. compressa* were air-dried without exposure to sunlight. The dried leaves (DL) were kept in plastic bags and refrigerated at 4 °C. The extract was prepared from 2.7 g DL that was soaked in 250 mL of boiling water (98 °C) for 10 min. The mixture was cooled to room temperature before filtration using 0.45-μm filter paper and then lyophilized. The freeze-dried solid extract was kept at −20 °C in plastic tubes, sealed with Parafilm and protected from light. Freeze-dried solid extracts (SE) of mate and green tea were prepared following the same standardized procedure as that described for AC. The SEs were redissolved in ddH_2O, filtered with a 0.22-μL syringe top filter, prior to use in all assays. To standardize the phenolic content, the total polyphenol content of SE from every preparation was measured.

Total Polyphenol Content and Characterization of Phenolic Compounds. The total polyphenol contents of the freeze-dried materials were measured as described by the modified Folin-Ciocalteu method (*15*). Briefly, 1 mL of 1 N Folin−Ciocalteu reagent was added to a 1 mL sample (GT, MT: 108 mg/L; AC: 540 mg/L), and this mixture was allowed to stand for 2−5 min before the addition of 2 mL of 20% Na_2CO_3. The solution was then allowed to stand for 10 more minutes before reading at 730 nm in a Beckman DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). The total polyphenol content was expressed as milligram equivalents to the standard used per milliliter of aqueous extract or gram of DL. Equations obtained for standard curves were $y = 0.0125x - 0.0758$, $r^2 = 0.99$; $y = 0.0267x - 0.0966$, $r^2 = 0.98$; and $y = 0.0269x - 0.0500$, $r^2 = 0.98$; for EGCG; gallic acid; and (+)-catechin, respectively.

The characterization of phenolic compounds was performed using a 1050 Hewlett-Packard (Palo Alto, CA) gradient liquid chromatograph, equipped with a 1050 HP auto sampler, a 1050 HP gradient pump, a 1050 HP photodiode array detector (PDA) and helium sparge. A C_{18} RP guard column and a C_{18} RP Phenomenex Prodigy ODS column (250- × 4.6-mm × 5-μm), were used. This method was chosen because it gave the best resolution among several methods that were developed. The column temperature was ambient, and the elution (1 mL/min) was performed using a solvent system comprising solvents A (water/methanol/formic acid, 79.7/20/0.3) and B (methanol/formic acid, 99.7/0.3) mixed using a gradient starting with 100% A, linearly decreasing to 48% A in 52 min, to 20% A in 5 min, and held at 20% A for 3 min, then a linear increase to 100% A in 5 min. The initial condition was held for 5 min before the next run. Mate was analyzed using the same method at a flow rate of 0.9 mL/min. For green tea, the analysis was started with 100% A for 10 min, suddenly decreased to 90% A and held for 15 min, then to 70% A, 40% A, and 20% A at 10 min intervals, and held at 20% A for 2 min before returning to the initial conditions in 5 min. The injection volume was 5−50 μL. Sample concentration was 0.3 mg/mL for pure standards and 0.84 μg equivalent of (+)-catechin for AC, GT, and MT. The PDA detector was set in the range of 195−450 nm, with outputs at 260, 280, 330, and 360 nm. The partial identification of AC phenolic compounds was based on standard retention times, spike standards, and spectra comparisons. A spectral library was built up from 25 of the standards mentioned in the Chemicals section. Calibration curves were generated for GA, ECG, and ardisin by plotting concentrations versus peak areas. Regression equations were used to calculate percentage recovery for GA ($y = 2020.96x + 5.51$, $r^2 = 0.999$), ECG ($y = 1423.47x - 7.12$, $r^2 = 0.999$), and ardisin ($y = 1064.12x + 334.86$, $r^2 = 0.997$).

Antioxidant Capacity. Total antioxidant capacity (ORAC assay (*16*)) was determined by measuring the protection of the tea samples on β-phycoerythrin (b-PE) fluorescent in the presence of free radicals generated by AAPH. The assay was carried out in black-walled 96-well plates (Fisher Scientific, Hanover Park, IL). Each well had a final volume of 200 μL. The following reactants were added in the order: 25 μL of 75 mM phosphate buffer pH 7; either 25 μL Trolox standard (1 mM final concentration) or sample (1.0−3.0 μg eq (+)-catechin/mL); 100 μL of b-PE (1.52 nM final concentration); and 50 μL of AAPH (41.6 mM final concentration). As a blank, 25 μL of 75 mM phosphate buffer pH 7 was added instead of Trolox or samples. Immediately after addition of AAPH, plates were placed in an FL × 800 fluorescence plate reader (Bio-Tek Instruments, Winooski, VT), set with excitation filter 530/25 nm and emission filter 590/35 nm, and then read every 2 min for 2 h to reach a 95% loss of fluorescence. Final fluorescence measurements were expressed relative to the initial reading. Results were calculated based on the differences in the area under the b-PE decay curve between the blank and a sample and expressed as millimoles of Trolox equivalents (TE)/g dry leaves (DL). Trolox (1−4 μM) was used as a standard ($y = 3.35x + 0.42$, $r^2 = 0.984$).

Quinone Reductase (QR) Activity. *Cell Culture.* Hepa1c1c7 murine hepatoma cells were grown in α-minimum essential medium (MEM), enriched with 10% heat and charcoal-inactivated fetal bovine serum and maintained at 37 °C in 95% ambient air and 5% CO_2. The cells were split every 4 days with a split ratio of 7. Cells with 80−90% confluence were plated into 96-well plates (Costar 3595, Corning Inc, Corning, NY), 1×10^4 cells per well, and incubated for 24 h in antibiotic-enriched media (100 units/mL penicillin, 100 μg/mL streptomycin).

QR Induction Activity. The QR induction activities of different teas were determined by means of QR assay (*17*). The cells were grown in 96-well plates (Costar 3595, Corning Inc, Corning, NY) for 24 h and then exposed to the different samples (GT, 2.5−12.5 μg eq (+)-catechin/mL; MT, 2.5−10.5 μg eq (+)-catechin/mL; and AC, 4.5−80.0 μg eq (+)-catechin/mL) for 24 h. Growth media and 1 μM β-naphthoflavone were used as negative and positive controls, respectively. Treated cells were rinsed with phosphate buffer pH 7.4, lysed with 50 μL 0.8% digitonin in 2 mM EDTA, incubated and agitated for 10 min. A 200-μL aliquot of mixed solution (74 mL of 25 mM Tris buffer; 50 mg of BSA; 0.5 mL of 1.5% Tween-20 solution; 0.5 mL of thawed cofactor solution (92.7%, 150 mM glucose-6-phosphate; 6.15%, 4.5 mM NADP; 1.14%, 0.75 mM FAD in Tris buffer); 150 units of

Table 1. Total Polyphenol Contents of Green, Mate, and Ardisia Teas[a]

	ardisia		green tea		mate	
polyphenol	mg/mL	mg/g DL	mg/mL[e]	mg/g DL[f]	mg/mL	mg/g DL
EGCG	1.20 ± 0.03[b]	81.19 ± 2.33	4.58 ± 0.12[c]	306.63 ± 12.73	2.68 ± 0.06[d]	190.89 ± 0.59
gallic acid	0.58 ± 0.02[b]	39.07 ± 1.11	2.22 ± 0.06[c]	148.77 ± 6.11	1.33 ± 0.03[d]	94.91 ± 4.18
(+) catechin	0.54 ± 0.02[b]	36.78 ± 1.07	2.05 ± 0.06[c]	137.19 ± 5.79	1.15 ± 0.03[d]	82.13 ± 3.83

[a] Values are the average of three independent tea preparations. All CVs are less than 5.0%. Abbreviations: DL, dried leaves; EGCG, epigallocatechin gallate. [b–d] Different superscripted letters in a row indicate statistical differences ($P < 0.001$). [e] Concentration of phenolic compounds expressed as milligram equivalents of EGCG, gallic acid, or (+)-catechin per milliliter of aqueous extract. [f] Concentration of phenolic compounds expressed as milligram equiv of EGCG, gallic acid, or (+)-catechin per gram of dry leaves.

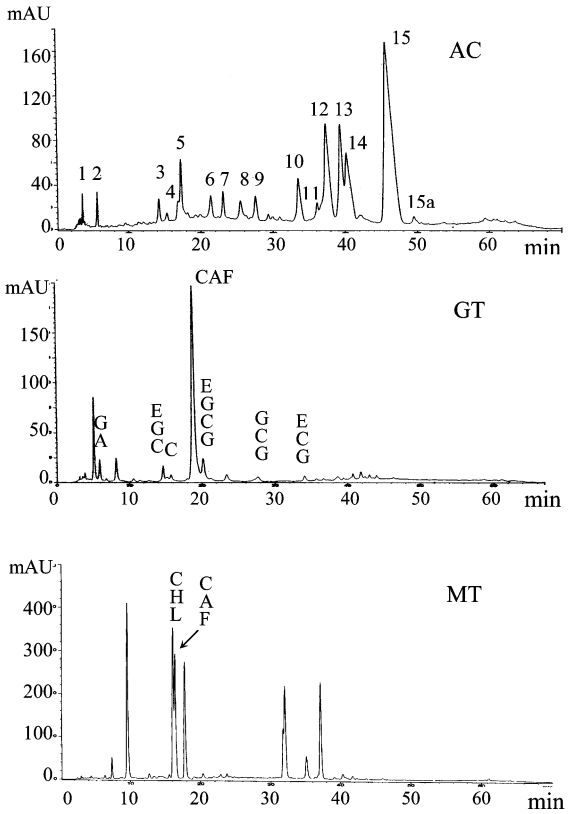

Figure 1. Comparative HPLC chromatogram of Ardisia, mate, and green teas at 280 nm. HPLC systems are described in the text. See **Table 2** for maximum wavelengths of AC peaks. AC peaks: gallic acid (2), proanthocyanidins (3, 4, 5, 7), ECG (6), flavonol, flavanone, or dihydroflavonol (10, 11, 13, 15), quinone derivative (12), ardisin (14), and kaempferol (15a).

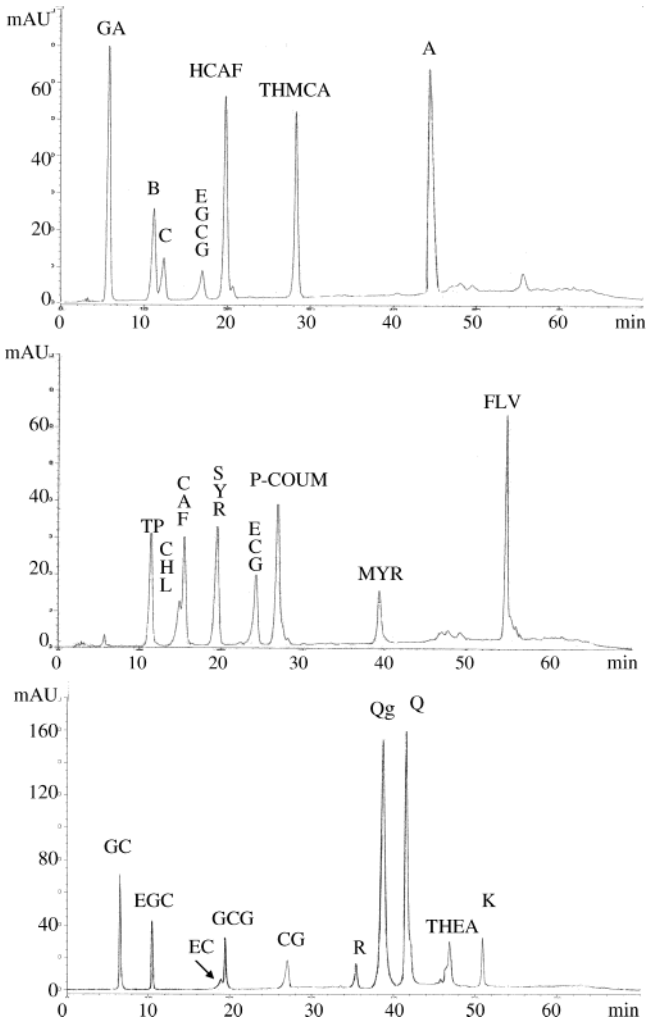

Figure 2. Chromatograms of 25 phenolic standards at 280 nm. Result of HPLC analysis on Phenomenex Prodigy column with conditions as described in the text. For abbreviations, see **Table 2**.

glucose-6-phosphate dehydrogenase; 22.5 mg of MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide); and 75 μL of 50 mM menadione in acetonitrile) was added into lysed cells. Readings were made at five time points, 50 s apart, using a μQuant microplate reader (Bio-Tek Instruments, Winooski, VT) at 610 nm. Immediately after completion of the readings, 50 μL of 0.3 mM dicumarol in 25 mM Tris buffer was added into each well, and the plate was read again (five time points, 50 s apart). Total protein content was measured by BioRad assay (*18*). QR induction activity was expressed as the specific activity (nmol MTT reduced/mg/min) ratio of treated to control cells. IC_{90} values were calculated by analysis of the percent cell inhibition of each tea at 0.5−20 μg equiv (+)-catechin/mL for MT and GT, and 35−80 μg eq (+)-catechin/mL for AC.

Statistical Analysis. A one-way ANOVA, with Dunnett and linear trend post test was used for statistical analysis. A probability (P) value of <0.05 indicated a significant difference.

RESULTS AND DISCUSSION

Table 1 presents the total polyphenol content of AC in comparison to green and mate teas. Values are expressed both as milligram equiv/L of either EGCG or gallic acid or (+)-catechin and as grams per dry leaves. AC, GT, and MT had significantly different total polyphenol contents ($P < 0.001$). AC showed the lowest value (81.19 mg equiv EGCG/g DL), and GT showed the highest (306 mg eq EGCG/g DL). The total phenolic content of GT was within the range of phenolic contents previously reported (*19, 20*). The Folin−Ciocalteu method is relatively simple; however, it is not specific. Heterogeneity of phenolic compounds and the presence of easily

Table 2. Maximum Wavelength of HPLC Peaks[a] of *Ardisia compressa* and Selected Standards

AC peak number/compound	max wavelength (nm)	peak number/ compound	max wavelength (nm)
1	285	catechin gallate (CG)	280
2	273	chlorogenic acid (CHL)	300 sh, 327
3	279	epicatechin (EC)	280
4	278	epicatechin gallate (ECG)	277
5	279	epigallocatechin (EGC)	273
6	280	epigallocatechin gallate (EGCG)	275
7	277	flavone (FLV)	293 sh, 297, 310 sh
8	259, 283	gallic acid (GA)	273
9	280, 337sh	gallocatechin (GC)	272, 280 sh
10	267, 342	gallocatechin gallate (GCG)	277
11	263 sh, 350, 365	kaempferol (K)	267, 285 sh, 320 sh, 365
12	253, 298 sh, 343 sh,358	myricetin (Myr)	255, 304, 374
13	282, 343	*p*-coumaric acid (p-coum)	300 sh, 313
14	256, 267, 295 sh, 353	Q-glycoside (Qg)	246, 295 sh, 356
15	280, 340	quercetin (Q)	257, 302 sh, 372
15a	267, 285 sh, 320, 365	rutin (R)	258, 267 sh, 295 sh, 355
ardisin (A)	256, 267, 295 sh, 353	syringic acid (SYR)	275
bergenin (B)	274, 314 sh	theaflavine (THEA)	275, 375
caffeic acid (HCAF)	297 sh, 325	theophyllin (TP)	273
caffeine (CAF)	275	*trans*-4-hydroxy-3- methoxy cinnamic acid (THMCA)	297 sh, 325
catechin (C)	279		

[a] Based on **Figures 1** and **2**. Peaks 2, 6, 14 and 15a were identified as gallic acid, ECG, ardisin and kaempferol, respectively. Peaks 3, 4, 5, and 7 represent proanthocyanidins. Peaks 10, 11, 13, and 15 represent flavonol, flavanone, or dihydroflavonol, and peak 12, a quinone derivative.

oxidized substances other than phenols can interfere with the result (*21*). Moreover, the total phenolic content based on certain standards may underestimate or overestimate the real total phenolic value. Although EGCG, gallic acid, and catechin are present in green tea (*22*), these phenolic compounds are not present in mate tea (*23*). However, to compare relative values of total polyphenols, mate tea was also reported as equivalents of EGCG, gallic acid, or catechin.

A preliminary characterization of AC phenolic compounds was conducted to compare the phenolic composition, or fingerprint, of AC in relation to that of GT and MT (**Figure 1**). Chromatograms of 25 standards are shown in **Figure 2**. Methods for the characterization of AC polyphenols were developed using several C_{18} reversed-phase columns in combination with different solvent composition. Phenomenex Prodigy and Waters Xterra with mobile phases water/methanol/formic acid (79.7/20/0.3) and water/formic acid (99/1), respectively, performed a comparable quality separation (data not shown). Phenomenex Prodigy with methanol as mobile phase was chosen. Previously reported major phenolic compounds in GT (*24*, *25*) and MT (*23*) were identified using this method. On the basis of the results of the present investigation, the composition of AC phenolic compounds is totally different from those of GT and MT.

The aqueous extract of AC had the most complex mixture of compounds. Retention times, standard spikes (90−100% recovery), and spectra comparison suggested the presence of gallic acid (peak 2), epicatechin gallate (peak 6), ardisin (peak 14), and kaempferol (peak 15a). Bergenin, caffeic acid, caffeine, catechin, catechin gallate, chlorogenic acid, *p*-coumaric acid, epicatechin, epigallocatechin, epigallocatechingallate, flavone, gallocatechin, gallocatechin gallate, myricetin, quercetin, quercetin-3-glucoside, rutin, syringic acid, theaflavine, theophylline, and *trans*-4-hydroxy-3-methoxy cinnamic acid were not identified as part of the main compounds in AC tea (**Figure 1**). Some of these compounds, such as quercetin, may be present as derivative or isomeric forms and consequently could not be identified because they lacked a perfect UV spectra comparison and standard spike match. The UV spectra of peaks 3, 4, 5, and 7 suggested the presence of proanthocyanidins such as those found in green tea (*26*, *27*). Peaks 10, 11, 13 and 15 indicate the presence of flavonols (band II 250−280, band I 330−385), flavanone or dihydroflavonols (band II 275−295, band I 300−330 sh) (*28*). Moreover, the similarity of the UV spectra of peak

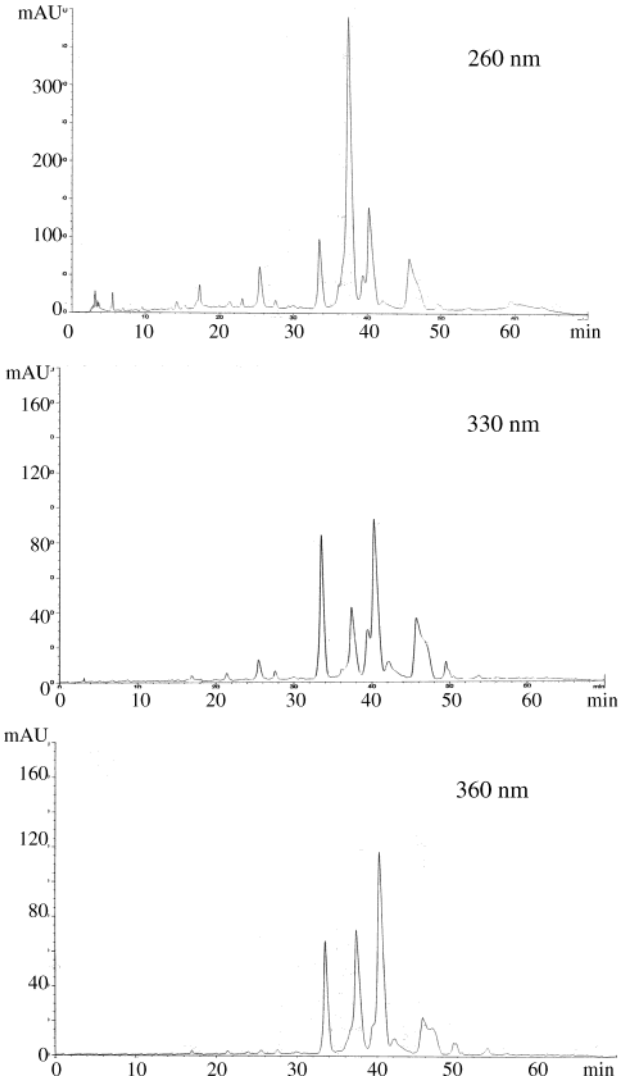

Figure 3. HPLC chromatogram of Ardisia tea at 260, 330, and 360 nm.

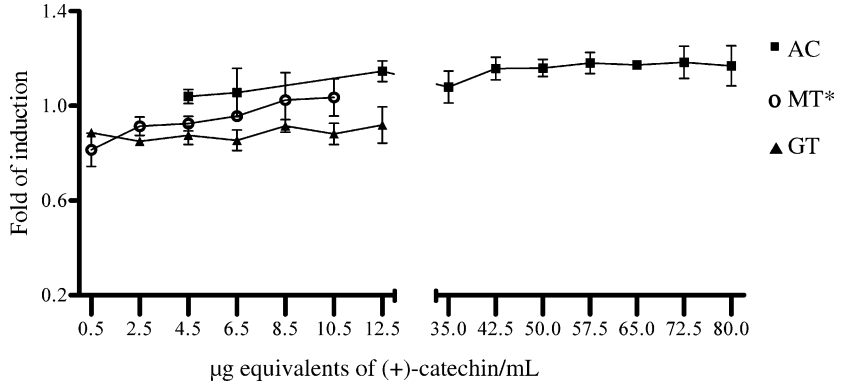

Figure 4. Effects of Ardisia, mate, and green teas on induction of quinone reductase activity in Hepa1c1c7 cells. Values are expressed as a ratio of QR specific activity of 24 h treated cells over a negative control. The positive control (1 μM βNF) was 3.90 ± 0.02. Mean ± SD (n = 6). Indicates significant linear trend (P < 0.05).

Table 3. Antioxidant Capacity of Mate, Green, and Ardisia Teas

	ORAC value[a]		
tea sample	nmol TE/μg equiv gallic acid	μmol TE/mL	μmol TE/g DL
mate	13.1 ± 0.6[c]	17.4 ± 0.8[c]	1238.9 ± 55.7[c]
green	9.1 ± 0.4[b]	20.1 ± 0.9[c]	1345.9 ± 60.0[c]
ardisia	8.5 ± 0.2[b]	4.9 ± 0.1[b]	333.5 ± 8.2[b]

[a] Antioxidant capacity relative to 1 μM Trolox. Values are the average of three independent tea preparations. Abbreviations: TE, Trolox equiv; DL, g dried leaves. [b−c] Values with different letters in the same column are statistically different, P < 0.001.

12 to that of peak 14 (ardisin) suggests that this peak represents a quinone derivative. Maximum wavelengths of AC chromatographic peaks and standards are listed in **Table 2**. Studies to isolate and further identify additional AC phenolic compounds are under way in our laboratory.

Major compounds in AC were retained in the column during the first half run (**Figure 3**). Under reversed-phase conditions, the results suggested that major compounds in AC were relatively less polar. Folin−Ciocalteu method measures total polyphenol content based on the reduction of the reagent, which depends on the availability of readily oxidized compounds. Thus, AC, which has less polar phenolic compounds than GT, gave a lower total polyphenol value using this method. *Ardisia compressa* tea is low in catechin type polyphenols, however, it also contains other more hydrophobic phenolic compounds.

The antioxidant capacities of AC, MT, and GT are summarized in **Table 3**. Mate tea showed the highest antioxidant capacity per μg gallic acid equivalent (13.1 nmol TE/μg) (P < 0.001), followed by green tea (9.1 nmol TE/μg), and AC (8.5 nmol TE/μg). However, when the antioxidant capacity was expressed as μmol TE/mL or as μmol TE/g DL, green tea, with the highest total polyphenol content, showed the highest antioxidant capacity as ORAC values. AC (333.5 μmol TE/g DL or 4.9 μmol TE/mL) had significantly lower ORAC values than mate (1238.9 μmol TE/g DL or 17.4 μmol/mL) and green tea (1345.9 μmol TE/g DL or 20.1 μmol TE/mL). In general, antioxidant capacity correlated positively with total polyphenol content per gram of dry leaves (r^2 = 0.86, P < 0.01). The ORAC values obtained in this study agree with the range of ORAC values reported for other teas (235-1526 μmol TE/g DL) (*16*). An improved ORAC assay has been reported (*29*) using fluorescein, a more stable compound than β-phycoerythrin. The use of fluorescein can also prevent nonspecific protein binding

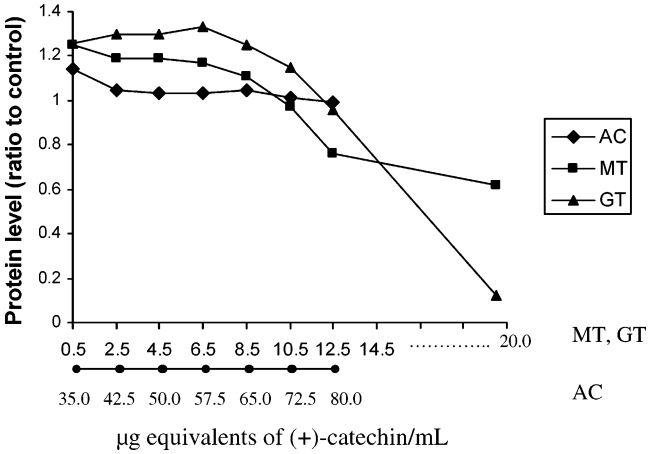

Figure 5. Protein level of Hepa1c1c7 cells treated with Ardisia, mate, and green teas.

that may happen with β-PE, which may affect the antioxidant capacity of the polyphenols. It was indicated that this improved method gave from 1.65 to 3.5 times higher ORAC values for several phenols studied. Despite these considerations, ORAC values in this study can still be used for comparison of AC's antioxidant capacity with those of green and mate teas.

As shown in **Figure 4**, AC (4.5−12.5 μg/mL) induced QR enzyme, in Hepa1c1c7 cells, up to 15%. However, at higher concentrations (42.5−80 μg/mL) the induction increased only an additional 3%. MT showed very low induction (<5.0%) at concentrations of 8.5 and 10.5 μg/mL, while GT showed no induction at the tested concentrations (0.5−12.5 μg/mL). MT, however, showed a significant linear trend with increasing concentrations (P < 0.05). This was not observed for either AC or GT. To obtain appropriate responses without damaging the Hepa1c1c7 cells, the concentrations used in this investigation (expressed as μg eq (+)-catechin/mL) were different among the tested extracts. Hepa1c1c7 presented cytotoxicity when exposed to GT and MT with IC_{90} = 12.88 eq (+)-catechin/mL, and IC_{90} = 10.96 eq (+)-catechin/mL, respectively. In contrast, AC was not toxic to the cells up to a concentration of 80 μg/mL (**Figure 5**). The differences in cellular sensitivity indicated that the phenolic compounds measured as total polyphenols of AC were different from those of MT and GT. The inability of GT to induce QR was in accord with its phenolic composition. The main phenols in GT were catechins, with EGCG as the major compound. Flavanols have been reported as being poor QR inducers in the Hepa1c1c7 model; while flavonols, such as

myricetine, quercetin, kaempferol, and galangin, are effective QR inducers with a maximal induction level from 1.6 to 3.6 (*30*). Studies on human liver cell lines (Chang liver cells) also showed that EGCG is a poor inducer for QR; although, in this model, green tea aqueous extract, green tea polyphenols, and several other catechins caused QR induction (*10*). The effect of AC and mate in inducing QR activity in Hepa1c1c7 cells was much lower than that of sulforaphane, which at a concentration of 2.5 μM causes a maximum of 3.1-fold induction over control (*31*). The concentrations used in this study were too low to cause significant induction compared with the negative control; however, this was limited by the apparent cytotoxicity on cell line Hepa1c1c7.

In conclusion, this study revealed that in *Ardisia compressa* tea, catechins were not the main phenolic compounds. The higher quinone reductase activity and lower antioxidant capacity of AC in comparison with mate and green tea suggest non-antioxidative mechanisms of protection behind its biological activity. The phenolic characterization also suggests that other compounds, still to be determined, may be involved in AC's mechanism of action. Further studies are needed to explore the mechanism and the potential of this herbal tea as a chemopreventive and therapeutic agent.

ACKNOWLEDGMENT

Special thanks to Dr. Elizabeth Jeffery, Dr. Keith R. Cadwallader, John P. Jerrel, Dr. Walter L. Hurley, Marco Ramirez-Mares, Nathan Matusheski, Marian Victoria Eberhardt, and Tanoj Singh for their valuable assistance in conducting this study.

LITERATURE CITED

(1) Ramirez-Mares, M. V.; Fatell, S.; Villa-Trevino, S.; De Mejia, E. G. Protection of extract from leaves of *Ardisia compressa* against benomyl-induced cytotoxicity and genotoxicity in cultured rat hepatocytes. *Toxicol. In Vitro* **1999**, *13*, 889−896.

(2) Gonzalez de Mejia, E.; Ramirez-Mares, M. V.; Nair, M. G. Topoisomerase I and II enzyme inhibitory aqueous extract of *Ardisia compressa* and ardisin protect against benomyl oxidation of hepatocytes. *J. Agric. Food Chem.* **2002**, *50*, 7714−7719.

(3) Rietveld, A.; Wiseman, S. Antioxidant effects of tea: evidence from human clinical trials. *J. Nutr.* **2003**, *133*, 3285S-3292S.

(4) Cai, Y. J.; Ma, L. P.; Hou, L. F.; Zhou, B.; Yang, L.; Liu, Z. L. Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes. *Chem. Phys. Lipids* **2002**, *120*, 109−117.

(5) Hour, T. C.; Liang, Y. C.; Chu, I. S.; Lin, J. K. Inhibition of eleven mutagens by various tea extracts, (−)epigallocatechin-3-gallate, gallic acid and caffeine. *Food Chem. Toxicol.* **1999**, *37*, 569−579.

(6) Tobi, S. E.; Gilbert, M.; Paul, N.; McMillan, T. J. The green tea polyphenol, epigallocatechin-3-gallate, protects against the oxidative cellular and genotoxic damage of UVA radiation. *Int. J. Cancer* **2002**, *102*, 439−444.

(7) VandeJagt, T. J.; Ghattas, R.; VanderJagt, D. J.; Crossey, M.; Glew, R. H. Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. *Life Sci.* **2002**, *70*, 1035−1040.

(8) Bracesco, N.; Dell, M.; Rocha, A.; Behtash, S.; Menini, T.; Gugliucci, A.; Nunes, E. Antioxidant activity of a botanical extract preparation of *Ilex paraguariensis*: prevention of DNA double-strand breaks in *Saccharomyces cerevisiae* and human low-density lipoprotein oxidation. *J. Altern. Complement. Med.* **2003**, *9*, 379−387.

(9) Gugliucci, A. Antioxidant effects of *Ilex paraguariensis*: induction of decreased oxidability of human LDL in vivo. *Biochem. Biophys. Res. Commun.* **1996**, *224*, 338−344.

(10) Steele, V. E.; Kelloff, G. J.; Balentine, D.; Boone, C. W.; Mehta, R.; Bagheri, D.; Sigman, C. C.; Zhu, S.; Sharma, S. Comparative chemopreventive mechanisms of green tea, black tea, and selected polyphenol extracts measured by in vitro bioassays. *Carcinogenesis* **2000**, *21*, 63−67.

(11) Khan, S. G.; Katiyar, S. K.; Agarwal, R.; Mukhtar, H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. *Cancer Res.* **1992**, *52*, 4050−4052.

(12) Jaiswal, A. K. Regulation of genes encoding NAD(P)H: quinone oxidoreductases. *Free Radical Biol. Med.* **2000**, *29*, 254−262.

(13) Stipanuk, M. H. *Biochemical and Physiological Aspects of Human Nutrition*; W. B. Saunders: Philadelphia, **2000**.

(14) Prochaska, H. J. Screening strategies for the detection of anticarcinogenic enzyme inducers. *J. Nutr. Biochem.* **1994**, *5*, 360−368.

(15) Nurmi, K.; Ossipov, V.; Haukioja, E.; Pihlaja, K. Variation of total phenolic content and individual low-molecular-weight phenolics in foliage of mountain birch trees (*Betula pubescens* ssp. *tortuosa*). *J. Chem. Ecol.* **1996**, *22*, 2023−2040.

(16) Prior R. L.; Cao, G. Antioxidant capacity and polyphenolic components of teas: implications for altering in vivo antioxidant status. *Proc. Soc. Exp. Biol. Med.* **1999**, *220*, 255−261.

(17) Prochaska, H. J.; Santamaria, A. B. Direct measurement of NAD(P)H: quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **1988**, *169*, 328−336.

(18) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(19) Astill, C.; Birch, M. R.; Dacombe, C.; Humphrey, P. G.; Martin, P. T. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *J. Agric. Food. Chem.* **2001**, *49*, 5340−5347.

(20) Beecher, G. R. Overview of dietary flavonoids: nomenclature, occurrence and intake. *J. Nutr.* **2003**, *133*, 3248S-3254S.

(21) Singleton V. L.; Rudolf, O.; Rosa, M. L. Analysis of total polyphenol and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. *Methods Enzymol.* **1974**, *299*, 152−178.

(22) Lakenbrink, C.; Lapczynski, S.; Maiwald, B.; Engelhardt, U. H. Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J. Agric. Food Chem.* **2000**, *48*, 2848−2852.

(23) Filip, R.; Lopez, P.; Giberti, G.; Coussio, J.; Ferraro, G. Phenolic compounds in seven South American *Ilex* species. *Fitoterapia* **2001**, *72*, 774−778.

(24) Zuo, Y.; Chen, H.; Deng, Y. Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black, and pu-erh teas using HPLC with a photodiode array detector. *Talanta* **2002**, *57*, 307−316.

(25) Lin, Y. S.; Tsai, Y. J.; Tsay, J. S.; Lin, J. K. Survey of catechins, gallic acid, and methylxanthines in green, oolong, pu-erh, and black teas. *J. Agric. Food Chem.* **1998**, *46*, 3635−3642.

(26) Nonaka, G.-I.; Sakai, R.; Nishioka, I. Hydrolyzable tannins and proanthocyanidins from green tea. *Phytochemistry* **1984**, *23*, 1753−1755.

(27) Nonaka, G.-I.; Kawahara, O.; Nishioka, I. Tannins and related compounds. XV. A new class of dimeric flavan-3-ol gallates, theasinensins A and B, and proanthocyanidin gallates from green tea leaf. *Chem. Pharm. Bull.* **1983**, *31*, 3906−3914.

(28) Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: New York, 1982.

(29) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(30) Uda, Y.; Price, K. R.; Williamson, G.; Rhodes, M. J. Induction of the anticarcinogenic marker enzyme, quinone reductase, in murine hepatoma cells in vitro by flavonoids. *Cancer Lett.* **1997**, *120*, 213-216.

(31) Matusheski, N. V.; Jeffery, E. H. Comparison of the bioactivity of two glucoraphanin hydrolysis products found in broccoli, sulforaphane, and sulforaphane nitrile. *J. Agric. Food Chem.* **2001**, *49*, 5743−5749.

Received for review October 28, 2003. Revised manuscript received March 16, 2004. Accepted March 22, 2004.

JF0352632