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

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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 µg/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).
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 Hepal1c1c7 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). Biodar protein reagent was from Bio-Rad (Hercules, CA). Tween 20 and methanol (HPLC grade) were obtained from Fisher Scientific (Hanover Park, IL). Quercetin-3-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 (98% HPLC grade), (→)-gallocatechin gallate, 98% epigallocatechin gallate (98% 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 at 0.45-°C, was kept at 0.45-°C, and then read every 2 min for 2 h to reach a 95% loss of fluorescence. The 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).

**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/mL; AC: 540 mg/mL), and this mixture was allowed to stand for 2–5 min before the addition of 2 mL of 20% NaCO3. 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 millilitre of aqueous extract or gram of DL. Equations obtained for standard curves were y = 0.0125x − 0.00785, r2 = 0.99; y = 0.0267x − 0.0966, r2 = 0.98; and y = 0.0269x − 0.0500, r2 = 0.98; for EGCg; gallic acid; and (→)-catechin, respectively.

**Antioxidant Capacity.** Total antioxidant capacity (ORAC assay (16)) was determined by measuring the protection of the beta-carotene in low-density lipoprotein (LDL) from β-carotene degradation (14). 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 reagents 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 equivalents (→)-catechin/mL); 100 μL of b-PE (1.52 mM final concentration); and 50 μL of APAP (41.6 mM final concentration). As a blank, 25 μL of 75 mM phosphate buffer pH 7 was added instead of Trolox or samples.

**Quinone Reductase (QR) Activity.** Hepal1c1c7 murine hepatoma cells were grown in d-minimum essential medium (MEM), enriched with 10% heat and charcoal-inactivated fetal bovine serum and maintained at 37 °C in 95% humidified air and 5% CO2. 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 × 104 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 tea 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 mM 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 μL 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

**J. Agric. Food Chem., Vol. 52, No. 11, 2004 Chandra and Gonzalez de Mejia**
glucose-6-phosphate dehydrogenase; 22.5 mg of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); and 75 µM f50m M menadione in acetonitrile) was added into lysed cells. Readings were made at five time points, 50 s apart, using a Quant 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. IC90 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 equiv (+)-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.

<table>
<thead>
<tr>
<th>polyphenol</th>
<th>mg/mL</th>
<th>mg/g DL</th>
<th>mg/mL</th>
<th>mg/g DL</th>
<th>mg/mL</th>
<th>mg/g DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>1.20 ± 0.03b</td>
<td>81.19 ± 2.33</td>
<td>4.58 ± 0.12b</td>
<td>306.63 ± 12.73</td>
<td>2.68 ± 0.06b</td>
<td>190.89 ± 0.59</td>
</tr>
<tr>
<td>gallic acid</td>
<td>0.58 ± 0.02b</td>
<td>39.07 ± 1.11</td>
<td>2.22 ± 0.06b</td>
<td>148.77 ± 6.11</td>
<td>1.53 ± 0.03b</td>
<td>94.91 ± 4.18</td>
</tr>
<tr>
<td>(+)-catechin</td>
<td>0.54 ± 0.02b</td>
<td>36.78 ± 1.07</td>
<td>2.05 ± 0.06b</td>
<td>137.19 ± 5.79</td>
<td>1.15 ± 0.03b</td>
<td>82.13 ± 3.83</td>
</tr>
</tbody>
</table>

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.

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
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 C18 reversed-phase columns in combination with different solvent composition. Phenomenex Prodigy and Waters Xterra with mobile phases water/methanol/formic acid (79.7/20.3) 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, gallo catechin 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 represent flavonol, flavanone, or dihydroflavonol, and peak 12, a quinone derivative.

<table>
<thead>
<tr>
<th>AC peak number/compound</th>
<th>max wavelength (nm)</th>
<th>peak number/compound</th>
<th>max wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>285</td>
<td>catechin gallate (CG)</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>273</td>
<td>chlorogenic acid (CHL)</td>
<td>300 sh, 327</td>
</tr>
<tr>
<td>3</td>
<td>279</td>
<td>epicatechin (EC)</td>
<td>280</td>
</tr>
<tr>
<td>4</td>
<td>278</td>
<td>epicatechin gallate (ECG)</td>
<td>277</td>
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<tr>
<td>5</td>
<td>279</td>
<td>epigallocatechin (EGC)</td>
<td>273</td>
</tr>
<tr>
<td>6</td>
<td>280</td>
<td>epigallocatechin gallate (EGCG)</td>
<td>275</td>
</tr>
<tr>
<td>7</td>
<td>277</td>
<td>flavone (FLV)</td>
<td>293 sh, 297, 310 sh</td>
</tr>
<tr>
<td>8</td>
<td>259, 283</td>
<td>gallic acid (GA)</td>
<td>273</td>
</tr>
<tr>
<td>9</td>
<td>280, 337 sh</td>
<td>gallocatechin (GC)</td>
<td>272, 280 sh</td>
</tr>
<tr>
<td>10</td>
<td>287, 342</td>
<td>gallo catechin gallate (GCG)</td>
<td>277</td>
</tr>
<tr>
<td>11</td>
<td>263 sh, 350, 365</td>
<td>kaempferol (K)</td>
<td>267, 285 sh, 320 sh, 365</td>
</tr>
<tr>
<td>12</td>
<td>253, 298 sh, 343 sh, 358</td>
<td>myricetin (Myr)</td>
<td>255, 304, 374</td>
</tr>
<tr>
<td>13</td>
<td>282, 343</td>
<td>p-coumaric acid (p-coun)</td>
<td>300 sh, 313</td>
</tr>
<tr>
<td>14</td>
<td>256, 267, 295 sh, 353</td>
<td>Q-glycoside (Qg)</td>
<td>246, 295 sh, 356</td>
</tr>
<tr>
<td>15</td>
<td>280, 340</td>
<td>quercetin (Q)</td>
<td>257, 302 sh, 372</td>
</tr>
<tr>
<td>15a</td>
<td>267, 285 sh, 320, 365</td>
<td>rutin (R)</td>
<td>258, 267 sh, 295 sh, 355</td>
</tr>
<tr>
<td>ardisin (A)</td>
<td>256, 267, 295 sh, 353</td>
<td>syringic acid (SYR)</td>
<td>275</td>
</tr>
<tr>
<td>bergenin (B)</td>
<td>274, 314 sh</td>
<td>theaflavine (THEA)</td>
<td>275, 375</td>
</tr>
<tr>
<td>caffeic acid (HCAF)</td>
<td>297 sh, 325</td>
<td>theophyllin (TP)</td>
<td>273</td>
</tr>
<tr>
<td>caffeine (CAF)</td>
<td>275</td>
<td>trans-4-hydroxy-3-methoxy cinnamic acid (THMCA)</td>
<td>297 sh, 325</td>
</tr>
</tbody>
</table>
| catechin (C)            | 279                 | **Table 2. Maximum Wavelength of HPLC Peaks$^a$ of Ardisia compressa and Selected Standards**

$^a$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.
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 (<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 IC90 = 12.58 eq (+)-catechin/mL, and IC90 = 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
myricetin, 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 (30). 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 Matsumeshki, Marian Victoria Eberhardt, and Tanoj Singh for their valuable assistance in conducting this study.

LITERATURE CITED


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

JF0352632