Effect of Yerba Mate (*Ilex paraguariensis*) Tea on Topoisomerase Inhibition and Oral Carcinoma Cell Proliferation

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Tea flavonoids have antitopoisomerase activity and can inhibit cell proliferation. The objectives of this study were to determine the phenolic content of yerba mate tea products (MT) (*Ilex paraguariensis*) and evaluate their capacity to inhibit topoisomerase I (Topo I) and II (Topo II) activities and oral carcinoma cell proliferation. Total polyphenols of aqueous extracts of dried MT leaves were measured by the Folin–Ciocalteau assay, using chlorogenic (CH) and gallic (GA) acids as standards. Topoisomerase inhibition was determined by a clone-forming assay, which uses yeast (*Saccharomyces cerevisiae*) strains as a model. Controls included dimethyl sulfoxide (1.66%); camptothecin (50 μg/mL), a Topo I inhibitor; and amsacrine (100 μg/mL), a Topo II inhibitor. Cytotoxicity studies were conducted using a nontumorigenic human keratinocyte cell line HaCaT and two human squamous cancer cell lines (SCC-61 and OSCC-3). MT was found to be a rich source of phenolic compounds. Total polyphenol content of various commercially available traditional MT products ranged from 236 to 490 mg equiv of CH/g of dry leaves. Such levels were significantly different among products depending on their origin ($P < 0.001$). Higher anti-topoisomerase II activity was observed against JN394t$_{2-4}$ strain for Nobleza Gaucha MT (IC$_{50}$ = 0.43 μg equiv of CH) in comparison to GA (IC$_{50}$ = 112 mM) and CH (IC$_{50}$ > 1500 mM). MT showed catalytic anti-topoisomerase activity against Topo II but not against Topo I. In addititon, MT exhibited dose-dependent cytotoxicity against all squamous cell lines tested. In comparison to premalignant cell line HaCaT [28 μg equiv of (+)-catechin mL^{-1}], the cell line SCC-61 [21 μg equiv of (+)-catechin mL^{-1}] was the most sensitive to MT, resulting in 50% inhibition of net cell growth. It is concluded that MT is rich in phenolic constituents and can also inhibit oral cancer proliferation. The effect on cancer cell proliferation may be mediated via inhibition of topoisomerase II. The lack of correlation between polyphenol content and the inhibition of topoisomerases suggests that the effect of MT on topoisomerase inhibition may be due to other still unidentified biologically active phytochemicals constituents.

KEYWORDS: Mate tea; polyphenol; topoisomerase; gallic acid; chlorogenic acid

INTRODUCTION

Mate or yerba mate (*Ilex paraguariensis*) (MT) is a tea-like infusion commonly consumed in several South American countries such as Argentina, Uruguay, and southern Brazil (*1*). MT contains flavonoids, caffeine, minerals (P, Fe, and Ca), and vitamins C, B$_1$, and B$_2$ (*2*). Flavonoids are phenolic compounds widely distributed in plants, and their consumption has been associated with the prevention of age-related chronic conditions including cancer and cardiovascular diseases (*3*, *4*). The reported medicinal properties of MT include central nervous system stimulation and diuretic (*5*) and antioxidant effects (*6*). MT has

also been used for treatments of arthritis, slow digestions, liver diseases, headache, rheumatism, and obesity (*7*). In addition, aqueous extracts of mate can reduce in vivo oxidation of human plasma low-density lipoproteins (LDL) (*8*) and protect against DNA oxidation and in vitro LDL lipoperoxidation (*9*). In general, a higher antioxidant activity has been observed in MT infusions than in green and black teas (*10*).

Various phytochemical constituents, such as flavonoids, have been isolated from mate plants and related species, and some of these compounds may be responsible for the observed antioxidant activity (*11*). Chandra and de Mejia (*12*) found that some of the bioactive antioxidant constituents in MT are caffeic and chlorogenic acids. Recently, we have found in our laboratory that MT extracts can be not only cytotoxic to human hepatoma cells (HepG2) but also catalytic inhibitors of topoisomerase II

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Table 1. Traditional Mate Tea Products

mate product	origin in Argentina	mg equiv of CH/mL	mg equiv of GA/mL	texture	appearance/color	aroma	observations
1. Cruz de Malta	Buenos Aires	3.36 ± 0.68	1.26 ± 0.13	rough	dark green powder	soft	fine powder of stem and leaf particles; in bags (recommended 3 g/250 mL)
2. Nobleza Gaucha Classic	Buenos Aires	7.37 ± 0.05	2.54 ± 0.02	rough	grassy light green	bitter	medium size leaves and higher stem content
3. Nobleza Gaucha Special Selection	Buenos Aires	7.01 ± 1.04	2.43 ± 0.32	soft	grassy dark green	bitter	fine powder of leaf and stem particles (in bags)
4. Taragui	Corrientes	5.45 ± 0.46	1.96 ± 0.14	soft	grassy dark green	bitter	fine selection of leaves with fewer stem particles; low ash content
5. Taragui Special Selection	Corrientes	7.13 ± 0.48	2.58 ± 0.30	soft	grassy dark green	bitter	fine powder of stem and leaves (in bags)
6. Union	Corrientes	5.35 ± 0.65	1.92 ± 0.20	soft	grassy dark green	bitter	fine selection of leaf and stem particles; low ash content
7. Amanda Classic	Misiones	6.70 ± 0.60	2.34 ± 0.18	soft	grassy dark green	bitter	fine powder of stem and leaf particles (in bags)
8. Kraus-Organic Classic	Misiones	6.78 ± 1.07	2.36 ± 0.32	rough	dark green	bitter	large amount of stems and leaves, organic; no additive
9. Romance Classic	Misiones	4.87 ± 1.16	1.78 ± 0.35	soft	grassy dark green	bitter	medium size leaf and stem particles
10. Rosamonte Classic	Misiones	6.61 ± 0.62	2.31 ± 0.19	rough	grassy light green	bitter	medium size leaves and higher stem content
11. Rosamonte, Soft	Misiones	6.50 ± 1.20	2.28 ± 0.37	rough	dark green	soft	medium size leaves and higher stem content' softer flavor
12. Rosamonte Special Selection	Misiones	5.73 ± 1.18	2.04 ± 0.36	soft	grassy dark green	bitter	medium size leaf and stem particles; low ash content
13. Oro Verde Classic	Misiones	5.95 ± 0.03	2.11 ± 0.01	soft	grassy dark green	bitter	fine powder of stem and leaves (in bags)

(Topo II) (*13*). Furthermore, data from in vitro and in vivo studies suggest a potential beneficial effect of tea polyphenols against cancer at most stages of development (*14−17*).

In contrast to the above reports, some clinical and epidemiological studies have also associated MT consumption with the development of cancer of the oral cavity, pharynx, and esophagus (*18, 19*). Despite this conflicting information, MT is widely consumed in South America, and it is gaining commercial penetration in various parts of the world. The increasing consumption and availability of MT demands further studies to gain a better understanding of its role in oral cancer, including the possibility of exploiting its chemopreventive properties. Unlike green tea, the potential of MT in the prevention or amelioration of oral cancer has not been fully investigated.

Various assays and protocols have been developed to examine the ability of a compound to modulate biochemical events, presumed to be, mechanistically linked to carcinogenesis. One of these assays exploits inhibition of DNA topoisomerases as biomarker of cancer cell proliferation. The objectives of this investigation were therefore to assess the total polyphenol content of MT (*I. paraguariensis*) and its in vitro capacity to inhibit DNA topoisomerase I and II activities and oral carcinoma cell proliferation.

MATERIALS AND METHODS

Experimental Approach. Several commercially available MT products from different sources were first screened for their phenolic contents and anti-topoisomerase activity. On the basis of this evaluation, a selected MT product was further tested for its cytotoxic capacity on oral carcinoma cells.

Chemicals. Dulbecco's Modified Eagle Medium (DMEM)/Nutrient Mixture F-12 Ham, fetal bovine serum (FBS), hydrocortisone, camptothecin (CPT), amsacrine (mAMSA), dimethyl sulfoxide (DMSO), adenine, EDTA, Folin−Ciocalteu's phenol reagent, sodium bicarbonate, (+)-catechin, gallic acid (GA), (−)-epigallocatechin gallate (EGCG), and chlorogenic acid (CH) were purchased from Sigma Chemical Co. (St. Louis, MO). Peptone bacto, yeast extract, agar bacto, and dextrose were purchased from Difco (Sparks, MD). EGCG, GA, and CH were chosen as common representatives of polyphenol components in teas.

Mate Tea Products. Twenty-four different MT products in the form of finely ground dried leaves were obtained from different regions in Argentina and the United States. On the basis of their ingredient composition, they were classified either as traditional or as nontraditional tea products (**Tables 1** and **2**).

Traditional MT are mate products containing only leaves without additional components or extracts. Nontraditional MT are mate leaves and stems combined with other ingredients including small dried fruit pieces, dehydrated milk powder, sugar, and natural herbs and flavors.

Biological Materials. *Yeast Strains.* A yeast *Saccharomyces cerevisiae* strain clone-forming assay was used as a model to measure Topo I and II inhibition. The *S. cerevisiae* yeast strains used in this study were JN362a (DNA repair proficient: *Matα ura3-52 leu2 trp1 his7 adel-2 ISE2*), JN394 (DNA repair deficient: *Matα ura3-52 leu2 trp1 his7 adel-2 ISE2 rad52::LEU2*), JN394t$_{-1}$ (JN394 with TOP 1 disruption: *Matα ura3-52 leu2 trp1 his7 adel-2 ISE2 rad52::TRP1 Δtop1::LEU2*), JN394t$_{2-4}$ (JN394 with temperature-sensitive allele of TOP2: *Matα ura3-52 top2-4 rad52::LEU2*), JN394t$_{2-5}$ (JN394 with temperature-sensitive allele of TOP2: *Matα ura3-52 leu2 trp1 his7 adel-2 ISE2 top2-5 rad52::LEU2*). They were kindly provided by Dr. John Nitiss of St. Jude Children's Research Hospital, Memphis, TN. DMSO (1.66%), CPT (50 μg/mL), a Topo I inhibitor, and m-AMSA (100 μg/mL), a Topo II inhibitor, were used as controls (*20*).

Oral Cancer Cells and Human Keratinocytes. The human squamous carcinoma lines OSCC-3 and SCC-61 and the HaCaT premalignant human skin keratinocytes used in this study were a gift from Dr. Mark Lingen (University of Chicago). HaCaT cells are a spontaneously transformed, nontumorigenic human skin keratinocyte cell line that was originally described by Boukamp et al. (*21*). HaCaT cells are premalignant and therefore are frequently used as a model to compare with malignant cell lines. The SCC-61 line is characterized by its radiosensitivity and is a p53 wild type. Both OSCC-3 and HaCaT cell lines were cultured in DME/F12 (1:1) medium (Sigma Chemical Co.) containing 10% FBS. The SCC-61 cells were cultured in DME/F12 (1:1) medium containing 10% FBS and hydrocortisone (0.4 μg/mL). All cells were maintained at 37 °C in 5% CO_2 atmosphere and 95% humidity. The culture medium was changed every other day.

Preparation of Herbal Tea. The MT products were kept in plastic bags and refrigerated at 4 °C until use. The aqueous extracts of MT were prepared as described previously (*12*). Briefly, each extract was prepared from 2.7 g of dry tea leaves (DL) that were first 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 freeze-dried in a Labconco, Shell Freeze System (Fisher Scientific, Pittsburgh, PA). The freeze-dried solid extract (SE) was kept at −20 °C in plastic tubes, sealed with Parafilm, and protected from light. Prior to use, these solid extracts were redissolved in double-distilled water (ddH_2O) and filtered with a 0.22-μL syringe top filter. The total phenolic

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Table 2. Nontraditional Mate Tea Products[a]

mate product	origin	mg equiv of CH/mL	mg equiv of GA/mL	texture	appearance/composition	aroma	observations
14. Lemon Taragui	Corrientes, Argentina	1.75 ± 0.09	0.59 ± 0.03	rough	grassy green leaves, dried lemon	lemon	artificial flavor, 1.2%; dried lemon particles, 2%, in 50 g of product
15. Lemon Union	Corrientes, Argentina	1.27 ± 0.01	0.45 ± 0.00	rough	grassy green leaves and dried lemon	lemon	artificial flavor, 1.2%; dried lemon particles, 2%, in 50 g of product
16. Peach Union soft	Corrientes, Argentina	1.40 ± 0.01	0.49 ± 0.00	rough	grassy green leaves and dried peach	peach	1% natural flavor, 2% dried peach and orange skin
17. Adelgamate with natural herbs	Misiones, Argentina	1.64 ± 0.10	0.56 ± 0.03	soft	mate leaf mixture and stem with additional herbs	regular soft	additional leaves: sea weeds, sen bush, *Cymtopogon citratus, Minthostchys mollis,* green tea
18. Grapefruit Taragui	Misiones, Argentina	1.48 ± 0.02	0.51 ± 0.01	rough	grassy green leaves and dried grapefruit	fruity	0.6% natural flavonoid, 2% dried grapefruit skin
19. CBSe	Santa Fe, Argentina	0.81 ± 0.32	0.31 ± 0.10	rough	mixture of stems and leaves with sugar	sweet	contains sugar
20. La Virginia, El Litoral dehydrated milk mate	Santa Fe, Argentina	0.02 ± 0.00	0.07 ± 0.0	soft	lighter green powdered milk and mate powder	no aroma	powdered milk, sugar, instant mate, 19 g of proteins per 100 g of product
21. Morning Thunder with caffeine	USA	1.58 ± 0.44	0.54 ± 0.13	soft	fine selection of processed leaves	strongly bitter	100% natural—all natural herbs and flavors and no artificial colors added; roasted mate and black tea
22. Tazo Tropic mate	USA	1.63 ± 0.27	0.55 ± 0.08	soft	fine selection of processed leaves	citric	contains caffeine
23. Tropical mate Zinger with caffeine	USA	0.56 ± 0.19	0.23 ± 0.06	soft	fine selection of processed leaves	strongly bitter	mate content (70%); other ingredients (30%): hibiscus, Chinese black berry leaves, natural flavors, vanilla bean
24. Verde mate Mist with caffeine	USA	1.05 ± 0.13	0.38 ± 0.04	soft	dark brown, fine selection of processed leaves	mango	natural peach flavor, Chinese blackberry leaves, and dried peaches

[a] As stated on the labels, standard commercial mate tea compositions: *Ilex* leaves, 55% minimum; dust, 20% maximum; stems, 25% maximum; H_2O, 10% maximum; aqueous extract, 25% minimum; ash, 8% maximum; ash insoluble in 10% HCl, 2% maximum; caffeine, 0.70% minimum Average chemical composition of MT prepared at 84 °C per 100 g of mate in ∼100 mL: total calories, 23.64 kcal; carbohydrates, 3.61 g; glucose, 0.59 g; sucrose, 2.77 g; protein, 2.14 g; caffeine, 0.85 g; vitamin C, 5.11 mg; vitamin B1, 1.48 mg; niacin, 1.27 mg; vitamin B, 0.94 mg; calcium, 80.9 mg; phosphorus, 45.9 mg; iron, 2.2 mg. magnesium, 58.6 mg; sodium, 14.1 mg; potassium, 100.6 mg.

content of each preparation was measured and standardized accordingly, by sample dilution, before each assay.

Total Phenolic Content of Mate Tea Products. The total phenolic content of the fresh and freeze-dried materials was measured according to the Folin−Ciocalteau method as described by Nurmi et al. (*22*). This method is based on the reduction of Folin−Ciocalteau reagent by the electrons from the phenols. Briefly, 1 mL of 1 N Folin−Ciocalteau reagent and 1 mL of MT sample were mixed and allowed to stand for 2−5 min. Subsequently, 2 mL of 20% Na_2CO_3 solution was added and allowed to stand for 10 min before the absorbance was measured at 730 nm by using a Beckman DU 640 spectrophotometer. Chlorogenic acid (CH) and gallic acid (GA) were used as standards. Total polyphenol content was expressed, respectively, as milligram equivalents of CH or GA per milliliter of aqueous extract of fresh tea (FT) or milligram equivalents of CH or GA per milligram of dried leaves (DL) or milligram equivalents of CH or GA per milligram of freeze-dried solid extract (SE). The equations of the standard curves used were CH ($y = 0.0052x − 0.006$, $r^2 = 0.99$) and GA ($y = 0.0171x − 0.108$, $r^2 = 0.92$), where y = absorbance at 730 nm and x = polyphenol concentration.

Anti-topoisomerase Assay. Sensitivity tests of tea extracts, m-AMSA, CPT, and DMSO (vehicle control) were carried out in a medium containing yeast extract, peptone, dextrose, and agar (YPDA). In all instances, cells at the logarithmic stage (JN362a, JN394, JN394t$_{-1}$, JN394t$_{-4}$, or JN394t$_{2-5}$) were adjusted to an initial cell titer of 2×10^6 cells/mL. In all cases, cells were pregrown at the same temperature that was used to measure drug sensitivity. After dilution and standardization, the different MT samples (1.683 mg/mL), m-AMSA (100 μg/mL), CPT (50 μg/mL), or DMSO (1.66%) were added to the cultures and the cells incubated for 24 h. The concentrations of the teas used in this assay were based on the solubility factor of the SE in DMSO.

Viable counts were determined by duplicate plating on YPDA medium solidified with 1.75% agar. Plates were incubated at the optimal temperature for cell growth to determine viable titer (25 °C for temperature sensitive *top2* mutants, 30 °C otherwise). The percent yeast cell survival was determined by comparison of the number of colonies counted in the no-treatment control culture with those in the test-treated culture. The IC_{50} is the concentration of the test substance that reduces the number of colonies by 50% as compared with cells grown in the absence of the test substance. The IC_{50} was determined using different dilutions of each MT sample, GA and CH (163, 326, 490, 653, or 817 μg/mL). All experiments were conducted in duplicate.

Cytotoxicity Assay. Cytotoxicity was determined following protocols established by NCI (*23*). The plate density for all of the cell lines was 5×10^3 cells/well. This ensured a linear relationship between absorbance at 450 nm and cell number when analyzed by the cell-counting kit-8 (CCK-8, Dojindo Molecular Technologies, Inc.). After 24 h, the cells were treated with serial concentrations of MT extract or EGCG (positive control, AG Scientific, San Diego, CA), which was added directly into the cells (eight replications). MT or EGCG was first dissolved in ddH_2O (1 mg/100 μL) and further diluted in serum-free medium to produce five concentrations. One hundred microliters of each concentration was added to the plate wells to obtain final concentrations between 0.05 and 1000 μg mL^{-1} (dry weight). The highest concentration of water as solvent for MT extract and EGCG was 5%, and the final volume in each well was 200 μL. Control cultures received the same amount of serum-free medium without treatment, and blank wells contained 100 μL of serum-free medium with no cells. After the samples were added, the plates were incubated under the same conditions for 48 h. At the end of the incubation, the medium containing the various concentrations of the tea extracts or EGCG was discarded, and the cells were washed twice with PBS. The viability of the cells was determined by CCK-8 according to the kit manufacturer's protocol. The cytotoxicity of the tested crude MT extracts was determined by comparing the response of the cells with that of the PBS control. IC_{50} values were calculated by analysis of the percent inhibition of each tea extract or EGCG at 11 concentrations. The following parameters were used to determine cytotoxicity:

IC_{50} (inhibitory concentration, 50%) = concentration resulting in 50% inhibition of net cell growth; IC_{50} = %T/C (OD of treated cells/OD of control cells) × 100.

GI_{50} (growth inhibition, 50%) = concentration resulting in a 50% reduction in the net cell growth in comparison to untreated cells; GI_{50} = $100 \times [(T - T_0)/C - T_0)]$.

TGI (total growth inhibition) = the concentration required to achieve complete halting of treated cell growth; TGI = T_0.

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LC_{50} (lethal concentration, 50%) = the concentration lethal to 50% of treated cells; $LC_{50} = 100 \times [(T - T_0)/T_0]$.

OD = optical density, C = control optical density, T = test optical density, T_0 = optical density at time zero.

Statistical Analysis. Results are expressed as the mean ± SD of values obtained in at least duplicate measurements from three different experiments. A one-way ANOVA with Dunnett and linear trend post test were used for statistical analysis. A probability (P) value of <0.05 indicated a significant difference.

Analysis on sigmoidal dose−response was performed by nonlinear regression (curve fit) using GraphPad Prism software.

RESULTS AND DISCUSSION

Polyphenol Content. Tables 1 and **2** present the total polyphenol concentrations in the liquid fresh extracts, expressed as CH and GA, of traditional and nontraditional MT products, respectively. It can be observed that the total polyphenolic concentrations are higher in traditional than in nontraditional MT products. It is remarkable to observe that the total polyphenol content in traditional products ranged from 3.4 to 7.4 mg of CH/mL of fresh tea and from 0.02 to 1.80 mg of CH/mL of fresh tea in nontraditional ones. The source of this difference is related to the presence, in nontraditional tea products, of additional ingredients such as sugars, fruit pieces, amino acids, vitamins, and flavoring agents. The same trend can be seen for traditional and nontraditional MT products when the values are expressed as GA.

When expressed as CH, total polyphenol content was significantly higher than when expressed as GA. This is due to the fact that CH is one of the main phenolic constituents present in yerba mate tea (*24*). Because GA represents only a small percentage of the total phenolic compounds, GA standardization may underestimate the true total phenolics in MT. It is therefore advisable that when working with MT, total phenolic compounds should be reported as milligram equivalents of CH.

In general, total polyphenol values of MT ranged from 236 to 490 mg equiv of CH when expressed as grams of dried leaves, whereas the respective range expressed as gallic acid varied from 90 to 176 mg equiv of GA/g of dried leaves.

In the group of traditional products, Nobleza Gaucha Classic, Kraus-Organic, Taragui Special, and Nobleza Gaucha Special had the highest polyphenol concentrations. Among nontraditional MT products, Lemon Taragui, Adelgamate with natural herbs, Morning Thunder, and Tazo tropic mate had the highest polyphenol concentrations (P < 0.001). Our results are in agreement with polyphenol values reported by Astill et al. (*25*) for nontraditional MT.

Anti-topoisomerase Assay (Mutant *S. cerevisiae* Model). Because this assay is sensitive to antimicrobial compounds, MT samples were first tested on the JN362a strain, a DNA repair-proficient strain sensitive to antimicrobial agents. This test ensured that the reduction in the number of observed colonies by the other yeast strains was in fact caused solely by the topoisomerase activity of the MT extracts and not by any potential antimicrobial properties of the products. There was no antimicrobial inhibition on JN362a strain by most of the tested MT products except for Kraus-organic, Peach Union soft, Grapefruit Taragui, and Rosamonte Soft (see **Tables 1** and **2** for identification), which showed slight antimicrobial properties. MT products that showed JN362a inhibition were not used for further topoisomerase study. It was also observed that some of the nontradtional mate samples containing high concentrations of added ingredients, such as sugar and proteins from dehydrated milk, promoted the growth of this strain. Strain JN394 was used for preliminary screening of anti-topoisomerase activity. This

Table 3. Percent Inhibition of Mate Tea and Tea Phenolic Treatments on the Survival of Different Mutant Strains of *S. cerevisiae* As Determined by the Topoisomerase Inhibition Assay[a]

mate product	JN394t$_{2-5}$ (25 °C)	JN394t$_{2-4}$ (25 °C)	JN394t$_{2-4}$ (30 °C)
Cruz de Malta	8 ↑	89 ↓	91 ↓
Rosamonte Classic	9 ↑	47 ↓	93 ↓
Romance	14 ↑	13 ↓	99 ↓
Nobleza Gaucha Classic	35 ↑	59 ↓	90 ↓
Taragui, Special	3 ↑	185 ↑	185 ↑
Nobleza Gaucha, Special	20 ↑	116 ↑	196 ↑
gallic acid	0	0	99 ↓
amsacrin	48 ↓	99 ↓	93 ↓
camptothecin	99 ↓	80 ↓	99 ↓

[a] DMSO (1.66%) was used as a control, and all of the results were referred to this value. ↓, decrease in growth; ↑, increase in growth.

strain is more sensitive to drugs, because it carries *rad52* and *ise2* mutations that result in DNA repair deficiency and higher cell membrane permeability (*26*). Growth inhibition of this strain after MT treatment is an indicator of anti-topoisomerase activity of MT. The strain JN394 was hypersensitive to CPT (99%), the control for a Topo I poison, and to m-AMSA (99%), the control for Topo II poison. MT samples Rosamonte Classic, Nobleza Gaucha Classic, Lemon Taragui, Lemon Union, and Romance showed 11, 18, 20, 29, and 73% growth inhibition, respectively. The rest of the samples did not show a significant growth inhibition (data not shown).

Table 3 depicts the anti-topoisomerase activity of MT on different strains of *S. cerevisiae*. A modest increase in the colony count was observed when JN394t$_{2-5}$ was exposed to all MT extracts. For instance, an 8% increase in the colony was observed with the extract of Cruz de Malta. Strain 394t$_{2-5}$ carries a *top2* allele that is resistant to multiple classes of Topo II poisons at its permissive temperature (25 °C). This observed increase in colony count indicates that MT extracts do not exhibit a true prominent Topo I inhibition. On the other hand, a different phenomenon was observed when JN394t$_{2-4}$ was subjected to different MT extracts, at both 25 and 30 °C. In this case, there was a marked decrease in the colony number, indicating up to 89 and 99% inhibition, respectively. These observations suggest that topoisomerase inhibition of yerba mate is by way of catalytic inhibition of Topo II and not of Topo I. The yeast strain JN394t$_{2-4}$ expresses the temperature-sensitive *top2-4* mutant in place of the wild-type *top2* gene. Romance (99%), Rosa Monte Classic (93%), Cruz de Malta (91%), and Nobleza Gaucha (90%) MT were strong growth inhibitory agents of JN394t$_{2-4}$ yeast cells at 30 °C.

DNA topoisomerases are important nuclear enzymes that regulate DNA metabolism and affect replication, transcription, recombination, chromatin assembly, possibly DNA repair, and cell division (*27*). Three mammalian topo enzymes (I, IIα, and IIβ) have significant consequences for cancer and cancer chemotherapy via their antiproliferative or cell-differentiating action. Differentiated cells express very low levels of topo II, whereas highly proliferative and tumor cells often express 25−300 times the levels of quiescent cells (*28*). Currently, the exact mechanism in carcinogenesis has not been clarified for compounds exhibiting anti-topoisomerase activities. Nonetheless, many anticancer compounds manifest topoisomerase inhibition, and therefore this property can be exploited for drug screening and discovery for anticancer compounds. The possibilities described above can be scrutinized by utilizing a yeast strain JN394t$_{2-4}$. This yeast strain expresses the temperature-sensitive

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top2-4 mutant in place of the wild-type *top2* gene (*26*). The top 2-4 protein shows wild-type activity at 25 °C, whereas its activity is reduced to ∼5−10% of that of the wild type at the semipermissive temperature of 30 °C. Therefore, if MT extracts function as Topo II "poison", the reduction in enzyme activity should greatly diminish the incidence of induced cell death. Conversely, if the cytotoxicity is correlated to the ability to impair the catalytic function of the enzyme, cells with decreased levels of Topo II activity should become hypersensitive.

On the basis of the topoisomerase inhibition results, it can be concluded that MT extracts act as catalytic Topo II inhibitors. This conclusion is supported by the fact that the sensitivity to inhibition was observed when yeast cultures were incubated at 25 and 30 °C. Unlike Topo II poisons, catalytic inhibitors are also capable of inducing sensitivity in yeast at 30 °C. Whereas JN394t$_{2-4}$ was sensitive to both conditions, a greater sensitivity or hypersensitivity was observed at the semipermissive temperature (30 °C). The increased toxicity toward cells that contain decreased levels of Topo II activity strongly indicates that the catalytic activity of Topo II is the primary physiological target of MT. The overcoming of the cell-killing action against this strain by MT samples Nobleza Gaucha Classic, Rosamonte Classic, Cruz de Malta, and Romance suggests that all of these treatments result in DNA impairment.

The strain JN394t$_{-1}$ is isogenic to JN394 and contains a disrupted *top1* gene; the absence of this gene results in diminished cytotoxicity of antitopo I drugs. Whereas Rosamonte Classic and Lemon Union failed to reduce the growth of these mutant cells (meaning that Topo I was not the cellular target of these MT samples), no resistance was observed when top-1 cells were treated with m-AMSA (99%), Nobleza Gaucha (22.2%), and Lemon Taragui (46.7%).

Figure 1 shows the topoisomerase inhibition capacity of Nobleza Gaucha MT (IC$_{50}$ = 0.43 μg equiv of CH) and Rosamonte MT (IC$_{50}$ = 0.63 μg equiv of CH) teas as function of their concentration, using the JN394t$_{2-4}$ strain. They both showed a higher catalytic Topo II inhibition (90−93%), in comparison to GA (IC$_{50}$ = 112 mM) and CH (IC$_{50}$ > 1500 mM), and a positive relationship between concentration and inhibition response.

Oral Cancer Cytotoxicity Assay. Due to the fact that Romance MT showed the highest topoisomerase inhibition, it was chosen for the study of oral cancer cytotoxicity. **Table 4** presents the cytotoxicity of Romance on oral carcinoma cells, in comparison to the positive control EGCG. The IC$_{50}$ values of EGCG against oral squamous cell lines were 116.1 μM (HaCaT), 166.0 μM (OSCC-3), and 123.0 μM (SCC-61). A previous investigation determined that the IC$_{70}$ of EGCG against YCU-H-891, a nasopharynx carcinoma cell line, was 21.8 μM after exposure of cells with this compound for 72 h (*29*). The apparent discrepancy with our finding might have resulted from differences in duration of chemical exposure, type of assay, and, most importantly, cell types. For instance, Chen et al. (*30*) reported that the IC$_{50}$ of EGCG against HT-29, a colon epithelial adenocarcinoma cell line, was ∼100 μg mL^{-1} and also found a wide range (40−200 μM) in the IC$_{50}$ for different cell types.

Although the IC$_{50}$ of MT against SCC-61 [74.8 μM equiv of (+)-catechin or 21.5 μg equiv of (+)-catechin mL^{-1}] was lower than that of HaCaT [99.9 μM equiv of (+)-catechin or 28.7 μg equiv of (+)-catechin mL^{-1}], other cytotoxicity values between these two cell lines were similar (**Table 4**). On the other hand, a high concentration [76.4 μg equiv of (+)-catechin mL^{-1}] of MT extract was necessary to observe 100% growth inhibition of OSCC-3. The value of IC$_{50}$ was also >3 times that of SCC-
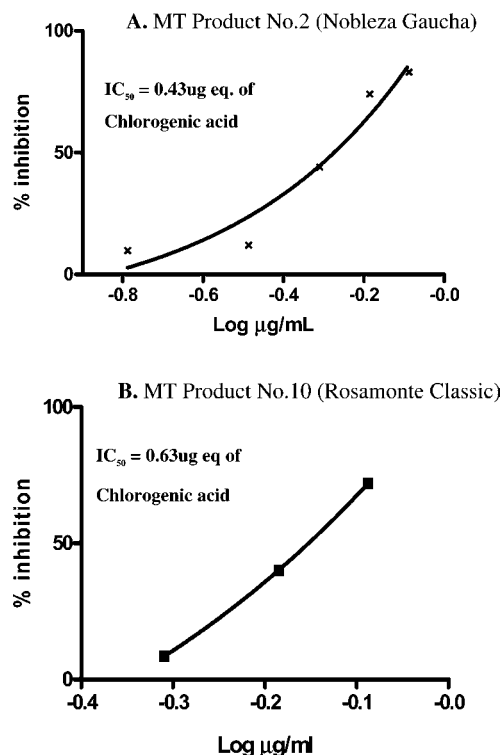

Figure 1. IC$_{50}$ of (**A**) Nobleza Gaucha and (**B**) Rosamonte Classic mate teas. Strain JN394t$_{2-4}$ was treated at 30 °C with increasing concentrations of MT. After 24 h, two aliquots of cells were taken, plated in YPDA, and grown for 3–4 days to determine the titer. Percent survival was determined by comparison with the control. Plots represent an average of two independent experiments. IC$_{50}$ was calculated from the antilog of the *x*-axis value.

Table 4. Cytotoxicity^a of Mate Tea Extract (Romance) and (−)-Epigallocatechin-3-gallate after 48 h of Exposure to Different Oral Cells

cell type		EGCG (μM)	Yerba mate [μM equiv of (+)-catechin]	Yerba mate [μg equiv of (+)-catechin/mL]	Yerba mate (μg of SE^b/mL)
HaCaT	IC$_{50}$	116.1	99.9	28.7	298.5
	GI$_{50}$	100.0	84.2	24.2	251.1
	TGI	177.9	94.7	27.2	281.8
	LC$_{50}$	237.1	118.7	34.1	354.8
OSCC-3	IC$_{50}$	166.0	266.0	76.4	794.3
	GI$_{50}$	141.3	237.1	68.1	707.9
	TGI	173.8	266.0	76.4	794.3
	LC$_{50}$	251.1	298.7	85.8	891.3
SCC-61	IC$_{50}$	123.0	74.8	21.5	223.9
	GI$_{50}$	79.4	94.3	27.1	281.8
	TGI	133.4	105.8	30.4	316.2
	LC$_{50}$	177.8	118.7	34.1	354.8

^{a} Cytotoxicity has been defined with the following parameters, calculated as presented under Materials and Methods: IC$_{50}$, inhibitory concentration, 50%; GI$_{50}$, growth inhibition, 50%; TGI, total growth inhibition; LC$_{50}$, lethal concentration, 50%. ^{b} SE, the freeze-dried solid extract.

61. These differences, in sensitivity against MT extracts, indicate that these two cell lines may be at different stages of cancer malignancy. Interestingly, the cytotoxicity of MT against cell lines used in this study exhibited a narrow range. For instance, for HaCat cells, 50% growth inhibition (GI$_{50}$), total growth inhibition (TGI), and 50% lethal concentration (LC$_{50}$) were between 24.2 and 34.1 μg mL^{-1} [84.2 and 118.7 μM equiv of (+)-catechin]. The cytotoxicity values of EGCG obtained against

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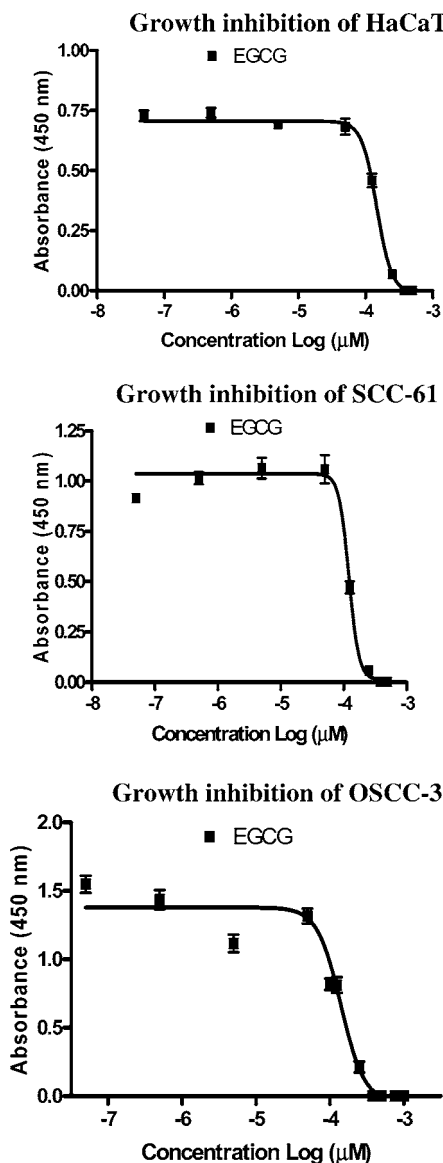*J. Agric. Food Chem.,* Vol. 53, No. 6, 2005 **1971**

Figure 2. Cytotoxicity of EGCG against HaCat, SCC-61, and OSCC-3 human cells. Cells were treated with various concentrations of *I. paraguariensis* for 48 h. Cell viability was determined by CCK-8 assay as described under Materials and Methods. Values are the average of three independent experiments. IC_{50} was calculated from the antilog of the *x*-axis value at the inflection point of each sigmoid-curve fit.

Figure 3. Cytotoxicity of MT (Romance) against HaCat, SCC-61, and OSCC-3 human cells. Cells were treated with various concentrations of *I. paraguariensis* for 48 h. Cell viability was determined by CCK-8 assay as described under Materials and Methods. Values are the average of three independent experiments. IC_{50} was calculated from the antilog of the *x*-axis value at the inflection point of each sigmoid-curve fit.

HaCaT ranged from 100 to 237.1 μM, contrasting to the findings with MT extracts. HaCaT cells responded to treatments with EGCG in a dose-dependent manner, and the IC_{50} obtained fell within the range known to activate extracellular signal-regulated kinase, p38, and c-Jun NH_2-terminal kinase (*31*), although EGCG is also known to diminish 2,2′-azobis(2-amidinopropane) dihydrochloride-induced cyclooxygenase-2 expression and p38 activation in HaCaT (*32*).

As observed in **Figure 2**, EGCG exhibited dose-dependent cytotoxicity against all oral squamous cell lines used in this study, although the concentration required for 100% cell growth inhibition varied among cell lines. **Figure 3** shows the narrow range in MT cytotoxicity, which was most pronounced in treatments with SCC-61. A similar trend was found with OSCC-3, the other carcinoma cell line, whereas a gradual decline in efficacy was found with HaCaT. These findings indicate that the cytotoxicity of MT is narrower for malignant oral squamous cell lines than for premalignant cells. This finding is of particular
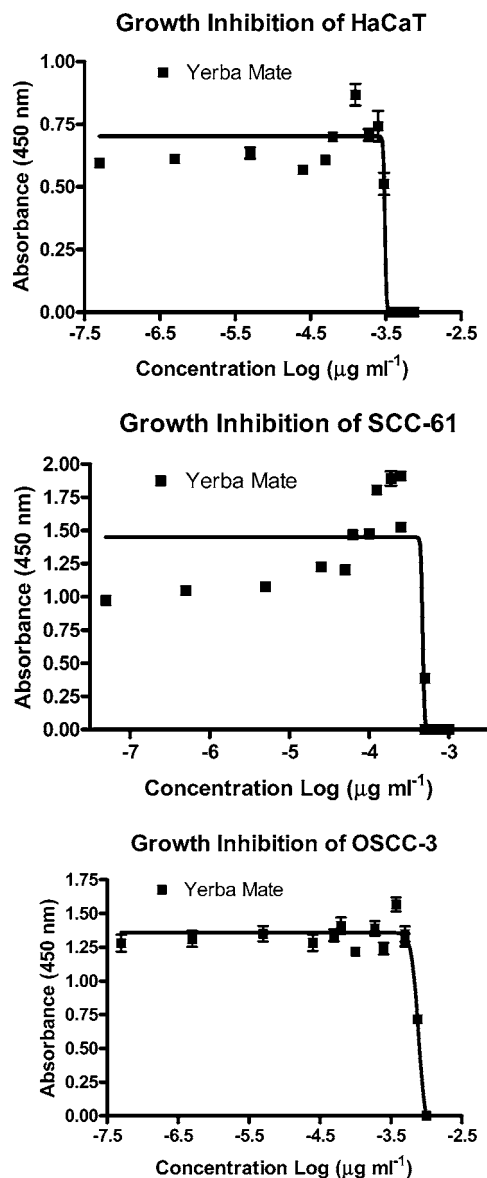
interest because MT extracts can be potentially exploited for its cytotoxicity against malignant oral carcinoma cells while causing little damage to normal or premalignant cells in the cancerous lesion. Similar observations have been reported by Tanaka et al. (*33*) and Lee et al. (*34*).

An intriguing observation in this study was that cell growth appeared to be promoted by MT treatments at intermediate concentrations (**Figure 3**). For instance, a tendency in the promotion of HaCaT cell growth was observed among cells treated with MT at various concentrations (250, 188, and 125 μg of SE mL^{-1}). However, higher MT concentrations (375, 500, 750, and 1000 μg of SE mL^{-1}) resulted in complete growth inhibition. A tendency to increase cell growth was also observed for SCC-61 and OSCC-3 at similar concentrations. These data indicate that the chemopreventive activity of MT against oral carcinogenesis depends on the dose level, as it has also been shown by tea catechins that promote tumor cell survival

pathways (*35*). More research is needed to clarify the mechanism of promotion of cell growth at intermediate MT concentrations.

The absorption and bioavailability of tea phytochemicals including (−)-epicatechin-3-gallate (ECG) and (−)-epigallo-catechin-3- gallate (EGCG), found in green tea, have been previously investigated, and the absolute availability of the former was determined in the rat to be as low as between 1.0 and 3.3% for doses of 12.5−50 mg/kg (*36*, *37*). Similarly, the concentration of tea catechin in plasma reached the highest values from 1.5 to 2.6 h after single consumption of tea, although returning to basal levels after 24 h (*38*). Although bioavailability of tea constituents is low, higher concentrations (375, 500, 750, and 1000 μg of SE mL^{-1}) used in the present study are relevant to normal tea drinking because in addition to the direct contact between oral squamous cells and MT, there may also be a more prolonged cellular interaction via the systemic circulation.

In conclusion, the content of total polyphenols in MT is greater in traditional products with no added ingredients in comparison to nontraditional MT products containing flavoring agents. Moreover, total polyphenol content of MT products should be preferentially expressed as milligram equivalents of chlorogenic acid, due to the high concentration of this compound in MT, compared to other phenolics. The data obtained from the freeze-dried MT-SE showed an average polyphenol content of 8.2 g equiv of CH, when 2 teaspoons of the solid powder is dissolved in a cup of water (250 mL). In contrast, the average polyphenol content extracted from fresh mate infusion was ∼1.8 g equiv of CH per 250 mL of fresh tea. These results are important because chlorogenic acid, among other tea phenols, is extensively metabolized in humans (*39*). The MT products tested in our study showed topoisomerase inhibitory activity only of Topo II. This is a relevant finding because certain types of cancer respond only to Topo II inhibitors. Furthermore, the polyphenolic content of the different MT did not correlate with the degree of topoisomerase inhibition. This finding suggests that the inhibition of topoisomerase and oral carcinoma cell proliferation may probably be due to the presence of other unidentified phytochemicals in MT.

The fact that MT also inhibits oral cancer cell proliferation makes this botanical product a potential source of still unknown active substances that can be added to the arsenal of compounds that could be used in cancer prevention and treatment. Therefore, MT deserves further studies to determine efficacy and safety in in vivo studies.

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