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Isolation and Identification of Strawberry Phenolics with Antioxidant and Human Cancer Cell Antiproliferative Properties

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Studies suggest that consumption of berry fruits, including strawberries (*Fragaria* × *ananassa* Duch.), may have beneficial effects against oxidative stress mediated diseases such as cancer. Berries contain multiple phenolic compounds, which are thought to contribute to their biological properties. Comprehensive profiling of phenolics from strawberries was previously reported using high-performance liquid chromatography with mass spectrometry (HPLC-MS) detection. The current study reports the isolation and structural characterization of 10 phenolic compounds from strawberry extracts using a combination of Amberlite XAD16-resin and C18 columns, HPLC-UV, and nuclear magnetic resonance (NMR) spectroscopy methods. The phenolics were cyanidin-3-glucoside (**1**), pelargonidin (**2**), pelargonidin-3-glucoside (**3**), pelargonidin-3-rutinoside (**4**), kaempferol (**5**), quercetin (**6**), kaempferol-3-(6′-coumaroyl)glucoside) (**7**), 3,4,5-trihydroxyphenyl-acrylic acid (**8**), glucose ester of (*E*)-*p*-coumaric acid (**9**), and ellagic acid (*10*). Strawberry crude extracts and purified compounds **1**–**10** were evaluated for antioxidant and human cancer cell antiproliferative activities by the Trolox equivalent antioxidant capacity (TEAC) and luminescent ATP cell viability assays, respectively. Among the pure compounds, the anthocyanins **1** (7156 μM Trolox/mg), **2** (4922 μM Trolox/mg), and **4** (5514 μM Trolox/mg) were the most potent antioxidants. Crude extracts (250 μg/mL) and pure compounds (100 μg/mL) inhibited the growth of human oral (CAL-27, KB), colon (HT29, HCT-116), and prostate (LNCaP, DU145) cancer cells with different sensitivities observed between cell lines. This study adds to the growing body of data supporting the bioactivities of berry fruit phenolics and their potential impact on human health.

KEYWORDS: Strawberries; polyphenols; antioxidant; antiproliferative

INTRODUCTION

Oxidative damage is thought to be one of the major mechanisms involved in chronic human diseases such as cancer and heart disease, the leading causes of mortality in the United States. An overwhelming and large body of studies suggests that a phytochemical-rich diet, which includes colorful fruits and vegetables, may reduce the risk of chronic human diseases. Phenolic compounds (commonly referred to as "flavonoids" or "polyphenols") are ubiquitous phytochemicals present in plant foods with numerous biological activities including antioxidant properties. Phenolics exert antioxidant properties through various mechanisms of actions including the scavenging of free radicals and inhibition of the generation of reactive species during the course of normal cell metabolism, thereby preventing damage to lipids, proteins, and nucleic acids and eventually cell damage and death (*1*). It has been shown that antioxidant-rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the onset of carcinogenesis (*2*), and the impact of antioxidants on mutagenesis and carcinogenesis has been well established (*3, 4*).

Strawberry (*Fragaria* × *ananassa* Duch.) fruits are popularly consumed in fresh forms, as processed food products, and as botanical extracts for dietary supplements. Strawberries have high antioxidant activity, which has been linked to their content of phenolic compounds (*5*–*7*). Strawberry juice extracts exhibited high levels of antioxidant capacity against superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen free radicals (*8*). The contents of phenolics in strawberries have been associated with the total antioxidant capacity for low-density lipoproteins of the fruit extracts (*5*).

Our group is currently investigating the pharmacokinetics and bioavailability of strawberry phenolics in animals and healthy human volunteers, and chemical standards are needed for evaluations of plasma and urinary metabolites. In addition, we are interested in evaluating the biological actions of phenolics

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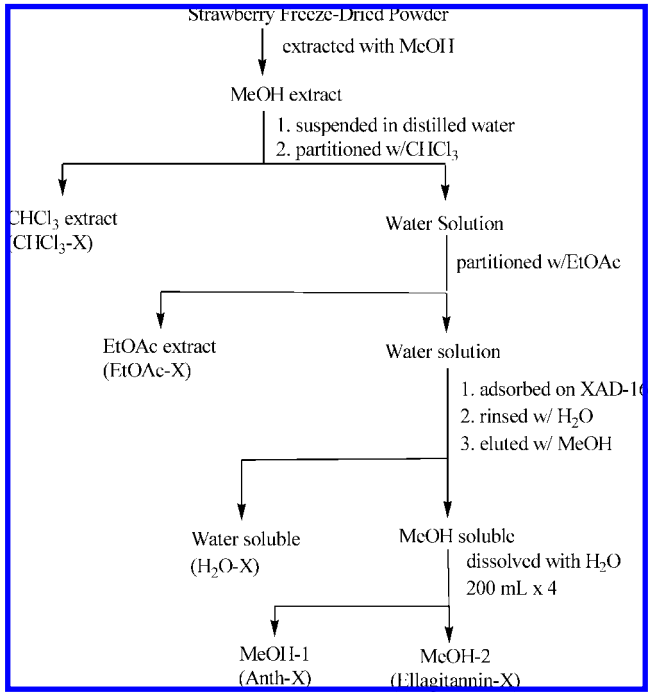

Figure 1. Generation of crude strawberry crude extracts for bioassays. $CHCl_3$, chloroform; EtOAC, ethyl acetate; MeOH, methanol.

as singly purified compounds compared to their combined "food-matrices" forms (*9, 10*). Our previous work reported the comprehensive profiling of phenolics from strawberries using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) methods (*11*). Strawberries were found to contain a wide variety of phenolics such as ellagic acid, ellagic acid glycosides, ellagitannins, gallotannins, flavonols (quercetin and kaempferol glucuronides and glycosides), anthocyanins (pelargonidin and cyanidin glycosides), flavanols, and coumaroyl glycosides (*11*). The objectives of the current study were (1) to isolate and structurally characterize individual phenolic constituents of strawberry fruits and (2) to evaluate the strawberry extracts generated, and their purified phenolic compounds, for biological activities in vitro. Although extracts of strawberries have previously been evaluated for antioxidant and human cancer cell antiproliferative properties (*12–14*), this is the first report of the isolation and biological investigation of these purified phenolic constituents from strawberry fruits.

MATERIALS AND METHODS

Reagents. All solvents were of high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific Co. (Tustin, CA). Amberlite XAD-16 resin was purchased from Sigma-Aldrich Co. (St. Louis, MO). C_{18} silica gel was obtained from DyChrom (Sunnyvale, CA).

General Experimental Procedures. The nuclear magnetic resonance (NMR) spectra were recorded on Bruker instruments operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, respectively. Chemical shifts for compounds **1**, **3**, **4**, and **7** were recorded in CD_3OD/CF_3COOD (19:1, v/v), and chemical shifts for compound **9** were recorded in DMSO-d_6. Chemical shifts are presented in δ (parts per million) and the coupling constants (J) in hertz.

Generation and Composition of Strawberry Crude Extracts. A freeze-dried whole strawberry fruit powder (SFP), provided by the California Strawberry Commission (Watsonville, CA), has been previously described (*11*). The SFP was subjected to an extraction protocol as outlined in **Figure 1**. Briefly, a portion of SFP (845 g) was extracted by cold percolation with methanol (6 L) under room temperature for 12 h to yield a methanol extract (624 g, 73.8% yield). The methanol extract was then subjected to a sequence of solvent−solvent partitioning to yield a chloroform extract ($CHCl_3$-X, 16.4 g; enriched in sterols and fatty acids), an ethyl acetate extract (EtOAc-X, 6.4 g; enriched in flavonoids and phenolic acids), and an aqueous portion. The aqueous portion was further adsorbed on an XAD-16 (Amberlite Resin, Sigma, St. Louis, MO) column and eluted with water followed by acidic methanol (adjusted with 6 N hydrochloric acid, HCl, to a pH of 3) as previously described (*11*) to yield a water extract (H_2O-X; enriched in natural fruit sugars and organic acids) and an acidic methanol fraction (25 g, polyphenol-enriched fraction), respectively. The acidic methanol fraction was further purified by suspending the extract in 200 mL of distilled water and filtering to yield a water-soluble fraction (MeOH-1, 14 g, dark red powder; enriched with anthocyanins) and a water-insoluble fraction (MeOH-2, 10.9 g; enriched in ellagitannins, ellagic acid, and other polymeric molecules). Crude extracts and their relative composition used in our experiments were (1) $CHCl_3$-X (sterols, fatty acids), (2) EtOAc-X (flavonols, small phenolic compounds), (3) H_2O-X (natural fruit sugars and acids), (4) Anth-X (anthocyanin enriched), and (5) ellagitannin-X (ellagitannin enriched).

Purification and Structural Elucidation of Strawberry Phenolics. Pure compounds **1**–**10** were obtained as follows. Briefly, medium-pressure liquid chromatography (MPLC) C_{18} columns were used to separate the anthocyanins, and silica gel MPLC and preparative-scale thin layer chromatography (TLC) were employed to purify the other phenolic compounds.

The Anth-X (anthocyanin enriched, 5 g) fraction was subjected to a C_{18} column and eluted with acidic methanol/water (pH 3 adjusted with HCl) gradient (1:9 v/v, 500 mL; 2:8 v/v, 500 mL; 3:7 v/v, 100 mL; 5:5 v/v, 500 mL; and 100% methanol, 500 mL). The color bands were collected; otherwise, 200 mL aliquot fractions were collected. A total of four colored fractions (AnthX1−X4), noncolored fractions (AnthX0), and a pure methanol eluted fraction (AnthX5) were obtained. The colored fractions were further purified by preparative HPLC with UV detector by using a C_{18} column and elution with methanol/water (7:3, v/v). Single peaks detected at 520 nm (i.e., the characteristic absorption wavelength of anthocyanins) were collected as compounds **1** (21 mg), **2** (15.3 mg), **3** (296 mg), and **4** (16.5 mg), respectively, according to retention times. A pale solid that precipitated from fraction AnthX5 yielded compound **10** (140 mg), which was further purified after filtration and recrystallization from methanol.

A portion of the EtOAc-soluble fraction (EtOAc-X, 2 g) was subjected to a silica gel MPLC column and eluted with a chloroform/acetone (C/A) solvent system under gradient conditions (C/A 9:1 v/v, 500 mL; C/A 4:1 v/v, 500 mL; C/A 3:1 v/v, 500 mL; C/A 2:1 v/v, 500 mL; C/A 1:1 v/v, 500 mL; C/A 1:2 v/v, 500 mL; acetone, 250 mL; methanol, 250 mL), affording 15 fractions (200 mL each, A1−A15). Fractions A4−A6 were further purified by preparative-scale TLC by collecting a band at a retention factor (R_f) of 0.45 (hexane/acetone, 8:2, v/v) to yield compound **6** (6.6 mg). Fraction A7 was precipitated with acetone to yield pure compound **5** (17 mg) as a pale yellow precipitate. Pale yellow needles, which precipitated from fraction A5, were filtered and recrystallized from acetone to yield pure compound **7** (44 mg).

A portion of the EtOAc-soluble fraction (EtOAc-X, 2 g) was subjected to a C_{18} MPLC column eluting with methanol/water (M/W) solvent system under gradient conditions (M/W 9:1 v/v, 500 mL; M/W 4:1 v/v, 500 mL; M/W 7:3 v/v, 500 mL; M/W 2:1 v/v, 500 mL; M/W 1:1 v/v, 500 mL; methanol, 500 mL), affording 12 fractions (M1−M12, 200 mL each, plus a methanol-eluted fraction M13). Compound **8** (11 mg) was precipitated as a needle form M4 fraction. Compound **9** was precipitated from fraction M2 (10 mg) as a white needle precipitate.

Compound 7 (kaempferol-3-(6′-coumaroyl)glucoside): ^{1}H NMR (CD_3OD) δ 7.98 (2H, dd, J = 9.0, 2.1 Hz, H-2′, 6′), 7.39 (1H, d, J = 16.0 Hz, H-8‴), 7.29 (2H, dd, J = 8.6, 1.84, H-2‴, 6‴), 6.81 (2H, dd, J = 9.0, 2.1 Hz, H-3′, 5′), 6.78 (2H, dd, J = 8.6, 1.84 Hz, H-3‴, 5‴), 6.29 (1H, d, J = 2.12 Hz, H-8), 6.12 (1H, d, 2.12 Hz, H-6), 6.07 (1H, d, J = 16.0 Hz, H-7‴), 5.24 (1H, d, J = 7.68 Hz, H-1′), 4.31 (1H, dd, J = 11.80, 2.20 Hz, Hα-6′), 4.19 (1H, dd, J = 11.80, 8.30 Hz, Hβ-6′), 3.50 (1H, m, H-5′), 3.47 (2H, m, H-2′, 4′), 3.35 (1H, m, H-3′); ^{13}CNMR δ 179.5 (C-4), 168.9 (C-9‴), 166.0 (C-7), 163.1 (C-5), 161.6(C-4′), 161.3 (C-4‴), 159.4 (C-2), 158.5(C-9), 146.7 (C-7‴),

135.4 (C-3), 132.4 (C-2′, 6′), 131.3 (C-2‴, 6‴), 127.2 (C-1‴), 122.8 (C-1′), 116.9 (C-3′, 5′), 116.2 (C-3‴, 5‴), 114.9 (C-8‴), 105.7 (C-10), 104.1 (C-1″), 100.1 (C-6), 95.0 (C-8), 78.1 (C-3″), 75.9 (C-2″, C-5″), 71.8 (C-4″), 64.5 (C-6″).

Compound **9** *[glucose ester of (E)-p-coumaric acid]:* ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H, -OH), 7.64 (1H, d, J = 15.9 Hz, H-7), 6.40 (1H, d, J = 15.9 Hz, H-8), 7.58 (2H, d, J = 8.6 Hz, H-2, 6), 6.80 (2H, d, J = 8.6 Hz, H-3, 5), 5.46 (1H, d, J = 7.96 Hz, H-1′), 3.65 (1H, dd, J = 12.00, 5.6 Hz, H-6′), 3.44 (1H, dd, J = 12.0, 5.6, H-6′). 3.37–3.48 (4H, m, H-2′, H-3′, H-4′, H-5′).

Trolox Equivalent Antioxidative Capacity (TEAC). The TEAC assay was performed as previously reported (*15*). Briefly, 2′,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cations were prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS$^{\bullet+}$ (20 mL using a 75 mM Na/K buffer of pH 7). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of Vvitamin E, was used as an antioxidant standard. A standard calibration curve was constructed for Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 μM concentrations. Samples were extracted in methanol/water (1:1, v/v) (10 mg/mL concentrations) by vortexing for 30 min, sonicating for 5 min, and centrifuging for 10 min at 2000*g*. Samples were diluted appropriately according to antioxidant activity in Na/K buffer pH 7. Diluted samples were mixed with 200 μL of ABTS$^{\bullet+}$ radical cation solution in 96-well plates, and absorbance was read (at 750 nm) after 5 min in a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA). Samples were assayed in six replicates. TEAC values were calculated from the Trolox standard curve and expressed as Trolox equivalents (TE, in micromolar per milligram).

Cell Culture Materials. All cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD). KB oral cancer cells and MCF-7 breast cancer cells were grown in minimum essential medium (MEM); CAL-27 oral cancer cells were grown in Dulbecco's minimum essential medium (DMEM); LNCaP and DU145 prostate cancer cells were grown in RPMI 1640; HT-29 and HCT116 colon cancer cells were grown in McCoy's 5A medium, modified. All media contained 10% fetal bovine serum (FBS) in the presence of 100 units/mL penicillin and 0.1 g/L streptomycin. Cells were incubated at 37 °C with 95% air and 5% CO_2. All cells were maintained below passage 20 and used in experiments during the linear phase of growth.

Cell Proliferation Assay. Proliferation was measured utilizing the CellTiter-Glo Luminescent Cell Viability Assay (Technical Bulletin 288, Promega Corp., Madison, WI). When added to cells, the assay reagent produces luminescence in the presence of ATP from viable cells. Cells were plated in 96-well plates at a density of 5000 cells/well and incubated for 24 h. Test samples were solubilized in deionized water by sonication, filter sterilized, and diluted with media to the desired treatment concentration. Cells were treated with 100 μL of media or test samples and incubated for 48 h drug exposure duration. Extracts were tested at 250 μg/mL, and pure compounds were tested at 100 μg/mL concentrations. At the end of 48 h, plates were equilibrated at room temperature for 30 min, 100 μL of the assay reagent was added to each well, and cell lysis was induced on an orbital shaker for 2 min. Plates were incubated at room temperature for 10 min to stabilize the luminescence signal, and results were read on an Orion Microplate Luminometer (Bertholds Detection Systems, Pforzheim, Germany). All plates had control wells containing medium without cells to obtain a value for background luminescence that was subtracted from the test sample readings. Data are expressed as percentage of untreated cells, mean ± SE, for three replications.

Statistical Analysis. Cell proliferation data were analyzed by either Student's t test or one-way ANOVA followed by Dunnett's multiple-range test (α = 0.05) with Graph Pad Prism 3.0 (Graph Pad Software Inc.) as appropriate. TEAC values were determined in six replicates, and the mean values ± SD are reported.

RESULTS AND DISCUSSION

Isolation and Identification of Strawberry Phenolics. The compounds were identified by comparison of their nuclear magnetic resonance (NMR) data with published literature values

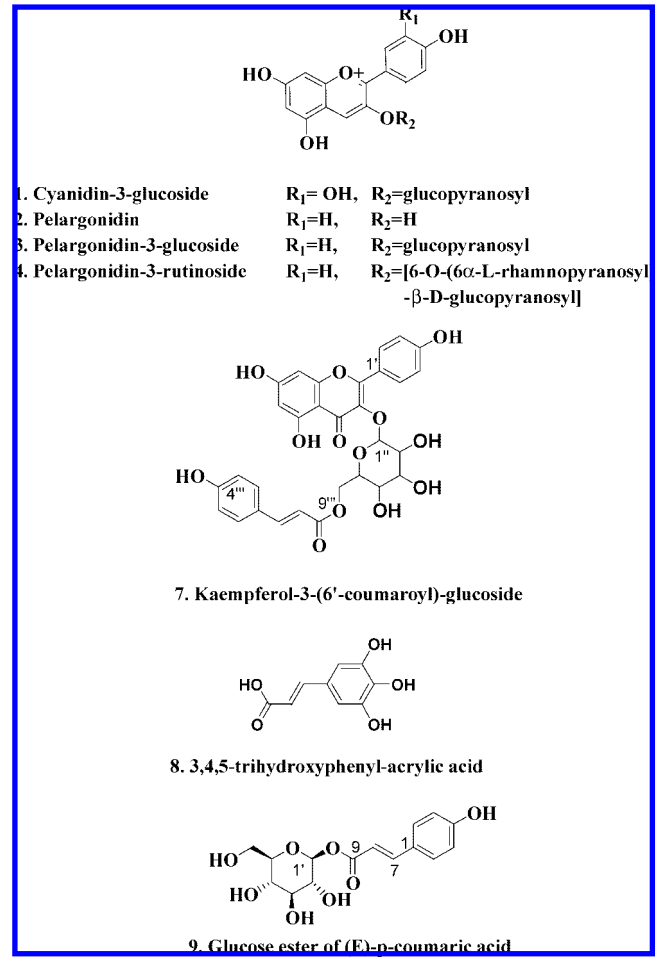

Figure 2. Chemical structures of phenolic compounds **1–4**, **7**, and **9**, isolated from strawberries.

and by comparison of their HPLC-UV retention times to those of authentic standards when available. **Figure 2** shows the chemical structures of the phenolics isolated and identified from strawberries: cyanidin-3-glucoside (**1**), pelargonidin (**2**), pelargonidin-3-glucoside (**3**), pelargonidin-3-rutinoside (**4**), kaempferol (**5**), quercetin (**6**), kaempferol-3-(6′-coumaroyl)glucoside (**7**), 3,4,5-trihydroxyphenyl-acrylic acid (**8**), glucose ester of (*E*)-*p*-coumaric acid (**9**), and ellagic acid (**10**).

A brief description of the structural elucidation of the compounds **1**, **3**, **4**, **7**, and **9** follows. Compound **1** was identified as cyanidin-3-glucoside, and its NMR data corresponded to previously published values (*16*). Similarly, the NMR data for compounds **3** and **4** corresponded to the reported data for pelargonidin-3-glucoside and pelargonidin-3-rutinoside, respectively (*17–19*). The ^{1}H NMR data of compounds **1**, **3**, and **4** are summarized in **Table 1**.

The ^{1}H NMR spectrum of compound **7** exhibited typical signals for a kaempferol moiety [δ 7.98 (2H, dd, J = 9.0, 2.1 Hz), 6.81 (2H, dd, J = 9.0, 2.1 Hz), 6.29 (1H, d, J = 2.1 Hz), 6.12 (1H, d, J = 2.1 Hz)] and a trans coumaroyl moiety [δ 7.39 (1H, d, J = 16.0 Hz), 7.29 (2H, d, J = 8.6 Hz), 6.78 (2H, d, J = 8.6 Hz), 6.07 (1H, d, J = 16.0 Hz)] and signals for an anomeric proton of a sugar at δ 5.24, d, J = 7.7 Hz. The coupling constant of J = 7.7 Hz indicated a β-configuration for the glucose moiety. Because the H-6″ proton signal of the glucose moiety was shifted downfield at δ 4.31 (1H, dd, J = 11.8, 2.2 Hz), 4.19 (1H, dd, J = 11.8, 8.3 Hz), it was concluded that the coumaroyl moiety was attached to the C-6 of glucose in the trans configuration. The structure of compound **7** was

Table 1. ^{1}H Nuclear Magnetic Resonance Data for Compounds **1**, **3**, and **4** (in MeOH-d_4 Containing 1% TFA-d)[a]

	1	3	4
H-4	9.01, 1H, s	9.02, 1H, s	9.00, 1H, s
H-6	6.65, 1H, d, J = 1.90	6.63, 1H, d, J = 1.96	6.63, 1H, br d, J = 1.64
H-8	6.89, 1H, d, J = 1.90	6.89, 1H, d, J = 1.52	6.93, 1H, br d, J = 1.20
H-2′	8.03, 1H, d, J = 2.30	8.55, 1H, d, J = 9.08	8.60, 1H, d, J = 12.08
H-3′		7.01, 1H, d, J = 9.08	7.05, 1H, d, J = 12.08
H-5′	7.01, 1H, d, J = 8.70	7.01, 1H, d, J = 9.08	7.05, 1H, d, J = 12.08
H-6′	8.25, 1H, dd, J = 8.70, 2.30	8.55, 1H, d, J = 9.08	8.60, 1H, d, J = 12.08
H-1″	5.30, 1H, d, J = 7.70	5.28, 1H, d, J = 7.68	5.27, 1H, d, J = 7.80
H-2″	3.68, 1H, dd, J = 8.96, 7.72	3.64, 1H, dd, J = 9.08, 7.72	
H-3″	3.55, 1H, t, J = 9.04	3.55, 1H, t, J = 9.04	
H-4″	3.45, 1H, dd, J = 9.60,8.96	3.44, 1H, t, J = 9.44	
H-5″	3.57, 1H, ddd, J = 9.44, 5.68, 2.12	3.58, 1H, ddd, J = 9.68,5.96, 2.12	
Hα-6″	3.92, 1H, dd, J = 12.12, 2.00	3.94, 1H, dd, J = 12.08, 2.16	4.07, 1H, dd, J = 11.24,1.68
Hβ-6″	3.72, 1H, dd, J = 12.12, 6.12	3.72, 1H, dd, J = 12.08, 6.04	

[a] δ in parts per million; J in hertz.

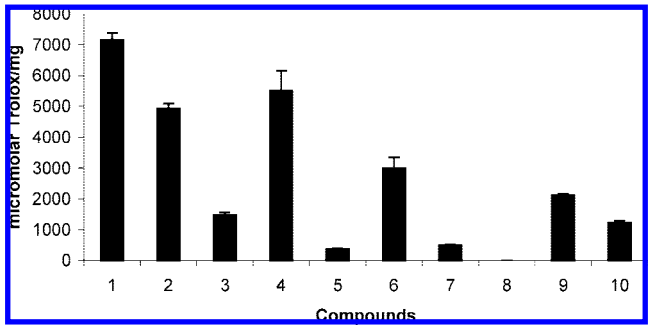

Figure 3. TEAC results of the pure compounds isolated from strawberry powder.

confirmed as kaempferol-3-(6′-coumaroyl)glucoside by comparison with published NMR data (*20*).

The LC-ESI-MS of compound **9** gave an $[M - H]^-$ ion at *m/z* 325 and indicated that the molecular formula of compound **9** was $C_{15}H_{18}O_8$. The characteristic signals for 1,4-disubstituted benzene protons at δ 7.58 (2H, d, J = 8.6 Hz) and 6.80 (2H, br d, J = 8.6 Hz) and the pair of trans-olefinic proton signals at δ 7.64 (1H, d, J = 16.0 Hz) and 6.40 (1H, d, J = 16.0 Hz), which were conjugated with a carbonyl group, clearly indicated that there is a *trans*-coumaroyl moiety in the structure. The anomeric proton signal at δ 5.46 (1H, d, J = 8.0 Hz) of the sugar unit demonstrated a β-configuration. Therefore, compound **9** was identified as 1-[3-(4-hydroxyphenyl)-2-propenoate]-β-D-glucopyranoside that corresponded with published NMR data (*21*).

Compounds **2**, **5**, **6**, **8**, and **10** were identified as pelargonidin, kaempferol, quercetin 3,4,5-trihydroxyphenyl-acrylic acid, and ellagic acid, respectively, first by comparison with correspondence standards on HPLC and were further confirmed by LC-ESI-MS/MS.

Antioxidant Activities. The antioxidant activity using the TEAC assay reached 7156 μM Trolox/mg for the pure compounds as shown in **Figure 3**. Among the purified phenolics, the anthocyanins **1** (7156 μM Trolox/mg), **2** (4922 μM Trolox/mg), and **4** (5514 μM Trolox/mg) were the most potent antioxidants. Anthocyanins have been reported to have potent antioxidant properties (*10*), which corroborate the results reported here. Structure–activity-related (SAR) studies have been conducted for anthocyanins, with respect to their antioxidant activities (*22, 23*), and these structural differences may explain the TEAC values observed for the anthocyanins in this study.

Antiproliferative Activities. Strawberry crude extracts were evaluated for their ability to inhibit the growth of human colon

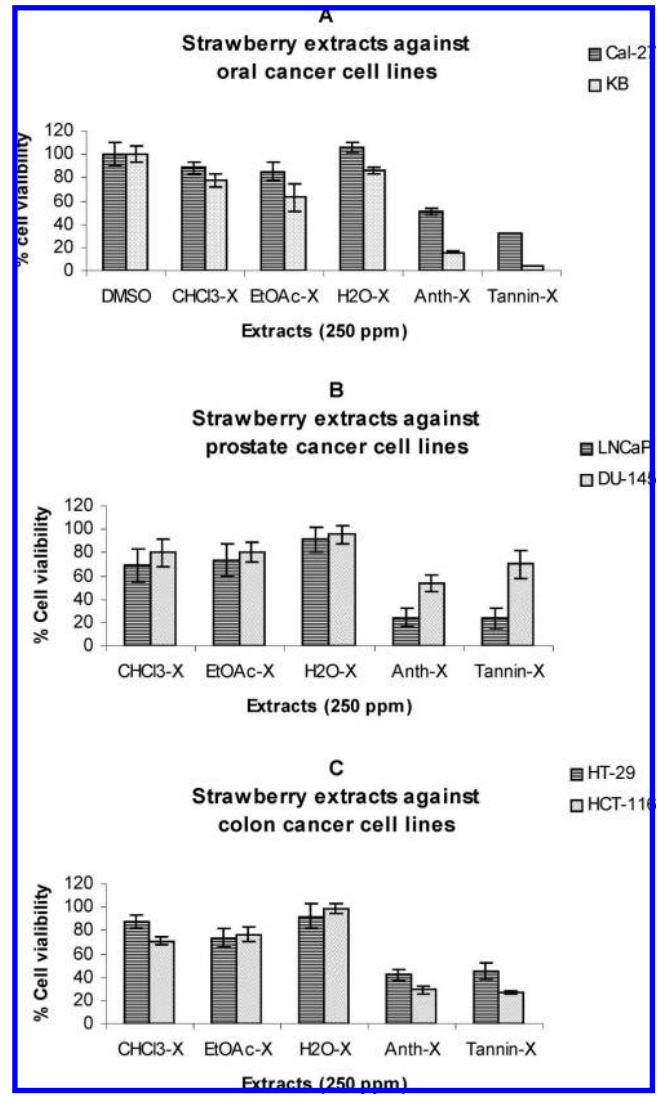

Figure 4. Inhibition of proliferation of human tumor cell lines by crude strawberry extracts: (**A**) KB (oral) and CAL-27 (oral); (**B**) LNCaP (prostate) and DU145 (prostate); (**C**) HT-29 (colon) and HCT-116 (colon). Cells were exposed to extracts at 250 μg/mL for 48 h.

(HT-29 and HCT-116, **Figure 4A**), prostate (LNCaP and DU145, **Figure 4B**), and oral (KB and CAL-27, **Figure 4C**) tumor cell lines. Pure compounds **1**–**10** were also evaluated for their ability to inhibit the growth of human colon (HT-29 and HCT-116, **Figure 5A**), prostate (LNCaP and DU145, **Figure**

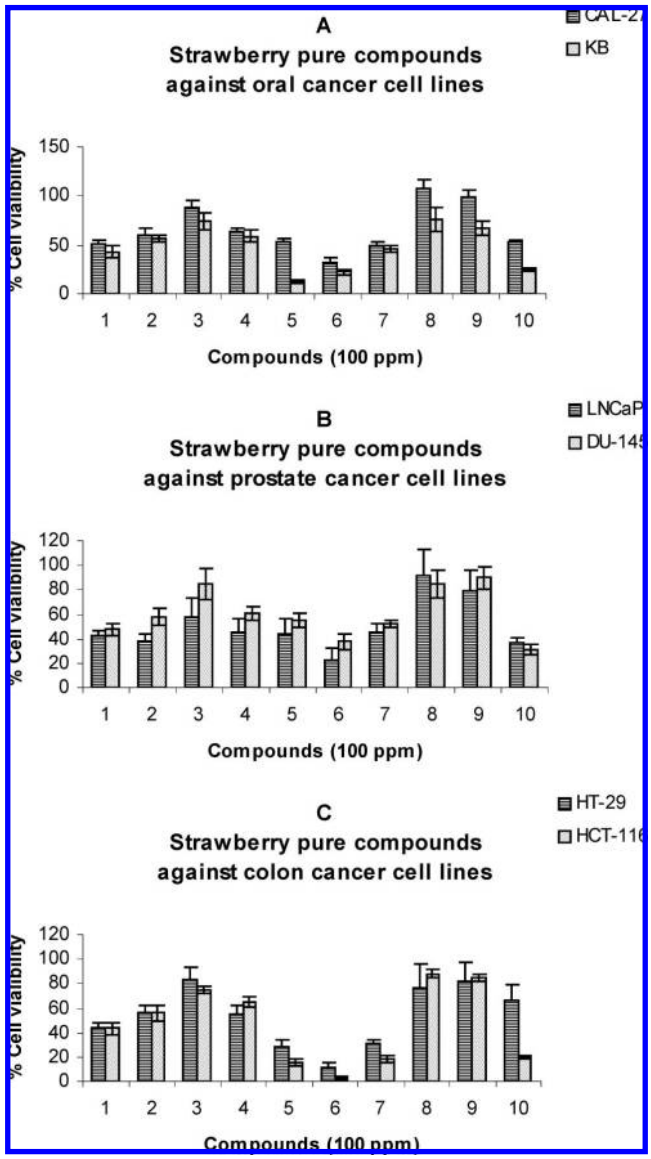

Figure 5. Inhibition of proliferation of human tumor cell lines by pure compounds isolated from strawberries: (**A**) KB (oral) and CAL-27 (oral); (**B**) LNCaP (prostate) and DU145 (prostate); (**C**) HT-29 (colon) and HCT-116 (colon). Cells were exposed to compounds at 100 μg/mL for 48 h.

5B), and oral (KB and CAL-27, **Figure 5C**) tumor cell lines. It is noteworthy that the anticancer effects of individual phytochemical constituents of strawberries, as well as whole strawberry extracts, have been previously demonstrated (reviewed in ref *6*). In agreement with previously published studies (*6*), these results confirm that strawberry extracts and their purified compounds inhibit human cancer cell growth in a dose-dependent manner with various degrees of potency.

In conclusion, the current study contributes to the growing body of literature which demonstrates that strawberry extracts, and their purified phenolic compounds, show antioxidant and human tumor cell antiproliferative activities in vitro. Other berry fruit extracts, similar to the strawberry, have also been reported to inhibit the growth of human tumor cell lines in vitro (*24*). It is noteworthy that the in vitro doses used for the antioxidant and antiproliferative assays in this study are not physiologically relevant. Nevertheless, the main objective of this study was to isolate and identify phenolic compounds from the strawberry fruit to serve as chemical standards for planned animal and human studies. There is an urgent need for continued and future in vivo studies before the impact of strawberry consumption on human health and disease can be thoroughly evaluated (*25*).

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