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Antioxidant, Prooxidant, and Cytotoxic Activities of Solvent-Fractionated Dandelion (*Taraxacum officinale*) Flower Extracts in Vitro

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This study was conducted to investigate the chemical antioxidant and bioactive properties of the water (WF) and ethyl acetate fractions (EAF) derived from dandelion (*Taraxacum officinale*) flower extract (DFE). HPLC analysis showed the presence of both luteolin and luteolin 7-glucoside in the DFE, which contributed to noted in vitro antioxidant and Caco-2 cell cytotoxic activities. Both WF and EAF of DFE exhibited free radical scavenging activities in a stable 2,2-diphenyl-1-picrylhydrazyl radical model and reduced the breakage of supercoiled DNA strand induced by both non-site-specific and site-specific hydroxyl radical. Oxidation of structured phosphatidylcholine liposome induced by peroxyl radical was reduced in the presence of both EAF and WF. EAF had greater ($p < 0.05$) affinity to scavenge peroxyl radical than WF, as measured by the formation of conjugated diene. At low concentration, prooxidant activity of both fractions was observed in Cu^{2+}-induced structured liposome and hLDL oxidation models, thus indicating that the reducing power of the DFE had resulted in generation of reactive cuprous ion. However, at high concentrations the EAF did not promote oxidation in the presence of Cu^{2+}, suggesting that the free radical scavenging activity of this fraction was sufficient to minimize the potential oxidative mechanism attributed to the metal ion reducing activity associated with prooxidant activity.

KEYWORDS: Dandelion; antioxidant; reactive oxygen species; cytotoxicity

INTRODUCTION

Phytochemicals derived from fruits, vegetables, and herbs have been extensively studied both in vitro and in vivo to characterize potential antioxidant and bioactive properties. Plant phenolic compounds have a significant capacity to scavenge free radicals and sequester transitional metal ions (*1*−*3*), which is based on physicochemical features that include the number and position of hydroxyl substituents. Reactive oxygen species (ROS) include a variety of oxygen derivatives generated from metabolism, smoking, and environmental stress (*4*−*6*). Hydroxyl radicals represent an ROS that can abstract hydrogen atoms from polyunsaturated fatty acids and induce free radical chain reaction (*7*). Peroxyl radicals derived from oxidation reaction contribute to food spoilage and in vivo membrane damage attributed to lipid oxidation (*7*, *8*). Examples of plant phenolics that have repeatedly shown an affinity to quench ROS include the tea catechins (*9*), flavonoids from *Gingko biloba* (*10*), flaxseed lignan (*11*), anthocyanins from fruits (*12*, *13*), and ginsenosides from ginseng (*14*).

Dandelion (*Taraxacum officinale*) is a plant well-known for use in North American folk medicine remedies and home recipes. Although considered by many to be a troublesome weed, it is also acclaimed as a nontoxic herb with special value used in traditional Chinese medicine for a variety of health benefits (*15*). Flavonoids that have been identified from the dandelion plant include caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside (*16*). However, relatively less is known about the antioxidant and bioactive properties of this plant and, more specifically, the flower component. The purpose of this study was to characterize the in vitro antioxidant properties (*17*, *18*) of dandelion flower and assess potential cytotoxic activity in a human colon colorectal adenocarcinoma cell line, that is, the Caco-2 cell.

MATERIALS AND METHODS

Materials. Electrophoresis grade agarose was obtained from Bio-Rad Laboratories (Richmond, CA). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). Luteolin and luteolin 7-glucoside were purchased from Indofine Chemical Co. Inc. (Somerville, NJ). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Human colon colorectal adenocarcinoma cells (Caco-2 cell) were purchased from American Type Culture Collection (Manassas, VA). Other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Prior to use, buffers were passed through a Chelex-100 metal chelation resin column to minimize the occurrence of transition metal ions.

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Sample Preparation. Dandelion (*T. officinale*) flowers were obtained from local areas within British Columbia and Nova Scotia (Naturally Nova Scotia, Dartmouth, NS), Canada, during early summer. Flowers were rinsed with water prior to freeze-drying. Freeze-dried dandelion flower was refluxed 40 times (v/w) with a 70% (v/v) ethanol/water solution for 8 h. The 70% (v/v) ethanol extract was further fractionated according to the following procedures. The dandelion flower crude extract solution was rotary evaporated (<40 °C) to one-third of original volume and stored at 4 °C overnight prior to filtration (Whatman no. 4). The filtrate was then extracted with 3 volumes of ethyl acetate. The ethyl acetate phases were combined and rotary-vacuum evaporated to dryness to obtain an ethyl acetate fraction (EAF) of DFE. The water phase residue was also collected and dried under vacuum. This fraction was referred to as the water fraction (WF) of DFE.

DPPH Radical Scavenging Capacity of EAF and WF of DFE. DFE fractions EAF and WF were evaluated for activity to scavenge the stable DPPH radical (0.1 mM 2,2-diphenyl-1-picryhydrazyl) according to our previous study (*17*). The affinity of test material to quench DPPH free radical was evaluated according to the equation

$$\text{scavenging \%} = \frac{A_c - A_s}{A_c} \times 100$$

where A_s and A_c are absorbance at 519 nm of the reaction mixture with samples and without sample, respectively.

Antioxidant Activities of EAF and WF of DFE in Peroxyl Radical- and Cu^{2+}-Induced Liposome Oxidation. The affinity of both EAF and WF to prevent the formation of conjugated diene in the peroxidation of soybean phosphatidylcholine-based liposome was performed as previously reported (*18*). Briefly, liposome peroxidation was initiated by the addition of 2 mM of AAPH or in a separate experiment, 100 μM $CuSO_4$, to the 0.1 mg/mL of liposome with different amounts of DFE fractions (0, 0.5, 1.0, and 2.5 μg/mL). The formation of conjugated diene hydroperoxide at 37 °C was measured at 234 nm (Unicam UV-2 UV−vis spectrometer, equipped with an eight-cuvette holder and temperature circulator) for 100 min. The formation of conjugated diene hydroperoxide was calculated using a molar extinction coefficient $\epsilon = 29500\ M^{-1}\ cm^{-1}$ (*19*). Generation of conjugated diene was plotted against time, and the antioxidant activities of different fractions were compared by examining both the reaction lag phase duration and the rate of propagation. Lag phase duration was defined as the time duration (minutes) of the intersection point of two best-fit linear regression lines for both the initiation and propagation phases. The rate of propagation was expressed as the formation of conjugated diene per minute (micromoles of conjugated diene per gram of phosphatidylcholine per minute).

Evaluation of Antioxidant Activities of EAF and WF of DFE in Cupric Ion-Induced Human Low-Density Lipoprotein Oxidation. EDTA-free human LDL (hLDL) was obtained by dialysis against a continuously nitrogen-treated 10 mM phosphate-buffered saline (PBS, 150 mM NaCl, pH 7.4) at 4 °C (*17*). Oxidation was initiated by adding 10 μmol/L $CuSO_4$ to the hLDL solution (0.5 mg of protein/mL) in PBS at 37 °C for 16 h. The reaction was stopped by adding 100 μM EDTA to the reaction mixture. The change of negative charge on hLDL was evaluated by using agarose (0.6%, w/v) electrophoresis in a horizontal gel electrophoresis apparatus (EC Apparatus Corp., St. Petersburg, FL) with 50 mM barbital buffer (pH 8.6). hLDL was then stained with Sudan black.

In a separate experiment, a spectrophotometric method was used to examine the formation of conjugated diene during the oxidation of hLDL (100 μg of protein/mL) induced by 2 μM $CuSO_4$ at 37 °C for 100 min.

Evaluation of Antioxidant Activities of EAF and WF of DFE Using a DNA Scission Assay. Antioxidant activities of EAF and WF of DFE were examined for the relative affinity to prevent both hydroxyl radical- and peroxyl radical-induced supercoiled DNA nicking (*18*). Briefly, the peroxyl radical-induced DNA scission was conducted using 2 μL of plasmid DNA (0.1 μg/mL, pBR322 plasmid DNA in PBS), and 5 mM AAPH was introduced to a final volume of 12 μL in 10 mM PBS (pH 7.4). The reaction mixtures with or without samples were incubated at 37 °C for 2 h.

In the hydroxyl radical-induced DNA scission model, both non-site-specific and site-specific hydroxyl radicals (*20*) were used to initiate DNA nicking. The reaction mixture of non-site-specific hydroxyl radical contained 100 μM EDTA, 10 μM $FeCl_3$, 10 μM ascorbic acid, and 100 μM H_2O_2. EDTA was replaced by 10 mM PBS in the site-specific hydroxyl radical generation assay system. Reaction mixtures (12 μL) including DNA, sample, and hydroxyl radicals were incubated at 37 °C for 1 h.

At the end of incubation, 2 μL of 6× gel loading buffer was added, and the resultant mixture was loaded onto a 0.7% (w/v) agarose gel. Gel electrophoresis was conducted in TAE buffer (40 mM Tris acetate and 2 mM EDTA, pH 8.5) using a horizontal electrophoresis apparatus at 3 V/cm. DNA was stained with 0.5 μg/mL ethidium bromide and visualized under UV; the image was also photographed by a Polaroid instant camera. The relative concentration of supercoiled DNA strand was quantified by using a densitometer (GS-670, Bio-Rad, Richmond, CA) with Molecular Analyst (version 1.3, Bio-Rad Laboratories, Richmond, CA). Retention of supercoiled DNA strand was calculated according to the equation

$$\text{\% retention} = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

where A_s and A_c represent the amount of supercoiled DNA treated with sample and without antioxidant and ROS, respectively

Reduction of Cu^{2+}. The formation of Cu^+ was traced over 100 min in a mixture consisting of Cu^{2+}, neocuproine, and the addition of DFE sample at 37 °C. The concentration of Cu^+ was calculated according to the extinction coefficient of Cu^+−neocuproine $\epsilon = 7.95 \times 10^3\ M^{-1}\ cm^{-1}$ at a wavelength of 454 nm (*21*).

Cytotoxicity Test. A human colon adenocarcinoma cell line Caco-2 was used to evaluate the cytotoxicity of DFE and known flavonoids found in dandelion according to the procedure of Jing and Kitts (*22*). DFE fractions, along with luteolin 7-glucoside and luteolin, were cultured at 37 °C with 5% CO_2 for 48 h in a 96-well plate containing 0.75×10^3 cells/well in DMEM supplemented with 10% fetal bovine serum. Cytotoxicity was evaluated by using the MTT protocol (Boehringer Mannheim), and cell viability was calculated according to the following equation:

$$\text{cell viability (\%)} = \frac{\text{absorbance}_{\text{sample,570nm}}}{\text{absorbance}_{\text{control,570nm}}} \times 100$$

HPLC Analysis for the Total Phenolic Compounds. HPLC consisted of two Waters M-45 pumps, a U6K injector, and a UV−vis detector Lambda Max 481. The Axxi-Chrom 717 chromatography data system was used for computer control and data analysis. Phenolic compounds of DFE were separated on a Phenomenex C_{18} (Phenomenex, Torrance, CA) reverse phase column (4.6 × 250 mm, 5 μm) at room temperature using a linear gradient elution [solvent A = acetonitrile/0.1% phosphoric acid (10:90, v/v); solvent B = acetonitrile/0.1% phosphoric acid (25:75, v/v)]. Solvent B increased from 0 to 100% in 30 min and was kept at 100% for 5 min. Samples were dissolved in the mobile phase, and 5 μL was injected. The wavelength of the UV−vis spectrophotometer detector was set at 350 nm, and the flow rate of the mobile phase was 1.0 mL/min. Caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside were used as external standards. Phenolic components were calculated from the area under respective peaks according to individual standard curves.

Statistics. All data were expressed as mean ± standard deviation (SD) of three individual experiments. One-way ANOVA was used to evaluate the difference between treatments with significant difference set at 0.05 level (SPSS for Windows 10.0, SPSS Inc.).

RESULTS

Effect of Dandelion Fractions on DPPH Radical Scavenging. EAF and WF derived from dandelion flower both exhibited significant ($p < 0.05$) free radical scavenging capacities for stable DPPH radical (**Table 1**). At equal concentrations, EAF

Table 1. Free Radical (DPPH) Scavenging Percentage of Ethyl Acetate Fraction and Water Fraction of Dandelion Flower Extract and Trolox Standard[a]

concn (μg/mL)	WF	EAF	Trolox
5.33	ND	ND	47.5 ± 0.9a
10.67	ND	ND	96.6 ± 0.2a
13.33	15.7 ± 2.4	67.2 ± 5.0**a	ND
26.67	23.6 ± 2.2a	90.7 ± 1.9**a	ND
53.33	51.9 ± 2.2a	94.4 ± 0.2**a	ND

[a] Values represent mean ± SD; **, significant difference ($p < 0.01$) between WF and EAF of DFE at the same concentration; a, significant difference ($p < 0.05$) versus control test in which no antioxidant was added to the reaction. ND, not determined.

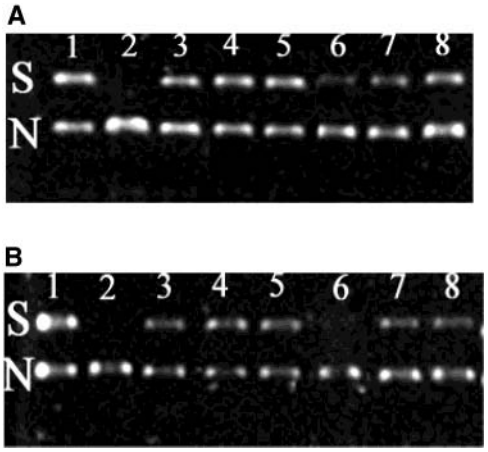

Figure 1. DNA breakage under hydroxyl radical stress recovered by EAF and WF of DFE: (A) non-site-specific hydroxyl radical; (B) site-specific hydroxyl radical; (lane 1) DNA + PBS; (lane 2) DNA + hydroxyl radical; (lane 3) DNA + EAF (10 μg/mL) + hydroxyl radical; (lane 4) DNA + EAF (50 μg/mL) + hydroxyl radical; (lane 5) DNA + EAF (100 μg/mL) + hydroxyl radical; (lane 6) DNA + WF (10 μg/mL) + hydroxyl radical; (lane 7) DNA + WF (50 μg/mL) + hydroxyl radical; (lane 8) DNA + WF (100 μg/mL) + hydroxyl radical. S, supercoiled DNA; N, nicked DNA.

had greater ($p < 0.01$) free radical scavenging activity compared to the WF but less activity than Trolox.

Effect of Dandelion Fractions on Preventing DNA Nicking Induced by either Peroxyl or Hydroxyl Radical. The hydroxyl radical generated by the Fenton reaction rapidly reacts with both deoxyribose (*20*) and DNA, the latter resulting in loss of DNA integrity (*5*). Generation of hydroxyl radicals in both non-site-specific (**Figure 1A**) and site-specific (**Figure 1B**) Fenton reaction resulted in the disappearance of supercoiled DNA strand on the agarose gel electrophoresis (lanes 2 of **Figure 1**). Supercoiled strand was partially protected in the presence of both dandelion WF and EAF extracts (lanes 3−8 of **Figure 1**). In both assays, EAF protected more DNA from base scission at lower concentration ($p < 0.05$) than the WF (**Table 2**). Although less protection by DFE was observed in the site-specific assay, a concentration-dependent protection was found for both WF and EAF in preventing DNA from hydroxyl radical-induced nicking (**Table 2**). In comparison, 4 μg/mL Trolox protected 83.5 ± 0.5% ($p < 0.001$) and 42.6 ± 2.5% of supercoiled DNA strand, respectively, from nicking in the non-site-specific and site-specific hydroxyl radical models. Similarly, 1.8 μg/mL luteolin 7-glucoside resulted in 64.0 ± 3.9% and 83.1 ± 2.9% protection of supercoiled DNA from the non-site-specific and site-specific hydroxyl radical-induced oxidation, respectively.

Table 2. Retention Percent of Supercoiled DNA Strand with the Addition of Ethyl Acetate Fraction and Water Fraction of Dandelion Flower Extract in Non-Site- and Site-Specific Hydroxyl Radical-Induced DNA Nicking[a]

concn (μg/mL)	WF	EAF
	A. Non-Site-Specific	
10	6.3 ± 3.9	60.9 ± 11.6***a
50	43.6 ± 1.5a	72.1 ± 4.1**a
100	70.3 ± 13.6a	78.1 ± 11.0a
	B. Site-Specific	
10	7.3 ± 0.6	35.5 ± 7.2*a
50	9.3 ± 2.4a	49.4 ± 4.9**a
100	52.6 ± 3.7a	50.8 ± 4.5a

[a] *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ by *t* test for both fractions at the same concentration; a, significant difference versus control test ($p < 0.05$).

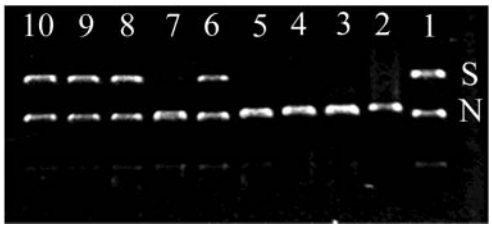

Figure 2. Effect of EAF and WF of DFE in DNA scission induced by peroxyl radical generated by thermolysis of AAPH at 37 °C: (lane 1) DNA + PBS; (lane 2) DNA + AAPH; (lane 3) DNA + WF (5 μg/mL) + AAPH; (lane 4) DNA + WF (10 μg/mL) + AAPH; (lane 5) DNA + WF (20 μg/mL) + AAPH; (lane 6) DNA + WF (50 μg/mL) + AAPH; (lane 7) DNA + EAF (5 μg/mL) + AAPH; (lane 8) DNA + EAF (10 μg/mL) + AAPH; (lane 9) DNA + EAF (20 μg/mL) + AAPH; (lane 10) DNA + EAF (50 μg/mL) + AAPH. S, supercoiled DNA; N, nicked DNA.

Table 3. Retention Percent of Supercoiled DNA Strand with the Addition of Dandelion Fractions in Peroxyl Radical-Induced Nicking[a]

concn (μg/mL)	WF	EAF
5	ND	1.9 ± 0.9
10	ND	23.6 ± 5.3a
20	ND	65.0 ± 2.0***a
50	60.0 ± 2.2**a	81.0 ± 0.5***a

[a] *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ by t test for both fractions at the same concentration; a, significant difference versus control test ($p < 0.05$). ND, not determined.

Generation of peroxyl radical by thermal decomposition of AAPH led to similar breakage of supercoiled DNA obtained from the Fenton reaction (**Figure 2**). The addition of both DFE EAF and WF protected against supercoiled DNA nicking, however, to different extents. For example, the EAF of dandelion extracts exhibited a higher protection ($p < 0.05$) on DNA against nicking than the WF at the same concentration (**Table 3**). In comparison, 2 μg/mL Trolox protected 92.6 ± 2.6% ($p < 0.001$) and 4.5 μg/mL luteolin 7-glucoside protected 66.1 ± 4.9% of supercoiled DNA strand from nicking.

Effect of Dandelion Fractions on the Oxidation of Phosphatidylcholine Liposome. Peroxyl radical generated by thermal decomposition of AAPH also led to a typical lipid peroxidation in a soybean phosphatidylcholine-based liposome model, with both clearly defined oxidation initiation and propagation phases (**Figure 3**). The enhanced formation of conjugated dienes was suppressed by the addition of DFE. The addition of dandelion fractions led to prolonged lag phase durations and a lower rate of propagation (**Table 4**). The

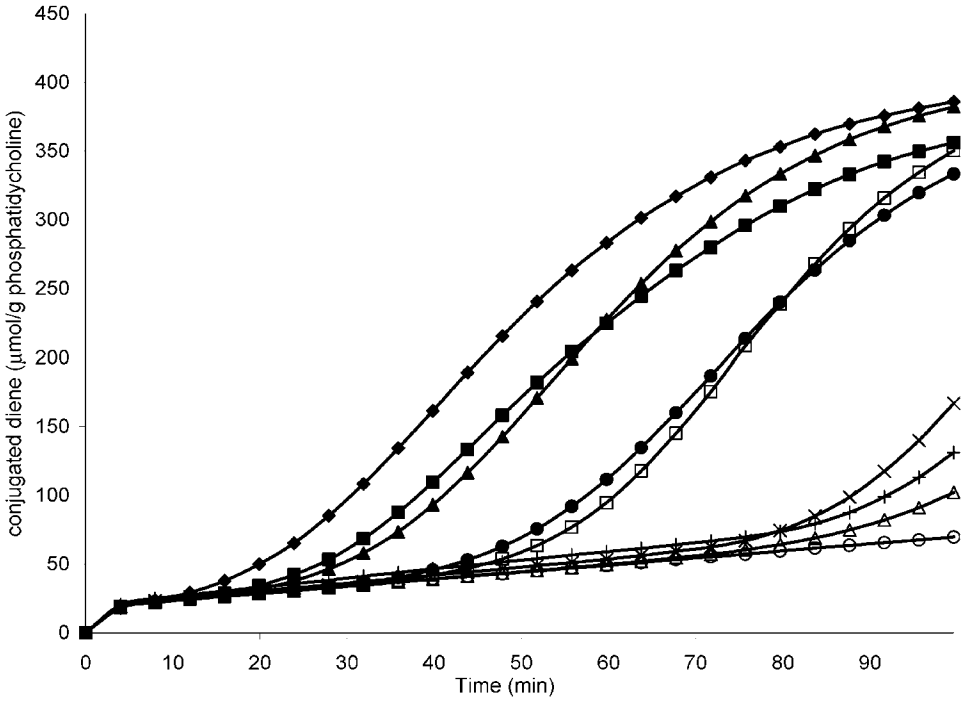

Figure 3. Antioxidant activities of EAF and WF of DFE in peroxyl radical-induced phosphatidylcholine liposome peroxidation at 37 °C: (◆) control; (■) 0.5 μg/mL WF; (▲) 1.0 μg/mL WF; (●) 2.5 μg/mL WF; (□) 0.5 μg/mL EAF; (△) 1 μg/mL EAF; (○) 2.5 μg/mL EAF; (×) 1 μg/mL Trolox; (+) 1 μg/mL luteolin 7-glucoside.

Table 4. Lag Phase Duration (Minutes) and Rate of Propagation (Micromoles of Conjugated Diene per Gram of Phosphatidycholine per Minute) of Phosphatidylcholine Liposome Oxidation Induced by Peroxyl Radical or Cupric Ion at 37 °C[a]

	peroxyl radical		Cu^{2+}	
	lag phase	rate of propagation	lag phase	rate of propagation
control	21.6 ± 2.6	0.021 ± 0.001	62.4 ± 7.9	0.007 ± 0.005
WF, 0.5 μg/mL	23.7 ± 2.2	0.020 ± 0.003	47.5 ± 4.9	0.014 ± 0.000
WF, 1 μg/mL	31.8 ± 2.8*	0.019 ± 0.001	39.4 ± 1.4*	0.015 ± 0.000*
WF, 2.5 μg/mL	47.3 ± 2.5***	0.017 ± 0.002	24.3 ± 2.1*	0.021 ± 0.002*
EAF, 0.5 μg/mL	51.1 ± 1.0***	0.019 ± 0.001	30.8 ± 3.8*	0.020 ± 0.002*
EAF, 1 μg/mL	80.0 ± 1.2***	0.006 ± 0.001***	28.9 ± 1.2*	0.023 ± 0.001*
EAF, 2.5 μg/mL	>100***	NA	43.4 ± 1.5	0.025 ± 0.002**
Trolox, 0.1 μg/mL	22.9 ± 0.2	0.019 ± 0.000	43.9 ± 1.4	0.026 ± 0.002**
Trolox, 1 μg/mL	84.7 ± 2.8**	0.018 ± 0.005	20.7 ± 0.2*	0.042 ± 0.00
luteolin 7-glucoside, 0.1 μg/mL	23.5 ± 0.2	0.022 ± 0.001	56.3 ± 1.5	0.003 ± 0.001
luteolin 7-glucoside, 1 μg/mL	86.0 ± 0.7***	0.012 ± 0.000***	48.9 ± 0.2	0.004 ± 0.000

[a] Values represent mean ± SD of three measurements *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ versus control within the same column. NA, not applicable.

protective effect of EAF of DFE was more significant than that of WF ($p < 0.05$) at the same concentration, reflecting the hydrophobic character of EAF possibly affiliated with the lipid fraction of liposome after the water-soluble peroxyl radical initiated the free radical chain reaction. Trolox and luteolin 7-glucoside also exhibited similar affinities to prevent peroxyl radical-induced liposome peroxidation ($p < 0.01$) at 1 μg/mL, but not at 0.1 μg/mL.

The oxidation of liposome was also initiated by the addition of transition metal ion Cu^{2+} (**Table 4**). Contrary to the antioxidant activities observed in peroxyl radical-induced peroxidation, prooxidant activities were seen with the addition of dandelion fractions in the Cu^{2+}-dependent liposome peroxidation ($p < 0.05$) (**Figure 4**; **Table 4**). Similar prooxidant activities occurred with the addition of higher Trolox and luteolin 7-glucoside concentrations as evidenced by shorter lag phase and greater propagation rate (**Table 4**).

The relative affinity of dandelion fractions to reduce the cupric ion was investigated, and a [Cu^{+}] time course is shown as **Figure 5**. When added to the mixture, the EAF from the dandelion flower exhibited a higher ($p < 0.05$) affinity to reduce Cu^{2+} to Cu^{+} than the WF at equivalent concentrations. The EAF gradually reduced >25% of the Cu^{2+} at a concentration of 2.5 μg/mL. The Cu^{2+} reduction by Trolox and luteolin 7-glucoside was also evident in this assay, suggesting that the reduction of Cu^{2+} by Trolox occurred more rapidly than that of luteolin 7-glucoside. The kinetic progress of luteolin 7-glucoside standard was similar to that of dandelion fractions.

Effect of Dandelion Flower Fractions on Preventing LDL Oxidation Induced by Cu^{2+}. For the $CuSO_4$-induced hLDL oxidation assay, only the high concentration of dandelion EAF prevented both the formation of conjugated dienes and the negative charge of hLDL (**Figure 6**). The hydrophilic fraction (WF) of DFE had no antioxidant activity in this metal-induced oxidation of hLDL, but a concentration-dependent prooxidant activity was shown to result from the transition metal-induced hLDL oxidation (**Figure 6A**).

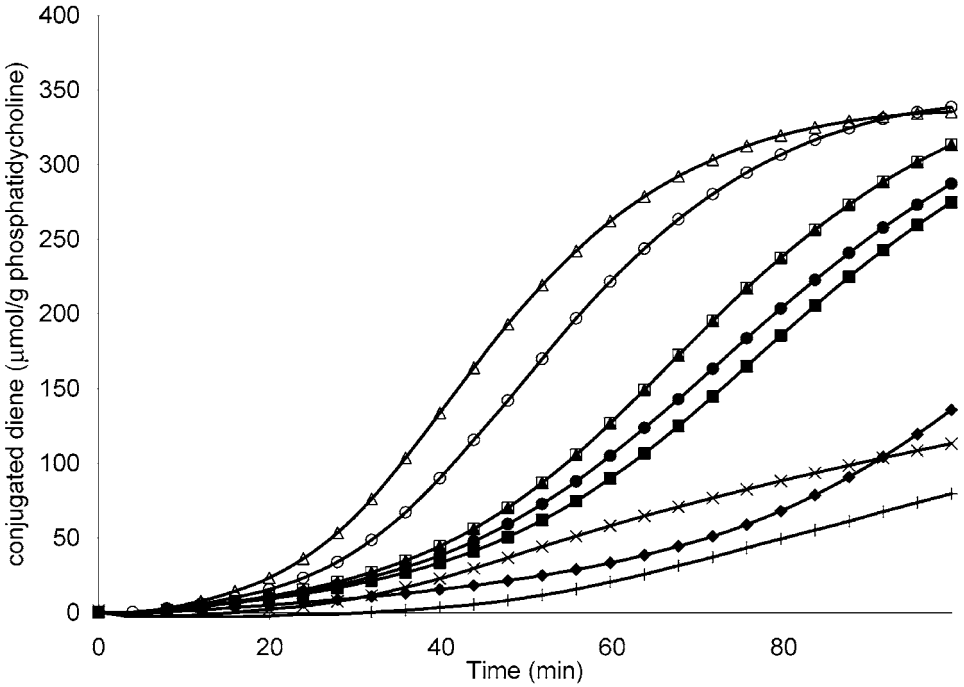

Figure 4. Effect of EAF and WF of DFE on the oxidation of $CuSO_4$-induced phosphatidylcholine liposome peroxidation at 37 °C: (◆) control; (■) 0.5 μg/mL WF; (▲) 1.0 μg/mL WF; (●) 2.5 μg/mL WF; (□) 0.5 μg/mL EAF; (△) 1 μg/mL EAF; (○) 2.5 μg/mL EAF; (×) 1 μg/mL Trolox; (+) 1 μg/mL luteolin 7-glucoside.

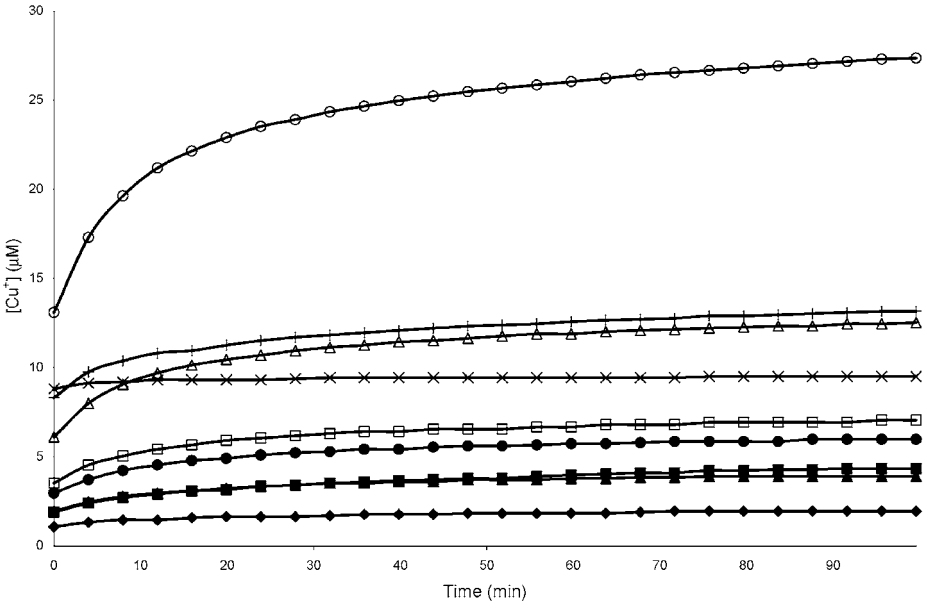

Figure 5. [Cu^+] time course of cupric ion incubated with neocoprione and different concentrations of EAF and WF of DFE at 37 °C: (◆) control; (■) 0.5 μg/mL WF; (▲) 1.0 μg/mL WF; (●) 2.5 μg/mL WF; (□) 0.5 μg/mL EAF; (△) 1 μg/mL EAF; (○) 2.5 μg/mL EAF; (×) 1 μg/mL Trolox; (+) 1 μg/mL luteolin 7-glucoside.

Cytotoxicity Test. Cell viability of Caco-2 cells was significantly ($p < 0.05$) reduced with the presence of WF and EAF at a relatively high concentration of 0.1 mg/mL. The relative LD_{50} values of luteolin (0.002 mg/mL) and luteolin 7-glucoside (0.025 mg/mL) standard reflect the greater cytotoxicity of the WF (0.3 mg/mL) as compared with the EAF (0.25 mg/mL) (**Figure 7**)

HPLC Profile. Our HPLC profile shows the presence of luteolin 7-glucoside, luteolin, caffeic acid, and chlorogenic acid (**Figure 8**). The concentrations of these specific phenolic compounds were found to be relatively higher in EAF than in WF (**Table 5**). More specific was the apparent absence of luteolin in the WF. These differences in plant phenolics in WF and EAF could contribute to the relatively higher antioxidant activity, free radical scavenging capacity, and cytotoxicity of the EAF.

DISCUSSION

In the present study, dandelion flower was shown to contain two known bioactive plant phenolics, caffeic acid and chlorogenic acid (*23*), and the flavones luteolin and luteolin 7-glucoside. Of these detected compounds, luteolin 7-glucoside was the most abundant compound in both the hydrophilic (WF) and

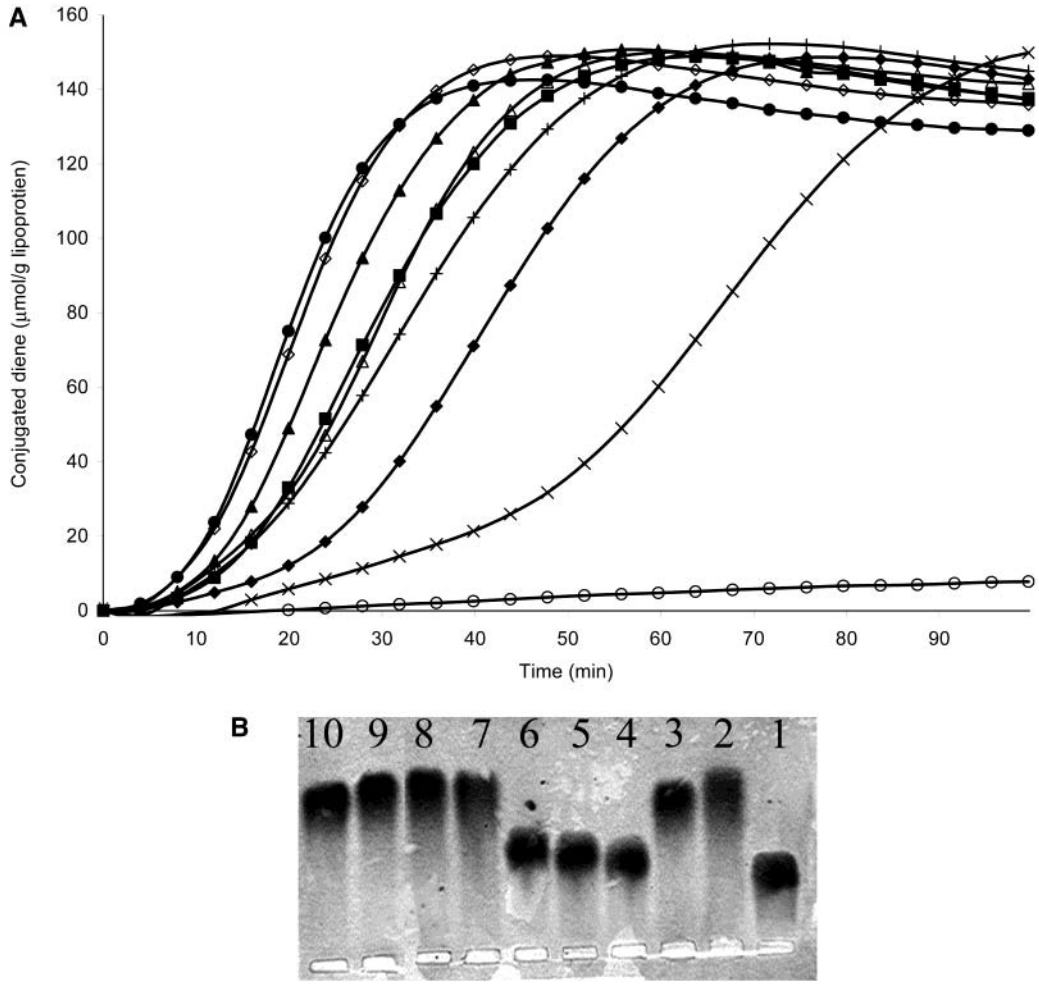

Figure 6. (A) Formation of conjugated dienes in $CuSO_4$-induced LDL (0.1 mg of protein/mL) oxidation and the effects of EAF and WF of DFE: (◆) control; (■) 0.5 μg/mL WF; (▲) 1.0 μg/mL WF; (●) 2.5 μg/mL WF; (□) 0.5 μg/mL EAF; (△) 1 μg/mL EAF; (○) 2.5 μg/mL EAF; (×) 1 μg/mL Trolox; (+) 1 μg/mL luteolin 7-glucoside. (B) Electrophoretic mobility of oxidative LDL (0.5 mg/mL of protein) initiated by $CuSO_4$ and the effects of EAF and WF of DFE: (lane1) LDL; (lane 2) LDL + 10 μM $CuSO_4$; (lane 3) LDL + 25 μg/mL EAF + 10 μM $CuSO_4$; (lane 4) LDL + 50 μg/mL EAF + 10 μM $CuSO_4$; (lane 5) LDL + 100 μg/mL EAF + 10 μM $CuSO_4$; (lane 6) LDL + 250 μg/mL EAF + 10 μM $CuSO_4$; (lane 7) LDL + 25 μg/mL WF + 10 μM $CuSO_4$; (lane 8) LDL + 50 μg/mL WF + 10 μM $CuSO_4$; (lane 9) LDL + 100 μg/mL WF + 10 μM $CuSO_4$; (lane10) LDL + 250 μg/mL luteolin 7-glucoside + 10 μM $CuSO_4$.

hydrophobic (EAF) fractions. Furthermore, luteolin was not detected in the dandelion flower WF, reflecting relatively lower hydrophilic character as compared to its glycoside.

Composition standardization of both the EAF and WF of DFE prior to assessing antioxidant activities facilitated the interpretation of data, showing relative differences in free radical scavenging activity of different dandelion flower fractions in different in vitro model systems. For example, both EAF and WF of DFE exhibited affinities to scavenge peroxyl radical in both the liposome peroxidation and DNA scission assays. Although generation of peroxyl radicals precedes lipid oxidation, it is also common that carbon-centered radicals are produced when a hydrogen atom is abstracted by a hydroxyl radical. This in turn will lead to the generation of a peroxyl radical and, on reaction with O_2, will initiate a major chain-propagating step in lipid peroxidation (*7*). In this study, peroxyl radical was generated through thermolysis of AAPH at 37 °C (*24*), and efforts were made to evaluate the affinity of DFE to prevent peroxyl radical-induced damage in both lipophilic (i.e., soybean phosphatidylcholine-constructed liposome oxidation) and hydrophilic (i.e., DNA nicking tests) model systems. In both instances, the fractionated EAF and WF constituents of dandelion flower at a concentration of 1 μg/mL dramatically increased (e.g., 2.7 and 0.5 times, respectively) the duration of the lag phase that characterizes liposome peroxidation. This result indicated that the DFE exhibited effective chain-breaking antioxidant activity toward the free radical chain reaction, reported earlier for many polyphenolic flavonols (*9*). Moreover, our conclusion that dandelion flower constituents can be categorized as scavengers of aqueous phase radicals and a chain-breaking antioxidant is confirmed by the observation that the presence of dandelion fractions effectively quenched the DPPH radical.

Oxidants elicit a wide variety of DNA damage that is relevant to strand breakage, sister chromatid exchange, and DNA−DNA and DNA−protein cross-links in addition to base modifications (*25*). The net result of these changes can eventually lead to mutagenesis and carcinogenesis (*26*). In both hydroxyl radical- and peroxyl radical-induced DNA supercoiled breakage, dandelion fractions elicited different degrees of protection against free radical-induced DNA damage. It was interesting to note that a major flavone glycoside of dandelion flower, namely, luteolin 7-glucoside, provided a protective effect against hydroxyl radical-induced DNA scission. Luteolin 7-glucoside also effectively retarded the peroxyl radical-induced liposome peroxidation, demonstrating that luteolin 7-glucoside in particular

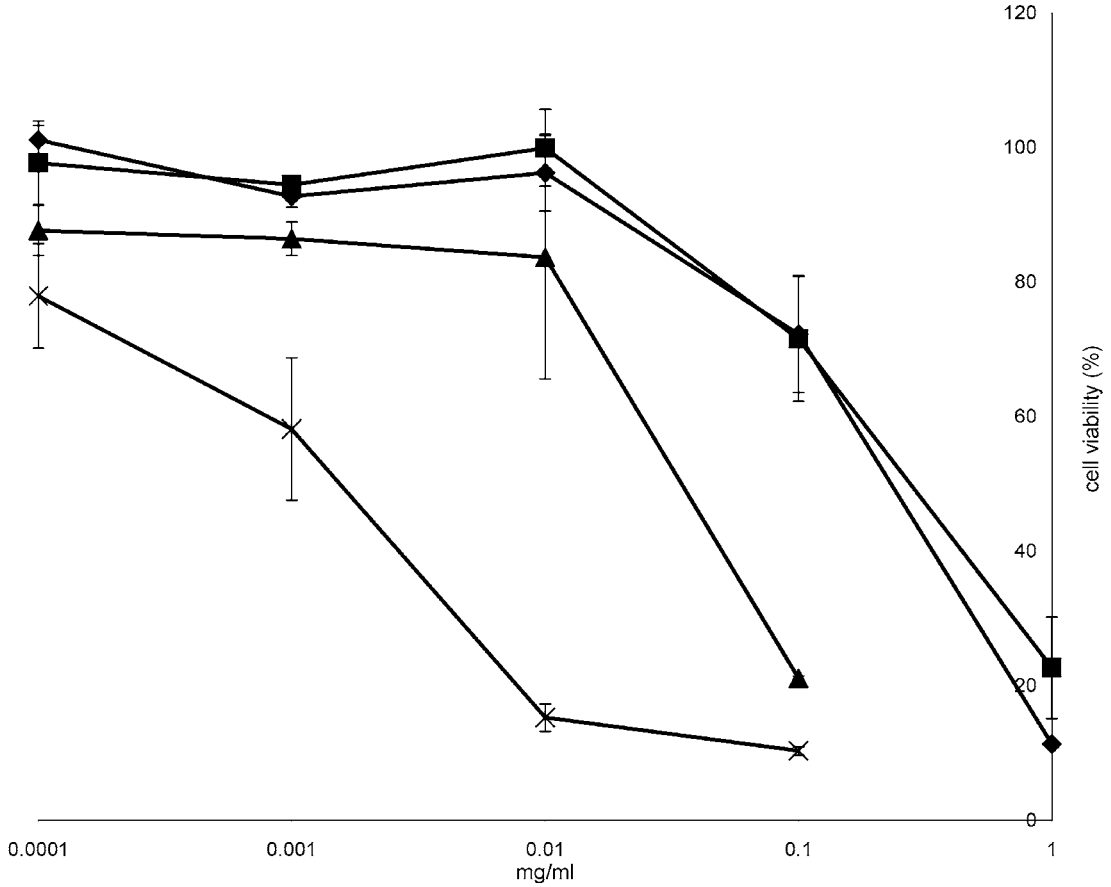

Figure 7. Cytotoxicity of dandelion flower fraction, luteolin, and luteolin 7-glucoside in human intestine cell line Caco-2 cell: (◆) EAF; (■) WF; (▲) luteolin 7-glucoside; (×) luteolin.

is a primary antioxidant component of dandelion flower. The underlying mechanisms for antioxidant activity of different flavonoids have been well characterized in various model systems (*1*, *2*, *27*, *28*). It is known that the formation of semiquinone intermediates occurs in a univalent redox step during the scavenging of free radicals by flavonoids (*29*). The univalent one-electron redox potential of flavonoids (including luteolin) with a 4′-hydroxyl group has been studied by pulse radiolysis, and results have indicated that the 4′-hydroxyl group is a primary site for generating semiquinones (*30*). A similar situation likely exists with luteolin 7-glucoside preventing peroxyl radical-induced peroxidation of liposome, which preceded the affinity of this constituent to neutralize the active radical and consequently break the free radical chain reaction.

There is strong evidence to show that oxidative modification of human low-density lipoprotein is associated with atherosclerosis (*6*, *31*), because the down-regulatory pathway for modified hLDL is different from that of native LDL and oxidative modified hLDL deposits in the vessel endothelia. Previous studies have demonstrated that α-tocopherol has the capacity to reduce hLDL oxidation (*6*). Herbal and food extracts, which include anthocyanins from grape and products (*12*, *13*) and tea catechins (*9*), also elicit a protective effect against hLDL oxidation in vitro. In the present study, we demonstrated the dandelion flower EAF suppressed the enhanced negative charge on hLDL that characterizes an oxidatively modified lipoprotein induced by the presence of cupric ion or peroxyl radical. This fraction also extended the lag phase of both the phosphatidylcholine liposome oxidation that was initiated by peroxyl radical generator AAPH. The significance of this finding indicated that the hydrophobic fractions of the DFE contributed greatly to the free radical scavenging activity.

It is important to acknowledge in this study the prooxidant activity observed for both fractions of the DFE in the oxidation mediated by the Cu^{2+} ion. This finding was not entirely unexpected because many natural antioxidants (e.g., many flavonoids and ascorbic acid) that can reduce the Cu^{2+} ion to the Fenton reaction favorable form Cu^{+} have prooxidant activity (*32*, *33*). In both cases, ROS such as hydroxyl radical and hydrogen peroxide were generated during the transformation of Cu^{+} to Cu^{2+} and the presence of molecular oxygen in which oxidation is promoted (*34*). The formation of a complex between neocuproine and Cu^{+} when dandelion fractions were added confirmed that the possibility of prooxidant activity of dandelion fractions was associated with the reduction of Cu^{2+}. In addition, this Cu^{2+}-reduction-dependent prooxidant was dependent on concentration. The actual physiological significance of prooxidant activity displayed by many antioxidants in vitro is, however, uncertain, because transition metal ions in vivo are more likely to be bound to protein instead of existing in a free state, which is required for prooxidant activity (*5*).

The prooxidant activity observed in the present study with the addition of cupric ion undoubtedly reflected the cupric ion reducing activity of dandelion. It is therefore of interest that higher concentrations of EAF actually protected against Cu^{2+}-induced hLDL oxidation, as evidenced by the negative charge on hLDL. This result is similar to previous studies with ascorbic acid that have shown the free radical scavenging activity will

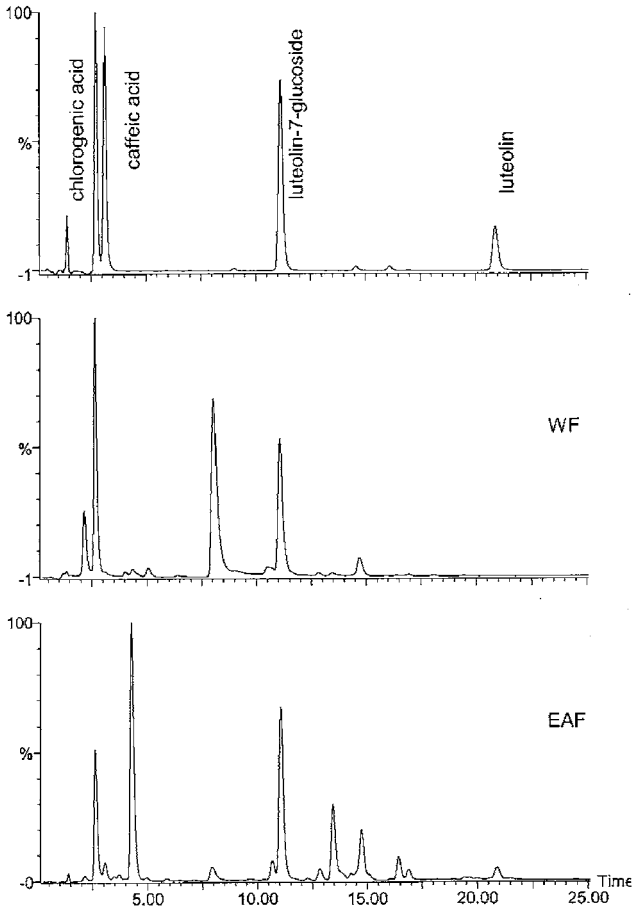

Figure 8. HPLC profile of DFE.

Table 5. Concentration of Identified Phenolic Acid and Flavones (Micrograms per Milligram) in Water Fraction and Ethyl Acetate Fraction of Dandelion Flower Extract[a]

	WF	EAF
chlorogenic acid	35.75 ± 0.05	47.62 ± 0.19
caffeic acid	0.85 ± 0.09	7.26 ± 0.03
luteolin 7-glucoside	26.55 ± 1.11	83.25 ± 0.16
luteolin		25.20 ± 0.56

[a] Values represent mean ± SD of three determinations.

exceed prooxidant activity originated from reducing power when the concentration of antioxidant constituents reaches a critical concentration (*35*). The formation of Cu^{+} from Cu^{2+} by the trace amount of lipid peroxide present in hLDL (*31*) to trigger the hLDL oxidation reaction was catalyzed in our study by the reducing activity of dandelion flower constituent, resulting in the free radical chain reaction of hLDL oxidation. On the other hand, free radical quenching activity of dandelion was observed at higher concentrations of dandelion flower EAF that suppressed the Cu^{2+}-induced oxidation of hLDL. An explanation for this observation could involve the metal chelating power of dandelion flavonoids, which at a critical concentration act to suppress metal ion-induced hLDL oxidation by sequestering the metal ion that otherwise interacts with the hLDL particle. Numerous phenolic components have been characterized to provide chain-breaking antioxidant activity (*9*, *36*) as well as transition metal chelation activity (*2*). For example, the di-*o*-hydroxyl group of the B-ring provides luteolin 7-glucoside (5,3′,4′-trihydroxyflavone-7-glucoside) with a higher stability of flavone radical intermediates during the process of quenching free radicals (*1*). We propose herein that the different antioxidant activities observed between hydrophobic and hydrophilic fractions of dandelion flower prevented Cu^{2+}-induced hLDL oxidation in the present study. Moreover, the fact that greater protection was observed with the more lipophilic EAF derived from dandelion flower, compared to the WF, confirms that an important characteristic for eliciting this response involved greater localization of EAF constituents with the membrane surface. Establishing contact with free radicals in this phase would provide scavenging activity to aqueous free radicals that may lead to a reduction in consumption of endogenous α-tocopherol, which is an important chain-breaking lipid peroxyl radical quencher located within hLDL (*37*).

Although in vitro data have provided evidence for the antioxidant activity of natural plant extracts in biological macromolecules, in many cases in vitro and in vivo data do not provide a consistent conclusion as to the significance of antioxidant properties. For example, tea polyphenols show the enhanced resistance of hLDL to Cu^{2+}-induced peroxidation (*38*), but consumption of green or black tea (6 cups/day for 4 weeks) had no such effect ex vitro for smokers (*39*) and healthy subjects (600 mL/day black tea for 4 weeks) (*40*). On the other hand, consumption of green tea decreases the DNA oxidative marker (8-OHdG in white cell and urine) for both smokers and nonsmokers (*41*). Epidemiological evidence also exists that higher level consumption of flavonoids from vegetables, fruits, tea, and wine will result in a lower risk of stroke (*42*). In the present study, we also evaluated the bioactive properties of DFE on cytotoxic response in cultured Caco-2 intestinal cells. The antioxidant patterns observed in the various chemical model systems for the most part agree with the cell culture data. For example, our results showed that both the EAF and WF from dandelion flower exhibited cytotoxicity of Caco-2 cells. This activity, however, was markedly weaker than that displayed by luteolin and luteolin 7-glucoside. Luteolin was relatively more effective at eliciting cytotoxicity than the glycoslyated form luteolin 7-glucoside. These findings can also be explained in part by the structure−function aspects of luteolin. For example, the *o*-dihydroxy structure on the B-ring of this flavonoid is important for protecting endothelial cells against linoleic acid hydroperoxide-induced toxicity (*43*). Other papers have also indicated that 5,7-dihydroxyflavones, such as luteolin, and chrysin induced UGT1A1 and SYP1A1 in hepatic cell lines of HepG2, suggesting that the induction of flavonoid with this structural feature provides a unique non-aryl hydrocarbon receptor-mediated mechanism (*44*). The implication of elevated expression of UGT1A2 involves the increased glucuronidation activity of colon carcinogen *N*-hyroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and the protection against carcinogenesis (*45*).

In conclusion, our results show for the first time that dandelion flower fractions possess both antioxidant and cytotoxic properties which can in part be attributed to the presence of luteolin and luteolin 7-glucoside. Although the hydrophobic fraction of DFE displayed a greater affinity than the hydrophilic counterpart in scavenging stable free radical and preventing water-soluble active radical-induced liposome and DNA oxidation, a higher potential of prooxidant activity was also identified due to reducing the transition metal ion. From these results, we concluded that dandelion flower represents a potentially valuable source of natural antioxidant and bioactive material. Further work for establishing the same conclusion from in vivo studies is warranted.

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