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# Variations in Free Radical Scavenging Capacity and Antiproliferative Activity Among Different Genotypes of Autumn Olive (*Elaeagnus umbellata*)

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Original Paper

**Abstract**

Fruit from six genotypes of autumn olive (*Elaeagnus umbellata* Thunb.) ('Brilliant Rose', 'Delightful', 'Jewel', Natural 1, Natural 2, and 'Sweet Tart') were evaluated for antioxidant capacity and anti-cancer properties. Based on data from electron spin resonance (ESR) measurements, autumn olive contained potent free radical scavenging activities for hydroxyl ($^{\cdot}OH$) and superoxide ($O_2^{\cdot -}$) radicals. Among the six genotypes, 'Brilliant Rose' and 'Jewel' had the highest levels of antioxidant activity. Pretreatment of JG6 $P^+$ mouse epidermal cells with autumn olive extracts inhibited the activation of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB) induced by either 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or ultraviolet-B (UVB). Extracts of all autumn olive genotypes inhibited proliferation of human leukemia HL-60 cancer cells and human lung epithelial cancer A549 cells and induced apoptosis of HL-60 cells. In particular, 'Brilliant Rose' and 'Jewel' had relatively potent activities compared to other genotypes. These results indicate that consuming autumn olive fruit may be beneficial to human health, although further studies are needed for confirmation.

**Key words**
Autumn olive · antioxidant capacity · antiproliferation · apoptotic cancer cells · Oleaster (Elaeagnaceae) · *Elaeagnus umbellata* Thunb.



## Introduction

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [1]. Fruits contain numerous phytonutrients which are good sources of antioxidants [2], [3]. The phytochemicals in fruits responsible for antioxidant activity are largely phenolic compounds such as anthocyanins, carotenoids, and other flavonoid compounds. These compounds may act independently or in combination as anticancer or cardioprotective agents by a variety of mechanisms. Autumn olive (*Elaeagnus umbellata* Thunb.) is native to Southern Europe and Central Asia and was introduced to the USA in the 1830's from East Asia as an ornamental plant [4]. Autumn olives produce an abundance of small deep-red colored, sweet-tart fruit which are considered suitable for human consumption. Autumn olive fruit can be used for preserves, condiments, fruit rolls, juice, flavoring, and other food products [5].

Fordham et al. [5] showed that autumn olive fruit contains carotenoids such as lycopene, α-cryptoxanthin, β-cryptoxanthin, β-carotene, lutein, phytoene, and phytofluene. The lycopene concentrations of red autumn olive fruit are considerably higher than those of fresh tomato fruit, and are similar to that of tomato paste [5]. The antioxidant activity of carotenoids enables them to act as quenchers for singlet oxygen ($^1O_2$) [6] and peroxyl radicals [7]. β-Carotene is a powerful quencher for singlet oxygen ($^1O_2$) and a low concentration is effective to protect membrane lipids

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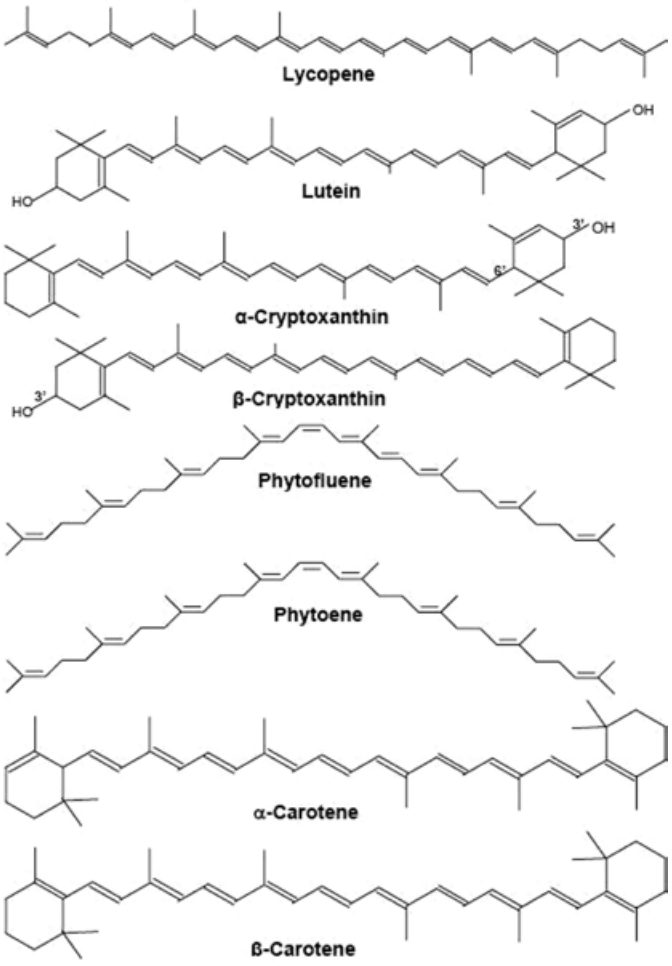


Fig. **1** The chemical structures of major carotenoids in autumn olive fruit.

from peroxidation reactions of $^1O_2$ radicals [8], [9]. β-Carotene has also shown a capacity to scavenge $O_2^{x-}$ [10], [11]. Lutein, the dihydroxy form of α-carotene, is more readily available in the human body than β-carotene [12]. Epidemiological studies indicate that people with a high intake of all three, α-carotene, β-carotene, and lutein, have a low risk of lung cancer [12]. β-Cryptoxanthin has been shown to suppress skin tumor promotion in mice [13]. Phytoene has been proven to suppress tumorigenesis in the skin [14] and the antioxidant activity of phytoene may play an important role in its action mechanism. Lycopene has been shown to have an exceptionally high singlet oxygen quenching ability [15]. Epidemiological studies have shown that the increased consumption of foods rich in carotenoids is correlated with a diminished risk of several diseases [16], [17], [18].

However, there has been little research conducted on autumn olive fruit, and thus minimal information is available on its health benefits. Activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB) are transcription factors associated with carcinogenesis [19]. Many stimuli induce the binding of AP-1 to the promoter region of various genes that govern cellular processes such as inflammation, proliferation, and apoptosis [20]. Inhibition of AP-1 activity has been shown to lead to suppression of cell transformation [21]. NF-κB is also an important regulator in deciding cell fate, such as programmed cell death and proliferation control, and is critical in tumorigenesis [20]. Reactive oxygen species (ROS) stimulate transcription by activating transcription factors such as AP-1 and NF-κB. AP-1 and NF-κB signal transduction pathways are important in neoplastic transformation and tumor promotion [19].

The purpose of this study is to evaluate six genotypes of autumn olive berry ('Brilliant Rose', 'Delightful', 'Jewel', Natural 1, Natural 2, and 'Sweet Tart') with regards to antioxidant capacity, and in-

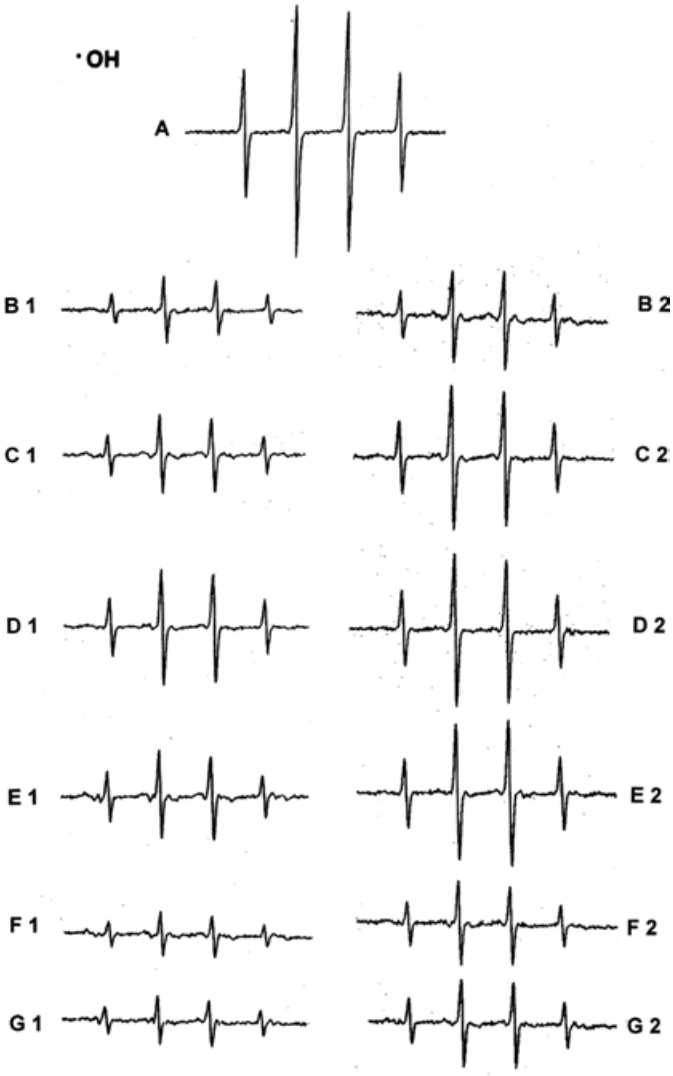


Fig. **2** The effectiveness of autumn olive extracts as ·OH radical scavengers. The scavenging effects of six different genotypes of autumn olive fruit extracts on the ·OH radical is shown. ESR spectra were recorded 3 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 10 mM DMPO and the following reactants: (A) 1.0 mM $FeSO_4$ and 1.0 mM $H_2O_2$; (B) 1.0 mM $FeSO_4$, 1.0 mM $H_2O_2$, and 5 mg/mL (B1) or 1 mg/mL (B2) 'Sweet N Tart' fruit extract; (C) 1 mM $FeSO_4$, 1 mM $H_2O_2$ and 5 mg/mL (C1) or 1 mg/mL (C2) Natural 2 fruit extract; (D) 1 mM $FeSO_4$, 1 mM $H_2O_2$ and 5 mg/mL (D1) or 1 mg/mL (D2) 'Delightful' fruit extract; (E) 1 mM $FeSO_4$, 1 mM $H_2O_2$ and 5 mg/mL (E1) or 1 mg/mL (E2) Natural 1 fruit extract; (F) 1 mM $FeSO_4$, 1 mM $H_2O_2$ and 5 mg/mL (F1) or 1 mg/mL (F2) 'Brilliant Rose' autumn olive extract; (G) 1 mM $FeSO_4$, 1 mM $H_2O_2$ and 5 mg/mL (G1) or 1 mg/mL (G2) 'Jewel' fruit olive extract. The ESR spectrometer settings were: receiver gain, $2.52 \times 10^4$; time constant, 20 ms; modulation amplitude, 0.5 G; scan time, 60 s; and magnetic field, 3486 ± 100 G.

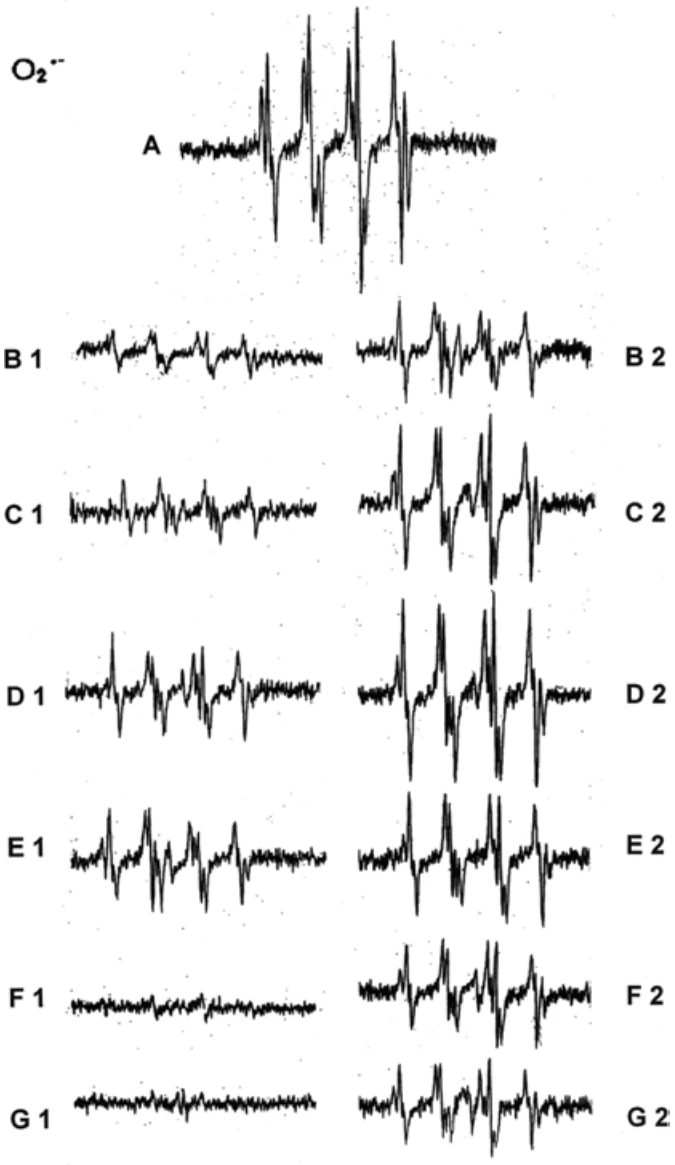


Fig. **3** The effectiveness of autumn olive extracts as $O_2^{\cdot-}$ radical scavengers. The scavenging effect of six different genotypes of autumn olive fruit extracts on the $O_2^{\cdot-}$ radical is shown. ESR spectra were recorded 1 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (A) 3.5 mM xanthine and 2 U/mL xanthine oxidase; (B) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 5 mg/mL (B1) or 1 mg/mL (B2) 'Sweet N Tart' fruit extract. (C) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 50 mg/mL (C1) or 10 mg/mL (C2) Natural 2 fruit extract. (D) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 50 mg/mL (D1) or 10 mg/mL (D2) 'Delightful' fruit extract. (E) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 50 mg/mL (E1) or 10 mg/mL (E2) Natural 1 fruit extract. (F) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 50 mg/mL or 10 mg/mL 'Brilliant Rose' fruit extract. (G) 3.5 mM xanthine, 2 U/mL xanthine oxidase, and 50 mg/mL (G1) or 10 mg/mL (G2) 'Jewel' fruit extract. The ESR spectrometer settings were: receiver gain, $2.52 \times 10^4$; time constant, 20 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3486 ± 100 G.

hibitory effects on AP-1 and NF-κB activation induced by either 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or ultraviolet-B (UVB). TPA and UVB are carcinogens and can produce ROS and stimulate AP-1 and NF-κB activity [22], [23]. The potential therapeutic activity of autumn olive fruit extracts on inhibition of cell proliferation and induction of apoptosis in human cancer cells was also investigated.

## Materials and Methods

### Cell lines and reagents

The JB6 $P^+$ mouse epidermal cell line, stably transfected with AP-1-luciferase or NF-κB-luciferase reporter plasmid (JB6/AP/κB) [24], was cultured in Eagle's MEM (EMEM) containing 5% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin-streptomycin. Human lung carcinoma A549 and human leukemia HL-60 cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). Human lung carcinoma A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and human leukemia HL-60 cells were cultured in RMPI 1640 and all contained 10% FBS, 50 units/mL penicillin, and 50 μg/mL streptomycin in 75 $cm^2$ T-flasks at 37 °C, 85% humidity, and 5% $CO_2$ atmosphere.

EMEM and DMEM were obtained from Whittaker Biosciences (Walkersville, MD, USA). EDTA (ethylenediaminetetracetic acid, disodium salt, dehydrate, $Na_2EDTA2\,H_2O$), FBS, gentamicin, L-glutamine, and trypsin were purchased from Life Technologies, Inc. (Gaithersburg, MD, USA). Luciferase assay substrate was obtained from Promega (Madison, WI, USA). RMPI 1640, Chelex 100, $FeSO_4$, $H_2O_2$, xanthine, and xanthine oxidase were purchased from Sigma (St. Louis, MO, USA). β-Apo-8′-carotenal, *N*,*N*-diisopropylethylamine and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) were purchased from Aldrich (Milwaukee, WI, USA).

### Sample material

Fruit from genotypes of autumn olive berry ('Brilliant Rose', 'Delightful', 'Jewel', and 'Sweet Tart') were hand-harvested at a commercially mature stage from Hidden Spring Nursery (Cookeville, TN, USA). Natural 1 and Natural 2 were hand-harvested from the plants grown in rural area of Beltsville, Maryland. Approximately 800 g to 1 kg of fruit were harvested per genotype. The fruit was picked around 09 : 00 in the fall and shipped with dry ice overnight to the Beltsville, Maryland, frozen upon receipt and stored at –80 °C until used for chemical analyses. The voucher specimens for 'Brilliant Rose', 'Delightful', 'Jewel', and 'Sweet Tart' are CELA 23, CELA 21, CELA 20 and CELA 19, respectively. The voucher specimens are maintained by the National Germplasm Repository (Corvallis, OR, USA).

### Sample preparation for assay

For electron spin resonance (ESR) measurement of $^{\cdot}OH$ and $O_2^{\cdot-}$ and the assays of AP-1/NF-κB activation, cell proliferation and cell apoptosis, autumn olive fruit extracts were prepared by mixing 100 g of fruit tissue with 100 mL of distilled deionized $H_2O$ and blended at high speed. The blended homogenates were strained, centrifuged at 6000 × *g* at 4 °C for 20 min and the supernatants were filtered. The supernatants were transferred to vials, and stored at –80 °C until analysis.

### Extraction, isolation HPLC analysis

For HPLC analysis [5], triplicate samples of 5 g fruit were extracted with pre-cooled 10 mL/g tetrahydrofuran containing 0.05% butylated hydroxytoluene (BHT), 10% magnesium carbonate, and 10% celite using a Polytron homogenizer (Brinkmann Instru-

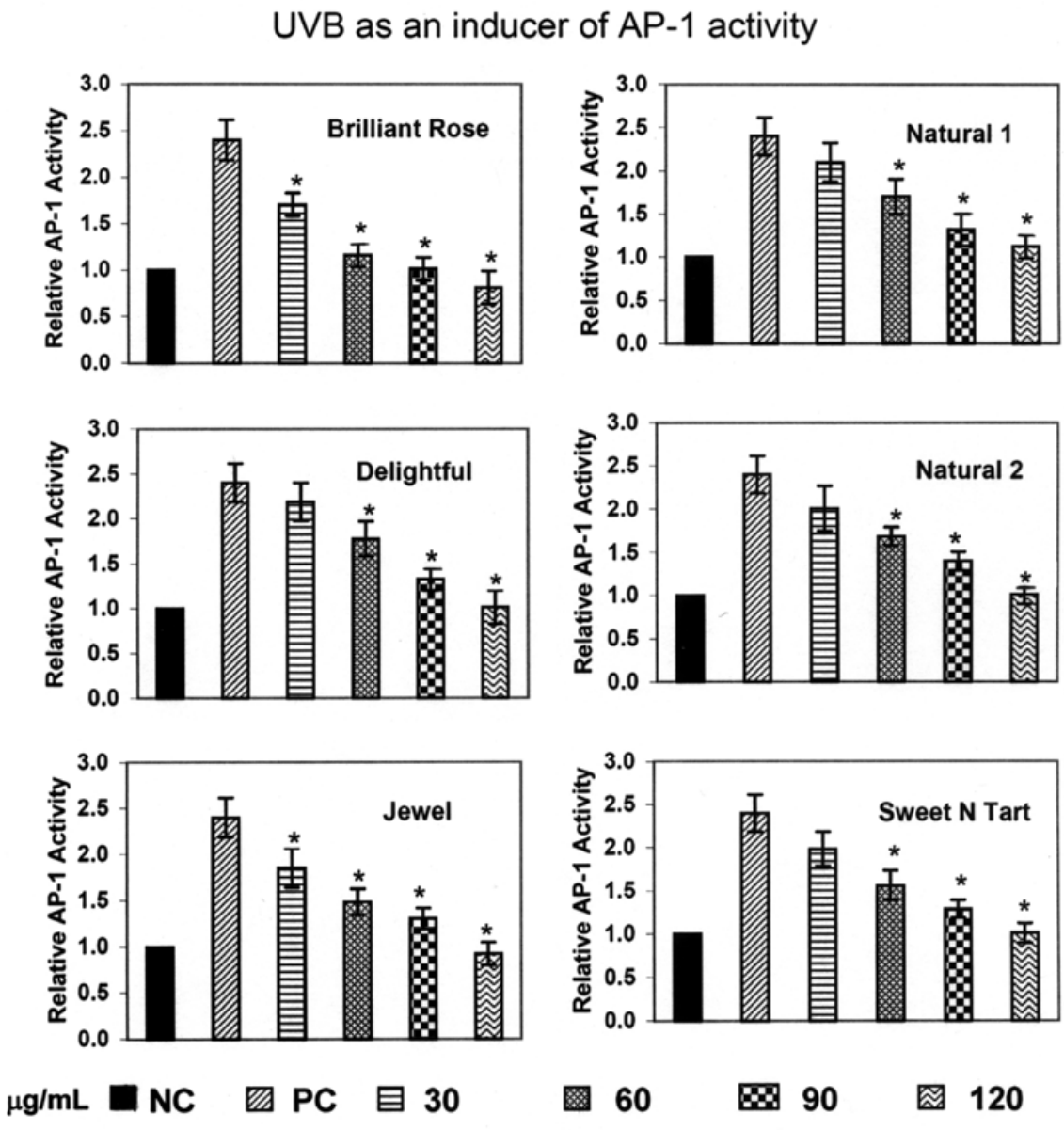


Fig. **4** **A** Autumn olive fruit extracts suppress UVB-induced AP-1 activity. JB6 $P^+$ mouse epidermal cells that were stably transfected with AP-1 luciferase reporter plasmid were cultured as described in the Materials and Methods. The cells were pretreated with or without various concentrations of autumn olive fruit extracts for 1 h, and then were exposed to UVB (4 kJ/$m^2$ ) and cultured for an additional 48 h. AP-1 activity was determined by luciferase assay. Results, presented as relative AP-1 induction compared to untreated control cells, were expressed as means and standard errors from three wells. The experiment was repeated three times. * Indicates significant inhibition of UVB-induced AP-1 activation by autumn olive extracts ($p \leq 0.05$).

ments, Inc.; Westbury, NY, USA) for one min. The mixture was filtered through Whatman No. 1 paper and the filtrates were combined and concentrated to 5 mL using a Buchler Evapomix (Fort Lee, NJ, USA) in a water bath at 30 °C. The concentrated samples were dissolved in 25 mL of methanol and partitioned into methylene chloride and saturated salt water; the organic layer was removed and dried over anhydrous sodium sulfate, filtered, and the volume reduced to near dryness. The concentrate was dissolved and the volume brought to 100 mL with methylene chloride containing 0.01 % BHT. The samples were diluted and 0.4 mL of $\beta$-apo-8′-carotenal was used as an internal standard and dried under nitrogen and dissolved in 0.4 mL of HPLC solvent (65 % acetonitrile, 25 % methylene chloride, 10 % methanol, 1 g/L BHT and 0.1 mL/L of *N*,*N*-diisopropylethylamine) and 50 $\mu$L were injected onto a revised $C_{18}$ column (Microsorb-MV, 250 mm × 4.6 mm, particle size 5 $\mu$m; Varian Analytical Instruments; Walnut Creek, CA, USA) with a guard column ($C_{18}$, 30 × 4.6 mm, particle size 5 $\mu$m) (Microsorb-MV; Varian Analytical Instruments). The samples were analyzed using a Waters (Waters Associated, Millipore; Milford, MA, USA) HPLC system equipped with two pumps (600 E system Controller). Carotenoids were eluated under isocratic conditions. The mobile phase was 65 % acetonitrile, 25 % methylene chloride, 10 % methanol, 1 g/L BHT and 0.1 mL/L of *N*,*N*-diisopropylethylamine. The flow rate was 0.8 mL/min. The carotenoids in fruit extracts were identified by their UV spectra recorded with a diode-array-detector, by chromatographic comparison with authentic markers and co-injection with authentic standards, and also verified using Standard Reference Material

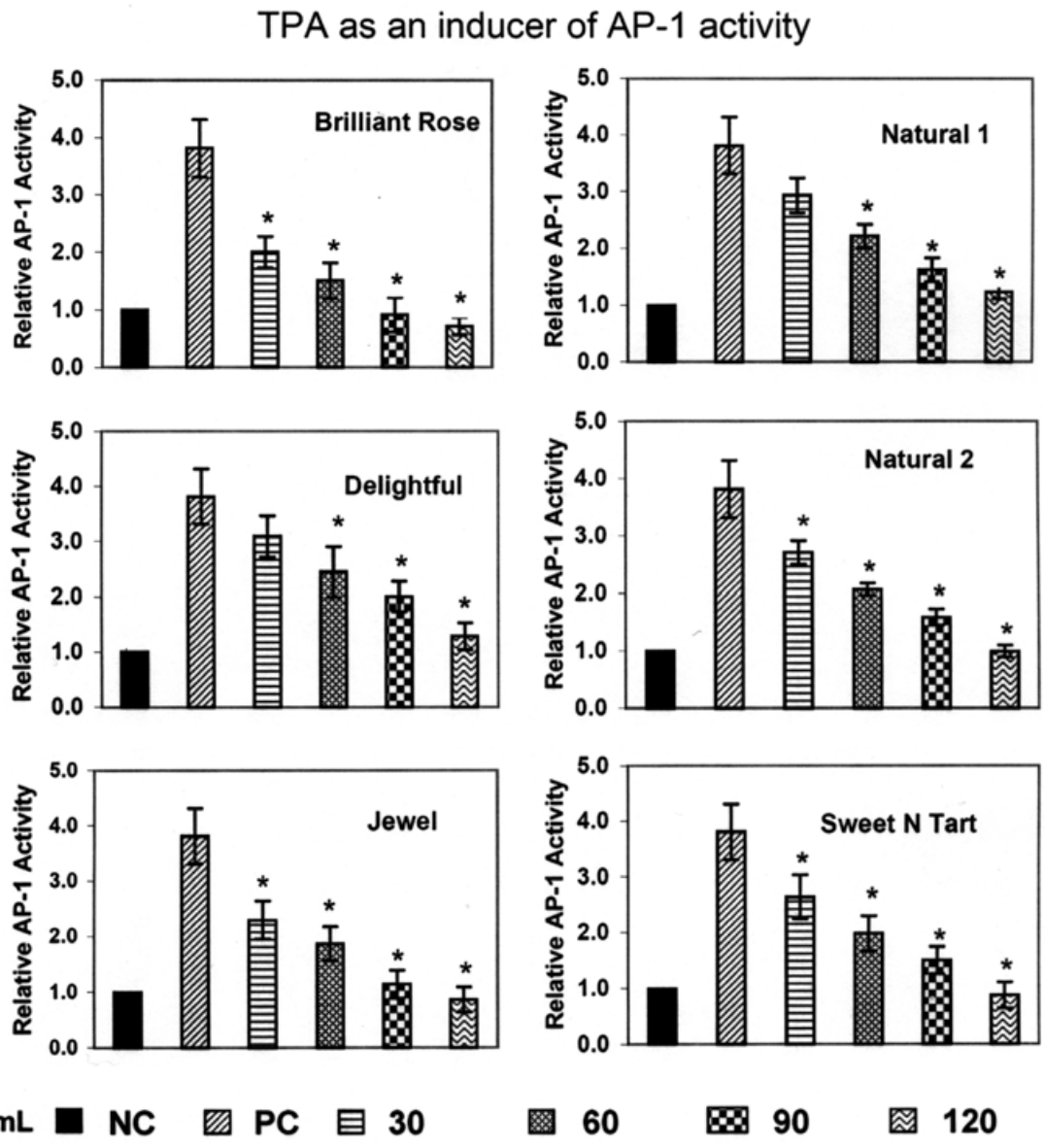


Fig. **4** **B** Autumn olive fruit extracts suppress TPA-induced AP-1 activity. JB6 $P^+$ mouse epidermal cells that were stably transfected with AP-1 luciferase reporter plasmid were cultured as described in the Materials and Methods. The cells were pretreated with or without various concentrations of autumn olive fruit extracts for 1 h, and then were exposed to TPA (20 ng/mL) and cultured for an additional 48 h. AP-1 activity was determined by luciferase assay. Results, presented as relative AP-1 induction compared to untreated control cells, were expressed as means and standard errors from three wells. The experiment was repeated three times. * Indicates significant inhibition of TPA-induced AP-1 activation by autumn olive extracts ($p \leq 0.05$).

968b (National Institute of Standards and Technology; Gaithersburg, MD, USA). Individual carotenoids were quantified by comparison with an external standard of carotenoids. Scanning between 300 and 450 nm was performed and data were collected by the Waters 990 3-D chromatography data system.

**Electron spin resonance (ESR) for measurement $^{\cdot}OH$ and $O_2^{\cdot -}$**

ESR measurements were conducted using a Varian E9 ESR spectrometer (Bruker Instruments; Billerica, MA, USA) and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using $K_3CrO_8$ and DPPH as reference standards. The EPRDAP 2.0 program was used for data acquisition and analysis [25]. The scavenging efficiency of $^{\cdot}OH$ or $O_2^{\cdot -}$ radicals by autumn olive extracts was measured by ESR using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) as the spin trap according to the procedures described previously [25]. Experiments were performed at room temperature and under ambient air.

**Cell culture and assay of AP-1 and NF-κB activities *in vitro***

JB6 $P^+$ mouse epidermal cells were cultured as described above and a confluent monolayer of cells was trypsinized, and $5 \times 10^4$ viable cells (suspended in 1 mL of EMEM supplemented with 5% FBS) were added to each well of a 24-well plate. Plates were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$ for twelve hours. After incubation, cells were cultured in EMEM supplemented with 0.5% FBS for 12 – 24 h to minimize basal AP-1 activity or NF-κB activity before treatment for 1 h with or without autumn olive fruit extracts at the concentrations indicated. The cells were then exposed to TPA (20 ng $mL^{-1}$) or UVB (4 kJ $m^{-2}$) ir-

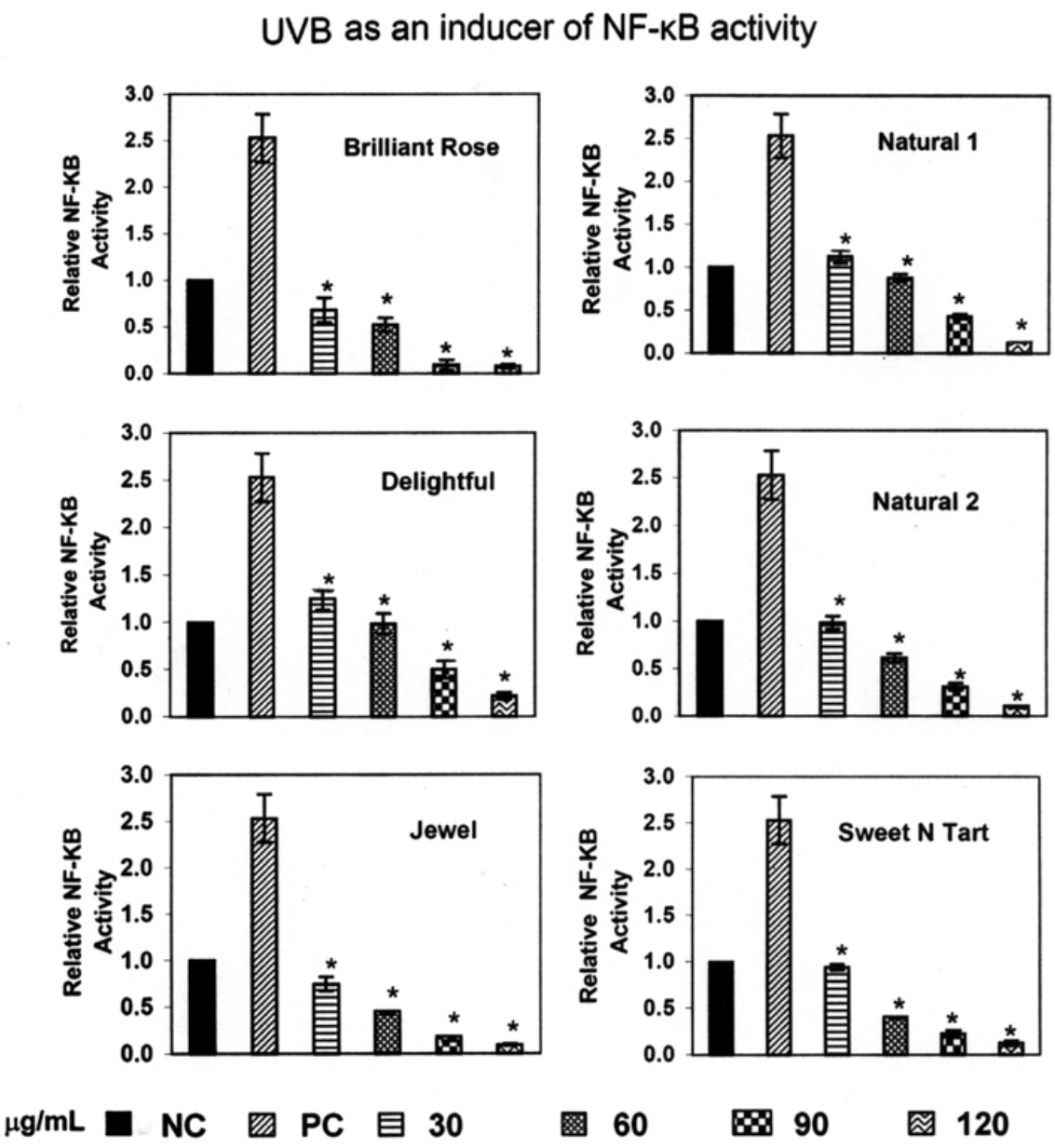


Fig. **5** **A** Autumn olive extracts suppress UVB-induced NF-κB activity. JB6 $P^+$ mouse epidermal cells that were stably transfected with NF-κB luciferase reporter plasmid were cultured as described in the Materials and Methods. The cells were pretreated with or without various concentrations of autumn olive fruit extracts for 1 h, and then were exposed to UVB (4 kJ/$m^2$ ) and cultured for an additional 48 h. NF-κB activity was determined by luciferase assay. Results, presented as relative NF-κB induction compared to untreated control cells, were expressed as means and standard errors from three wells. The experiment was repeated three times. * Indicates significant inhibition of UVB-induced NF-κB activation by autumn olive fruit extracts ($p \leq 0.05$).

radiation in the same medium for additional hours to monitor the effects on AP-1 or NF-κB activation. The cells were extracted with 200 μL of lysis buffer provided in the luciferase assay kit by the manufacturer. Luciferase activity was measured using a Monolight luminometer, model 3010. The results were expressed as relative AP-1 or NF-κB activity compared with untreated controls.

**Inhibition of proliferation of cancer cells**

Two cell lines, human lung cancer A549 cells, and human leukemia HL-60 cells, were cultured as described above. Subcultures were carried out every 2–3 days using a 0.25% trypsin and 0.02% EDTA solution. Briefly, A549 and HL-60 cells were plated in their growth medium at a density of $1 \times 10^4$ cells/well in 96-well flat-bottomed cell culture plates and incubated at 37 °C. Twenty-four hours after plating, various doses of autumn olive fruit extracts (30 to120 μg/mL) were added to each well (except for control wells). Following 48 h incubation, 10 μL MTT solution were added in each well to form formazan salt crystals and the plates were further incubated for 4 h. Then 100 μL solubilization solution (10% SDS in 0.01 M HCl) were added and the plate was incubated overnight at 37 °C. The amount of formazan produced was proportional to the number of viable cells [26]. After incubation, the MTT-formazan was solubilized in 2-propanol and the optical density was measured at a wavelength of 575 nm and a reference wavelength of 690 nm using a Microplate Spectrophotometer (Spectra MAX™ 250; Molecular Devices; Sunnyvale, CA, USA), where higher OD values indicated more cell proliferation.

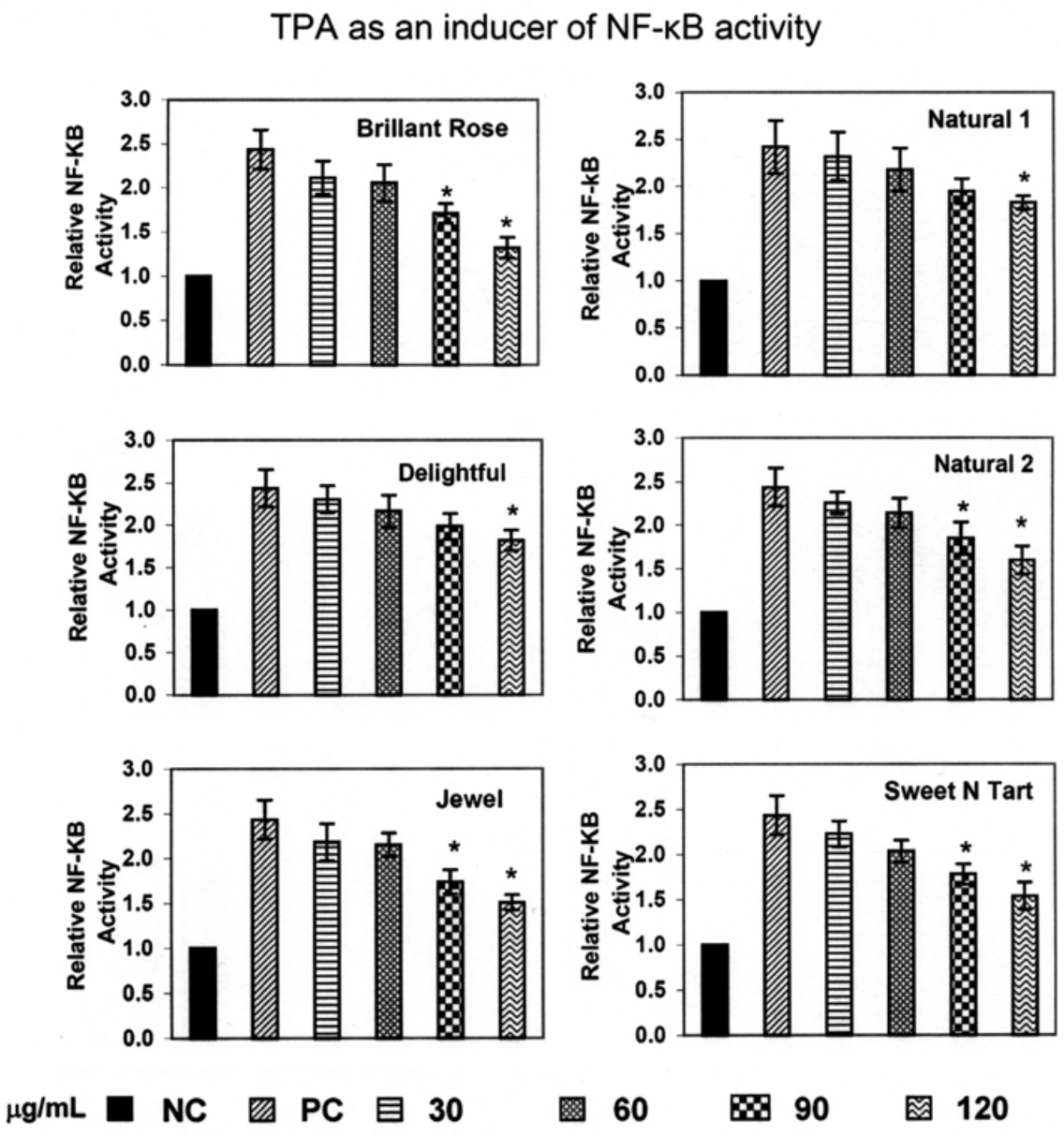


Fig. **5** **B** Autumn olive extracts suppress TPA-induced NF-κB activity. JB6 P+ mouse epidermal cells that were stably transfected with NF-κB luciferase reporter plasmid were cultured as described in the Materials and Methods. The cells were pretreated with or without various concentrations of autumn olive fruit extracts for 1 h, and then were exposed to TPA (20 ng/mL) and cultured for an additional 48 h. NF-κB activity was determined by luciferase assay. Results, presented as relative NF-κB induction compared to untreated control cells, were expressed as means and standard errors from three wells. The experiment was repeated three times. * Indicates significant inhibition of TPA induced NF-κB activation by autumn olive fruit extracts ($p \leq 0.05$).

Proliferation was expressed as a percentage of cell growth in wells that received no extract. Cyanidin 3-glucoside (50 μM) was used as a positive control. Data are expressed as mean ± S.E. of three samples.

**Induction of apoptosis in cancer cells**

The human leukemia HL-60 cell line and JB6 P+ mouse epidermal cell line were used in this study. To test whether autumn olive fruit extracts possessed any apoptotic induction on cancer cells, HL-60 cells and JB6 P+ were treated with autumn olive fruit extracts (30 to120 μg/mL) for 18 h. Cells were assessed for typical apoptotic morphology by staining with 10 μmol/L bisbenzimide Hoechst 33258 fluorochrome (Molecular Probes) for 30 min. Apoptotic cells were counted with a fluorescence microscope, and photographed using a digital video camera (Pixera; Los Gatos, CA, USA). Approximately 200 – 400 cells per group were assessed in randomly selected fields to avoid experimental bias.

**Statistical analysis**

All experiments were conducted at least three times independently. Results were given as mean ± standard deviation of six independent determinations. All statistical analyses were performed with NCSS Statistical Analysis System [27]. One-way analysis of variance (ANOVA) was used to compare the means, and differences were considered significant at $p \leq 0.05$.

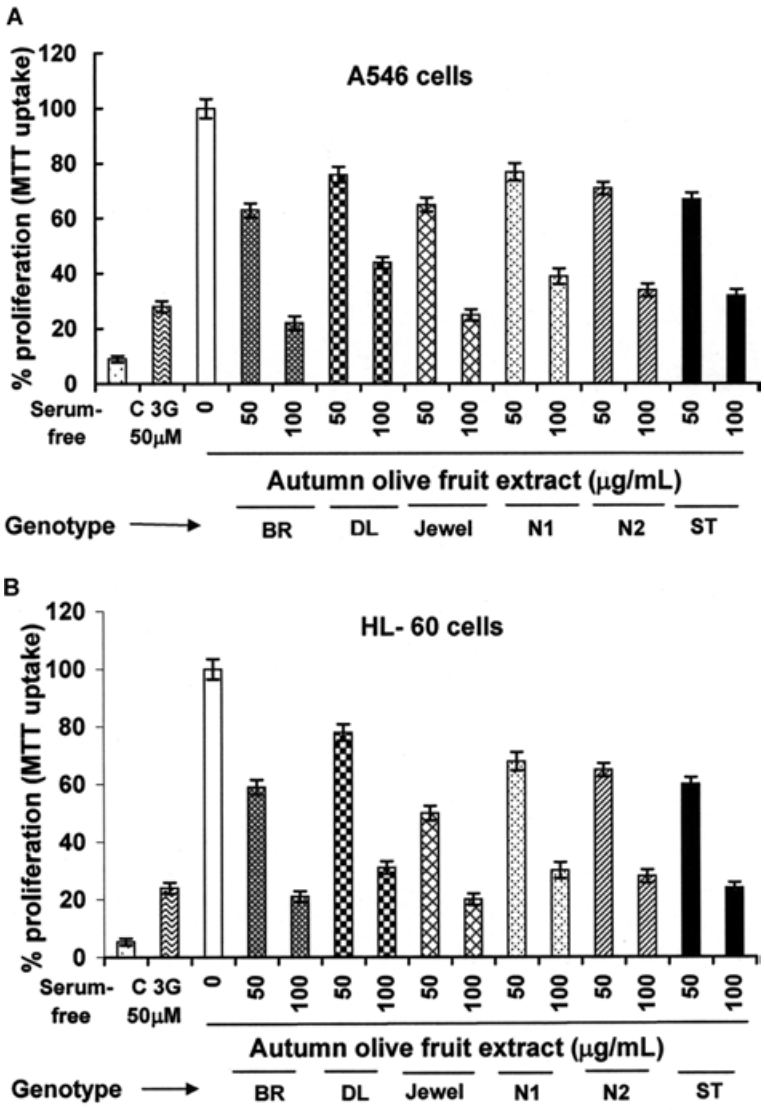


Fig. **6** Inhibition of proliferation of cancer cells by autumn olive fruit extracts. (**A**) human lung cancer A549 cells and (**B**) human leukemia HL-60 cells. Cells ($1 \times 10^4$) were incubated with the indicated fruit extracts (50 $\mu$g/mL) for 48 h and cell proliferation was determined by the MTT assay. Proliferation was expressed as a percentage of cell growth in wells that received no extract. Cyanidin 3-glucoside (C 3G) at 50 $\mu$M was used as positive control. Data are expressed as mean ± S.E. of three samples. The abbreviations for BR, DL, N1, N2 and ST were 'Brilliant Rose', 'Delightful', Natural 1, Natural 2, and 'Sweet Tart', respectively.

## Results and Discussion

The chemical structures of major carotenoids in autumn olive fruit such as lycopene, lutein, $\alpha$-cryptoxanthin, $\beta$-cryptoxanthin, $\alpha$-carotene, $\beta$-carotene, phytofluene and phytoene are shown on Fig. **1**. Lycopene was the dominant carotenoid in autumn olive fruit ranging from 30.58 to 46.23 mg FW 100 $g^{-1}$. These represent 75.41 to 81.57 % of total carotenoids. 'Jewel' (46.23 mg FW 100 $g^{-1}$), 'Brilliant Rose' (39.37 mg FW 100 $g^{-1}$), and 'Sweet N Tart' (37.95 mg FW 100 $g^{-1}$) had higher lycopene contents than other cultivars (30.58 – 33.54 mg FW 100 $g^{-1}$). $\alpha$-Cryptoxanthin ranged from 2.38 to 5.41 mg FW 100 $g^{-1}$ and $\beta$-cryptoxanthin ranged from 3.42 to 7.35 mg FW 100 $g^{-1}$. 'Jewel' and 'Brilliant Rose' were also found to have the highest $\alpha$-cryptoxanthin and $\beta$-cryptoxanthin contents and 'Delightful' had the lowest. Other carotenoids such as lutein, $\alpha$-carotene, $\beta$-carotene, phytofluene and phytoene in autumn olive fruit ranged from 0.01 to 0.72 mg FW 100 $g^{-1}$. The total carotenoids in 'Brillant Rose', 'Delightful', 'Jewel', Natural 1, Natural 2 and 'Sweet N Tart' were 52.21, 37.49, 59.03, 40.73, 43.28 and 47.54 mg FW 100 $g^{-1}$, respectively. These values were comparable with those reported by Fordham et al. [5].

ESR was utilized to measure the ability of autumn berry extract to scavenge $^{\cdot}OH$ and $O_2^{\cdot -}$ radicals. As shown in Fig. **2** and **3**, various genotypes of autumn olive extracts showed inhibition of $^{\cdot}OH$ and $O_2^{\cdot -}$ radicals in a dose-dependent manner. 'Brilliant Rose', 'Jewel' and 'Sweet N Tart' extracts showed higher inhibition of $^{\cdot}OH$ radicals and $O_2^{\cdot -}$ radicals than 'Delightful', Natural 1 and Natural 2. These data suggest that autumn olive fruit could be an excellent food source for scavenging oxygen species in the human diet.

AP-1 and NF-κB are two eukaryotic transcription factors that regulate genes implicated in reactive oxygen species (ROS)-induced responses, and both factors are targets of oxidative stimuli [28]. AP-1 and NF-κB play a critical role in tumorgenesis induced by carcinogens and they are also associated with the stimulation of cancer proliferation. High AP-1 and NF-κB activity have been shown to be involved in tumor promotion [29], and progression of various types of cancers, such as lung [30], breast [31] and skin cancer [32]. Cancer cells with greater metastatic capability have greater AP-1 and NF-κB activity. Inhibition of AP-1 and NF-κB activity has been shown to lead to suppression of cell transformation and tumor promotion [33], [34]. The effects of autumn olive extracts on AP-1 and NF-κB activation are shown in Fig. **4** and **5**.

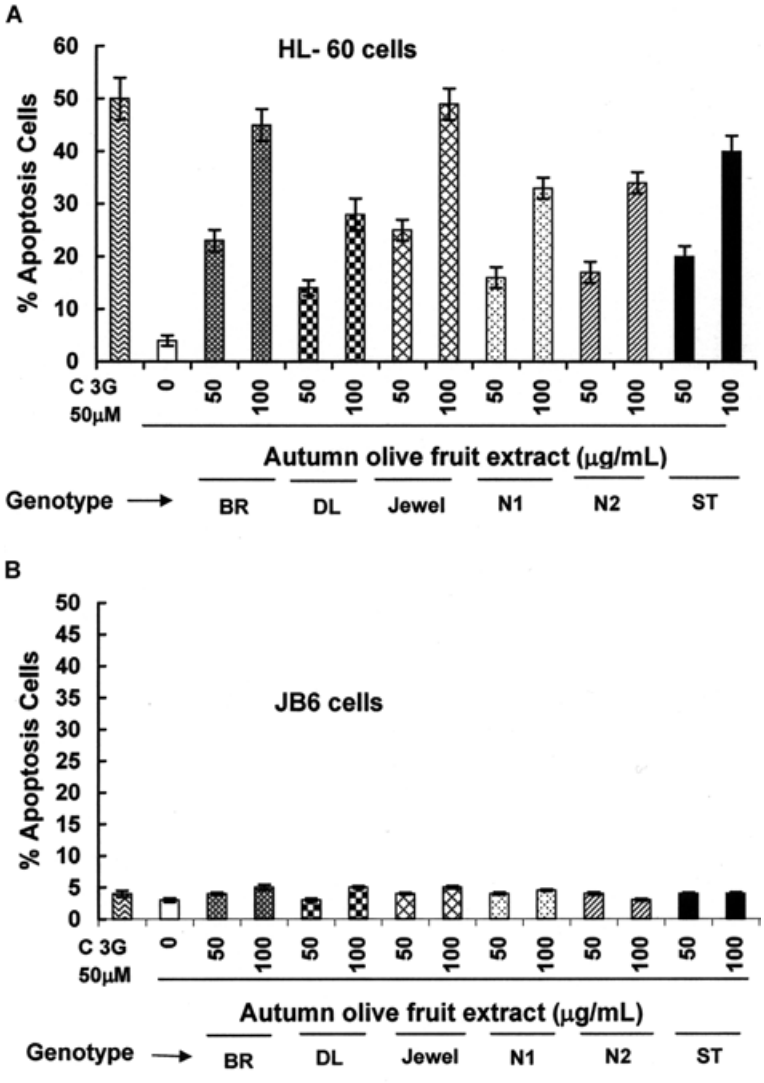


Fig. **7** Autumn olive fruit extracts induced apoptosis in human leukemia HL-60 cells, but not in JB6 $P^+$ mouse epidermal cells. Human leukemia HL-60 cells and JB6 cells were incubated with the indicated fruit extracts (50 $\mu$g/mL) for 18 h. The apoptotic HL-60 cells were detected by Hoechst 33 258 staining and the percentage of apoptotic cells was calculated by determining the number of cells with nuclear morphology change divided by the total number of cells. Cyanidin 3-glucoside (C 3G) at 50 $\mu$M was used as a positive control. Data are expressed as mean ± S.E. of three samples. The abbreviations for BR, DL, N1, N2 and ST were 'Brilliant Rose', 'Delightful', Natural 1, Natural 2, and 'Sweet Tart', respectively.

Pretreatment of JB6 cells with autumn olive extracts resulted in inhibition of AP-1 activation induced by either UVB or TPA (Figs. **4A** and **4B**). AP-1 activity induced by either UVB or TPA was inhibited 26 – 66 % or 36 – 81 %, respectively, with autumn olive extracts in the dose range of 30 to 120 $\mu$g/mL (Figs. **4A** and **4B**). Similarly, NF-κB activity induced by UVB or TPA was also inhibited by varying concentrations of autumn olive extracts. (Figs **5A** and **5B**). These results suggest that autumn olive extracts have a protective role in cancer development.

Uncontrolled cell proliferation is a major feature of cancer, and cancer cell proliferation is one of the key events in the progression of a cancer tumor [35]. Several fruit and vegetable components such as carotenoids, have also been shown to inhibit cancer cell proliferation *in vitro* [36], [37], [38], [39], [40]. Antiproliferative activities of different genotypes of autumn olive extracts on the growth of human lung epithelial cancer A549 and human leukemia HL-60 cells *in vitro* are shown in Fig. **6**. Furthermore, autumn olive extracts also induced apoptosis in human promyelocytic leukemia HL-60 cells in a dose-dependent manner (Fig. **7**). In contrast, the fruit extracts did not cause apoptosis in non-tumor JB6 cells (Fig. **7**). Among the six selected autumn olive genotypes, 'Brilliant Rose' and 'Jewel' showed relatively potent antiproliferative activities on A549 and HL-60 cell growth and inductive apoptosis in human promyelocytic leukemia HL-60 cells compared to other genotypes. There is a good correlation between antiproliferative and antioxidant activities in autumn olive with $R^2$ values equal to 0.9207 and 0.9158 for $O_2^{C-}$ and $^{\cdot}OH$, respectively. The correlation between apoptosis and antioxidant activities was also evident with $R^2$ values equal to 0.8869 and 0.9452 for $O_2^{C-}$ and $^{\cdot}OH$, respectively.

In summary, our results indicate that autumn olive berry had high content of carotenoids and high scavenging free radical activities for $O_2^{C-}$ and $^{\cdot}OH$. Autumn olive extracts inhibited AP-1 and NF-κB activity as well as cell proliferation and increased the induction of apoptosis in cancer cells. The levels of these activities were different among the autumn olive genotypes. Dietary supplementation with various fruits and vegetables including autumn olive berry could have benefits to human health.

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