Phytochemical profiles and antioxidant activities of wine grapes

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ABSTRACT

Grapes are rich in phenolics, flavonoids and resveratrol, which have been suggested to be responsible for their health benefits. The concentrations of phenolic, flavonoid, anthocyanins and resveratrol of 14 grape varieties grown in the Finger Lakes area of New York State were examined. Among the varieties tested, Cabernet Franc and Pinot Noir contained the highest total phenolic content with 424.6 ± 3.8 and 396.8 ± 12.4 mg/100 g, respectively. The total flavonoid content of Pinot Noir (301.8 ± 6.2 mg/100 g) was around 3.1-fold higher than that of Baco Noir. Baco Noir had the highest resveratrol content (571 ± 30 µg/100 g) of the varieties tested. Cabernet Franc possessed the highest antioxidant activity. Total antioxidant activities of grape extracts are well correlated with total phenolic content. The proliferation of Caco-2, HepG$_2$ and MCF-7 human cancer cells was significantly inhibited in a dose-dependent manner after exposure to Pinot Noir, Cabernet Franc, Chardonnay, Catawba, Concord, Sheridan, Niagara and Riesling. The results suggest that phytochemicals in the selected wine grapes have potent antioxidant and antiproliferative activities.

1. Introduction

Grapes, one of the most popular fruits and the most widely cultivated throughout the world, contain large amounts of phytochemicals including anthocyanins and resveratrol, which offer health benefits (Pezzuto, 2008). There are about 60 species of *Vitis*, which are mainly found in the temperate zones of the Northern Hemisphere and almost equally distributed between America and Asia (Mullins, Bouquet, & Williams, 1992). Approximately 80% of all grapes are used in winemaking, and 13% are consumed as table grapes. The *Vitis vinifera* grapes are commonly used for wine production around the world, principally distributed in Europe. In the United States, species such as *Vitis labrusca*, *Vitis riparia*, *Vitis aestivalis*, *Vitis rupestris* and *Vitis rotundifolia* are also used in wine-making. Grape phenolics, especially high in the grape peel (Singleton, 1982), are classified into two groups: the flavonoids and nonflavonoids. The flavonoids include flavan-3-ols (catechin), flavonols (quercetin) and anthocyanins. The nonflavonoids encompass hydroxybenzoates (gallic acid), hydroxycinnamates and stilbenes (resveratrol). The traditional Western diet provides roughly 1 g/day of mixed flavonoids. Besides antioxidant activity, flavonoids have many biological activities such as the inhibition of plasma platelet aggregation and cyclooxygenase activity, the suppression of histamine release and SRS-A biosynthesis *in vitro*,

potent nitric oxide radical scavenging activity and exhibiting antibacterial, antiviral, anti-inflammatory and antiallergenic effects (Cook & Samman, 1996). In the grape berry, the flavonoids are mainly localised in the skins, such as the anthocyanins and resveratrol, while the flavan-3-ols (catechins and proanthocyanidins) are present both in the skins and in the seeds. However, the composition and concentration of phenolics in grapes vary with variety, species, season and environmental and management factors such as soil conditions, climate and crop load.

Grapes are one of the major dietary sources of anthocyanins, which are responsible for the colouring of black, red and purple grapes; however, they are lacking in white grapes. In particular, anthocyanins mostly accumulate in the skins, whereas procyandins are located in the seeds. It was found that the Lomanto and Colobel hybrid grape cultivar had the highest anthocyanin content with 603 mg/100 g; Midsouth cultivar contained the lowest content with 5.5 mg/100 g (Mazza, 1995). The anthocyanins in grape skins are predominately the 3-O-glucosides of malvidin, cyanidin, delphinidin, peonidin and petunidin (Wrolstad, 2000). Malvidin, the reddest of all anthocyanins, is the major one in dark red *vinifera* grapes, with higher proportions of cyanidin in red grapes. Cyanidin 3-monoglucoside and delphinidin 3-monoglucoside are the major anthocyanins in Concord grapes (Singleton, 1982). Anthocyanins possess antioxidant activity, which is considered to be an important physiological function. Additively, anthocyanins are reported to have anti-inflammatory activity, anticancer activity, apoptotic induction effect, α-glucosidase inhibition activity, vision benefits and effects on collagen, blood platelet aggregation and capillary

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permeability and fragility (Hou, 2003). Thus, anthocyanins, as naturally occurring bioactive compounds and pigments, have attracted interest due to their safety and health benefits.

Resveratrol (3,4′,5-trihydroxystilbene, RSV), which is synthesised by some plants in response to adverse conditions such as pathogenic attack and environmental stress, is found in various food products. It is particularly high in grape skins, seeds and in red wine. RSV was first found in grapevines (V. vinifera) in 1976 (Langcake & Pryce, 1976) and then reported in wine in 1992 (Siemann & Creasy, 1992). The 'French Paradox' has suggested that RSV might be the major bioactive component in red wine (Frankel, Kanner, German, Parks, & Kinsella, 1993). Thus RSV has attracted considerable attention due to its cardioprotective and cancer chemopreventive activities (Jang et al., 1997), which provide great interest in grapes, wines and dietary products containing RSV. The proposed mechanisms related to RSV's health effects can be summarised as scavenging intracellular ROS, inhibiting the oxidation of LDL, preventing platelet aggregation, suppressing cell proliferation via steps in the signal transduction pathways, inducing apoptotic cell death through activation of mitochondria-dependent pathways, exhibiting anti-inflammatory activity via down-regulation of proinflammatory cytokines, promoting cellular differentiation, exhibiting antioestrogenic activity and inhibiting CYP1 enzymes (Chang, Chen, & Lee, 2001).

The beneficial health-related effects of phenolics in grapes are of importance to consumers, breeders and the grape industry. There is limited knowledge about the phytochemical profiles, antioxidant and antiproliferative activities in both V. vinifera and nonV. vinifera wine grapes grown in the Finger Lake area of New York State. The objectives for this study were: (1) to determine the profiles of total phenolics, total flavonoids, total anthocyanins and resveratrol in selected grapes; (2) to measure the total antioxidant activity and (3) to determine the antiproliferative activity of grape extracts against human colon, liver and breast cancer cells in vitro.

2. Materials and methods

2.1. Materials

Sodium nitrite, (+)-catechin, Folin-Ciocalteu reagent (FCR), hydrochloric acid, glucagon, hydrocortisone, insulin, α-keto-γ-methiolbutyric acid (KMBA) and tran-RSV were purchased from Sigma Chemical Co. (St. Louis, MO). Aluminium chloride, sodium hydroxide, methanol and acetone were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid was purchased from ICN Biomedical Inc. (Costa Mesa, CA). 2,2′-Azobis (amidinopropane) (ABAP) was purchased from Wako Chemicals (Richmond, VA).

Fourteen wine grape varieties were provided by Grapeyard located in Branchport, NY. General descriptions of the grape varieties are given in Table 1. The wine grapes were harvested upon ripening in the 2003 and 2004 vintages. Grapes free from visible blemish or disease were selected. Three separate batches of grapes from different sites were used to prepare triplicate samples. For quantitative analysis, 50–70 grape berries, randomly selected from each grape variety, were collected for extraction. All data collected for each grape variety were reported as mean ± SD for at least three replications.

2.2. Extraction of total phenolic compounds

Total phenolics were extracted from fresh grapes by the modified method reported previously in our laboratory (Yang, Meyers, van der Heide, & Liu, 2004). Briefly, 100 g of grapes were blended for 1 min in 100 g of 80% acetone using Waring blender with

medium speed to remove seeds. After removal of the seeds and adding an additional 100 g of 80% acetone, the grapes were blended for 3 min using a Waring blender with high speed. The mixture was then homogenised in a Virtis High Speed Homogeniser for 3 min and filtered with vacuum under an ice bath. The acetone in the filtrate was evaporated using a rotary evaporator at 45 °C until the weight of the evaporated filtrate was less than 10% of the weight of the original filtrate. All extracts were stored at −40 °C until use. All extractions were performed in triplicate.

2.3. Determination of total phenolic content

The total phenolic content in the grapes was determined using the Folin-Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999), which was modified by our laboratory (Yang et al., 2004). Briefly, all extracts were diluted 1:10 with distilled water to obtain readings within the standard curve ranges of 0.0–600.0 µg of gallic acid/mL. The grape extracts were oxidised by the Folin-Ciocalteu reagent and the reaction was neutralised with sodium carbonate. The absorbance was measured at 760 nm after 90 min at room temperature by a MRX II Dynex plate reader (Dynex Technologies Inc., Chanilly, VA). The absorbance values were then compared with those of standards with known gallic acid concentrations. All values were expressed as the mean (milligrams of gallic acid equivalents per 100 g of fresh sample) ± SD for three replications.

2.4. Determination of total flavonoid content

The total flavonoid content of the grape extract was determined using a modified colourimetric method (Jia, Tang, & Wu, 1999; Yang et al., 2004). Briefly, 0.25 mL of 1:10 diluted grape extracts was mixed with 1.25 mL of distilled water and subsequently with 0.075 mL of 5% sodium nitrite solution and was allowed to react for 5 min. Then a 0.15 mL of 10% aluminium chloride was added and allowed to further react for 6 min before 0.5 mL of 1 M sodium hydroxide was added. Distilled water was added to bring the final volume of the mixture to 3 mL. The absorbance of the mixture was immediately measured at a 510 nm wavelength against a prepared blank using a MRX II DYNEX spectrophotometer. The flavonoid content was determined by a catechin standard curve and expressed as the mean (milligrams of catechin equivalents per 100 g of fresh sample) ± SD for the triplicate extracts.

2.5. Quantification of anthocyanin content

The monomeric anthocyanin content of the grape extract was measured using a modified pH differential method (Boyles & Wrolstad, 1993). The grape extracts were mixed thoroughly with 0.025 M potassium chloride buffer pH 1 in a 1:2 ratio of extract to buffer. The grape extracts were then mixed similarly with a sodium acetate buffer pH 4.5. A Beckman DU640B spectrophotometer was used to measure absorbance at 510 and 700 nm against a buffer blank at pH 1.0 and 4.5. Absorbance readings were converted to total milligrams of cyanidin 3-glucoside (C3G). The anthocyanin content was calculated as follows:

Total monomeric anthocyanins (mg/100 g)

$$= \Delta A \times MW \times 1000/(\varepsilon \times C)$$

$$\Delta A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

where A is absorbance, MW (449.2) is molecular weight for C3G, ε(26,900) is the molar absorptivity of C3G and C is the concentration of the grape extract in milligrams per millilitre. The anthocyanin

Table 1
The total phenolic, total flavonoid,and total anthocyanin contents in 14 grape varieties.

Cultivar	Colour	Total phenolics (mg/100 mL)	Total flavonoids (mg/100 mL)	Total Anthocyanin (mg/100 mL)
Vinifera				
Cabernet Franc	Dark purple/blue	424.6 ± 3.8[a]	180.9 ± 15.3[b]	58.7 ± 4.4[de]
Chardonnay	Green	201.1 ± 4.9[e]	166.4 ± 20.4[bcd]	
Pinot Noir	Dark purple/blue	396.8 ± 12.4[a]	301.8 ± 6.2[a]	49.8 ± 4.3[e]
Riesling	Green	255.8 ± 8.8[d]	133.5 ± 13.7[def]	
Hybrid				
Baco Noir	Dark purple	217.0 ± 14.1[e]	97.8 ± 9.8[f]	101.6 ± 2.5[c]
Catawba	Pink	311.7 ± 9.1b[c]	180.9 ± 5.4[b]	8.1 ± 0.8[f]
Cayuga White	Green	206.3 ± 8.2[e]	176.1 ± 10.7[b]	
Chancellor	Dark purple/blue	325.8 ± 21.7[bc]	140.0 ± 18.8[cde]	198.7 ± 4.8[b]
Concord	Red, purple	334.0 ± 13.6[b]	168.2 ± 6.0[bc]	80.6 ± 2.4[cd]
DeChaunac	Dark blue	293.5 ± 21.6[c]	113.9 ± 12.0[ef]	239.6 ± 25.4[a]
Marechal Foch	Dark purple	312.5 ± 10.9[bc]	127.0 ± 14.2[ef]	174.1 ± 15.3[b]
Niagara	Green	229.6 ± 3.9[de]	173.1 ± 11.3[bc]	
Sheridan	Red, purple	331.4 ± 8.2[b]	166.8 ± 1.38[bcd]	54.4 ± 7.7[e]
Vidal Blanc	Green	228.0 ± 5.5[e]	100.7 ± 9.4[f]	

[a–f]Bars with no letters in common are significantly different ($p < 0.05$) in the same column.

content was expressed as milligrams of C3G equivalents per 100 g of fresh grape for the tripicate extracts.

2.6. Reverse-phase HPLC analysis of resveratrol

A 3-mL grape sample was extracted in a test tube with 5 mL of ethyl ether, and then the mixture was put into a shaker with 200 rpm for 15 min. The organic phase was transferred into a new test tube. The residues were extracted with 5 mL of ethyl acetate twice using the same conditions. The organic solvent in the new test tube was evaporated by flushing with N_2. The dry residue was dissolved in 1 mL methanol, and the aliquots were then analysed by RP-HPLC.

Stock solution containing 14.4 mg/mL of resveratrol in methanol was prepared. The solution was stored at $-4\ °C$ in the dark after elimination of oxygen with N_2 to avoid the oxidation or decomposition of the phenolic compounds. Resveratrol in the grape extracts was quantified using a RP-HPLC procedure employing a Supelcosil LC-18-DB, 150 mm × 4.6 mm and 3 μm column. Samples of 20 μL standard or grape methanolic extracts were directly injected into the column. Elution was carried out with a mobile phase delivered using a Waters 515 HPLC pump (Waters Corp., Milford, MA) at a flow rate of 1.2 mL/min according to the following gradient: the initial mixture was acetonitrile – water (9:91) adjusted to pH 2 with triflouroacetic acid for 10 min; linear gradient to (25:75) in 10 min, hold for 10 min; linear gradient to (70:30) in 1 min, hold for 12 min. A Waters 2487 wavelength absorbance detector (Waters Corp., Milford, MA) was used for UV detection of analytes at 307 nm. Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999) (Waters Corp., Milford, MA). Three HPLC injections were performed for each extract. Peak heights were used for all calculations. The recoveries for RSV analyses were 104.78 ± 4.87% (n = 4).

2.7. Determination of total antioxidant capacity

The total antioxidant capacity of grape extracts was measured using a total oxyradical scavenging capacity (TOSC) assay (Winston, Regoli, Duga, Fong, & Blanchard, 1998) as modified in our laboratory (Yang et al., 2004). In this assay, peroxy radicals produced from 2,2′-azobis-amidinopropane (ABAP) oxidise α-keto-γ-methiolbutyric acid (KMBA) to form ethylene gas, which was measured by a gas chromatographic headspace analysis. Briefly, the antioxidant activity was quantified after 15, 30, 45 and 60 min for four different grape extract concentrations and a control. The amount of

ethylene generated by the reaction was expressed as peak area. The TOSC value corresponding to each extract concentration was calculated by integrating the area under the kinetic curve and assessed as the following equation: $TOSC = 100 - (\int SA / \int CA) \times 100$, where $\int SA$ is the integrated area from the sample reaction, and $\int CA$ is the integrated area from the control reaction. The median effective dose (EC_{50}) was determined for each grape variety from the dose–response curve of grape concentration versus TOSC value. The TOSC value is expressed as μmol of vitamin C equivalents per gram of sample. All values were presented as the mean ± SD for at least three replicates.

2.8. Measurement of inhibition of Caco-2, HepG2 and MCF-7 cell proliferation

The antiproliferative activity of different grape extracts was assessed by measurement of the inhibition of Caco-2, $HepG_2$ and MCF-7 (The American Type Culture Collection, ATCC, Rockville, MD) human cancer cell proliferation. Antiproliferative activities were determined by the colourimetric MTS assay (MTS-based cell titre 96 nonradioactivity cell proliferation assay) (Promega, Madison, WI) reported previously (Yang et al., 2004). $HepG_2$ – human liver cancer cells were cultured in Williams medium E (WME), containing 10 mM Hepes, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 2 μg/mL glucagon, 5% foetal bovine serum (Gibco, Life Technologies, Grand Island, NY), 50 U/mL penicillin, 50 μg/mL streptomycin and 100 μg/mL gentamicin. Caco-2 – human colon cancer cells were maintained in DMEM, containing 10 mM Hepes, 5% FBS, 50 U/mL penicillin, 50 μg/mL streptomycin and 100 μg/mL gentamicin. MCF-7 human breast cancer cells were maintained in Alpha Minimum Essential Medium (MEM-a), containing 10 mM Hepes, 0.01 mg/mL insulin, 50 U/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL gentamicin and 10% foetal bovine serum (Gibco, Life Technologies, Grand Island, NY). $HepG_2$, Caco-2 and MCF-7 cells were maintained in a 5% $CO_2/37\ °C$ incubator. A total of 2.5×10^4 $HepG_2$, Caco-2 or MCF-7 cells in growth media were placed in each well of a 96-well flat-bottomed plate. Cell proliferation was measured by the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2H-tetrazolium (MTS) to formazan. After 4 h of incubation, the growth medium was removed and media containing various concentrations (2, 5, 10, 20, 30, 40, 50, 75 and 100 mg/mL) of grape extracts were added to the cells. Control cultures received the extraction solution minus the grape extracts, and blank wells contained 100 μL of growth medium without cells. Cell

proliferation (percent) was determined at 96 h from the MTS absorbance (490 nm) reading for each concentration compared to the control, using at least three replications for each sample. The effective median dose (EC_{50}) was determined and expressed as milligrams of grape extracts per millilitre ± SD.

2.9. Statistical analysis

Statistical analysis was performed using Minitab Student Release 12 (Minitab Inc., State College, PA) and SigmaStat Version 8.0 (Jandel Corp., San Raphael, CA). Results were subjected to ANOVA, and differences between means were located using Tukey's multiple comparison test. Correlations between various parameters were also investigated. Significance was determined at $p < 0.05$. All data were reported as the mean ± SD of three replications.

3. Results and discussion

3.1. Total phenolic content

Total phenolic contents of 14 wine grapes are presented in Table 1. Among all the grape varieties analysed, Cabernet Franc and Pinot Noir had the highest total phenolic content (424.6 ± 3.8 and 396.8 ± 12.4 mg of gallic acid equivalents/100 g of grape, respectively), followed by Concord, Sheridan, Chancellor, Marechal Foch, Catawba, DeChaunac, Riesling, Niagara, Vidal Blanc, Baco Noir, Cayuga White and Chardonnay. Significant differences were found in total phenolic content in comparisons between Cabernet Franc and Concord, Pinot Noir and Sheridan, Chancellor and Riesling, and DeChaunac and Niagara ($p < 0.05$); however, significant differences in total phenolic content were not found between Cabernet Franc and Pinot Noir, between Riesling and Niagara, among Concord, Sheridan, Chancellor, Marechal Foch and Catawba, or among Vidal Blanc, Baco Noir, Cayuga White and Chardonnay ($p > 0.05$). The results show that the red grape varieties except Baco Noir contain high concentrations of total phenolics. In contrast, the green grapes have less phenolic content. The phenolic distribution in juice, pulp, skins and seeds are approximately 5%, 1%, 30% and 64%, respectively (Singleton, 1982; Singleton & Esau, 1969). The average level of phenolic compounds for seeded *V. vinifera* varieties from California is about 4000 mg gallic acid equivalents (GAE)/kg for white grapes and 5500 mg GAE/kg for red (Singleton & Esau, 1969). Mattivi, Zulian, Nicolini, and Valenti (2002) have studied 25 high-quality red grape cultivars (*V. vinifera*), and found that the Schiava variety contained the lowest phenolic content with 1358 mg/kg, and the Sagrantino variety had the highest phenolic content with 4628 mg/kg, resulting in a variability factor of 2.7 between the average phenolic concentrations of the varieties. Kanner, Frankel, Granit, German, and Kinsella (1994) also found a variation in phenolic content among Thomson seedless, Flame seedless and Black seedless grapes, ranging from 260 to 930 mg/kg of fresh weight. In this study, there was a 2.1-fold difference in total phenolic content between the highest and lowest ranked varieties, Cabernet Franc and Chardonnay ($p < 0.05$). It is well known that both genetic and agronomic or environmental factors play important roles in phenolic composition and thus nutritional quality of crops. Generally, the total phenolic of red grape skins is greatly higher than that of white grapes due to the loss of the ability to produce anthocyanins in the skins of white grapes. However, our results showed that the phenolic content of different grapes depends mainly on the varietal differences, not on grape skin colour. For example, Baco Noir variety had lower contents of total phenolics and flavonoids than Riesling, Niagara and Vidal Blanc.

3.2. Total flavonoid content

Total flavonoids of the 14 grape extracts were measured (Table 1). The Pinot Noir presented the highest flavonoid content (301.8 ± 6.2 mg of catechin equivalents/100 g of fresh grapes, $p < 0.05$), followed by Catawba, Cabernet Franc, Cayuga White, Niagara, Concord, Sheridan, Chardonnay, Chancellor, Riesling, Marechal Foch, DeChaunac, Vidal Blanc and Baco Noir. The flavonoid content of Pinot Noir, Catawba, Chancellor and Baco Noir was significantly different from each other ($p < 0.05$). However, significant differences in the total flavonoid content were not found in comparisons among Catawba, Cabernet Franc, Cayuga White, Niagara, Concord, Sheridan and Chardonnay, and among Chancellor, Riesling, Marechal Foch, DeChaunac, Vidal Blanc and Baco Noir ($p > 0.05$). An approximately 3.1-fold difference in total flavonoid content was found between the highest and lowest ranked varieties, Pinot Noir and Baco Noir ($p < 0.05$).

3.3. Total anthocyanin content

Total anthocyanin contents of nine red grape extracts were determined (Table 1). DeChaunac had the highest total anthocyanin content (239.6 ± 25.4 mg of cyanidin 3-glucoside equivalents/100 g of grapes, $p < 0.05$), followed by Chancellor, Marechal Foch, Baco Noir, Concord, Cabernet Franc, Sheridan, Pinot Noir and Catawba. There was significant difference ($p < 0.05$) in anthocyanin content between DeChaunac, Chancellor, Baco Noir, Cabernet Franc and Catawba. However, significant differences in the anthocyanin content were not observed between Chancellor and Marechal Foch, between Baco Noir and Concord or among Cabernet Franc, Sheridan and Pinot Noir ($p > 0.05$). In addition, anthocyanins were not detected in any of the green grape varieties. Generally, the total anthocyanin content of red grapes is from about 30 to 750 mg/g of fresh weight of ripe berries (Mazza, 1995). Munoz-Espada, Wood, Bordelon, and Watkins (2004) reported the total anthocyanin contents in the skin of three non*V. vinifera* grapes, which were 258 ± 37 mg/100 g of wet weight for Marechal Foch, 888 ± 78 mg/100 g for Norton and 326 ± 5.9 mg/100 g for Concord. Upon malvidin 3-monoglucoside chloride as a standard, the lowest anthocyanin content in Primitivo variety was found to be 250 mg/kg whereas the maximum amount was found in a Teroldego grape with 2323 mg/kg (Mattivi et al., 2002). In this study, the DeChaunac variety contained the highest total anthocyanin content, whereas Catawba had the lowest, indicating almost a 30-fold difference. The concentration and composition of the anthocyanins in red grapes vary greatly with the variety, species, maturity, production area and climate. In assessing red grape varieties for total anthocyanin content, we found that broad variability exists. This is not surprising since these secondary metabolites are synthesised under genetic control in different varieties. As a characteristic associated with the variety, the level of anthocyanins in grapes may serve as an estimate of the red pigments and be useful for the classification of grape varieties and of relevant wines. Therefore, anthocyanins have been proposed as chemical markers to differentiate grape varieties and red wines.

3.4. Resveratrol content

The resveratrol contents of 14 grape extracts were quantified (Fig. 1). The Baco Noir variety contained the highest RSV content (571 ± 30 µg/100 g of fresh sample), followed by Pinot Noir (421 ± 54), Vidal Blanc (263 ± 16), Marechal Foch (130 ± 8), Cabernet Franc (119 ± 8), Chancellor (117 ± 7), Sheridan (112 ± 10), Riesling (80 ± 7), DeChaunac (75 ± 6), Chardonnay (73 ± 9), Catawba (72 ± 5), Concord (65 ± 8), Niagara (53 ± 4) and Cayuga White (38 ± 2). There were significant differences ($p < 0.05$) in RSV
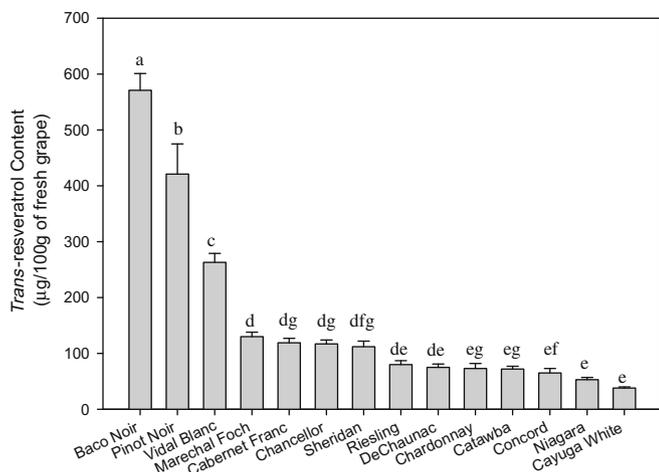
Fig. 1. Resveratrol content of 14 grape varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

content among Baco Noir, Pinot Noir, Vidal Blanc, Marechal Foch and Chardonnay. However, no significant differences in RSV content were found among Marechal Foch, Cabernet Franc, Chancellor, Sheridan, DeChaunac and Riesling, or among Chardonnay, Catawba, Concord, Niagara and Cayuga White ($p > 0.05$). RSV is synthesised particularly in the leaf epidermis and the skin of grapes and only trace amounts are present in the fruit flesh. Grape skins and red wines contain 50–100 μg RSV/g and 1.5–3 mg/L, respectively (Joe et al., 2002). This study presented that Baco Noir grape extract contained the highest RSV content, while Cayuga White variety was the lowest, showing a 15-fold difference.

3.5. Total antioxidant activity

Total antioxidant activities of the 14 grape varieties, expressed as micromoles (μmol) of vitamin C equivalents per gram of fresh grape, are shown in Fig. 2. Phytochemical extracts of Cabernet Franc had the highest antioxidant activity ($149.0 ± 10.0$ μmol/g, $p < 0.05$), followed by Pinot Noir ($122.4 ± 5.7$), Concord ($106.0 ± 6.0$), Sheridan ($106.6 ± 3.6$), Chancellor ($102.8 ± 6.0$), Marechal Foch ($100.2 ± 6.0$), Catawba ($98.0 ± 4.6$), DeChaunac ($96.3 ± 6.1$), Riesling ($79.8 ± 4.3$), Niagara ($65.3 ± 3.8$), Vidal Blanc
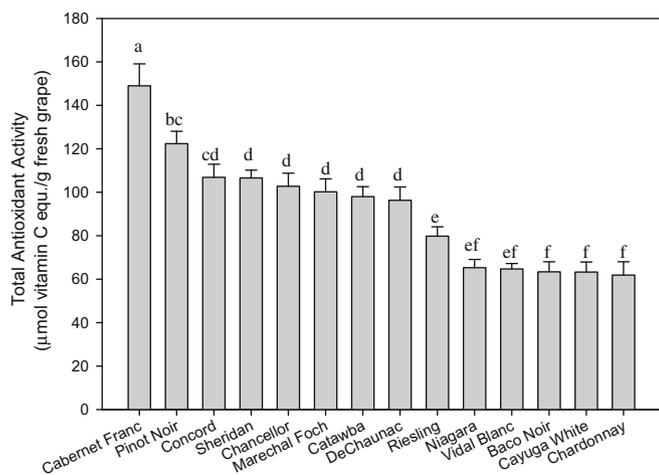
(64.7 ± 2.5), Baco Noir (63.4 ± 4.6), Cayuga White (63.3 ± 4.6) and Chardonnay (61.9 ± 6.1). A statistically significant difference ($p < 0.05$) was found among Cabernet Franc, Pinot Noir, Chancellor, Riesling and Chardonnay. The total antioxidant activities of Pinot Noir and Concord were similar ($p > 0.05$) but lower ($p < 0.05$) than that of Cabernet Franc. No significant difference ($p > 0.05$) was found among Concord, Sheridan, Chancellor, Marechal Foch, Catawba and DeChaunac, among Riesling, Niagara and Vidal Blanc, or among Vidal Blanc, Baco Noir, Cayuga White and Chardonnay grapes. The varieties containing high total phenolic contents had higher antioxidant activities. The present study reveals a strong correlation between total antioxidant activity and total phenolics ($R^2 = 0.9829$, $p < 0.05$). Kanner et al. (1994) found that antioxidant effect was found in all grape varieties, which corresponded to the concentration of phenolics in a number of different systems. The antioxidant activity was measured by the inhibition of human low-density lipoprotein (LDL) oxidation. *In vitro* oxidation positively correlated with the content of total phenolics, anthocyanins and flavonols after investigation of phenolic extracts from 14 different types of fresh grapes (Meyer, Yi, Pearson, Waterhouse, & Frankel, 1997). There was a positive correlation between anthocyanin content and antioxidant activities of red grape extracts (Meyer et al., 1997), grape juices (Frankel, Bosanek, Meyer, Silliman, & Kirk, 1998) and red wines (Burns et al., 2000). Frankel et al. (1993) were the first to demonstrate that *trans*-RSV reduced the copper-catalysed oxidation of human LDL. The LDL peroxidation was more blocked by RSV than by a phenolic extract from red wine. However, both the anthocyanin and RSV contents did not correlate with total antioxidant activity of grapes in this experiment. This could be because particular compounds may act additively or synergistically with other compounds and the total expressed antioxidant activity may be dependent on the relative proportions of each compound in the system.

3.6. Inhibition of human cancer cell proliferation

The effects of 14 wine grape varieties on the growth of Caco-2 human colon cancer cells, HepG$_2$ human liver cancer cells and MCF-7 human breast cancer cells *in vitro* are summarised, respectively, in Fig. 3. Grape extracts had potent antiproliferative activity against human colon, liver and breast cancer cells in a dose-dependent manner. Among the 14 grape varieties tested, Cabernet Franc, Pinot Noir, Chardonnay, Catawba, Concord, Sheridan, Niagara and Riesling show relatively high antiproliferative activities towards both Caco-2 and HepG$_2$ cells. Cabernet Franc and Catawba exhibit relatively strong antiproliferative activities towards MCF-7 cells.

Fig. 4 presents the EC$_{50}$ of the antiproliferative activity of different grape varieties. Lower EC$_{50}$ values represent higher antiproliferative activities. The phytocemical extracts of Pinot Noir and Cabernet Franc possess the greatest antiproliferative activity towards Caco-2 cells with the lowest EC$_{50}$ of $9.5 ± 1.4$ and $10.0 ± 1.0$ mg/mL ($p < 0.05$), respectively, followed by Chardonnay, Catawba, Concord, Sheridan, Niagara, Riesling, DeChaunac, Cayuga White, Baco Noir, Chancellor, Vidal Blanc and Marechal Foch. The EC$_{50}$ doses were significantly different among Pinot Noir, Concord, Niagara, Baco Noir and Marechal Foch ($p < 0.05$). The phytochemical extract of Marechal Foch exhibited a weak antiproliferative activity towards Caco-2 cells at a higher dose with an EC$_{50}$ of $37.2 ± 1.5$ mg/mL.

The antiproliferative activities of grape extracts towards HepG$_2$ human liver cancer cells were somewhat different from those towards Caco-2 cells. The phytochemical extracts of Pinot Noir and Chardonnay varieties exhibit the strongest antiproliferative effect towards HepG$_2$ cells with the lowest EC$_{50}$ of $17.0 ± 0.8$ mg/mL and $18.1 ± 0.1$ mg/mL ($p < 0.05$), respectively, followed by Cabernet Franc, Sheridan, Catawba, Riesling, Niagara, Concord, Cayuga

Fig. 2. Total antioxidant activity of phytochemical extracts of 14 grape varieties (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).
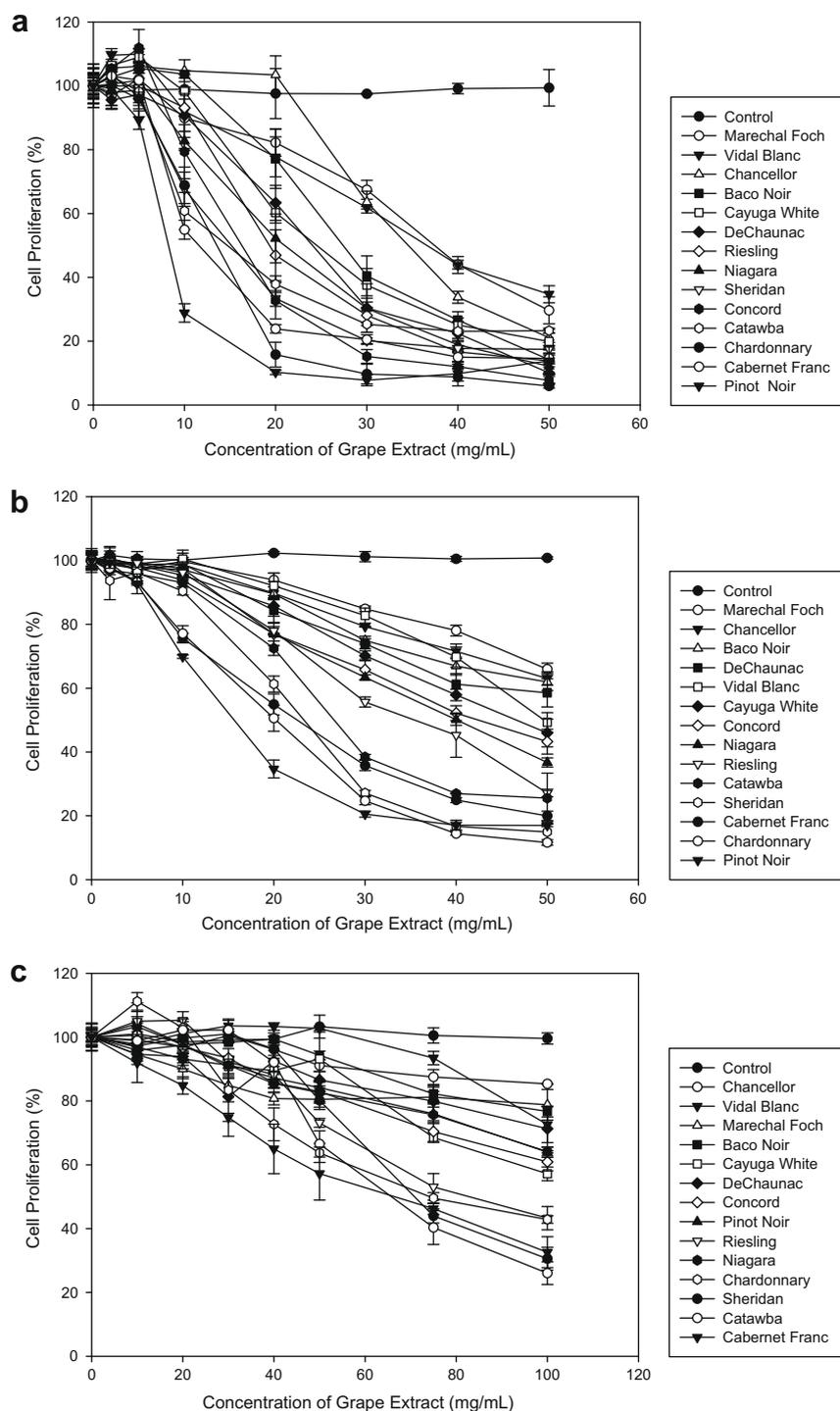
Fig. 3. Percent inhibition of Caco-2 (a), HepG$_2$ (b) and MCF-7 (c) cell proliferation by 14 grape extracts.

White, Vidal Blanc and DeChaunac. Cabernet Franc and Sheridan had similar EC$_{50}$ values ($p > 0.05$). The phytochemical extracts of Vidal Blanc and DeChaunac showed a weak antiproliferative activity at higher doses with the EC$_{50}$ of 52.1 ± 2.1 and 52.2 ± 3.0 mg/mL, respectively. Although the Baco Noir, Chancellor and Marechal Foch varieties demonstrated against HepG$_2$ cell proliferation, the EC$_{50}$ values could not be calculated at the maximum doses used in this experiment.

The antiproliferative activities of grape extracts on MCF-7 human breast cancer cells were different from both HepG$_2$ and Caco-2 cells. The phytochemical extract of the Cabernet Franc variety contained the lowest EC$_{50}$ at 64.0 ± 3.9 mg/mL, indicating the most antiproliferative effect towards MCF-7 cells of the varieties examined ($p < 0.05$). There were statistical differences among the EC$_{50}$ values of Catawba, Chardonnay and Riesling varieties ($p < 0.05$). Although they exhibited inhibition of MCF-7 cell proliferation, the EC$_{50}$ values of the Pinot Noir, Concord, DeChaunac, Cayuga White, Baco Noir, Chancellor, Vidal Blanc and Marechal Foch varieties could not be calculated at the maximum doses used in this experiment.

The 14 grape varieties possessed the greatest ability to inhibit Caco-2 colon cancer cell proliferation than HepG$_2$ and MCF-7
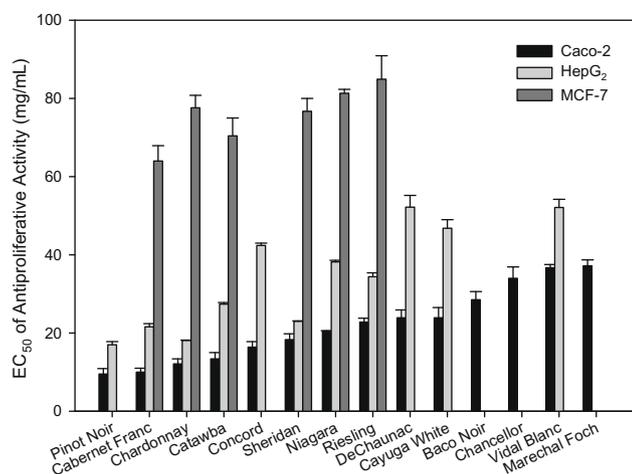
Fig. 4. EC_{50} of antiproliferative activity of phytochemical extracts of 14 grape varieties (mean ± SD, $n = 3$).

human cancer cell proliferation. Also, most grape extracts exhibited higher antiproliferative activity on $HepG_2$ than MCF-7. Although a dose-dependent manner, after exposure to the extracts of Cabernet Franc, Pinot Noir, Chardonnay, Catawba, Concord, Sheridan, Niagara and Riesling, was observed, there was a significant difference among the varieties with respect to antiproliferative activity towards different cell lines. The Pinot Noir and Cabernet Franc grape varieties contained the highest level of inhibitory action against Caco-2 human colon cancer cells, and Marechal Foch possessed the lowest level. The phytochemical extract of the Pinot Noir and Chardonnay varieties exhibited the highest antiproliferative effect towards $HepG_2$ human liver cancer cells with the lowest EC_{50}, and the DeChaunac and Vidal Blanc grapes had the highest EC_{50} values. The possible explanation may be that phytochemicals in grapes target different organs, showing different effects. Since antiproliferative activity in cells involves uptake, metabolism and transcriptional events, it is not surprising that different cell types exhibit different rates of inhibition when presented with the same array of phytochemicals. It was reported that anthocyanidins directly cause human promyelocytic leukemia cells (HL-60) to generate intracellular hydrogen peroxide and trigger apoptosis, possibly through an oxidative stress-involved JNK signalling pathway (Hou et al., 2003).

RSV is a well-known phenolic largely produced in grapevines. It has been reported to perform antiproliferative activity in different human cancer cell lines, such as MCF-7 (Basly, Marre-Fournier, Le Bail, Habrioux, & Chulia, 2000) and Caco-2 colorectal carcinoma (Schneider et al., 2000). The effects of RSV in breast cancer cell lines are inconsistent. Some researchers found that RSV enhances MCF-7 (Basly et al., 2000) cell growth. It was found that RSV induces growth inhibition, S-phase arrest, apoptosis and changes in biomarker expression in MCF-7, SW480, HCE7, Seg-1, Bic-1 and HL60 human cancer cell lines (Joe et al., 2002). On the other hand, RSV was reported to slow down the proliferation of several human malignant cell lines (Hsieh et al., 1999). The suppression effect of RSV in normal cell growth is accompanied by the accumulation of cells in S and G_2 phases (Hsieh et al., 1999). The anti-cancer properties of phenolics in Merlot grapes and wine were evaluated in their ability to modulate gene expression in an athymic nude mouse model transplanted with the oestrogen receptor-negative MDA-MB231 cells (Hakimuddin, Tiwari, Paliyath, & Meckling, 2008). The result presents the potential effects of grape phenolics on gene expression in estrogen receptor-negative tumours and its relation to inhibition of tumour growth. The abilities of RSV

and related compounds to interfere with cell proliferation and their uptake and effects on parameters of the cellular state in $HepG_2$ cells were studied (Colin et al., 2008). It was observed that RSV and related compounds induce changes in cell activity through autofluorescence *in situ* measurement, due to its association with an induction of detoxifying enzyme mechanisms, which is consistent with our previous finding (Yang & Liu, 2008). RSV exerts its antiproliferative effect on $HepG_2$ cells through inducing cell cycle arrest, NOS activation and NO production (Notas et al., 2006). The antiproliferative activities of RSV may be partly explained by the direct inhibition of ribonucleotide reductase, which supplies proliferating cells with deoxyribonucleotides required for DNA synthesis (Fontecave, Lepoivre, Elleingand, Gerez, & Guittet, 1998). However, the correlation between the RSV content and EC_{50} value of the antiproliferative activity for the three human cancer cell lines examined was not found in our study. The possible reason may be the low dosage of RSV in the grape extracts.

Correlations between total phenolics, total flavonoids, total antioxidant activity and cell proliferation EC_{50} values were analysed for the 14 grape varieties. A significant linear relationship was found between total phenolic and total antioxidant activity in the phytochemical extracts of the different grape varieties ($R^2 = 0.9775$, $p < 0.05$). The positive correlation indicates that the higher total phenolic content resulted in a higher total antioxidant activity.

No relationships were found between total antioxidant activity and antiproliferative activity against Caco-2, $HepG_2$ and MCF-7 cell lines ($p > 0.05$). Additionally, the total phenolic and flavonoid contents of grapes did not correlate to antiproliferative activity toward all three cell lines (data not shown), which may suggest that phytochemicals other than those tested in this experiment are responsible for inhibiting cell proliferation, or that the combination of different phytochemicals functions additively and synergistically or antagonistically accounting for the antiproliferative activity of grapes.

4. Conclusion

The oxidative stress arising from an imbalance in the human antioxidant status contributes to the pathology of chronic diseases (Ames, Shigenaga, & Hagen, 1993). Besides endogenous defenses, the consumption of dietary phenolic antioxidants contained in fruits and vegetables plays an important role in protecting against those pathological events. Previously, much attention has been paid to the antioxidant properties of ascorbic acid, tocopherol and β-carotene. In recent years, phytochemicals, especially phenolics, have attracted increasing attention for their antioxidant activities. Grapes provide phenolic antioxidants, which contribute to their potential health benefits. This work has shown that the phytochemicals present in grapes have potent antioxidant and antiproliferative activities and that the antioxidant activity in grapes is positively correlated with total phenolic content. Our results have also found that significant differences in phytochemical content can exist among grape varieties. However, information on the bioavailability and metabolism of phenolics in humans is still scarce; therefore, further studies need to be carried out.

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