Free Radical Scavenging Properties of Skin and Pulp Extracts of Different Grape Cultivars In Vitro and Attenuation of H₂O₂-Induced Oxidative Stress in Liver Tissue Ex Vivo

Indrani Singha · Subir Kumar Das

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Abstract Grapes are the richest source of antioxidants due to the presence of potent bioactive phytochemicals. In this study, the phytochemical contents, scavenging activities and protective role against H₂O₂-induced oxidative stress in liver tissue ex vivo of four grape (*Vitis vinifera*) cultivars extracts, namely Flame seedless (black), Kishmish chorni (black with reddish brown), Red globe (red) and Thompson seedless mutant (green), were evaluated. The total phenolics and flavonoids content in pulp or skin fractions of different grape cultivars were in the range of 47.6–310 mg gallic acid equivalent/g fresh weight (fw), and 46.6–733.3 µg catechin equivalent/g fw respectively. The scavenging activities in skin of different grape varieties against 2,2-diphenyl-1-picrylhydrazyl (44–58 %), hydrogen peroxide (15.3–18.6 %), and hydroxyl radicals (50–85 %), were higher than pulp of the corresponding cultivars. These scavenging activities of grape extracts were found to be significantly ($p < 0.01$) correlated with the levels of total phenols, flavonoids and ascorbic acid. Liver tissues from goat treated with H₂O₂ (500 µM) showed significantly decreased GSH content by 42.9 % and activities of catalase by 50 % and glutathione reductase by 66.6 %; while increased thiobarbituric acid reactive substances and nitric oxide level by 2.53- and 0.86-fold, respectively, and activity of glutathione *S*-transferase by 0.96-fold. Grape skin extracts showed the stronger protective activity against H₂O₂-induced oxidative stress in liver tissue ex vivo, than its pulp of any cultivar; and the Flame seedless (black) cultivar showed the highest potential. In conclusion, our study suggested that the higher antioxidant potential, phytochemical

contents and significant scavenging capacities in pulp and skin of grape extracts showed the protective action of grape extracts against H₂O₂-induced oxidative stress in liver tissue ex vivo.

Keywords Antioxidant · Grapes · Hydrogen peroxide · Liver · Scavenging activity · *Vitis vinifera*

Introduction

Reactive oxygen and nitrogen species (RONS), the by-products of cellular metabolic activity, are a wide range of small signaling molecules that include free radicals such as superoxide anion radical, hydroxyl radical, peroxynitrite (ONOO[−]), and non-free-radicals such as hydrogen peroxide and singlet oxygen [1, 2]. These are constantly generated in cells during normal oxidative processes through a variety of pathways, including both enzyme-catalyzed reactions and nonenzymatic reactions [3]. At physiological concentrations, it may not have harmful effects to cell function [4]. Low to moderate concentrations of RONS show beneficial effects and have physiological roles in cellular responses to anoxia, in defence against infectious agents, in a number of cellular signalling pathways, and the induction of a mitogenic response. The various RONS-mediated actions protect cells against ROS-induced oxidative stress and maintain “redox balance”, also termed “redox homeostasis” [5].

Harmonious cellular metabolic systems are characterized by the perfect balance between the oxidant challenge and antioxidant response [4]. Whenever the balance between RONS generation and the natural antioxidant defense system is lost, due to an increase in RONS production or a decrease in RONS scavenging capacity, this

I. Singha · S. K. Das (✉)
Department of Biochemistry, College of Medicine & JNM
Hospital, WBUHS, Kalyani, Nadia 741235, West Bengal, India
e-mail: drsubirkdas@yahoo.co.in

may leads to a series of events that may cause various pathological conditions to almost all vital organs [5, 6]. The resulting oxidative and nitrosative stress, implicated in several human diseases, subsequently damages the cellular bio-molecules such as nucleic acids, lipids and proteins, ultimately resulting in cell death [4, 7, 8].

Grapes, one of the most popular, widely cultivated, and consumed fruits in the world [9], are rich in phytochemicals, such as sugars, organic acids, mineral salts, vitamins and enzymes [10]. These phytochemicals are responsible for nutraceutical and health benefits [11]. India grows approximately 20 different cultivars of grapes, under diverse soil and climatic conditions including the Thompson seedless being the most popular cultivar. About 120,000 tonnes of Thompson seedless and its mutants, namely, Tas-A-Ganesh, Sonaka and Manik Chaman are dried for raisins. Some 20,000 tonnes of Bangalore Blue are crushed to make juice, and 10,000 tonnes of Bangalore Blue, Cabernet Sauvignon, Chenin Blanc, Chardonnay, Merlot, Pinot Noir and Uni Blanc are crushed to process into wine annually. In past few years, some of the introduced cultivars have been evaluated in different soil and climatic conditions which include Flame seedless, Red globe, Crimson seedless, Italia and Manjri Naveen [12]. However, no study reported on the scavenging properties and cellular protective actions of grape extracts of different cultivars against oxidative stress.

Aims and Objectives

The objective of the present study was to assess the scavenging properties of different grape (*Vitis vinifera*) cultivars in relation to their bioactive phytochemical constituents and also to determine their protective action against H₂O₂-induced oxidative stress in liver tissue *ex vivo*.

Materials and Methods

Chemicals

Catechin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), naphthylethylene diamine dihydrochloride (NEDD) from SIGMA Chemical Co; bovine serum albumin (BSA) (fraction V) from Loba Chemie; aluminum chloride-6 hydrate (AlCl₃, 6H₂O), 2-deoxy-D-ribose, m-phosphoric acid from Alfa-Aesar; 5,5'-dithiobisnitro benzoic acid (DTNB), nicotinamide adenine dinucleotide reduced (NADH), nicotinamide adenine dinucleotide phosphate reduced (NADPH), 5-methylphenazinium methosulfate (PMS), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR), 2,4-dinitrophenyl-

hydrazine (DNPH), from SRL Chemical Co. Folin–Ciocalteu's phenol reagent and gallic acid were purchased from Merck India Ltd. All other chemicals were purchased from SRL India.

Methods

Grape Analysis

Commonly available four grape (*V. vinifera* L.) cultivars, namely 'Thompson seedless' (green), 'Red globe' (red), 'Flame seedless' (black), and 'Kishmish chorni' (black with reddish brown) were purchased from local market, and were authenticated from the Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Nadia.

The skin and pulp of grapes were separated by squeezing the fruits. Proteins [13], sugar [14], ascorbic acid [15], polyphenols [16] and flavonoids [17] contents were determined.

Scavenging Activities *DPPH Radical Scavenging Activity Determination:* DPPH (2.4 mg) was dissolved in 100 ml of methanol and diluted to obtain an absorbance of about 0.98 ± 0.02 at 517 nm. An aqueous solution of 0.1 ml (50 mg/ml) of the extract was added in 3 ml of DPPH solution. After incubation for 15 min in dark; absorbance of the mixture was determined at 517 nm [18].

Hydrogen Peroxide Scavenging Activity Determination: A solution of 50 μ l grape extracts (20 mg/ml) was added to 0.6 ml H₂O₂ solution [40 mM H₂O₂ in phosphate buffer (0.1 M, pH 7.4)] and the total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 240 nm. Phosphate buffer without H₂O₂ was used as blank [19].

Hydroxyl Radical Scavenging Activity Determination: Reagent solution was prepared by sequential addition of ferric chloride (10 mM), 0.25 ml of 2-deoxyribose (2.8 mM) in 50 mM phosphate buffer (pH 7.4), 0.1 ml of 1 mM (1:1 v/v) EDTA solution and 0.1 ml of 10 mM H₂O₂. A solution of 0.1 ml (40 mg/ml) of the extract was added to the reagent solution. Then 0.1 ml of ascorbate (1 mM) was added and incubated at 37 °C for 1 h. In the mixture thiobarbituric acid (TBA) 0.5 % w/v in 1 ml of 50 mM NaOH and 1 ml 10 % w/v trichloroacetic acid (TCA) was added, cooled to room temperature after incubation in a boiling water bath for 15 min. Intensity of chromogen was read at 532 nm [18].

The scavenging activities were expressed as a percentage of DPPH•/OH•/H₂O₂ scavenged from the following equation: $\{(A_0 - A_s)/A_0 \times 100\}$, where A₀—absorbance of control and A_s—absorbance in presence of grape extract (sample).

Ex Vivo Studies

The goat liver was collected fresh from a local slaughter house, plunged into cold sterile phosphate buffered saline (PBS) and maintained at 4 °C till its use within 2 h of collection. One gram of goat liver slice of 1 mm thickness was mixed with 4.0 ml of sterile PBS in each of ten flasks. The oxidizing agent H₂O₂ (500 µM) and/or the grape extract (10 mg) from skin or pulp of each cultivar were added and incubated at 37 °C with mild shaking for 1 h. Appropriate controls were also set up. After incubation, the goat liver slices were homogenized in the incubation medium and each the homogenate was used for assaying protein [13], thiobarbituric acid reactive substances (TBARS) [20], nitrite [21], reduced glutathione (GSH) [22], as well as activities of catalase (EC 1.11.1.6) [23], glutathione peroxidase (GPx; EC 1.11.1.9) [24], GR (EC 1.6.4.2) [25] and glutathione *S*-transferase (GST; EC 2.5.1.18) [26].

Statistical Analysis

Analyses of grape phytochemicals were performed in triplicate, while those performed on the liver slices were replicated five times and the results were expressed as the mean ± standard error (SE). Statistical significance was established by the one-way analysis of variance (ANOVA), followed by Tukey test for individual differences using SPSS, version 13.0 (SPSS Inc., Chicago, IL). Pearson correlation was used to establish the association of phytochemicals with scavenging activities. A $p < 0.05$ was set to establish, the statistical significance.

Results

As shown in Table 1, a significant difference ($p < 0.05$) in grape phytochemical content between skin and pulp within the same cultivar was observed. The protein, ascorbic acid, polyphenols and flavonoid content were found in higher concentration in grape skin compared to its pulp; while carbohydrate content was found higher in concentration in grape pulp compared to its skin (Table 1). Among the grape skin of different cultivars, Flame seedless (black) cultivar showed highest concentration of protein, ascorbic acid, polyphenols and flavonoid, while Thompson seedless (green) cultivar showed lowest concentration. However, maximum carbohydrate content was found in Thompson seedless (green) pulp (Table 1). The total phenol and flavonoid contents varied from 47.6 to 310 mg gallic acid equivalent and 46.6–733.3 µg catechin equivalent, respectively in 100 g fresh weight (Table 1). Data analysis showed a

Table 1 Total protein, sugar, polyphenols, flavonoids and ascorbic acid content in different grape cultivars

	Thompson seedless (green)		Flame seedless (black)		Kishmish chorni (black with reddish brown)		Red globe (red)	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
Protein (mg/100 g fresh wt)	180 ± 10.06*	42 ± 2.88	389.67 ± 7.12*	90.67 ± 3.53	260.67 ± 26.62*	54.33 ± 4.66	164.67 ± 5.92*	30.67 ± 1.85
Sugar (g/100 g fresh wt)	13.67 ± 0.88*	21.67 ± 0.66	10.27 ± 0.48*	16.3 ± 1.1	9.27 ± 0.17	12.63 ± 0.28	9.6 ± 0.11**	13.6 ± 0.88
Polyphenols (GAE mg/100 g fresh wt)	220.67 ± 12.72*	47.67 ± 2.03	310 ± 14.42*	67.33 ± 2.6	296.67 ± 8.11*	59.33 ± 2.4	140.67 ± 9.26*	38.67 ± 2.6
Flavonoids (CE µg/100 g fresh wt)	638.67 ± 24.34*	63.67 ± 2.33	733.33 ± 37.56*	47.33 ± 2.4	708 ± 23.46*	46.67 ± 2.73	534 ± 15.4*	54.33 ± 2.03
Ascorbic acid (mg/100 g fresh wt)	27.67 ± 0.33*	3.03 ± 0.09	38.4 ± 0.3*	5.63 ± 0.26	25.67 ± 1.69*	5.1 ± 0.17	26.67 ± 1.45*	6.2 ± 0.25

Values are mean ± SE (standard error). *P* values: * <0.001, ** <0.05 compared between skin and pulp within the same grape cultivar

GAE gallic acid equivalent, CE catechin equivalent

significant correlation between phenol and flavonoid ($r = 0.947$, $p < 0.01$).

Though both skin and pulp extracts of grape possess scavenging properties, grape skin showed higher scavenging activity than its pulp. Among the parameters tested, skin of Flame seedless (black) cultivar showed highest scavenging activity (Table 2). Hydrogen peroxide scavenging activity varied from 4.6 to 18.6 %, at a concentration of 1 mg in 50 μ l grape extracts depending on grape cultivar and extract (Table 2). Hydroxyl radical and DPPH radical scavenging activities in grape skin or pulp were found in the order Flame seedless (black) > Kishmish chorni (black with reddish brown) > Thompson seedless (green) = Red globe (Table 2). These scavenging activities were found significantly ($p < 0.01$) correlated with total polyphenols, flavonoids or ascorbic acid (Table 3).

The scavenging properties in grapes were evaluated against H_2O_2 -induced oxidative stress in intact liver tissues ex vivo (Table 4). H_2O_2 treatment (500 μ M) significantly ($p < 0.001$) decreased GSH content by 42.9 % and activities of catalase by 50 % and GR by 66.6 %; while increased TBARS and nitric oxide level by 2.53- and 0.86-fold, respectively, and activity of GST by 0.96-fold (Table 4). While Flame seedless (black) cultivar skin treatment increased GSH content by 58.4 % ($p < 0.001$), Red globe cultivar skin increased GSH content by 33.6 % ($p < 0.001$), compared to H_2O_2 -treated liver slice (Table 4). While skin of Flame seedless (black) cultivar reduced TBARS and nitrite content by 59.4 and 31.3 %, respectively ($p < 0.001$), pulp of Thompson seedless (green) cultivar reduced TBARS and nitrite content by 25.7 and 12.2 % respectively ($p < 0.001$), compared to oxidant treated group (Table 4). Skin of Flame seedless (black) cultivar increased activities of catalase (Table 4) and GR (Table 4) by 50.8 and 101.8 %, while decreased GST activity 48.7 % (Table 4) compared to H_2O_2 treated group ($p < 0.001$). However, pulp of Red globe cultivar treatment non-significantly increased catalase activity by 17.4 % and GR activity by 15.1 % (Table 4).

Discussion

A large portion of the soluble solid in grapes is sugar that varied between 9.2 g to 21.6 g/100 g of fresh weight in this study (Table 1). The protein content varied between 30 and 390 mg/100 g fw depending on cultivar, and skin or pulp. While grape pulp contains high sugar content, other phytochemicals are abundant in skin fractions. However, the biological importance of grape extracts is mainly attributed to its antioxidant properties [27, 28]. Ascorbic acid, a cytosolic antioxidant that present in grapes at low concentrations in pulp than skin of the same cultivar, is reported to be rapidly oxidized [29]. Among the dietary

Table 2 Percent (%) scavenging activity of different grape cultivars

	Thompson seedless (green)		Flame seedless (black)		Kishmish chorni (black with reddish brown)		Red globe (red)	
	Skin	Pulp	Skin	Pulp	Skin	Pulp	Skin	Pulp
H_2O_2	15.67 $\pm$ 0.89*	4.67 $\pm$ 0.67	18.67 $\pm$ 1.02	13.33 $\pm$ 0.89	15.33 $\pm$ 1.45***	9.33 $\pm$ 0.89	17 $\pm$ 1.16	7.67 $\pm$ 2.09
Hydroxyl radical	50.33 $\pm$ 1.76	40.33 $\pm$ 1.45	85.33 $\pm$ 8.21*	56.33 $\pm$ 2.33	80.67 $\pm$ 2.03*	48.33 $\pm$ 2.03	76.67 $\pm$ 1.76*	42.33 $\pm$ 0.89
DPPH radical	44.33 $\pm$ 1.45**	28.67 $\pm$ 1.76	58 $\pm$ 2.65*	36 $\pm$ 1.16	54 $\pm$ 3.05*	33 $\pm$ 0.57	44.67 $\pm$ 1.76	38.67 $\pm$ 2.03

Values are mean $\pm$ SE (standard error). P values: * <0.001 , ** <0.01 , *** <0.05 compared between skin and pulp within the same grape cultivar

Table 3 Correlation between phytochemicals with scavenging activity of different radicals

	H ₂ O ₂	Hydroxyl radical	DPPH radical
Polyphenols	0.74* (<0.001)	0.762* (<0.001)	0.889* (<0.001)
Flavonoids	0.795* (<0.001)	0.761* (<0.001)	0.861* (<0.001)
Ascorbic acid	0.837* (<0.001)	0.784* (<0.001)	0.87* (<0.001)

Figures in the parentheses are showing the level of significance

* Correlation is significant at the 0.01 level (2-tailed)

antioxidants, the phenolic compounds, secondary metabolites occurring in plants, are the most abundant natural antioxidants [30]. They protect plants against harmful environmental conditions and are divided in two main categories, namely, flavonoids and nonflavonoids [31]. The significant correlation between the total polyphenol content and flavonoids content, as observed in this study, was also reported in other studies, in grape pomace extracts [32] and buckwheat extracts [33]. The phytochemical composition of grapes, however, varied not only among the different cultivars, but also on skin or pulp of the same cultivar.

The antioxidant potential of phytochemicals is generally linked to their ability to scavenge free radicals [34]. In the present study, the antioxidant properties of grape extracts were evaluated by hydrogen peroxide (H₂O₂), hydroxyl radical (OH•) and DPPH-scavenging assays. Studies have reported the free radical scavenging activity measured by DPPH assay and hydroxyl radicals and hydrogen peroxide scavenging by grape extracts [11, 35]. In this study, grape skin showed higher scavenging activity compared to its pulp, and Flame seedless cultivar exhibited highest activity. Earlier studies had also shown that grape skin was good source of phenolics, had a high antioxidant capacity, and could be used as a potential source of natural antioxidants [36]. Significant correlation between the scavenging activities of grape extracts with phytochemicals, such as total phenolics and flavonoids, as observed in the current study, is in agreement with the previous studies [16, 37, 38].

Hydrogen peroxide (H₂O₂), a non-radical ROS in living organisms, has the ability to penetrate cell membranes, inactivate enzymes by oxidation of thiol groups, and initiate lipid peroxidation [39]. It may give rise to hydroxyl radicals (OH•) and singlet oxygen by reacting with transition metal ions [40]. Hydroxyl radical has the potential of reacting with almost every cellular macromolecules and thereby inducing tissue damage [41, 42]. The scavenging activities of the grape extracts due to its phytochemicals

were evaluated for protection of biological systems against oxidants, such as H₂O₂. Substantial efforts have been made towards the development of alternative methods to safety studies using laboratory animals in accordance with international acceptance. Animal testing not only should cause as little suffering to animals as possible, but also animal tests should only be performed where necessary. Therefore, precision cut liver slices were used as an in vitro (ex vivo) appropriate model in the present study, mainly because of its simplicity, ease of preparation, retention to normal organ architecture, and the ability to obtain multiple slices from each organ [43].

The tissue GSH concentration reflects its potential for detoxification and is critical in preserving the proper cellular redox balance for its role as a cellular protectant [44]. The depletion in GSH sensitizes the tissue to oxidative injury and sets up a vicious cycle. Decreased level of GSH, and increased levels of TBARS and nitrite indicates the increased oxidative and nitrosative stress [23]. The reversal of these parameters by grape extracts of different cultivars and skin or pulp, in varying amount as observed in the present study, elicit a varying degree of protective response against the toxic affects of H₂O₂.

Hydrogen peroxide is detoxified to water by catalases and peroxidases. Catalase is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent in the cytosol [45]. Decreased catalase activity as observed in the current study might be due to loss of NADPH or generation of superoxide [46]. Catalase is more important when H₂O₂ levels are low. At higher concentrations of H₂O₂, GPx is responsible for detoxifying H₂O₂. During this process, GSH is oxidized to oxidized glutathione disulfide (GSSG) which is then reduced back to GSH by GR using NADPH as a co-factor [46]. Significant decrease in GPx activity in isolated liver tissue due to H₂O₂ treatment might be due to free radical-dependent inactivation of the enzyme or depletion of co-substrates, i.e. GSH and NADPH [46]. GST plays an essential role in liver by eliminating toxic compounds by conjugating them with GSH. Increased GST and decreased GR activities are important factors in H₂O₂-induced oxidative stress [46]. The liver tissues treated with grape extracts attenuate the H₂O₂-induced alterations in antioxidant status.

In conclusion, our study suggested that scavenging properties of extracts of grape skin and pulp and can attenuate H₂O₂-induced alterations in oxidative stress in liver tissue ex vivo. This study also indicated that grape skin possesses higher antioxidant potential than pulp of the same cultivar, and the presence of different phytochemicals contents varies among the different cultivars of grape.

Table 4 Effect of skin and pulp of different grape cultivars on reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and nitrite content and activities of catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) in H₂O₂-treated intact liver tissue ex vivo

	GSH (μ g/mg tissue)	TBARS (μ mole MDA formed/min/ 100 mg tissue)	Nitrite (nM/100 mg tissue)	Catalase (μ mole H ₂ O ₂ decomposed/ min/mg protein)	GPx (μ mole NADPH breakdown/min/ 100 mg protein)	GR (μ mole NADPH breakdown/min/ 100 mg protein)	GST (μ mole CDNB conjugate formed/ min/mg protein)
Control	2.19 $\pm$ 0.039	0.156 $\pm$ 0.007	0.462 $\pm$ 0.013	25.2 $\pm$ 0.86	0.39 $\pm$ 0.011	0.318 $\pm$ 0.009	0.308 $\pm$ 0.013
H ₂ O ₂ -treated	1.25 $\pm$ 0.016 ^a	0.552 $\pm$ 0.023 ^a	0.856 $\pm$ 0.04 ^a	12.6 $\pm$ 0.87 ^a	0.21 $\pm$ 0.007 ^a	0.106 $\pm$ 0.006 ^a	0.604 $\pm$ 0.018 ^a
H ₂ O ₂ + Thompson seedless treated							
Skin	1.73 $\pm$ 0.038 ^{ad}	0.308 $\pm$ 0.01 ^{ad}	0.66 $\pm$ 0.017 ^{ad}	15.4 $\pm$ 0.87 ^a	0.25 $\pm$ 0.005 ^a	0.206 $\pm$ 0.007 ^{ad}	0.38 $\pm$ 0.01 ^{cd}
Pulp	1.43 $\pm$ 0.019 ^{af}	0.41 $\pm$ 0.011 ^{ad}	0.752 $\pm$ 0.018 ^{af}	15.4 $\pm$ 0.81 ^a	0.21 $\pm$ 0.007 ^a	0.142 $\pm$ 0.006 ^a	0.454 $\pm$ 0.017 ^{ad}
H ₂ O ₂ + Flame seedless treated							
Skin	1.98 $\pm$ 0.058 ^{bd}	0.224 $\pm$ 0.01 ^{cd}	0.588 $\pm$ 0.018 ^{cd}	19 $\pm$ 0.45 ^{ad}	0.29 $\pm$ 0.008 ^{ad}	0.214 $\pm$ 0.006 ^{ad}	0.406 $\pm$ 0.014 ^{bd}
Pulp	1.54 $\pm$ 0.029 ^{ad}	0.342 $\pm$ 0.013 ^{ad}	0.672 $\pm$ 0.02 ^{ad}	16.2 $\pm$ 0.66 ^a	0.26 $\pm$ 0.008 ^{af}	0.164 $\pm$ 0.007 ^{ad}	0.468 $\pm$ 0.009 ^{ad}
H ₂ O ₂ + Kishmish chorni treated							
Skin	1.77 $\pm$ 0.03 ^{ad}	0.294 $\pm$ 0.008 ^{ad}	0.632 $\pm$ 0.013 ^{ad}	16.2 $\pm$ 0.58 ^a	0.27 $\pm$ 0.009 ^{ad}	0.174 $\pm$ 0.009 ^{ad}	0.368 $\pm$ 0.009 ^d
Pulp	1.45 $\pm$ 0.019 ^{ae}	0.366 $\pm$ 0.012 ^{ad}	0.702 $\pm$ 0.018 ^{ad}	15.2 $\pm$ 0.86 ^a	0.23 $\pm$ 0.007 ^a	0.152 $\pm$ 0.009 ^{af}	0.434 $\pm$ 0.019 ^{ad}
H ₂ O ₂ + Red globe treated							
Skin	1.67 $\pm$ 0.024 ^{ad}	0.288 $\pm$ 0.006 ^{ad}	0.65 $\pm$ 0.016 ^{ad}	18.2 $\pm$ 0.8 ^{ae}	0.26 $\pm$ 0.007 ^{af}	0.18 $\pm$ 0.01 ^{ad}	0.378 $\pm$ 0.01 ^{cd}
Pulp	1.48 $\pm$ 0.025 ^{ae}	0.38 $\pm$ 0.01 ^{ad}	0.738 $\pm$ 0.017 ^{af}	14.8 $\pm$ 0.97 ^a	0.23 $\pm$ 0.009 ^a	0.122 $\pm$ 0.008 ^a	0.428 $\pm$ 0.015 ^{ad}

Values are mean $\pm$ SE of 5 observationsP values: ^a <0.001, ^b <0.005, ^c <0.05 compared to control group; ^d <0.001, ^e <0.005, ^f <0.05 compared to H₂O₂-treated group

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References

- Ellis A, Triggler CR. Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. *Can J Physiol Pharmacol*. 2003;81:1013–28.
- Inoue M, Sato EF, Nishikawa M, Park AM, Kira Y, Imada I, Utsumi K. Cross talk of nitric oxide, oxygen radicals, and superoxide dismutase regulates the energy metabolism and cell death and determines the fates of aerobic life. *Antioxid Redox Signal*. 2003;5(4):475–84.
- Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. *Drug Resist Updat*. 2004;7(2):97–110.
- Reddy SV, Suchitra MM, Reddy YM, Reddy PE. Beneficial and detrimental actions of free radicals: a review. *J Global Pharma Technol*. 2010;2:3–11.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44–84.
- Thomas CE, Kalyanaraman B, editors. Oxygen radicals and the disease process. The Netherlands: Hardwood Academic Publishers; 1997.
- Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol*. 1990;186:1–85.
- Förstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract Cardiovasc Med*. 2008;5(6):338–49.
- la Cruz AA, Hilbert G, Mengin V, Rivière C, Ollat N, Vitrac C, Bordenave L, Decroocq S, Delaunay JC, Mérillon JM, Monti JP, Gomès E, Richard T. Anthocyanin phytochemical profiles and anti-oxidant activities of *Vitis candicans* and *Vitis doaniana*. *Phytochem Anal*. 2013;24(5):446–52.
- Waterhouse AL. Wine phenolics. *Ann NY Acad Sci*. 2002;957:21–36.
- De Nisco M, Manfra M, Bolognese A, Sofo A, Scopa A, Tenore GC, Pagano F, Milite C, Russo MT. Nutraceutical properties and polyphenolic profile of berry skin and wine of *Vitis vinifera* L. (cv. Aglianico). *Food Chem*. 2013;140(4):623–9.
- Shikhamany SD. Grape production in India. <http://www.fao.org/docrep/003/x6897e/x6897e06.htm>.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. *J Biol Chem*. 1951;193:265–75.
- Hedge JE, Hofreiter BT. Determination of total carbohydrate by anthrone method. In: Whistler RL, BeMiller JN, editors. Carbohydrate chemistry. New York: Academic Press; 1962. p. 17.
- Olliver M. Ascorbic acid estimation. In: Sobrell WH, Harris RS, editors. The vitamins. New York: Academic Press; 1967. p. 338.
- Weidner S, Powalka A, Karamać M, Amarowicz R. Extracts of phenolic compounds from seeds of three wild grapevines—comparison of their antioxidant activities and the content of phenolic compounds. *Int J Mol Sci*. 2012;13:3444–57.
- Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, Pieters L, Vlietinck AJ, Vanden Berghe D. Structure-activity relationship and classification of flavonoids as inhibitors of Xanthine oxidase and superoxide scavengers. *J Nat Prod*. 1988;61:71–6.
- Shabbir M, Khan MR, Saeed N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. *BMC Complement Altern Med*. 2013;13:143.
- Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. 1989;10:1003–8.
- Sinnhuber RO, Yu TC, Yu TC. Characterization of the red pigment formed in the thiobarbituric acid determination of oxidative rancidity. *Food Res*. 1958;23:626–30.
- Kleinbongard P, Rasaf T, Dejam A, Kerber S, Kelm M. Griess method for nitrite measurement of aqueous and protein containing sample. *Meth Enzymol*. 2002;359:158–68.
- Ellman GL. The sulphydryl groups. *Arch Biochem Biophys*. 1959;32:70–7.
- Das SK, Vasudevan DM. Modulation of lecithin activity by vitamin-B complex to treat on ethanol induced oxidative stress in liver. *Indian J Exp Biol*. 2006;44:791–801.
- Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxides. *J Lab Clin Med*. 1967;70:158–9.
- Goldberg MD, Spooner JR. Glutathione reductase. In: Bergmayer HU, Bergmayer J, Grabl M, editors. Methods enzyme analysis, vol. III, 3rd edn. Florida: Academic Press, Inc.; 1983. p. 258–65.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. *J Biol Chem*. 1974;249:7130–9.
- Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*. 1992;339(8808):1523–6.
- Soleas GJ, Diamandis EP, Goldberg DM. Wine as a biological fluid: history, production, and role in disease prevention. *J Clin Lab Anal*. 1997;11(5):287–313.
- Yang J, Xiao YY. Grape phytochemicals and associated health benefits. *Crit Rev Food Sci Nutr*. 2013;53(11):1202–25.
- Fiorentino A, D'Abrascia B, Pacifico S, Mastellone C, Piscopo V, Caputo R, Monaco P. Isolation and structure elucidation of antioxidant polyphenols from quince (*Cydonia vulgaris*) Peels. *J Agric Food Chem*. 2008;56:2660–7.
- Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr*. 2005;81(1):230S–42S.
- de Campos Luanda MAS, Fernanda VL, Rozangela CP, Sandra RS. Free radical scavenging of grape pomace extracts from Cabernet sauvignon (*Vitis vinifera*). *Bioresour Technol*. 2008;99:8413–20.
- Sun T, Ho CT. Antioxidant activities of buckwheat extracts. *Food Chem*. 2005;90:743–9.
- Garcia-Alonso FJ, Guidarelli A, Periago MJ. Phenolic-rich juice prevents DNA single-strand breakage and cytotoxicity caused by tert-butylhydroperoxide in U937 cells: the role of iron chelation. *J Nutr Biochem*. 2007;18(7):457–66.
- Correia HS, Batista MT, Dinis TC. The activity of an extract and fraction of *Agrimonia eupatoria* L. against reactive species. *BioFactors*. 2007;29(2–3):91–104.
- Bunea CI, Pop N, Babeş AC, Matea C, Dulf FV, Bunea A. Carotenoids, total polyphenols and antioxidant activity of grapes (*Vitis vinifera*) cultivated in organic and conventional systems. *Chem Cent J*. 2012;6(1):66.
- Caillet S, Salmieri S, Lacroix M. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. *Food Chem*. 2006;95:1–8.
- Lutz M, Jorquera K, Cancino B, Ruby R, Henriquez C. Phenolics and antioxidant capacity of table grape (*Vitis vinifera* L.) cultivars grown in Chile. *J Food Sci*. 2011;76(7):C1088–93.
- Zhang A, Fang Y, Wang H, Li H, Zhang Z. Free-radical scavenging properties and reducing power of grape cane extracts from 11 selected grape cultivars widely grown in china. *Molecules*. 2011;16:10104–22.

40. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd ed. London: Oxford University Press; 1999. p. 608–10.
41. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J*. 1984;219:1–4.
42. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem*. 2005;53:1841–56.
43. Catania JR, McGarrigle BP, Rittenhouse-Olson K, Olson JR. Induction of CYP2B and CYP2E1 in precision-cut rat liver slices cultured in defined medium. *Toxicol In Vitro*. 2007;21(1):109–15.
44. Mari M, Wu D, Nieto N, Cederbaum AI. CYP2E1-dependent toxicity and up-regulation of antioxidant genes. *J Biomed Sci*. 2001;8(1):52–5.
45. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care*. 2008;31(Suppl 2):S170–80.
46. Das SK, Vasudevan DM. Effect of ethanol on liver antioxidant defense systems: a dose dependent study. *Indian J Clin Biochem*. 2005;20(1):80–4.