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Free Radical Scavenging and Lipid Peroxidation Activity of the Shahani Black Grape

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Abstract: The present study was designed to evaluate antioxidant activity of different parts of Shahani black grape berries. The antioxidant activity of grape berry juice, seed and skin extracts were measured by the inhibition of lipid peroxidation (Ferric Ammonium Thiocyanate) and free radical scavenging activity (2, 2-diphenyl-1-picrylhydrazyl) methods. Vitamin E and Butylated Hydroxy Toluene (BHT) were used as reference values. The free radical scavenging capacity of grape extracts followed this order: seed methanol extract> skin extract> grape juice> seed hexane extract. Meanwhile, inhibitions of lipid peroxidation of seed methanol and hexane extracts were the highest, grape skin extract activity was intermediate and that of grape juice was the lowest. It seems that the antioxidant activity of samples from grape seed and skin extracts to be mainly based on inhibition of lipid peroxidation, whereas the Antioxidant Activity of grape juice is based on free radical scavenging activity. The results indicate that Shahani black grape has potent antioxidant activity specially on lipid peroxidation and has beneficial effects on human health and help to prevent disease which are caused by free radicals.

Key words: *Vitis vinifera*, Shahani black grape, lipid peroxidation, free radical scavenging, Vitaceae

INTRODUCTION

Antioxidants are hypothesized to play an important role in chronic disease prevention, because they might be able to prevent oxidative damage caused by reactive oxidant species to vital biomolecules such as DNA, lipids and proteins. Oxidative damage might be involved in several pathological conditions such as atherosclerosis, cancer and chronic inflammation (Halliwell, 1994). Plant phenols are antioxidants because of their chemical nature: hydroxyl groups attached to the phenyl ring. The antioxidant capacity of these plant phenolics is similar to or even higher than that of the well-known dietary antioxidant vitamin E (Rice-Evans *et al*., 1996). The potential relevance of the antioxidant properties of plant phenols in the prevention of cardiovascular disease is illustrated by experiments with isolated Low-density Lipoproteins (LDL). In these experiments a whole range of antioxidants can prevent the oxidation of LDL *in vitro*. Oxidised LDL is atherogenic and is considered to be a crucial intermediate in the development of cardiovascular disease (Steinberg *et al*., 1997).

Principal source of antioxidant chiefly include those of herbs, spices and medicinal plants. Grape (*Vitis vinifera* L. Vitaceae) is one of the world's largest fruit crops, which approximate an annual production of 58 million metric tons (Kotamballi, 2002). Grape seeds are a rich source of monomeric phenol compounds, such as (+)-catechins, (-) - epicatechin and (-) - epicatechin 3-O-gallate, dimeric and trimeric procyanidins and these compounds act as anti mutagenic and antiviral agents (Saito *et al*., 1998). There are reports of the possible use of grape phenols in preventing atherosclerosis and myocardial infarction (Bagchi *et al*., 2000; Fuhrman *et al*., 2005). Recognition of such health benefits of catechin and procyanidins has led to the use of grape seed extract as a dietary supplement (Sehirli, 2008). The phenols in grapes have been reported to inhibit human Low Density Lipoprotein (LDL) oxidation *in vitro* and *in vivo* (Teissedre *et al*., 1996; Jinming *et al*., 1998). Grape products (rich in polyphenols), inhibit platelet aggregation (PA), a risk factor for coronary artery disease and combining extracts of grape seed and grape skin (primary sources of grape polyphenols), which individually shown to inhibit PA, might enhance their individual antiplatelet effects (Shanmuganayagam *et al*., 2002). Consumption of raisin (dried grape) is very useful for kidney and bladder. Grape and raisin with seed are effective for gastric pain, but eating of fresh grape in large amount is not useful for bladder (Avicenna, 1991). There are some reports about antioxidant effects of different varieties of grape and grape seed methanol extract (Jayapvakasha *et al*., 2003; Kotamballi *et al*., 2002;

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Ruberto *et al*., 2007) to denote strong antioxidant activity of this fruit crop. Because of these useful properties of grape we decided to evaluate antioxidant activity of different parts of Shahani black grape from Chahar-Mahal Bakhtiari Province which grows in Ghazvin and Kermanshah Provinces too. This is the first report from antioxidant activity of this kind grape that cultivated in Iran.

MATERIALS AND METHODS

Plant material: *Vitis vinifera* L. (Shahani black grape) fruits (Fig. 1) were collected in Sep. 2006 from Shahrekord (Chahar-Mahal Bakhtiari Province), Iran. Grape berry seeds, skin and juice (obtained by fruits pressing) were used in this experiment. The voucher specimen is deposited in Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences.

Chemicals: Linoleic acid (approx. 95%), vitamin E and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Company, methanol, hydrochloric acid 37%, ammonium thiocyanate, ferrous chloride tetrahydrate, di-sodium hydrogen phosphate $12H_2O$, mono-sodium dihydrogen phosphate $2H_2O$, absolute ethanol, n-hexane and BHT were obtained from Merck company.

Extraction: Washed and dried grape seeds were powdered and extracted in a soxhlet extractor with hexane (6 h) for removal of the fatty matter (Seed hexane extract), then defatted seed powder was extracted for 10 h with methanol (Seed methanol extract). Grape berry skin was extracted in a soxhlet extractor for 10 h with methanol (Skin methanol extract). These extracts were evaporated at low pressure and temperature. The juice was prepared after pressing of the fruits and filtering. Dry matter of juice and berry skin extract was calculated by drying of the samples at 100°C for 2-4 h.

Fig. 1: Shahani black grape, an cultivated species of *Vitis vinifera* L. in Iran

Inhibition of lipid peroxidation: The Ferric thiocyanate (FTC) method, modified in our laboratory, was used (Yassa *et al*., 2005; Sharififar *et al*., 2003). A mixture of samples was prepared in a screw cupped tube from seed hexane extract at different concentrations in methanol (0.20, 0.10 and 0.05 mg mL^{-1}) (1 mL), an emulsion of 2.51% linoleic acid in absolute ethanol (0.5 mL) and 0.05 M sodium hydrogen phosphate buffer, pH = 7 (1 mL), shacked and incubated in oven at 40°C. The same reaction mixture without sample extract was used as the control. To 0.1 mL of these solutions were added 4.7 mL of 75% ethanol and 0.05 mL of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.05 mL of 0.02M ferrous chloride in 3.5% hydrochloric acid to the reaction mixtures, the absorbance was measured against a reagent blank at 500 nm, each 24 h (t) until 1 day after absorbance of the control reached a maximum (120 h). The same reaction was used for juice, seed and skin methanol extract. These samples were used at different concentrations for example: juice (7.92, 3.96 and 1.98 mg mL^{-1}); seed methanol extract (0.100, 0.005, 0.003 and 0.001 mg mL^{-1}) and skin methanol extract (0.830, 0.410 and 0.205 mg mL^{-1}). Vitamin E and BHT were included as standard antioxidant for the comparison. The degree of linoleic acid peroxidation was calculated during 120 h using the following formula:

$$AA\ (\%) = \frac{[1\text{-(absorbance of sample at 500 nm t)}]}{\text{(absorbance of control at 500 nm t)}} \times 100$$

Free radical scavenging: The DPPH method modified in our laboratory was used (Sánchez-Moreno *et al*., 1999). A mixture of sample from seed hexane extract (1 mL) at different concentration in methanol (25, 10, 5 mg mL^{-1}) was added to 2 mL of DPPH solution (4×10^{-5} g mL^{-1} MeOH). Control consist of sample was added to methanol up to 3 mL; Blank consist of 1 mL methanol without sample was added to 2 mL of DPPH solution.

Absorbance of mixture was measured at different times 0, 5, 10, 15, 20, 25 and 30 min by a Shimadzu, UV/VIS model 160A Spectrophotometer at 517 nm. The same reaction was used for juice, skin and seed methanol extracts. These extracts were prepared at different concentrations for example: juice (23.76, 7.92, 3.96 and 0.79 mg mL^{-1}); seed methanol extract (0.025, 0.015, 0.010 and 0.005 mg mL^{-1}) and skin methanol extract (16.4, 4.1 and 0. 82 mg mL^{-1}). The percentage of inhibition activity was calculated as follows:

$$\text{Inhibition (\%)} = 100 - \frac{(\text{Sample absorbance} - \text{Control absorbance})}{\text{Blank absorbance}} \times 100$$

Analyses of at least three samples were carried out in triplicate. For calculating of [IC_{50}] or [AA_{50}], in DPPH and FTC methods, the percentage of inhibition against sample concentration was plotted to obtain the amount of antioxidant necessary to cause 50% percent inhibition activity.

Statistical analysis: The results are expressed as Means±SD of IC_{50}. Student's t-test has been down to compare the data and all tests were considered statistically significant at $p < 0.05$. Results were processed by the Excel XP 2003. IC_{50} was calculated with CurveExpert 1.3.

RESULTS AND DISCUSSION

Lipid autoxidation is a free radical process which proceeds via a chain reaction including induction, propagation and termination steps. Two commonly used methods to evaluate the Antioxidant Activity (AA) in food and biological systems are the ferric thiocyanate (FTC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) procedures. The FTC method measures the AA by the inhibition of lipid peroxidation (peroxide compounds formed during lipid oxidation) and DPPH method measures the free radical scavenging capacity of the antioxidants (Yassa *et al*., 2005; Sharififar *et al*., 2003; Sánchez-Moreno *et al*., 1999). Taking into account of the antioxidant activity determined by these methods it may be possible to elucidate the predominant mechanism of each sample: inhibition of lipid peroxidation or free radical scavenging. Inhibition concentration (IC_{50}) of DPPH method in the standards (BHT and vitamin E) and grape extracts followed the order: seed methanol extract > vitamin E > BHT > skin extract > grape juice > seed hexane extract. This result was showed free radical scavenging power of seed methanol extract is higher than other samples even more than vitamin E and BHA (Table 1).

The methanol extract of grape seed contains proanthocyanidins which have antioxidant activity. Proanthocyanidins are anti-carcinogen (Kim *et al*., 2005) anti-atherogen (Nikitina *et al*., 2006) and anti-inflammation (Li *et al*., 2001) and may inhibit platelet aggregation (Keevil *et al*., 1998). Grape seed proanthocyanidins are also claimed to exert anti-diabetic effects by preserving pancreatic β-cell function (Abir *et al*., 2005). Grape seed extract may be useful as antibacterial agents to prevent the deterioration of food products (Baydar *et al*., 2004).

Table 1: Concentration of sample necessary to reduce by 50% inhibition of lipid peroxidation and free radical scavenging

Samples	FTC method[e] X[d]	DPPH method[e] X[d]
Seed methanol extract	3.96±1.30[a]	14.01±0.08[a]
Seed hexane extract	37.88±2.10[b]	6372.00±0.12[c]
Skin extract	168.92±1.98[c]	1090.70±0.03[c]
Juice	5734.00±3.50[c]	4356.00±0.14[c]
BHT	147.34±2.04	127.33±0.07
Vitamin E	25.53±2.20	20.92±0.08

[a]:Significant when vitamin E was compared to other extracts ($p < 0.05$), [b]:Significant when BHA was compare to other extracts ($p < 0.05$), [c]:Not significant when vitamin E and BHA were compared to other extracts ($p > 0.05$), [d]X: Mean values (n=3)±SD [e]: Expressed as μg dry sample/mL in the reaction mixture

In this research vitamin E, seed methanol and seed hexane extracts showed the highest inhibition of lipid peroxidation among other extracts, juice had the lowest and this property was intermediate for BHT and skin extract (Table 1). For grape juice, BHT and vitamin E, the sample concentration necessary to reduce by 50% the oxidation (IC_{50}) as determined by DPPH method is lower than that for inhibition of lipid peroxidation (Table 1), suggesting the free radical scavenging capacity was the predominant mechanism in the antioxidant activity of these samples. Against, for seed methanol, seed hexane and skin extracts, the IC_{50} (AA_{50}) as determined by FTC method is lower than the IC_{50} which determined by DPPH method, suggesting the inhibition of lipid peroxidation was the predominant mechanism in the antioxidant activity of these extracts. The IC_{50} for methanol seed extract in two methods was lower than hexane seed extract, meaning that the power of antioxidant activity of methanol seed extract is more than hexane seed extract. Thus the results of the present study indicate the selective extraction of antioxidants from natural sources by an appropriate solvent is very important for obtaining a fraction with high antioxidant activity and show the presence of compounds possessing high antioxidant activity in Shahani black grape.

In conclusion, fruits of Shahani black grape have potent effect on inhibition of lipid peroxidation and different activity of grape berry extracts (seed, juice and skin) can be ascribed to their different components or different amount of active compounds. It seems eating of this kind grape is useful for human health and disease prevention which caused by free radicals.

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