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Antioxidant Activities of Selected Grape Wastes from Egypt

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ABSTRACT

The methanolic extracts of six grape wastes were screened for their effect on 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid **(**ABTS) to determine their free radical scavenging activity, reducing power, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities. The activity of seed extract was greater than peel one. The grape seeds extract possessed a relatively high antioxidant activity and might be considered as a rich source of natural antioxidant.

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INTRODUCTION

Free radicals have been implicated in over a hundred disease conditions in humans, including arthritis, hemorrhagic shock, atherosclerosis, advancing age, ischemia and reperfusion injury of many organs, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis, and AIDS. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes. A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. However, the structure-activity relationship, bioavailability and therapeutic efficacy of the antioxidants differ extensively.

Dietary antioxidants are considered beneficial because of their potential protective role against oxidative stress, which is involved in the pathogenesis of multiple diseases such as cancer and coronary heart disease (Duthie et al., 2000). Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high contents of natural antioxidants, contribute to reduce mortality from cardiovascular and cerebrovascular diseases, although their protective effect on cancer risk is less conclusive (reviewed in Hertog et al., 1996; Yang et al., 2001). The potential antioxidant effect *in vivo* of individual food polyphenols (PP) or concentrated extracts has been widely investigated in cultured cells (Youdim et al., 2000; Schroeter et al., 2001), live animals (Pataki et al., 2002) and humans (Natella et al., 2001). However, it has been considerably more difficult to demonstrate the antioxidative effect of mixed diets rich in PP both in live animals (Youdim et al., 2000) and humans (OReilly et al., 2001; Van der Berg et al., 2001). In fact, some authors have reported controversial results about the positive or null antioxidant effect of a PP-rich or PP-supplemented diet on the biomarkers of the redox status (Van der Gaag et al., 2000; Young et al., 2002).

The search for cheap and abundant sources of natural antioxidants is attracting worldwide interest. Much research is needed in order to select raw materials; those of residual origin are especially promising due to their lower costs.

Over the last decades, various plant extracts have gained a lot of interest because of their beneficial effects on human health. Vegetables and fruits are substantial part of the Mediterranean diet. Grapes, in particular, are thought to possess health-related properties. It has been established that grape consumption is related to the prevention of chronic diseases such as cardiovascular diseases (Renaud and Lorgeril 1992) and cancer (Singh, et al., 2004).

The biological importance of grape extracts is mainly attributed to the antioxidant properties of the polyphenolic compounds they possess (Soleas et al., 1997). This is the main reason why polyphenolic compounds and plant extracts have been increasingly used as part of the diet or as nutritional supplements. Nevertheless, polyphenols may also act as prooxidants as they may induce free-radical production mainly via Fenton reaction (Halliwell, 2007). In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the plant raw materials.

Tons of grape pomace is produced while processing grapes, peels and seeds constitute a major proportion of pomace (Schieber *et al*., 2001). Grape peel and seeds are rich sources of functional components such as phenolics and anthocyanins which have antioxidant and radical scavenging activities (Negro *et al*., 2003; Yilmaz and Toledo, 2004; Pinelo *et al*., 2006). Phenolics may also act selectively at very low concentrations to inhibit LDL oxidation *in vitro* (Frankel *et al*., 1993; Teissedre *et al*., 1996). Antiradical and antioxidant activities of plant extracts have been confirmed by β-carotene linoleate and linoleic acid peroxidation methods (Jayaprakasha *et al*., 2001) as well as by 1, 1-diphenyl-2-picrylhydrazyl and phosphomolybdenum complex methods (Jayaprakasha *et al*., 2003).

Flavanols have also been shown to exhibit powerful antioxidant activities in different environments (Bors et al., 2000), and the antioxidant abilities of red grapes (Teissedre et al., 1996; Meyer et al., 1997), juices (Frankel et al., 1998) have always been correlated with the flavanol content.

The objective of this investigation is to evaluate the antioxidant capacity of different grape wastes by 5 different methods.

MATERIALS AND METHODS

Chemicals

Ammonium thiocyanate was purchased from E. Merck. Ferrous chloride, ascorbic acid, 1,1-diphenyl-2- picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2, 4-triazine (ferrozine), nicotinamide adenine dinucleotide (NADH), butylated hydroxy toluene (BHT), and trichloroacetic acid (TCA) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Nitroblue tertazolium salt was purchased from Fluka Bio Chemica.

Extraction of total phenolic compounds

Known weight of air dried waste powdered pomace (1 g) were extracted at room temperature with petroleum ether (40-60ºC) till complete extraction to remove lipoidal matter, waxes, pigments, sterols and non phenolic compounds. Then the mark was extracted with 80% EtOH several times (10 ml x 4) till complete extraction. The combined extract was transferred to measuring flask (100 ml) and made volume up to 100 ml.

Folin–Ciocalteu method was used to determine total phenols content as chlorogenic acid (sigma) according to the method described by Meda et al. (2005).

Concentration of the total phenol was plotted from the chlorogenic acid calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of chlorogenic acid equivalents /100 g of air dried waste sample).

Antioxidant activity

Six concentrations of tested grape wastes (25, 75, 150, 300, 600 and 1000 µg/mL) were prepared in methanol to determine their antioxidant activities using six methods compared with ascorbic and BHT.

DPPH radical-scavenging activity

The antioxidant activity was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging model (Mahakunakorn et al., 2004). The percentage inhibition of the DPPH radical was calculated according to the following formula:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

Where A is absorbance.

Ferric reducing antioxidant power (FRAP)

The ferric reducing power of the grape wastes was determined by using the potassium ferricyanide ferric chloride method (Ozcan et al., 2006).

Ferrous ion chelating ability assay

The ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and Rajini (Singh and Rajini 2004). The percentage inhibition of ferrous Fe^{+2} complex formation was calculated by using the formula:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample}) A \text{ control}] X 100$$

Where A = absorbance

ABTS radical scavenging activity

The determination of ABTS was performed according to the procedure described by Muller (1985) and was measured from the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [1-(AA/ AB] \times 100$$

Lipid Peroxidation- Ammonium Thiocyanate Method (LP-ATM)

Inhibition of lipid peroxidation by grape wastes was assayed according to the method of (Gülçin et al., 2004).

The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\% \text{ Inhibition} = [(A0 - A1) / A0] \times 100$$

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of extracts or standard compound.

Superoxide Anion Scavenging Activity

The superoxide scavenging ability of the extracts was assessed by the method of Winterbourn et al. (1975).

Decrease in absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Percent inhibition} = [(A0-A1)/A0] \times 100$$

Where A0 was the absorbance of the control (Ascorbic acid), and A1 was the absorbance of extract and Ascorbic acid or BHT.

Statistical Analysis

The experimental data was analysed using analysis of variance (ANOVA) and significant differences among means from a triplicate analysis at ($P<0.01$) were determined by SPSS software.

RESULTS AND DISCUSSION

Total phenolic content

Results in Table (1) revealed that, the total phenols in skin extracts were lower than in seeds one; the highest concentration of total phenolic was found with acidified EtOH extract in Grenache seeds, skin and Roomy seeds (28.5, 20.63 and 15.54 mg/g respectively).

Table 1. Total phenolic content (mg/g Dw) in different grape wastes using different extracting solvents

Grape wastes	Total phenolics			
	EtOH : HCl	50% EtOH	80% EtOH	Me_2CO
Roomy skin	14.61 ±0.3^{c}	1.81 ±0.07^{e}	1.95 ±0.1^{c}	1.54 ±0.2^{d}
Roomy seeds	15.54 ±0.6^{c}	14.64 ±0.37^{a}	14.19 ±0.9^{a}	13.21 ±0.5^{b}
Grenache skin	20.63 ±0.6^{b}	8.53 ±0.21^{c}	11.87 ±2.9^{a}	7.30 ±0.3^{c}
Grenache seeds	28.50 ±0.9^{a}	11.37 ±0.25^{b}	14.90 ±0.5^{a}	16.33 ±0.8^{a}
Thompson skin	1.13 ±0.3^{d}	3.83 ±0.46^{d}	6.47 ±0.9^{b}	2.37 ±0.1^{d}
Crimson skin	13.78 ±0.7^{c}	1.80 ±0.07^{f}	1.74 ±0.3^{c}	1.48 ±0.0^{d}

Data are represented as mean ± S.E.

Statistical analysis is carried out by one way analysis of variance using SPSS program.

Unshared letters between brackets were significant value between groups.

The choice of the proper solvents has been controlled by the properties of phenolic components of the concerned plants. The effect of different solvents on the extraction of some phenolic compounds from grape seeds was shown in Table (1)**,** which revealed the following statements. The yield of total phenolics is solvent dependent, where as the highest values have been attained using acidified EtOH compared with the other solvents investigated. Thompson skin may be considered the poorest source of the total phenolics compared with the other grape wastes.

Clear differences could be concluded among skin and seeds extracts for all solvent investigated. Grenache seeds are considered the highest source of total phenolics rather than those of skin for all solvents studied herein. For example, total phenolics values in Grenache skin reached 20.63 mg/g and increased to 28.5 in case of the seeds of the same cultivar.

The above mentioned data may focus some lights on the polar properties of the phenolics characterized grape wastes, and this may be confirmed by the less efficiency of acetone for extracting phenolics of grape wastes. The extractive capacity of phenolic components from grape wastes material is considerably depends on the type of solvents. The best extraction efficiency was achieved by ethanol: 0.1% HCl (acidified alcohol) followed by 80% EtOH.

These results go parallel with the data obtained by Goli*et al*., (2004) and Lapornik *et al*., (2005) who found that the extraction of phenolic compounds from a plant depends on the methods and type of extracting solvent. Results of previous studies showed that the extraction yield of phenolic and flavonoid content is greatly expressed in acidified alcohol and this holds with our results.

A high yield of phenolics can be extracted from sorghum leaf using water (Agbangnan *et al.*, 2012) while extraction of phenolics from wheat bran requires 80% aqueous ethanol (Verma *et al.*, 2008). In another example, an investigation dealing with effect of different solvents on extraction of phenolics from aerial parts of *Potentilla atrosanguinea* showed that 50% aqueous ethanol was more efficient than pure or 50% aqueous forms of methanol, and acetone (Kalpana*et al*., 2008). On the other hand, the highest levels of phenolics were extracted from *Vitis vinifera* wastes and sunflower meal using pure methanol and 80% aqueous acetone, respectively (Taha *et al.*, 2011).

Four concentrations of the tested extracts (5, 25, 75, and 100 µg/mL) prepared in ethanol were checked for their antioxidant activities using six methods (DPPH radical-scavenging activity (Mahakunakorn *et al*., 2004), Ferric reducing antioxidant power (Ozcan *et al*., 2006), Ferrous ion chelating ability assay (Singh and Rajini 2004), ABTS radical scavenging activity (Muller, 1985), Lipid Peroxidation- Ammonium Thiocyanate Method (Gülçin *et al*., 2004), and Superoxide Anion Scavenging Activity (Winterbourn *et al*. (1975). Ascorbic and BHT were used as positive standard.

(a) DPPH radical-scavenging activity

DPPH can provide an advantage if the tested antioxidants is more soluble in organic solvents. Therefore, this protocol provides a good selection of methods to use for antioxidant measurements, which can meet the needs of most researches dealing with fruits and fruit juices.

Table 2. Radical-scavenging activities (%) of different grape waste extracts against DPPH radical.

Grape wastes	Sample concentration (µg/ml)				
	5	25	50	100	IC_{50}
Roomy skin	15.18 ±0.97	75.92 ±1.58	80.69 ±2.47	81.94 ±2.69	16.5
Roomy seeds	17.91 ±0.84	89.55 ±2.18	92.56 ±2.32	93.31 ±1.27	14
Grenache skin	17.16 ±0.96	85.79 ±1.16	89.38 ±2.7	91.47 ±2.55	14.6
Grenache seeds	17.01 ±0.95	85.03 ±1.03	87.21 ±2.25	90.23 ±1.02	14.7
Thompson skin	16.97 ±0.52	84.87 ±2.54	86.29 ±2.13	89.05 ±2.04	14.7
Crimson skin	11.19 ±0.51	55.94 ±1.46	70.15 ±2.00	74.33 ±2.44	22.3
Ascorbic acid	14.46 ±0.63	72.28 ±2.24	86.17 ±2.17	90.15 ±2.5	17.3
BHT	15.38 ±0.58	76.91 ±1.04	87.46 ±1.54	94.1 ±2.03	16.3
LSD 0.01	1.9	4.2	5.0	3.2	

Data are represented as mean ± S.E.

Statistical analysis is carried out by two way analysis of variance using SPSS program.

The DPPH technique showed high reproducibility, simplicity, rapidly performed and showed the highest correlation with total phenolics. From Table (2), the data clearly shown that the antioxidant activity of different grape seeds extracts were concentration dependent, and all grape seeds powder extracts exhibited appreciable scavenging activity ranging between $IC_{50=}$ 14 and 22 µg/ml for Roomy seed sand Crimson skin respectively.

Higher antioxidant capacity was obtained by ascorbic acid and BHT at concentration 100 µg/ml. Other grapes produced antioxidant capacity of 93.31, 91.47, 90.23, 89.05 and 81.94 % for Roomy seeds, Grenache skin, Grenache seeds, Thompson skin and Roomy skin, respectively. The lowest antioxidant capacity was produced by Crimson skin with 74.33%.

(b) ABTS Radical Cation Scavenging Activity

The second method performed to determine the scavenging activity of grape wastes was ABTS. Table (3) represents the data obtained using different concentrations of grape wastes.

Table 3. Antioxidant capacity (%) of ethanolic extract of different grape wastes against ABTS radical.

Grape wastes	Extracts concentration (µg/ml)				
	5	25	50	100	IC_{50}
Roomy skin	12.9 ±3.21	64.52 ±0.7	68.98 ±1.01	70.57 ±0.39	21.2
Roomy seeds	11.3 ±0.69	56.31 ±0.8	71.08 ±0.18	79.08 ±0.6	20.2
Crimson skin	13.3 ±0.66	66.41 ±0.4	67.07 ±1.06	68.98 ±0.62	22.3
Thompson skin	13.7 ±0.63	68.34 ±0.8	70.25 ±1.15	72.17 ±0.97	20.6
Grenache skin	11.9 ±0.89	59.94 ±1.0	61.97 ±1.0	63.2 ±0.5	22.0
Grenache seeds	12.8 ±0.95	63.89 ±1.0	68.34 ±0.9	72.17 ±1.34	21.6
Ascorbic acid	14.2 ±0.79	55.0 ±0.75	72.0 ±0.98	83.6 ±0.88	19.7
BHT	14.6 ±0.73	60.0 ±0.46	90.0 ±0.28	99.5 ±0.6	18.8
LSD 0.01	3.3	1.9	2.26	2.03	

Data are represented as mean ± S.E.

Statistical analysis is carried out by two way analysis of variance using SPSS program.

From Table (3) it is obvious that grape wastes producing a scavenging capacity in concentration dependent manner from 5 to 100 µg/ml.

From Table (3) it is clear that all grape wastes extracts possesses radical scavenging activity more than 60% at 100 µg/ml but less than ascorbic acid and BHT as standard antioxidant (83.61 and 99.5%, respectively).

The scavenging activity can be arranged at 100 µg/ml in the following order: BHT, ASC, Grenache seeds, Roomy seeds, Thompson skin, Roomy skin, Crimson, Grenache skin which represent the lowest values; 63.2. No significant differences due to the scavenging activity by ABTS were found between Roomy skin, and Crimson skin extracts.

(c) Superoxide Anion Scavenging Activity

The third method is superoxide anion scavenging activity. The basis of this method is reducing the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. The antioxidants are able to inhibit the blue NBT formation.

Table (4) compiles the data obtained from different concentrations (5, 25, 50 and 100 µg/ml) of grape wastes extracts using superoxide anion scavenging activity.

Table 4. Superoxide scavenging (activity %) of different grape wastes extracts

Grape wastes	Extracts concentration (µg/ml)				
	5	25	50	100	IC_{50}
Roomy skin	5.2 ±0.66	26.0 ±0.33	30.0 ±1.46	36.0 ±0.44	417
Roomy seeds	6.2 ±0.75	31.1 ±0.92	42.2 ±0.94	47.7 ±0.72	105
Grenache skin	7.2 ±0.93	36.0 ±0.78	44.0 ±0.51	48.0 ±1.08	104
Grenache seeds	7.3 ±0.42	36.5 ±0.48	40.9 ±0.95	68.0 ±1.02	61.1
Thompson skin	8.0 ±0.66	40.0 ±1.01	42.0 ±1.71	48.0 ±0.65	104
Crimson skin	2.6 ±0.39	13.2 ±0.77	14.3 ±0.73	14.5 ±0.72	2124
Ascorbic acid	4.0 ±1.08	20.0 ±0.94	49.2 ±0.9	68.2 ±0.41	50.8
BHT	14.0±1.06	70.1 ±0.32	76.5 ±0.56	92.6 ±0.56	17.8
LSD 0.01	2.03	1.8	2.66	1.5	

Data are represented as mean ± S.E.

Statistical analysis is carried out by two way analysis of variance using SPSS program.

From the previous table, it is clear that, BHT had the strongest radical scavenging activity (IC_{50}=17.8) and ascorbic acid (IC_{50}; 50.8) compared with the other grape wastes extracts investigated in this study.

The antioxidant capacity of the extracts can be arranged in the following order according to the results of superoxide scavenging activity: Grenache seeds > Grenache skin = Thompson skin = Roomy seeds > Roomy skin > Crimson skin.

(d) Metal chelating activity

The fourth method applied to determine the metal chelating activity.

Table (5) compares the values obtained by using metal chelating activity. The inhibition values varied from 71 to 98% for Crimson skin to Grenache seeds at concentration of 100 µg/ml.

Table 5. The metal chelating activity (Inhibition %) of different grape waste extract

Grape wastes	Sample concentration (µg/ml)				
	5	25	50	100	IC_{50}
Roomy skin	11.56±0.84	57.82 ±0.78	73.44 ±0.85	85.94 ±0.8	21.6
Roomy seeds	12.5 ±0.67	62.5 ±0.46	74.54 ±1.04	86.25 ±0.84	20
Grenache skin	3.13 ±0.4	45.91 ±1.23	83.13 ±0.18	87.5 ±0.88	27.2
Grenache seeds	13.75 ±0.88	68.75 ±0.72	71.31 ±0.59	98.18 ±0.38	18.2
Thompson skin	2.81 ±1.09	15.63 ±0.42	45.32 ±1.17	78.14 ±0.3	80
Crimson skin	9.18 ±0.9	14.06 ±0.79	62.15 ±0.6	71.23 ±0.83	40.2
Ascorbic acid	3.6 ±0.62	45 ±1.00	58 ±0.31	90 ±0.56	27.7
BHT	4.42 ±0.44	35 ±0.16	46.23 ±0.3	72 ±0.61	54.1\
LSD 0.01	2.0	1.9	1.86	1.47	

Data are represented as mean ± S.E.

Statistical analysis is carried out by two way analysis of variance using SPSS program.

Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Chang *et al*., 2002; Halliwell, 1991). Metal chelating capacity is important since it reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh *et al*., 1999). It was reported that chelating agents, that form bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990).

The metal chelating effect of different grape wastes showed dose dependent manner. In this assay, all extract disrupted the ferrozine– Fe^{2+} complex, thus decreased the red colour.

The activities are arranged according to inhibition % at 100 ug/ml as follows: Grenache seeds > Roomy seeds > Roomy skin > Grenache skin > Crimson skin > Thompson skin.

The data obtained from Table (5) revealed that all waste extracts demonstrate a marked capacity for iron binding, suggesting that its main action as a peroxidation inhibitor may be related to its iron binding capacity. The IC_{50} values of Vit C and BHT were higher than those of Grenache seeds, Roomy seeds, Roomy skin and Grenache skin.

Metal chelating capacity was significant, since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. Trace metals significantly contribute to the free radical formation by decomposing lipid hydroperoxides into free radicals.

It was reported that compounds with structures containing C–OH and C=O functional groups can coordinate metal ions. Kazazica *et al*. (2006) demonstrated that flavonoids, such as kaempferol, chelated cupric ions (Cu^{2+}) and ferrous ions (Fe^{2+}) through the functional carbonyl group. The compounds with structures containing two or more of the following functional groups: -OH, -SH, -COOH, $-H_2PO_3$, C=O, $-NR_2$, -S- and -O- in a favorable structure– function configuration, can show metal chelation activity (Gülçin, 2006).

(e) Lipid peroxidation

The antioxidant effect of various extracts from grape wastes (in different concentration) in preventing the peroxidation of lionoleic acid measured by thiocyanate method was tabulated (Table 6).

Table 6. Inhibition % of linoleic peroxidation by grape wastes extracts at different concentrations.

Grape wastes	Extracts concentration (µg/ml)				
	5	25	50	100	IC_{50}
Roomy skin	16.17 ±0.38	85.84 ±1.02	88.81 ±0.78	86.87 ±0.87	15.46
Roomy seeds	17.76 ±0.39	93.8 ±0.69	98.74 ±0.52	99.32 ±0.38	14.08
Grenache skin	17.35 ±0.58	91.76 ±0.73	96.09 ±0.22	98.24 ±0.85	14.41
Grenache seeds	14.74 ±0.63	96.99 ±0.82	99.83 ±0.24	99.9 ±0.16	16.96
Thompson skin	15.72 ±0.74	83.61 ±0.56	89.93 ±0.27	93.5 ±0.79	15.9
Crimson skin	5.328 ±0.72	31.64 ±0.7	81.56 ±0.5	81.69 ±0.95	46.92
Ascorbic acid	16.1 ± 0.5	50 ± 0.3	67.06 ± 0.2	93.1 ± 0.1	25
BHT	11.1 ± 0.1	40 ± 0.3	52.3 ± 1.7	89 ±0.0	31.25
LSD 0.01	0.9	1.7	1.8	1.37	

Data are represented as mean ± S.E.

Statistical analysis is carried out by two way analysis of variance using SPSS program.

All extract induced significant inhibition of linoleic oxidation and varies according to extracts of wastes used and arranged according to degree of prevention lipid peroxidation in decreasing order as follows: Grenache seeds > Roomy seeds > Grenache skin > Thompson skin > Roomy skin > Crimson skin. Most of those grape wastes inhibited lipid peroxidation more than with ascorbic and BHT (93 and 89 inhibition at 100 ug/ml, respectively).

(f) Reducing Power Assay

The sixth method adopted to determine the antioxidant capacity of grape wastes is the reducing power assay. This method indicates that the antioxidant compounds are electron donors. Data in Fig. (1) illustrate the reducing power of different grape wastes.

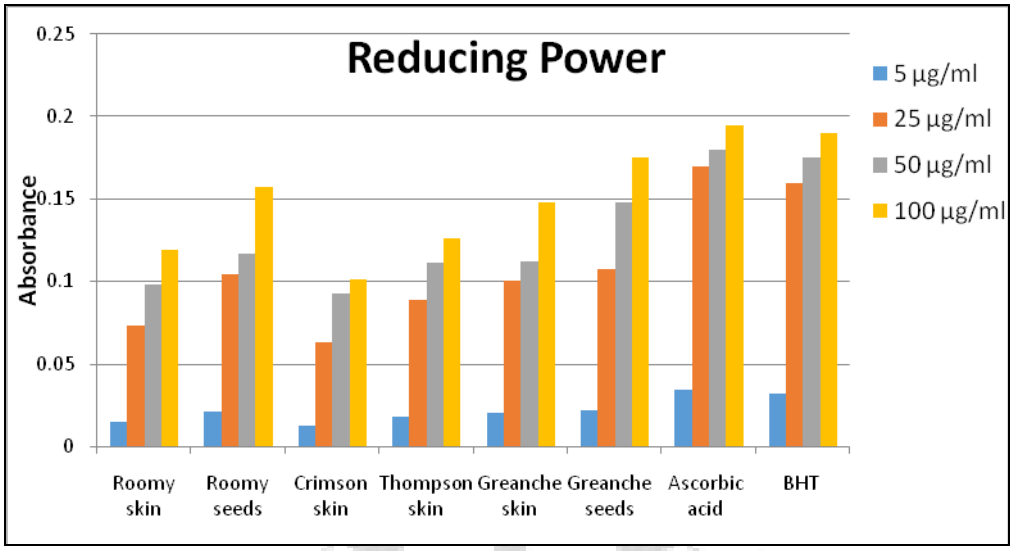

Fig. 1. The reducing power ability of different grape waste extracts at different concentrations. Ascorbic and BHT are used as positive control.

Figure (1) clearly represents the different reducing power, and can be arranged in following order: Grenache seeds > Roomy seeds > Grenache skin > Thompson skin > Roomy skin > Crimson skin.

The reducing capacity can be measured by the direct reduction of $Fe[(CN)_6]^3$ to $Fe[(CN)_6]^2$. Addition of free Fe^{3+} to the reduced product leads to the formation of the intense Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]^3$, which has a strong absorbance at 700 nm. An increase in absorbance of the reaction mixture would indicate an increase in reducing capacity due to an increase in the formation of the complex. The ferric ion reducing antioxidant power assay takes advantage of an electron transfer reaction in which a ferric salt is used as an oxidant (Wood *et al*., 2006).

From the above obtained results of the six methods adopted, the following could be concluded:

All tested extracts exerted significant antioxidant activities. The results are expressed as IC_{50} values. The lower IC_{50} value express as the highest antioxidant activity of the extract.
The total antioxidant capacity estimated by the ABTS and DPPH methods; reveal that sample extract showed marked inhibition in the both model but in DPPH assay more inhibition of free radical was observed as compared to ABTS assay.

Antioxidants may respond in a different manner to different radical or oxidant sources.

It should be emphasized that there is a great difference between "antiradical" and "antioxidant" activity and that they do not necessarily coincide. According to Tirzitis and Bartosz (2010) the antiradical activity characterizes the ability of compounds to react with free radicals (in a single free radical reaction), but antioxidant activity represents the ability to inhibit the process of oxidation (which usually, involves a set of different reactions). Consequently, all test systems using a stable free radical (for example, DPPH, ABTS, etc.) give information on the radical scavenging or antiradical activity, although in many cases this activity does not correspond to the antioxidant activity.

It must be noticed that there is no single antioxidant assay method can provide a complete picture of the antioxidant capacity of compounds that show complex kinetics, i.e., most complex natural products. Using at least two different antioxidant methods be needed to compare samples and to provide the opportunity to identify variations in response that may otherwise be missed.

(3) Correlation between antioxidant activity and phenolic content of grape wastes extract

The close correlation between antioxidant activity and phenolic content obtained from various natural sources has been demonstrated by Liu *et al*.(1997); Guendez *et al*. (2005); Verzelloni *et al*.(2007) and Erkan *et al*.(2008).

It was found that there is a correlation between lipid peroxidation, metal chelation, DPPH (Table 2) and super oxide scavenging (Table 4) activity of grape seed wastes and total phenolic content (Table 1). In addition, there is a moderate correlation between reducing power of grape seed wastes and total phenolic content.

A positive correlation has been well established with the EtOH extract of Roomy seeds and Grenache seeds where the highest values of polyphenols were found (14.2 and 14.9 mg/g

respectively Table 1) relevant to the DPPH radical scavenging activity results (93.31 and 90.23% respectively Table 2). While Roomy skin and Crimson skin had the lowest polyphenolic content and the highest IC_{50} values. This correlation between phenolics compounds and the antioxidant capacity (DPPH method) is in good agreement with the results of Mustafa *et al*. (2010); Surveswaran *et al*. (2007) and Janovik *et al*. (2011). Also, Bartolom *et al*. (2004) determined *in vitro* antioxidant activity of red grape skins against DPPH radical, and they found that, there are a statistically correlations between antioxidant activity and phenolic content.

In fact, phenolics are one of the major classes of natural antioxidants found in plants that remove such free radicals. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and to break down peroxides. It is clearly shown that the number of phenolic-OH groups present in the structure of an antioxidant molecule isn't always the only factor determining its antioxidant activity. Positions of phenolic-OH groups, presence of other functional groups in the whole molecule, such as double bonds and their conjugation to (-OH) groups and ketonic groups, also play important roles in antioxidant activities (Rice-Evans *et al.*, 1996).

It is noteworthy that the winery residues could be an alternative source for obtaining natural antioxidants, and are considered completely safe in comparison with synthetic ones such as butylated-hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT), compounds are largely used in the food industry now with undesirable effects on the enzymes of human organs (Nakatani, 1997).

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