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Characterization of the Antioxidant Properties of Seeds and Skins in Selected Turkish Grapes

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1,1-Diphenyl-2-picryl-hydrazyl (DPPH•), hydroxyl (HO•), superoxide anion ($O_2^{\bullet-}$) radicals scavenging activities and total antioxidant capacities; total phenolic, catechin and epicatechin contents were determined in ethyl acetate-water extract (EAWE) and methanol-water extract (MWE) extracts of selected six varieties of *Vitis vinifera* L. from Turkey grape seeds and skins. DPPH• scavenging capacities of 95 % were determined for 0.14 µg/mL of seed and 2.01 µg/mL of skin EAWEs of Eksi kara grape. $O_2^{\bullet-}$ scavenging capacities were observed only in EAWEs of all grape seeds. The highest HO• scavenging capacity was also reached to 98 % with 0.5 µg/mL of EAWE of Eksi kara grape seed. Total antioxidant capacities of seed extracts were generally higher than skin extracts. Total phenolic contents in seed EAWEs were ranged from 5.34 to 101.7 µg gallic acid/µg extract. Catechin and epicatechin levels in seeds and skins were higher in EAWEs by comparison with MWEs. The radical scavenging capacities of Eksi kara grape seed and skin extracts, which had the highest values,were decreased between 10-20 % after heating process although the increases of their total phenolic contents.

Key Words: Total antioxidant capacity, Catechin and epicatechin, Hydroxyl radical scavenging activity, Superoxide anion radical scavenging activity, Total phenolic content, Turkish grapes, 1,1-Diphenyl-2-picryl-hydrazyl radical scavenging activity.

INTRODUCTION

Living cells produce different reactive oxygen species (ROS) such as superoxide, hydroxyl, peroxyl free radicals and singlet oxygen and hydrogen peroxide molecules[1,2]. Reactive oxygen species are important damage factors on lipid, protein and DNA molecules in the cell and that's why cause to cellular degeneration[3]. Lipid peroxidation causes the damage of the membranes surrounding cell and cell organelles contain large amounts of polyunsaturated fatty acid side-chains[4-6]. Oxidized polyunsaturated fatty acids may also induce aging and carcinogenesis. Attack of ROS upon proteins produces

carbonyls and other amino acid modifications and cause to disorders of protein functions. ROS has also damage effects on the base and sugar units in DNA strand. The correlations between the levels of the bases modified by hydroxyl radicals and diseases are important research area[7,8]. Consequently, accumulation of potentially harmful ROSs increases in stress, disease and aging periods and can cause to loss of homeostatic control and organ function[9].

The biological damage that could result from these highly reactive compounds is controlled *in vivo* by the endogenous antioxidant defense mechanisms[10]. Many plant phenols other than antioxidant vitamins such as -C, -E and carotenoids exert powerful antioxidant effects. Therefore, consumption of foods containing plant phenolics besides antioxidant vitamins has increased the importance. Plant products are also known to possess potential for food preservation. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have restricted use in foods as these synthetic antioxidants are under great consideration lately for toxicological reasons[11]. In recent years, the importance of search for natural antioxidants, especially plant origin, has greatly increased[12].

Grapes (*Vitis vinifera*) which are the member of the family of Vitacea, are considered as the world's largest fruit crops, with an approximate annual production of 60 million metric tones[13]. Grape seeds and skins are rich sources of antioxidant compounds and these compounds act anti-mutagenic and antiviral agents[14]. In addition, to be rich of grape seeds and skins in view of plant phenols are get out foreground its pharmacological importance. However, the research on the antioxidant capacities of grape seeds and skins depend on species of grape and growth area is not widely investigated. Identifying of grape varieties with the highest antioxidant capacities is important for grape growing targets and contributions providing by grape products in pharmacological and medicinal sectors.

In this study, we investigated the antioxidant properties of grape seed and skin extracts from six species in various areas of west region of Turkey by employing various *in vitro* assay methods, such as; 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\bullet}$), hydroxyl ($HO^{\bullet}$), superoxide ($O_2^{\bullet -}$) anion radicals scavenging and total antioxidant capacities; total phenolic, catechin and epicatechin contents. These parameters were also investigated in one of them grape species after heating process for investigation of the applicability in food and pharmacological sectors.

EXPERIMENTAL

V. vinifera L. varieties Pembe germe (1), Eksi kara (2), Kozak siyahi (3), Yediveren (4, 5), Iri kara (6) grapes are cultivated in the Bergama and Kaynaklar-Izmir and Denizli states existent in the north-, middle- and south-regions of the west of Turkey, respectively. Information about the origin

and vineyard location is given in Table-1. The grape varieties used were harvested at optimum technological maturity. Grape berries are manually deseeded and the seeds and skins were dried and stored in the freezer (-20 ºC) until analyzed. The selected grapes were termed with the number in the present study as showed in Table-1.

TABLE-1
ORIGIN AND LOCATION OF THE VARIETIES OF SELECTED GRAPES

Grape no.	Origin	Location
1	Pembe germe	Kaynaklar-Izmir
2	Eksi kara	Denizli
3	Kozak siyahi	Bergama-Izmir
4	Yediveren	Denizli
5	Yediveren	Kaynaklar-Izmir
6	Iri kara	Izmir

Extraction: Dried grape seeds and skins were crushed in a blender for 2 min, but at 15 s intervals the process was stopped for 15 s to avoid heating of the sample. Powdered grape seeds and skins were extracted in a Soxhlet extractor with hexane (60-70 ºC for 1 h) for the removal of fatty material. The defatted grape seeds and skins powder were extracted after optimization of the conditions in a Soxhlet apparatus sequential with ethyl acetate: water (17:3, v/v) and then methanol:water (3:2, v/v) at 70-80 ºC, for 1 h. The extracts were concentrated in a vacuum evaporator to get dry materials and stored in a desiccator. Assays were performed in samples solved by methanol:water (3:2, v/v) medium. The obtained extracts in indicated solvents were expressed as ethyl acetate-water extracts (EAWE) and methanol-water extracts (MWE), respectively.

The same extraction processes were also applied in Eksi kara (2) grape seed and skin samples, which had the best results, after heating processes at 200 ºC for 0.75 h by putting into dough in pressed block form. The same assays were also performed in the obtained EAW and MW extracts from heat process and the results were compared with values of seed and skin extracts of grape 2.

Determination of 1,1-diphenyl-2-picryl-hydrazyl (DPPH$^{\bullet}$) radical scavenging capacity: The hydrogen atom or electron donation abilities of the of grape seed and skin extracts were measured from the bleaching of the purple-coloured methanol solution of the 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$)[15]. Half of milliliter of various dilutions of the extracts or ascorbic acid as a positive control was mixed with 1.5 mL of DPPH solution. The samples were incubated for 0.5 h at room temperature and the decreases in the absorbance values were measured at 517 nm.

Determination of superoxide anion ($O_2^{\bullet -}$) radical scavenging capacity: Superoxide anion radical scavenging capacity was measured according to Crosti method based on the inhibitory effect of samples on the spontaneous autoxidation of 6-hydroxydopamine at 490 nm[16]. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging capacity.

Determination of hydroxyl radical ($HO^{\bullet}$) scavenging capacity: Deoxyribose has often been used to measure the formation of $HO^{\bullet}$ in biochemical systems[17]. Reaction mixture contained in a final volume of 1.0 mL, following reagents at the final concentrations stated: deoxyribose (2.8 mM), $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM), ascorbate (100 μM) and extracts or BHA, as a positive control. If a Fe^{2+}-EDTA chelate is incubated with deoxyribose in phosphate buffer (20 mM) at pH 7.4, $HOs^{\bullet}$ are formed. Reaction mixture was incubated at 37 ºC for 1 h and colour developed with thiobarbituric acid (TBA). Then absorbance at 532 nm was measured as a pink malondialdehyde-TBA chromagen.

Determination of total antioxidant capacity: Total antioxidant capacities of the extracts and BHA as a positive control were determined according to the thiocyanate method[18]. Linoleic acid emulsion (0.02 M) in phosphate buffer (0.02 M, pH 7.0) was prepared by mixing linoleic acid with an equal amount of Tween 20 (0.02 M). Each extract was mixed with linoleic acid emulsion and incubated in the dark at 37 ºC. During linoleic acid oxidation, the formed peroxides oxidize Fe^{2+} to Fe^{3+} and it subsequently forms complex with SCN^-, which had maximum absorbance at 500 nm.

Determination of total phenolic contents: Total phenolic contents were measured by using the Prussian Blue Assay, based on oxidation and reduction of iron[19]. Gallic acid (0.0-1.7 μg/mL) was used as the standard and data were expressed as gallic acid equivalents (GAE) in (μg GAE/μg extract) dry material.

The extract (0.10 mL), 50.0 mL distilled water and 3.0 mL 0.10 M $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ (in 0.10 M HCl) were mixed. Exactly 20 min after the addition of the ferric ammonium sulphate, 3.0 mL 0.008 M $K_3[Fe(CN)_6]$ were added and mixed. After 20 min, the addition of ferricyanide absorbance was read at 720 nm against to blank.

Determination of catechin monomers using HPLC method: Quantification of monomeric flavonols was done by HPLC using (+)-catechin and (-)-epicatechin as external standards. Two Beckman Ultrasphere (C18) ODS (250 × 4.6 mm) columns placed in line and protected with a guard column packed with the same packing were used for all analysis. Flow rate was set at 1 mL/min and the chromatograms were monitored at 280 nm using a UV detector. The elution system consisted of two solvents, A: 2.5 % HOAc in H_2O, B:80 % CH_3CN in A and the following gradients; elution

starting with 7 % B in A isocratic for 5 min; 7 to 20 % B in A, 5 to 90 min; 20 to 100 % B in A, 90 to 95 min; 100 % B, 95 to 100 (isocratic); followed by washing (100 % B over 10 min) and reconditioning of the column (100 to 7 % B in A over 5 min).

Statistical analysis: Tukey test, one of the multiple comparisons, was used for statistical significance analyses. The values are the mean of three separate experiments (n = 3). The comparisons between antioxidant capacities in the extracts were made with Pearson correlation.

RESULTS AND DISCUSSION

DPPH• Scavenging capacity variations: DPPH• is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The free radical scavenging capacity of grape seed and skin extracts was evaluated with the change of absorbance produced by reduction of DPPH•. Table-2 shows the amounts of the two different extracts of six grape varieties (seeds and skins) for 95 % scavenging. The results were compared with the variations of DPPH• scavenging capacities in the presence of various amounts of vitamin C, as a reference compound. As seen from Table-2, 95 % scavenging of DPPH• by all grapes seed EAWEs except Kozak siyahi (3) and Iri kara (6), were provided with lower amount than MWEs. The highest scavenging capacities were determined as 0.14 ± 0.009 µg/mL for seed and 2.01 ± 0.19 µg/mL for skin EAWEs of Eksi kara (2) grape and they were 100 and 7.5 times higher than vitamin C, respectively.

TABLE-2
95 % SCAVENGING OF DPPH• WITH GRAPE EXTRACTS

Grape no.	95% scavenging of DPPH• with extracts				Vitamin C	
	Seeds (µg/mL)		Skins (µg/mL)		µg/mL	Scavenging (%) of DPPH•
	EAW	MW	EAW	MW		
1	0.38±0.025	0.59±0.032	2.05±0.18	16.96±1.19	2	26.15
2	0.14±0.009	0.42±0.028	2.01±0.19	21.76±1.10	3	31.82
3	0.74±0.042	0.89±0.061	2.12±0.11	47.33±3.40	4	35.58
4	0.28±0.022	0.39±0.025	2.49±0.095	30.38±2.19	5	50.53
5	0.88±0.052	0.93±0.065	3.48±0.23	27.41±1.15	10	69.96
6	2.76±0.150	1.09±0.250	3.11±0.25	23.97±1.25	13	87.21
2-Heated	0.16±0.010	0.47±0.030	2.23±0.17	25.14±1.22	15	94.92

EAW = Ethyl acetate-water; MW = Methanol-water

Extract amounts required for 95 % scavenging of DPPH• of Eksi kara (2) grape after heating process were increased significantly, in other words the radical scavenging capacities were decreased.

Superoxide anion radical scavenging variations: Superoxide anion radical scavenging activities were observed only in seed EAWEs (Table-3). The highest scavenging capacities of superoxide radicals were obtained by Eksi kara (2) and Denizli-Yediveren (4) grape seed extracts at 1 mg/mL in similarly values. Scavenging capacities of Kozak siyahi (3) and Iri kara (6) seed extracts were not significantly ($p > 0.01$). The scavenging capacity of $O_2^{\bullet-}$ by Eksi kara (2) grape, which heat treated, was decreased as 15.92 % and obtained to be 70.52 %.

TABLE-3
SUPEROXIDE ANION RADICAL SCAVENGING (%) BY OBTAINED ETHYL ACETATE-WATER EXTRACTS OF GRAPE SEEDS

Grape no.	1	2	3	4	5	6	2-Heated
Superoxide radical scavenging (%)	38.04	83.87	2.01	83.68	12.61	2.18	70.52

Hydroxyl radical scavenging capacity variations: The effects of grape seeds extracts on oxidative damage, induced by Fe^{3+}/ H_2O_2 on deoxyribose, were determined by the deoxyribose method. IC_{50} is the amount of extract providing 50 % inhibition of hydroxyl radical. Extract amounts providing IC_{50} values were found using the graph by plotting inhibition percentage against extract amount (Table-4).

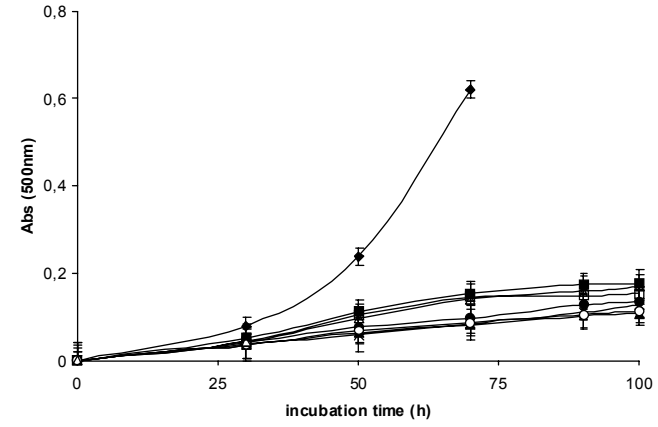
TABLE-4
IC_{50} VALUES OF GRAPES SEED AND SKIN EXTRACTS FOR HYDROXYL RADICAL SCAVENGING

Grape no.	Seed (µg/mL)		Skin (µg/mL)	
	EAW	MW	EAW	MW
1	0.51 ± 0.049	3.67 ± 0.25	0.96 ± 0.085	7.91 ± 0.57
2	0.20 ± 0.015	3. 35 ± 0.18	0.77 ± 0.055	9.52 ± 0.62
3	0.92 ± 0.067	3.75 ± 0.20	2.42 ± 0.190	20.22 ± 2.00
4	0.41 ± 0.022	3.33 ± 0.18	1.13 ± 0.095	12.31 ± 0.95
5	1.12 ± 0.095	4.12 ± 0.30	2.81 ± 0.018	9.64 ± 0.65
6	1.53 ± 0.200	4.81 ± 0.33	3.62 ± 0.250	10.92 ± 0.85
2-Heated	0.23 ± 0.015	3.87 ± 0.22	0.88 ± 0.065	10.79 ± 0.98

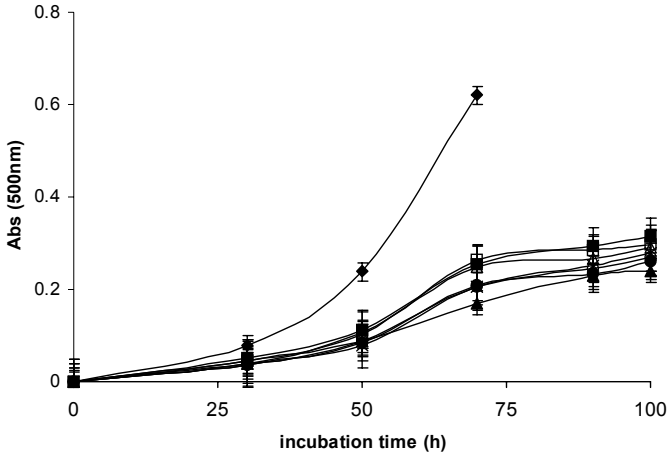
EAW = Ethyl acetate-water; MW = Methanol-water

A marginal inhibition was evident at the all grape seeds extracts. The highest $HO^{\bullet}$ scavenging capacity was reached to 98 % with 0.5 µg/mL of Eksi kara (2) grape seed EAWE. $HO^{\bullet}$ scavenging capacities of both extracts of skins were lower than seed extracts. The best IC_{50} values were determined in grape-2 except skin MWE. These values of Eksi kara (2) grape seed and skin after heating process were increased approximately 15.0, 15.5 % for EAWEs and 14.3, 13.3 % for MWEs.

Total antioxidant capacity variations: The antioxidant capacities in two extracts of grape seeds and skins from 6 different grape species, in preventing the peroxidation of linoleic acid, as measured by thiocyanate method, was shown in Fig. 1. The lowest total antioxidant absorbance value in MWEs as compared to EAWEs was detected. It was determined that absorbance values for grape-1 skin and seed MWEs and EAWEs were 0.345 and 0.225; 0.314 and 0.178, respectively. This difference between MWEs and EAWEs was showed similarity for all extract samples. Data of EAWEs which had been the higher total antioxidant capacities were depicted in Fig. 1 in order to avoid confusing data.



(a)



(b)

Fig. 1. Total antioxidant capacities of selected grape seeds (a) and skins ethyl acetate-water (b) at 100 µg/mL: control (-◆-), grapes-1 (-■-), -2 (-▲-), -3 (-×-), -4 (-●-), -5 (-○-), -6 (-□-), 2-heated

In assay condition, the oxidized products react with ferrous sulphate to form ferric sulphate, then to ferric thiocyanate of blood-red colour. The absorption values of control were increased up to 1.870 at 90 h and then they were decreased slightly ($p > 0.01$). According to the results, all grape seeds and skins extracts were exhibited effective total antioxidant capacities and the oxidation of linoleic acid was not observed up to incubation period of 70 h ($p > 0.01$). The significant decreases in antioxidant capacity were just about reached after incubation period of 90 h ($p < 0.01$). Generally, it was determined that total antioxidant capacities of seed extracts were higher than skin extracts and EAWEs of seed and skin had higher total antioxidant capacities than MWEs. It was also determined that antioxidant capacities in both extracts of Eksi kara grape were 10 % lower after heating process.

Total phenolic content variations: Total phenolic contents of selected grapes grown in north, middle and south areas of west of Turkey were determined as gallic acid equivalents (GAE). As can be seen from Table-5, generally total phenolic values in EAWEs for both seed and skin were higher than MWE values ($p < 0.01$). The highest total phenolic contents in EAWEs and MWEs of seeds were determined in Eksi kara (2) grape as 101.73 ± 4.85 and 3.32 ± 0.15 μg GAE/μg extract while the values of skin in Yediveren-Izmir (5) grape were 866.72 ± 20.23 and 15.52 ± 0.95 μg GAE/mg extract, respectively. Total phenolic contents were found to be higher levels in seed and skin extracts of Eksi kara (2) grape after heating process.

TABLE-5
TOTAL PHENOLIC VALUES OF SELECTED GRAPE EXTRACTS

Grape no.	Seed (μg GAE/μg extract)		Skin (μg GAE/mg extract)	
	EAW	MW	EAW	MW
1	7.39 ± 0.52	2.82 ± 0.12	51.92 ± 3.25	2.98 ± 0.12
2	101.73 ± 4.85	3.32 ± 0.15	63.13 ± 3.41	5.34 ± 0.23
3	8.30 ± 0.45	2.40 ± 0.11	92.31 ± 3.86	7.48 ± 0.51
4	12.73 ± 0.92	2.96 ± 0.15	78.16 ± 3.57	6.36 ± 0.91
5	6.72 ± 0.34	2.21 ± 0.12	866.72 ± 20.23	15.52 ± 0.95
6	5.34 ± 0.24	1.87 ± 0.09	156.44 ± 8.75	14.71 ± 0.92
2-Heated	206.25 ± 11.21	5.64 ± 0.23	94.21 ± 3.92	6.43 ± 0.42

EAW = Ethyl acetate-water; MW = Methanol-water

Catechin and epicatechin level variations: Catechin and epicatechin levels were determined in seeds and skins of grapes-2 and -5, which had been generally the highest and lowest antioxidant capacities, respectively (Table-6). The catechin and epicatechin levels of EAWEs were higher in seeds of both grape extracts with compared to skins. Catechin levels in all

extracts of both grapes were also higher than epicatechins. The highest total monomer level of catechins was determined in grape-2 seed extract as 505.5 ± 17.6 mg/100 mg dry sample.

TABLE-6
CONTENTS (mg/100 mg DRY SAMPLE) OF CATECHIN MONOMERS IN GRAPES-2 AND -5

Grapes / Catechins	2			
	Seed		Skin	
	EAW	MW	EAW	MW
(+)-Catechin	380.4 ± 13.2	147.2 ± 6.5	101.5 ± 4.1	71.3 ± 2.1
(-)-Epicatechin	125.1 ± 4.8	82.6 ± 2.9	35.4 ± 0.9	28.5 ± 0.8
Total monomer	505.5 ± 17.6	229.8 ± 11.5	136.9 ± 5.2	99.8 ± 3.8
	5			
(+)-Catechin	52.1 ± 1.6	23.4 ± 0.7	32.7 ± 1.2	21.3 ± 0.7
(-)-Epicatechin	28.6 ± 0.9	9.5 ± 0.4	19.2 ± 0.6	7.1 ± 0.4
Total monomer	80.7 ± 2.7	32.9 ± 0.9	51.9 ± 1.5	28.4 ± 0.9

EAW = Ethyl acetate-water; MW = Methanol-water

In living systems, ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanisms. The imbalance between the production of ROS and the antioxidant defense mechanisms leads to oxidative modification in cellular membrane or intracellular molecules. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. The role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may cause many diseases including cancer[1].

Grape seed and skin extracts, which prepared with different grape varieties grown in north, middle and south areas of west region of Turkey, were exhibited to various levels of radical scavenger, total antioxidant capacities and total phenolic contents. In generally, the levels of these investigated parameters in EAWEs were higher than MWEs. The results showed coherence with the other researches that ethyl acetate exhibited significant selectivity in extracting procyanidins as an effective antioxidant from natural products[20]. In addition, EAW mixture may extract more polar compounds such as trimers, tetramers and pentamers and increases permeability of seed and skin tissues which lead to a better mass transport by molecular diffusion. Therefore, the selective extraction of antioxidant from natural sources by an appropriate solvent mixture is very important in obtaining a fraction with high antioxidant activity.

Generally, the positive correlations were observed between total phenolic contents and the other parameters such as total antioxidant capacity, $DPPH^{\bullet}$, $O_2^{\bullet -}$ and $HO^{\bullet}$ scavenging capacities of the selected grapes extracts ($r = 0.405$, $r = 0.640$, $r = 0.612$, $p < 0.05$). The antioxidant effect is mainly on account of phenolic components, such as flavanoids, phenolic acids and phenolic diterpenes. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups[21]. Total phenolic contents of seed extracts were higher than skin extracts and their distribution levels in EAWEs and MWEs were generally showed a contrast among the selected grapes. The phenolic content of Eksi kara (2) grape seed was highest in the EAWE as 101.73 ± 4.85 μg GAE/μg extract. In addition, (+)-catechin and (-)-epicatechin levels as the procyanidin monomers in the both seed and skin extracts of grape-2 and -5 were determined. The highest (+)-catechin and (-)-epicatechin values of grape-2 seed were 380 and 125 mg/100 mg dry sample in EAWE. Catechin and epicatechin levels in EAWE of grape-2 were 7.3 and 4.5 times higher than those of grape-5. It was reported that (+)-catechin was the major flavan-3-ol monomer and the most effective compound[22]. The result showed that EAWE of grape-2 seed was exhibited the highest total phenolic contents as well as total catechin monomers. Therefore, it may be had higher antioxidant capacity than the selected grape samples. The catechin and epicatechin levels in EAWs of grape-2 seed were higher than those of Negoska and Merlot grapes[23].

The antioxidant capacities of these extracts were investigated in the $DPPH^{\bullet}$ assay, which primarily evaluates proton radical-scavenging capacity. The synthetic nitrogen-centered $DPPH^{\bullet}$ is used as an indicator compound in testing of hydrogen-donation capacity and thus antioxidant activity. The total scavenging capacities of selected grape seeds and skins, which were defined as the sum of the values of EAW and MW extracts, decreased in order; grape -2 > -4 > -1 > -3 > -5 > -6 and grape -1 > -2 > -6 > -4 = -5 > -3, respectively. While radical scavenging capacities of seed extracts were 40 times higher than those of skin extracts, Eksi kara (2) and Pembe germe (1) grapes were stood in the first ranks. Although Yediveren-Denizli and Yediveren-Izmir grapes were the same varieties, the reason of having higher scavenging capacity of Yediveren-Denizli can be explained by soil properties as well as light factor due to being in more southern of west region of Turkey. The obtained results for all extracts were significantly higher than vitamin C except for skin MWEs ($p < 0.01$). The $DPPH^{\bullet}$ scavenging capacity of these extracts may be mostly related to their phenolic hydroxyl groups. The $DPPH^{\bullet}$ scavenging capacities of EAWEs of grape seeds were also higher than those of *Hypericum hyssopifolium*, *Swiss chard* (β-*vulgaris*), *Origanum vulgaris* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L[24-26]. Generally, there was a linear correlation between the total

phenolic contents and the DPPH$^{\bullet}$ scavenging capacities for each extract (r = 0.405). These results were also supported by catechin and epicatechin levels which they were determined for grape-2 and -5. The obtained data were in coherence with some research results[22,25].

The relation between total phenolic contents and total antioxidant capacities in grape seed and skin extracts compared to control indicates the formation of the conjugate diene hydroperoxides from linoleic acid oxidation as results of free radical damage in cell. Antioxidant capacities of the selected grapes, which minimize the oxidation of lipid components in cell membranes, were higher than those of Bangalore blue grape, onion, Swiss card, *Phellinus baumii* (belonging to family, Hymenochaetaceae) and mushroom (*Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia)[25,27-30].

Hydroxyl radicals are biologically relevant and extremely reactive oxygen species[2]. The HO$^{\bullet}$ generated from the Fenton reaction was scavenged by phenols which act as antioxidants[21]. According to the results, grape -2, -4 and -1 were attracted for HO$^{\bullet}$ scavenging capacities. The highest HO$^{\bullet}$ scavenging capacity, which also included the highest level of total phenolic contents as well as catechin and epicatechin levels, was determined in EAWEs of Eksi kara (2) grape seed and skin in the presence of 0.20 and 0.77 µg/mL, respectively. These extracts have higher scavenging capacities on hydroxyl radical than potato peel, *Origanum vulgaris, Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris* and mushroom polysaccharide extracts[26,31,32].

In cellular oxidation reactions, $O_2^{\bullet-}$ is normally formed and its effects can be magnified because it produces either kinds of cell-damaging free radicals and oxidizing agents. In the present study, $O_2^{\bullet-}$ scavenging capacities were observed only in grape seed EA:WEs at varying degrees. The order of decreasing $O_2^{\bullet-}$ scavenging capacities of these extracts were grape- 2 = -4 > -1> -5 > -6 > -3. These results suggested that the antioxidant capacities of seed EAWEs were related to their ability to scavenge superoxide anion radicals. EAWEs of Eksi kara (2) and also Yediveren-Denizli (4), which had the highest percentage of $O_2^{\bullet-}$ scavenging, were much effective on superoxide radical than potato peel extract[31]. While $O_2^{\bullet-}$ was scavenged by grape seed EAWEs, but MWEs did not. This is originated from possible differentiation on phenolic component contents dependent extraction conditions. In some reports, it was shown that especially procyanidin B_2 3'-*o*-gallate from phenolic components was the most effective compound in trapping oxygen free radicals[33,34].

According to the obtained results, radicals scavenging abilities and total antioxidant capacities of grape seed EAWEs and MWEs were correlated with their total phenolic contents. Conversely, the obtained data were not

significant linkage between DPPH$^{\bullet}$, $O_2^{\bullet -}$ and HO$^{\bullet}$ scavenging capacities and total phenolic contents of grape skin extracts ($p > 0.01$). For instance, HO$^{\bullet}$ scavenging capacities of Pembe germe (1) grape skin extracts, which had lower total phenol values, were higher than the other extracts. In addition, the higher total phenol contents of Yediveren-Izmir (5) and Iri kara (6) grapes skin extracts might origin from coloured anthocyanin contents. These coloured components may not relate to antiradical capacity among grapes tested[35]. This situation was supported by lower catechin and epicatectin levels in grape-5.

In order to determine of the applicability of food processes as an additive substances of grape residues from wine sector; grape Eksi kara (2) seed and skin, which had the highest radicals scavenging capacities, were heated in dough at 200 ºC for 45 min. Later, it was established that their total phenolic values were increased but contrast to radicals scavenging capacities were decreased between 10-20 % at the same conditions. The decreases in antioxidant capacities of grape-2 seed and skin extracts after heating process were acceptable levels. Phenols are known to have a strong tendency to undergo polymerization reactions that promote important changes and, as a consequence, variations in their values and properties. Therefore, temperature has important roles on phenolic properties and values. In heat process, the denaturation in seed and skin tissues was caused, with dramatic effects on radical scavenging capacity and positive effect on total phenolic value[36].

The present study showed that Turkish grape varieties are strong radical scavengers and can be considered as good sources of natural antioxidants. This study suggested that grape seeds as well as skins are useful nutritional antioxidants as food additives and pharmacological usage. The natural food additive is advantage that maximum lawful levels for synthetic food additives are established from various toxicological parameters that need to be applicable to naturally occurring compounds[37].

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