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PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITIES IN GRAPE BERRY SKIN, SEED AND STEMS OF SIX WINE GRAPE VARIETIES GROWN IN TURKEY

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ABSTRACT

In this study, seeds, skins and stems of the red wine grape varieties Boğazkere, Cabernet Sauvignon, Merlot, Nero d'Avola, Sangiovese and Syrah grown in Turkey were analysed for their phenolic compounds. The highest total phenolic compound and DPPH were found in the stem of Boğazkere respectively as 62550 mg GAE/kg dw and 614 µmol/g dw; the highest $ABTS^{\bullet+}$, (+)-catechin, (–)-epicatechin were detected in the seed of Nero d'Avola respectively as 617 µmol trolox/g dw, 8650 mg/kg and 1902 mg/kg dw; the highest total anthocyanin and rutin were measured in the skin of Boğazkere respectively as 143.52 mg/kg dw and 9692 mg/kg dw; the highest quercetin was found in the seed of Boğazkere as 49.21 mg/kg dw and the highest *trans*-resveratrol was measured in the stem of Syrah as 61.56 mg/kg dw.

Key words: grape seed, grape skin, grape stem, *trans*-resveratrol, $ABTS^{\bullet+}$

INTRODUCTION

High-energy atoms or molecules with at least one non-paired electron in their outer orbitals are called "free radicals" [Nawar 1996]. Since free radicals are unstable due to their unmatched electrons, they tend to react with other substances and cause damage to their lipid, protein and DNA structures. The relationship between free radicals and diseases is explained by the term of "oxidative stress".

Various environmental pollutants, including pesticides, toxic chemical wastes, ambient cigarette smoke, exhaust, urban air pollutants of ozone and radiation and physical stress have similar toxic effects on human health [Bagchi et al. 2000]. As a result of all these negative factors mentioned above, the formation of free radicals increases. When the free radical-antioxidant balance within human body deteriorates, many diseases including inflammatory diseases, Alzheimer's disease, tumor formation, poor quality aging, ischemia and immune system diseases occur [Bagchi et al. 2000].

With the understanding of the relationship between free radicals and diseases, the public interest in antioxidants has increased. Antioxidants are compounds that inhibit the onset or progression of oxidation reactions by retaining oxygen in the environment and a antioxidant is defined as "any substance that, when present in low concentrations compared to that of an oxidizable substrate" [Halliwell and Gutteridge 1989]. The human body contains an antioxidant defense system to counteract damage caused by oxidative stres.

Secondary metabolites are not directly related to the vital activities of plants, but they regulate their in-

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teraction with the environment and their adaptation. The grape contains a large number of secondary metabolites, and the phenolic compounds are the richest member of this group qualitatively and quantitatively. It is known that the phenolic composition in grapes varies according to variety, environmental conditions and cultivation techniques. Grapes contain high amounts of phenolic compounds [Macheix et al. 1990]. While phenolic compounds in the vines influence aroma, color, bitterness, and mouth-feel properties of wines, they also protect wines against biotic and abiotic stress factors. Grape phenolics have been the most studied subjects in recent years with their antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial, antiarthritic, antiallergenic properties and most importantly antioxidative structures [Pastor et al. 2017].

Red grapes, in particular, are a valuable source of phenolic compounds with antioxidant activity: (+)-catechin and (–)-epicatechin (flavan-3-ols), rutin (flavonols) and *trans*-resveratrol (stilben) [Iapocini et al. 2008]. (+)-catechin and (–)-epicatechin are the most dominant phenolic compounds in grape seed. (+)-catechin shows antioxidant activity by delaying endogenous α-tocopherol and β-carotene degradation by inhibiting the oxidation of plasma lipids by [Lotito and Fraga 1997] and (–)-epicatechin shows antioxidant activity by scavenging free radicals [Moini et al. 2002]. Many studies have emphasized that quercetin and rutin provide protection against many diseases, especially rheumatoid arthritis. *Trans*-resveratrol (3,5,4 &-trihydroxy-*trans*-stilbene) is the most commonly known and studied phenolic compound for human health [Bath et al. 2001]. There is much research on the biological activity of this compound [Wood et al. 2004].

Phenolic compounds do not exhibit the same antioxidative properties in quality and quantity due to differences in cultivation techniques, harvest maturity, oenological techniques and grape variety [Pérez-Magariño and González-San José 2004].

Turkey has 416.906 ha of vineyards and grape production 4.2 million tons, according to data of 2017 [FAOSTAT 2018]. Although there have been numerous research on the phenolic content of wines to date, there are a few number of studies investigating both phenolic content and antioxidant activity levels in the seeds, skins and stems of wine grape varieties.

In this study, it was aimed to quantify total phenolic content and antioxidant activity levels of Cabernet Sauvignon, Merlot, Syrah, Sangiovese and Nero d'Avola varieties grown in Urla-Izmir, one of Turkey's most important vineyard regions and of Turkey's highest quality red wine grape variety Boğazkere. For this purpose, total phenolic and total anthocyanin contents and antioxidant activity levels of the seeds, skins and stems of the varieties were determined by spectrophotometric method; and (+)-catechin, (–)-epicatechin, rutin, quercetin and *trans*-resveratrol levels were determined by HPLC-DAD. This paper studies the selected phenolic composition known with health effects of the skins, seeds and especially stems of six white grape varieties, and compares them with those of four varieties of redgrapes, all widely grown and of recognized prestige.

MATERIAL AND METHODS

Chemicals. The standards of phenolic compounds and methanol, ethyl acetate, acetonitrile, potassium persulfate, sodium chloride, sodium phosphate monobasic and dibasic, (R)-(+)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Folin Ciocalteus phenol reagent, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 2,2′-diphenyl-1-pycrylhydrazyl radical (DPPH$^{\bullet}$) were obtained from Sigma-Aldrich (St Louis, Miss., USA). Hydrochloric acid, formic acid, sodium carbonate, sodium acetate, and potassium chloride were obtained from Merck (Darmstadt, Germany). Deionized waterwas obtained from a Milli-Q Element water purification system (Millipore, Bedford, MA, USA).

Plant Material. In this research, 5 varieties of international red wine grapes (Cabernet Sauvignon, Merlot, Syrah, Sangiovese and Nero d'Avola) and one national red wine grape variety (Boğazkere) were used as plant material. The varieties were provided from private sector vineyards (38°15'10.39"N, 26°44'14.11"E; elevation 38 m) in Urla-Izmir, one of Turkey's most important vineyard regions [Vintage 2012]. In order to obtain the same amount of product in vinestocks of all varieties included in the study, the numbers of buds left in the winter pruning were kept the same (20 buds/vinestock). The stems from 5 vine for each variety were harvested manually during physiological maturity periods and transferred to the Post-Harvest

Physiology Laboratory of the Department of Horticulture, Faculty of Agriculture, Ankara University in cooler boxes on the same day. Clusters for each variety (5 kg) were selected randomly and and rapidly separated into the seed, skin and stem with a scalpel, lyophilized at –87°C with Freeze Drier (Labconco Freezone 2.5 Liter, USA) for 72 h. Lyophilized samples were stored at –80°C in a light-proof manner until analysis.

Extraction

Extraction of Samples for Spectrophotometric Analysis. Lyophilized sample (0,5 g for seed, skin, stem samples) were weighed and milled with the help of a homogenizer (Ultra-Turrax T25, Ika-Labortechnik, Germany) for 3 min at 24,000 rpm with the addition of 10 mL of methanol. Then centrifuged at 3000 rpm for 10 min to remove the liquid fraction and methanol was evaporated at the rotary evaporator set to 40°C. Post-rotary extracts were made up to 25 mL with pure water (containing 0.01% HCl, v/v). Before spectrophotometric analysis, the extracts were passed through 0.45 Starm polyvinylidene difluoride (PVDF) filters (Sartorius, Goettingen, Germany). Total phenolic, total anthocyanin and antioxidant activity analyzes were performed using the Shimadzu brand 1700 model UV Vis Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Samples were extracted 3 times.

Extraction of Samples for HPLC-DAD Analysis. The extracts obtained for spectrophotometric analyses were purified by means of vacuum monifold system using C_{18C} Sep-Pak (Waters, Milford, MA, U.S.A.) cartridges. For this purpose, the cartridges were loaded with 5 mL ethyl acetate, 5 mL methanol (containing 0.01% HCl, v/v) and 2 mL aqueous 0.01% HCl (v/v) respectively. Then 1 mL of extract was loaded, then 5 mL of ethyl acetate was added to the cartridge to obtain pure extract. These pure extracts were dried under nitrogen gas (TurboVap LV, Caliper, Hopkinton, MA, USA) at 40°C, and aqueous 0.01% HCl ultrasonic bath was used to dissolve the phenolic compounds. The result extract was transferred from 0.45 µm PVDF filters (Sartorius, Goettingen, Germany] to amber color auto-sampler vials [Waterhouse 2005].

Spectrophotometric analyses

Total phenolic compounds. Total phenolic composition was determined according to Singleton and Rossi [1965] in the seeds, skins and stems of grape varieties. Measurement results are expressed in mg GAE/kg dry weight (dw). For this purpose, calibration curves were obtained using gallic acid at concentrations of 1200, 1100, 1000, 900, 800, 700, and 600 mg/L ($R^2 = 0.9948$). Measurements were performed at 765 nm. Triplicate analyses were performed for each sample.

Total anthocyanin. Total anthocyanin analyzes in the skins of grape varieties were performed by pH differential method developed by Giusti and Wrolstad [2001]. The total amount of anthocyanins was calculated in terms of malvidin-3-glucoside dominantly found in grape. The measurements were made at 520 and 700 nm and the results were calculated in mg/kg dw according to the following formula.Triplicate analyses were performed for each sample.

$$\text{Total anthocyanin content (mg/L)} =$$
$$= [(A) \times (MW) \times (DF) \times 1000]/[(\varepsilon) \times (L)];$$

where: A – difference of sample absorbance between pH 1.0 and 4.5; MW – molecular weight; DF – dilution factor; ε – molar absorption coefficient; L – pathlength (cm).

Antioxidant capacity. The antioxidant capacity in the seeds, skins and stems was also determined by the method of TEAC (Trolox Equivalent Antioxidant Activity) according to Re et al. [1999] and by the method of DPPH$^{\bullet}$ (2,2′-diphenyl-1-pycrylhydrazyl) according to Brand-Williams et al. [1995]. Triplicate analyses were performed for each sample.

ABTS$^{\bullet+}$. Antioxidant capacities of extracts were evaluated using the trolox equivalent antioxidant capacity assay based on the method of Re and others [1999]. First, ABTS (2.2′-azinobis-(3-etilenbenzotiazolin-6-sulfonik asit) diammonium salt)-98-Sigma A1888 solution was prepared by using 2.45 mM potassium persulfate. The radical solution was allowed to stand in the dark for 12 to 16 h at room temperature and was used within 2 d of preparation. During analysis, the solution was kept in +4°C. 0.1 M and phosphate buffered saline (PBS) buffer (pH 7.4) was prepared to dilute ABTS and extracts. Readings were executed in PBS solution and before each reading, the ABTS radical solution was diluted with PBS to an absorbance of 0.700 (±0.010) at 734 nm. The analyses were conducted with 10, 20, and 30 µL samples to obtain 3 differ-

ent inhibition rates at the end of 6 min. The standard curve (R^2 = 0.9996) obtained with the trolox standard (R-(+)-6-Hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid %98-Aldrich 391913) in 5, 10, 15, and 20 µM concentrations was used for calculations and the results are expressed as µmol trolox/g dw.

DPPH• The seed, skin and stem extracts and the 2,2′-diphenyl-1-pycrylhydrazyl (DPPH•) methanolic solution were stirred and allowed to stand in the dark for 30 min and the reaction was then measured at 517 nm. The results are expressed as µmol trolox/g dw [Brand-Williams et al. 1995].

HPLC-DAD analyses. For determination of (+)-catechin, (–)-epicatechin, quercetin, rutin and *trans*-resveratrol levels in the seeds, skins and stems, Shimadzu LC 10 AT VP system (Shimadzu Corp., Kyoto, Japan) HPLC device with diode array detector (DAD) was used and the analysis was performed according to Waterhouse [2005]. Gemini Phenomenex C18 (Calif., U.S.A.): 4.6 mm × 260 mm was used as column and 2 solvents were used as mobile phase: solvent A, water/ formic acid (99/1: v/v) and solvent B, acetonitrile (100/100: v/v). The identification of phenolic compounds was obtained out by using authentic standards and by comparing the retention times and their visible spectra, while quantification was performed by external calibration with standards. The limit of detection (LOD) was calculated with the equation LOD = 3 σ/S and the limit of quantification (LOQ) was calculated with the equation LOQ = 10 σ/S, where σ is the standard deviation of the y-intercepts of the calibration curves, and S is the average of the slopes of the concentration curves. The results were expressed as mg/kg sample dw. Calibration parameters are given in Table 1.

Statistical analysis

All analyses were run in triplicate and the results expressed as mean ± standard deviation (SD). Statistical analyses of the data were carried out using SPSS (SPSS Inc., Chicago, Illinois) statistical program version 11.5 and Duncan's multiple range tests were used to determine the significance level. A two tailed Pearson's correlation test was conducted to determine the correlations among means.

RESULTS AND DISCUSSION

Titratable acidity, pH, total soluble solids (Brix°) and harvest dates of grape varieties. Technological maturity level pH, titratable acidity and total soluble solids (Brix°) values of grape varieties at the time of harvest and harvest dates are given in Table 2. pH levels of the varieties ranged between 3.52–4.01; titratable acidityvalues between 2.99–5.59 mg/L and Brix° values between 23.8–26°. All of the varieties have reached technological maturity in September, the month in which the wine grape varieties in Urla-Izmir are mostly ripened. The earliest maturing variety was the Merlot variety harvested on 5th September and the latest maturing variety was the Cabernet Sauvignon variety which reached the maturity of harvest on 20th September.

Different letters within the same column indicate significant difference at $p < 0.05$ by Duncan's test. Values are mean ±SD values of three replicates. Titratable acids were expressed as miligrams tartaric acid equivalents/L .

Total phenolic compounds, total anthocyanin and antioxidant capacity of seeds, skins and stems. Table 3 shows the total phenolic compounds and

Table 1. Calibration parameters used for the HPLC-DAD determination of phenolic compounds

Phenolic compounds	RT (min)	λ (nm)	Calibration curve	R^2	LOD (mg/kg)	LOQ (mg/kg)
(+)-catechin	28.6	280	y = 15323x–160	0.9997	0.96	2.91
(–)-epicatechin	33.7	280	y = 33977x–7173	0.9999	0.69	2.09
Rutin	55.6	365	y = 20153x–44559	0.9999	0.46	1.53
Quercetin	43.2	365	y = 74629x–24943	0.9999	0.45	1.37
trans-resveratrol	54.9	306	y = 403404x–78716	0.9998	0.28	0.86

RT – retention time, λ – detection wavelength, R^2 – correlation coefficients, LOD – limit of detection, LOQ – limit of quantification

Table 2. pH, titratable acids, Brix° and harvest dates of grape varieties

Varieties	pH value	Titratable acidity	Brix°	Harvest date
Cabernet Sauvignon	3.81 ±0.03^{b}	3.22 ±0.09^{c}	26.0 ±0.5^{a}	20.09.2012
Merlot	4.01 ±0.03^{a}	3.57 ±0.06^{bc}	25.8 ±0.2^{a}	05.09.2012
Syrah	3.78 ±0.04^{c}	3.45 ±0.05^{c}	25.3 ±0.3^{ab}	14.09.2012
Boğazkere	3.98 ±0.07^{ab}	2.99 ±0.05^{c}	23.8 ±0.2^{c}	15.10.2012
Nero d'Avola	3.88 ±0.09^{bc}	5.59 ±0.91^{a}	24.4 ±0.5^{bc}	07.10.2012
Sangiovese	3.52 ±0.01^{d}	4.71 ±0.15^{ab}	24.2 ±0.3^{bc}	14.09.2012

Table 3. Total phenolics and antioxidant capacity determined by the ABTS$^{\bullet+}$ and DPPH assays of the grape seeds, skins and stems

Seed				
varieties	total phenolics (mg GAE /kg dw)*	antioxidant capacity		total anthocyanin (mg/kg dw)
		ABTS$^{\bullet+}$ (µmol trolox/g dw)	DPPH (µmol/g dw)	
Cabernet Sauvignon	56550 ±700^{b}	552 ±1.3^{b}	486 ±0.5^{b}	–
Merlot	45350 ±200^{c}	474 ±8.8^{d}	180 ±0.2^{d}	–
Syrah	31050 ±225^{d}	319 ±1.3^{e}	120 ±0.4^{e}	–
Boğazkere	60250 ±1100^{a}	495 ±2.4^{c}	378 ±0.3^{c}	–
Nero d'Avola	59950 ±100^{a}	617 ±0.1^{a}	525 ±0.1^{a}	–
Sangiovese	27400 ±325^{e}	477 ±5.5^{d}	219 ±1.1^{d}	–
skin				
varieties	total phenolics (mg GAE/kg dw)	antioxidant capacity		total anthocyanin (mg/kg dw)
		ABTS$^{\bullet+}$ (µmol trolox/g dw)	DPPH (µmol/g dw)	
Cabernet Sauvignon	21950 ±75^{d}	174 ±2.4^{de}	144 ±1.3^{de}	3815 ±11.5^{e}
Merlot	25950 ±475^{c}	297 ±1.4^{b}	296 ±1.0^{b}	6723 ±4.85^{d}
Syrah	26750 ±225^{c}	184 ±3.1^{cd}	157 ±3.0^{d}	8352 ±23.8^{b}
Boğazkere	37875 ±200^{a}	320 ±8.6^{a}	390 ±4.3^{a}	9692 ±63.4^{a}
Nero d'Avola	21175 ±50^{d}	164 ±4.2^{e}	134 ±1.1^{e}	6773.3 ±0^{d}
Sangiovese	31400 ±75^{b}	195 ±2.1^{c}	197 ±2.1^{c}	7762 ±28.6^{c}
stem				
varieties	total phenolics (mg GAE /kg dw)	antioxidant capacity		total anthocyanin (mg/kg dw)
		ABTS$^{\bullet+}$ (µmol trolox/g dw)	DPPH (µmol/g dw)	
Cabernet Sauvignon	37800 ±950^{d}	510 ±1.5^{b}	580 ±1.0^{b}	–
Merlot	46300 ±1050^{c}	310 ±0.5^{c}	496 ±0.8^{c}	–
Syrah	24075 ±1000^{f}	305 ±0.5^{c}	489 ±0.7^{c}	–
Boğazkere	62550 ±1000^{a}	605 ±1.0^{a}	614 ±1.05^{a}	–
Nero d'Avola	52050 ±1300^{b}	495 ±0.5^{b}	575 ±1.1^{b}	–
Sangiovese	33575 ±50^{e}	120 ±0.01^{d}	287 ±0^{d}	–

Different letters within the same column indicate significant difference at $p < 0.05$ by Duncan's test. Values are mean ±SD values of three replicates.
* GAE: gallic acid equivalent, dw: dry weight

antioxidant capacity levels of the grape varieties. According to the results of the study, total phenolic contents showed significant differences according to the varieties ($p < 0.01$). The seeds of Sangiovese varieties (27400 mg GAE/kg dw), Nero d'Avola (59950 mg GAE/kg dw) and Boğazkere (60250 mg GAE/kg dw) exhibited the highest; and the seeds of exhibited the lowest total phenolic compound values.

The antioxidant capacity $ABTS^{\bullet+}$ levels in the seeds ranged between 319–617 µmol trolox/g dw, and between 120–525 µmol/g dw according to the results of $DPPH^{\bullet}$. As for total phenolic contents, Nero d'Avola seeds also gave the highest values in antioxidant capacity parameters according to the results of both methods. Syrah seeds were found to be poorer than other varieties in terms of antioxidant capacity.

Total phenolic compound, total anthocyanin and antioxidant capacity values in the skins are given in Table 3. Total phenolic content was found to vary between 21 175–37 875 mg GAE/kg dw, total anthocyanin content between 3815–9692 mg/kg dw; and antioxidant capacity between 164–320 µmol trolox/g dw and 134–390 µmol/g dw. The highest values in all parameters were obtained from the skin tissue of Boğazkere variety, while the skins of Nero d'Avola showed the lowest values. Nero d'Avola seeds exhibited the highest values in total phenolic compound and antioxidant capacity parameters, while Nero d'Avola skinshad the lowest total phenolic content and antioxidant capacity compared to other varieties. It is understood that high total anthocyanin level of the Boğazkere variety, which is a very dark colored variety, has been effective in total phenolic contents and antioxidant capacity levels of the skins.

Total phenolic content varied between 62 550–24 075 mg GAE/kg dw; $ABTS^{\bullet+}$ antioxidant capacity between 605–120 µmol trolox/g dw and $DPPH^{\bullet}$ antioxidant capacity between 614–287 µmol/g dw. In terms of total phenolic content and antioxidant capacity of stems, the highest contents were measured in Boğazkere variety (Tab. 3). Sangiovese stemshad the lowest total phenolic content and antioxidant capacity.

Total phenolic content is an important quality parameter for wine grape varieties. Significant variations in the total phenolic content of different varieties of red wine have also been reported in previous studies [Rodriguez Montealegre et al. 2006]. These differences can be influenced by many factors such as climate, maturity and grape variety. As mentioned in previous studies, the extraction method of phenolic compounds from plant material is another factor that affects total phenolic content [Downey et al. 2007]. There were statistically significant differences in the total phenolic contents of the tissues of the varieties. In parallel to previous studies, the total phenolic content in all-examined varieties was higher in the seeds compared to the skins [Iacopini et al. 2008, Pantelić et al. 2016]. According to the results of the study, antioxidant activities of the tissues of the varieties were not only related to the total phenolic content; it was understood that the phenolic compounds could act in synergy, exhibit antagonism, or individually affect the antioxidant activity.

(+)-catechin, (–)-epicatechin, quercetin, rutin and *trans*-resveratrol contents of seeds, skins and stems. Table 4 shows the phenolic contents of the seeds belonging to 6 red wine grape varieties. Seeds were determined as the richest tissues with respect to (+)-catechin and (–)-epicatechin. (+)-catechin contents of the seeds showed values ranging from 2523 (Sangiovese) to 8650 (Nero d'Avola) mg/kg dw; (–)-epicatechin contents showed values ranging from 796 (Boğazkere) to 1902 (Nero d'Avola) mg/kg dw; *trans*-resveratrol contents showed values ranging from 8.09 (Syrah) to 25.54 (Cabernet Sauvignon) mg/kg dw. In this study, rutin and quercetin which are known to be higher in the skin were also determined in the seed. Quercetin was found only in the seeds of 3 varieties (Cabernet Sauvignon, Syrah, Boğazkere) and rutin was found only in the seed of Boğazkere variety (as 3.46 mg/kg dw).

Table 4 shows (+)-catechin, (–)-epicatechin, rutin, quercetin and *trans*-resveratrol contents of the skins of the 6 red grape varieties. (+)-catechin content of the skins ranged from 0.00 (Nero d'Avola) to 611.91 (Boğazkere) mg/kg dw; (–)-epicatechin content of the skins ranged from 23.60 (Syrah) to 70.07 (Sangiovese) mg/kg dw; rutin content of the skins ranged from 67.21 (Merlot) to 143.52 (Boğazkere) mg/kg dw; quercetin content of the skins ranged from 8.54 (Nero d'Avola) to 30.05 (Boğazkere) mg/kg dw; *trans*-resveratrol content of the skins ranged from 19.06 (Sangiovese) to 27.99 (Boğazkere) mg/kg dw. Rodríguez Montealegre et al. [2006] also measured high content of (+)-catechin and (–)-epicatechin in the skins.

Table 4. (+)-catechin, (–)-epicatechin, rutin, quercetin, *trans*-resveratrol content (mg/kg dw*) of the grape seeds, skins and stems

Varieties	(+)-catechin	(–)-epicatechin	Rutin	Quercetin	*trans*-resveratrol
			seed		
Cabernet Sauvignon	4051 ±25^{b}	989 ±10^{b}	nd**	24.90 ±0.01^{b}	25.54 ±2.83^{a}
Merlot	3160 ±49^{c}	1704 ±35^{a}	nd	nd	10.00 ±0.01^{b}
Syrah	3755 ±90^{b}	1687 ±53^{a}	nd	48.12 ±0.06^{a}	8.09 ±0.01^{b}
Boğazkere	2958 ±43^{d}	796 ±11^{b}	3.46 ±0.01^{a}	49.21 ±0.21^{a}	23.30 ±0.01^{a}
Nero d'Avola	8650 ±18^{a}	1902 ±40^{a}	nd	nd	22.31 ±0.10^{a}
Sangiovese	2523 ±21^{d}	1722 ±135^{a}	nd	nd	10.01 ±0.01^{b}
			skin		
Cabernet Sauvignon	106.05 ±0.01^{d}	54.08 ±0.01^{b}	142.49 ±0.70^{a}	18.75 ±0.50^{b}	27.13 ±0.02^{b}
Merlot	263.80 ±39.40^{b}	24.75 ±0.01^{c}	67.21 ±0.01^{b}	9.21 ±0.01^{c}	24.92 ±0.11^{d}
Syrah	127.65 ±6.67^{c}	23.60 ±0.01^{c}	71.12 ±0.01^{b}	8.96 ±0.01^{c}	25.60 ±0.07^{c}
Boğazkere	611.91 ±2.05^{a}	24.70 ±0.01^{c}	143.52 ±0.69^{a}	30.05 ±0.38^{a}	27.99 ±0.01^{a}
Nero d'Avola	nd	24.80 ±0.01^{c}	70.35 ±0.01^{b}	8.54 ±0.01^{c}	22.45 ±0.01^{e}
Sangiovese	173.90 ±1.30^{c}	70.07 ±1.28^{a}	68.35 ±0.01^{b}	9.12 ±0.01^{c}	19.06 ±0.01^{f}
			stem		
Cabernet Sauvignon	3217 ±44^{b}	126.57 ±1.51^{a}	45.95 ±0.18^{c}	21.40 ±0.18^{b}	26.64 ±0.27^{e}
Merlot	1240 ±27^{c}	76.257 ±0.21^{b}	45.79 ±0.68^{c}	19.23 ±0.01^{b}	24.98 ±0.08^{e}
Syrah	2987 ±50^{b}	nd	49.10 ±0.01^{b}	35.40 ±0.50^{a}	61.56 ±0.43^{a}
Boğazkere	1995 ±59^{c}	nd	nd	20.12 ±0.80^{b}	30.28 ±0.34^{d}
Nero d'Avola	2947 ±15^{b}	35.68 ±0.01^{c}	50.12 ±0.60^{b}	39.26 ±1.01^{a}	40.90 ±1.20^{c}
Sangiovese	4353 ±60^{a}	nd	68.34 ±0.21^{a}	40.21 ±1.01^{a}	43.75 ±1.15^{b}

Different letters within the same column indicate significant difference at $p < 0.05$ by Duncan's test. Values are mean ±SD values of three replicates
* dw: dry weight; ** nd: not detected

In their study, Iacopini et al. [2008] determined only resveratrol, rutin and quercetin levels in the skin to prevent interactions with other phenolic compounds by fluorescence using HPLC-UV. In this study, because of the use of HPLC-DAD, 5 phenolic compound levels were determined in all tissues belonging to the varieties, and generally, the amount of catechin and epicatechin in the skin was found to be lower compared to the seed. In parallel with previous research, very small amounts of rutin and quercetin were measured in the seed while their amounts were found to be higher in the skin [Pantelić et al. 2016].

The phenolic contents of the stems are given in Table 4. According to the analysis results, (+)-catechin in the stems ranged from 1240 (Merlot) to 4353 (Sangiovese) mg/kg dw; (–)-epicatechin ranged from 0.0 to 126.57 (Cabernet Sauvignon) mg/kg dw; rutin ranged from 0,0 to 68.34 (Sangiovese) mg/kg dw; quercetin ranged from 19.23 (Merlot) to 40.21 (Sangiovese) mg/kg dw and *trans*-resveratrol ranged from 24.98 (Merlot) to 61.56 (Syrah) mg/kg dw.

The researchers determined the total phenolic content of the stems in the range of 16 000–116 000 mg GAE/kg dw [Kállay and Kerényi 1999, Llobera and Cañellas 2007, Apostolou et al. 2013]; and antioxidant capacity of the stems in the range of 11.8–4.0 µg/mL ($ABTS^{\bullet+}$), 15–4.2 µg/mL ($DPPH^{\bullet}$) [Anastasiadi et al. 2012]. In their study, Apostolou et al. [2013] measured catechin content in the range of 9330–85 810 mg/kg dw; epicatechin content in the range of 0–13 320 mg/kg dw; rutin content in the range of 0–41 830 mg/kg dw; quercetin content in the range of 600–8210 mg/kg dw

and *trans*-resveratrol content in the range of 4850–17 560 mg/kg dw. Phenolic content of the stems of red varieties showed quite varying values. Grape stem which makes up about 25% of winery output is an important residue for the wine industry. According to the research results, the stem is an important source of phenolic compounds and antioxidants. In recent years, stem has been studied extensively for its antioxidant content. Today, the stem is used as sipirits, dietary fiber, plant protein concentrates and fertilizers [Arvanitoyanniset et al. 2006].

In the results of previous studies expressed as dry weight, total phenolic content ranged between 12.10 mg GAE/kg dw – 75 200 mg GAE/kg dw in the skin, between 1167.3 mg/kg dw – 154 600 mg/kg dw in the seed; total anthocyanin content ranged between 651.2 mg/kg dw – 24 500 mg/kg dw in the skin; antioxidant activity by $ABTS^{\bullet+}$ method ranged between 71.76 µmol troloks/g dw – 464.1 µmol troloks/g dw in the skin, between 76.33 µmol troloks/g dw – 649.85 µmol troloks/g dw in the seed, antioxidant activity by $DPPH^{\bullet}$ method ranged between 1.74–300.9 µmol/g in the skin, between 1.7–422.18 µmol/g dw in the seed; catechin ranged between 4.9 mg/kg dw – 7600 mg/kg dw in the skin, between 603 mg/kg dw – 23800 mg/kg dw in the seed; epicatechin ranged between 1 mg/kg dw – 1100 mg/kg dw in the skin, between 0 mg/kg dw – 16 880 mg/kg dw in the seed; quercetin ranged between 5 mg/kg dw – 10.7 mg/kg KA in the skin; rutin ranged between 400.5 mg/kg dw – 1690 mg/kg dw in the skin, between 0 mg/kg dw – 211.3 mg/kg dw in the seed; *trans*-resveratrol ranged between 36 mg/kg dw – 255 mg/kg dw in the skin andbetween 0 mg/kg dw – 28.5 mg/kg dw in the seed [Iacopini et al. 2008, Butkhupl et al. 2010, Xu et al. 2010, Rockenbach et al. 2011, Ky et al. 2014]. Such wide range is reported in the studies of other researchers as well. Therefore, the hypothesis that besides the extraction method, variety differences are also effective in determining the phenolic content is validated.

In the study, five phenolic compounds were determined in the seeds, skins and stems and these 5 phenolic compounds were selected for their biological and pharmacological effects. In similar previous studies, catechin and epicatechin were reported in both seed and skin tissues with higher quantity in the seed, as also supported by the results of this study [Rodriguez Montealegre et al. 2006].

In this study, the highest total phenolic content was measured in the stem (62550 mg GAE/kg dw), the highest $ABTS^{\bullet+}$ was measured in the seed (617 µmol trolox-/g dw), the highest $DPPH^{\bullet}$ was measuredin the stem (614 µmol/g dw), the highest catechin was measuredin the seed (8650 mg/kg dw), the highest epicatechin was measured in the seed (1902 mg/kg dw), the highest rutin was measured in the skin (143.52 mg/kg dw), the highest quercetin was measured in the seed (49.21) and the highest *trans*- resveratrol was measured in the stem (61.56 mg/kg dw).

The highest seed content of total phenolics, antioxidant activity, catechin and epicatechin were measured in Nero d'Avola variety and the highest seed content of rutin, quercetin and *trans*-resveratrol was determined in Boğazkere variety. All parameters except epicatechin were the highest in Boğazkere variety. The highest stem content of total phenolics and antioxidant activity was measured in Boğazkere variety, the highest stem content of catechin and rutin was determined in Sangiovese variety, the highest stem content of epicatechin was measured in Cabernet Sauvignon variety and the highest stem content of *trans*-resveratrol was determined in Syrah variety. Boğazkere and Nero d'Avola were the remarkable varieties in terms of the parameters examined in this study.

Correlations. A correlation analysis was done between the total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, (+) – catechin, and *trans*- resveratrol for the grape seeds; total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, total anthocyanin and (+)-catechin for the grape skins; total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and rutin for the grape stems for all measurement (n = 54) – Tables 5, 6 and 7. Correlation analyses were performed also for other parameters but were not presented in the table as they were statistically insignificant. Significant correlations among different antioxidant assays ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) were found in seeds, skins and stems. This result suggests that these two assays are almost comparable and interchangeable in characterising the grape antioxidant capacities. These results are in agreement with Xu et al. [2010].

In the seed, the highest correlation was measured between $DPPH^{\bullet}$ and *trans*-resveratrol ($r = 0.940^{**}$, $p < 0.01$). This was followed by the correlation

Table 5. Analysis of the correlation (r^2) between the total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, (+)-catechin, and *trans*-resveratrol in the grape seeds

	Total phenolic compounds	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	(+)-catechin	*trans*-resveratrol
Total phenolic compounds	1	0.727**	0.840**	0.492*	0.890**
$ABTS^{\bullet+}$		1	0.883**	0.591**	0.744**
DPPH			1	0.641**	0.940**
(+)-catechin				1	0.404*
trans-resveratrol					1

* Correlation is significant at the 0.05 level (n = 54); ** correlation is significant at the 0.01 level

Table 6. Analysis of the correlation (r^2) between the total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, total anthocyanin and (+)-catechin in the grape skins

	Total phenolic compounds	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	Total anthocyanin	(+)-catechin
Total phenolic compounds	1	0.684**	0.801**	0.794**	0.884**
$ABTS^{\bullet+}$		1	0.979**	0.499*	0.893**
$DPPH^{\bullet}$			1	0.586*	0.957**
Total anthocyanin				1	0.616**
(+)-catechin					1

* Correlation is significant at the 0.05 level (n = 54); ** correlation is significant at the 0.01 level

Table 7. Analysis of the correlation (r^2) between the total phenolic compounds, $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and rutin in the grape stems

	Total phenolic compounds	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	Rutin
Total phenolic compounds	1	0687^{**}	0.576^{*}	-0.724^{**}
$ABTS^{\bullet+}$		1	0.955^{**}	-0.775^{**}
$DPPH^{\bullet}$			1	-0.710^{**}
Rutin				1

* Correlation is significant at the 0.05 level (n = 54); ** correlation is significant at the 0.01 level

between total phenolic compound and *trans*-resveratrol (r = 0.890**), $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (r = 0.883**, p < 0.01), total phenolic compound and $DPPH^{\bullet}$ (r = 0.840**, p < 0.01), $ABTS^{\bullet+}$and *trans*- resveratrol (r = 0.744**, p < 0.01), total phenolic compound and $ABTS^{\bullet+}$ (r = 0.727**, p < 0.01), $DPPH^{\bullet}$ and catechin (r = 0.641**, p < 0.01), $ABTS^{\bullet+}$ and catechin (r = 0.591**, p < 0.01), total phenolic compound and catechin (r = 0.492*, p < 0.05). In the skin, the highest correlation was measured between $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (r = 0.979**, p < 0.01). This was followed by the correlation between $DPPH^{\bullet}$ and catechin (r = 0.957**, p < 0.01), rutin and quercetin (r = 0.923**, p < 0.01), $ABTS^{\bullet+}$ and catechin (r = 0.893**, p < 0.01), total phenolic compound and catechin (r = 0.884**, p < 0.01), total phenolic compound and $DPPH^{\bullet}$ (r = 0.801**, p < 0.01), total phenolic compound and total anthocyanin (r = 0.794**, p < 0.01), total phenolic compound and $ABTS^{\bullet+}$ (r = 0.684**, p < 0.01). Correlation analysis results in skin and seed are similar to those of previous studies [Xu et al. 2010]. In the stem, the highest correlations were measured respectively between $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ (r = 0.955**, p < 0.01), total phenolic compound and $ABTS^{\bullet+}$ (r = 0.687**, p < 0.01),

and total phenolic compound and DPPH$^{\bullet}$ ($r = 0.576^{**}$, $p < 0.05$).

The correlation analysis results indicate that phenolic compounds and antioxidant capacity may correlate positively or negatively and be questionable. It is suggested that the antioxidant effects of phenolic compounds should be elaborated by more detailed future research.

CONCLUSIONS

In this study, phenolic contents and antioxidant capacity levels were determined in the seeds, skins and stems of 6 red wine grape varieties grown in the same vineyard and correlation analyzes were performed between the results. According to the results of the study, significant statistical differences were observed between varieties and tissues. Boğazkere and Nero d'Avola were the remarkable varieties in terms of their phenolic contents and antioxidant capacity levels. In all tissues examined, catechin was the highest measured phenolic compound. Seed and skin are extensively studied materials for their phenolic contents and antioxidant capacity levels. Introducing the stems of the same varieties in the study has been a different aspect of this research. Especially the stem of Boğazkere variety was found to be a strong source of total phenolic compounds. The highest antioxidant capacity level, total phenolic, (+)-catechin, (–)-epicatechin and quercetin contents were measured in the seed. Rutin was measured as the highest in the skin and *trans*-resveratrol was measured as the highest in the stem. Phenolic compounds do not act according to exact parameters as demonstrated in previous studies, and they are easily affected by external factors and show quantitative differences. For this reason, unified modification of product load in all varieties for the purpose of eliminating the effect of pruning and product load-is an important aspect of this research. This research is considered to be a valuable reference for phenolic profiles of red wine grape varieties in Turkey in the forthcoming harvest periods.

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