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Nutraceutical and Functional Properties of Peel, Pulp, and Seed Extracts of Six 'Köhnü' Grape Clones

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Abstract: Grape production has a long history in the Elazig province and surrounding vicinity and produced grapes have been used for table consumption and also processed into traditional beverages, Şıra (special non-alcoholic grape juice) and wine. In the Elazig province, the main grape cultivars are 'Ağın Beyazı', 'Öküzgözü', 'Boğazkere', 'Şilfoni', 'Tahannebi', and 'Köhnü'. Among them, 'Köhnü' cultivar is highly preferred by consumers due to its black color and perfect berry characteristics. The cultivar has grown for centuries in different parts of Elazig and shows a great variability for most of its morphological and biochemical characteristics. In the present study, we aimed to determine morphological and biochemical traits in six 'Köhnü' clones sampled from Elazığ. The cluster weight of six clones was found between 334–394 g. The highest total phenolic content was observed in seeds followed by peel and pulp samples. The seed extract of Clone 2 had the highest total phenolic content at 254 mg gallic acid equivalent/100 g fresh weight. The results also showed that peel, pulp, and seed samples of 'Köhnü' grape clones had considerable amounts of antioxidant components determined by DPPH (1,1-Diphenyl-2-picryl-hydrazyl), FRAP (ferric reducing antioxidant power), and TEAC assays and might be rich sources of natural antioxidants. Among the six 'Köhnü' clones, Clone 3, and Clone 6 differed from the others in respect to the highest cluster weight, the highest concentrations of total phenolic content, and antioxidant activity. The results also implied that all clones could be used potentially as a readily accessible source of natural antioxidants and as a possible pharmaceutical supplement.

Keywords: grape; biological activity; skin; pulp; seed

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1. Introduction

Turkey, a bridge between Asia and Europe, is one of the most important countries for horticulture. Turkey ranks first in terms of production amount in some fruit species such as hazelnut, sweet cherry, apricot, sour cherry, and quince. The country has nine agricultural regions with different climate and soil conditions. This situation is quite remarkable for a country that does not have a large area. Each region has its own special fruit species, and more than 100 fruit species can be grown easily in Turkey. In terms of grape production, the country is the 6th most important grape producer in the world with a production value of approximately 4,000,000 tons [1–4].

Grapevine and viticulture have a culture that is as old as human history and can be found in mythologies and religious texts. In this sense, vineyard cultivation is not only an economic endeavor for Anatolian lands but also a factor ensuring cultural continuity. Anatolia is a central point in the journey of *Vitis* species. The South Caucasus

(Georgia–Azerbaijan–Armenia) and the Fertile Crescent regions are accepted as the vine's homeland [5,6].

Grapes have a very important place in Turkey's agriculture. It is accepted as one of the most important and economic horticultural crops. Almost all agricultural regions have grape production in Turkey, and each region has its own characteristic production system. In Turkey, grape berries are traditionally processed into several very special products such as 'pestil', 'pekmez', 'sucuk', 'koruk', 'köfter', etc. All these products are unique to Turkey. The used cultivars and obtained products from grapes also differ from one region to the other. Approximately 30% of the grapes produced in Turkey are used in fresh (table) production, 37% are dried, 30% are processed into molasses, juice (şıra in Turkish), cider, etc., and 3% are used for wine production. Around 27% of grape production consists of seedless grapes. Seedless raisins are common in the Aegean region, and table and wine grapes are common in the Marmara and Mediterranean region [7–11].

Grape species can be classified as the species used commercially only for fruit production, the species used only for rootstocks, and the species used for ornamental purposes. Among the species used for fruit production, the most common in the world contains the *Vitis vinifera* L. ssp. *sativa*. There are more than 10,000 grape cultivars that belong to this species and more than 90% of the production in the world is obtained from *Vitis vinifera*. It is believed that the species is obtained from its wild ancestor *Vitis vinifera* L. ssp. *sylvestris* Gmel [12].

Scientific studies conducted around the world in recent years have revealed that plants, especially horticultural crops (fruits, vegetables, and grapes), have very important functions in terms of human health. They include a high content of non-nutritive, nutritive, and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. They also have distinct flavor and taste, excellent medicinal value, and health care functions. Grape (*Vitis vinifera*) is a symbol of health, longevity, stamina, and spiritual resolve in particular in Mediterranean countries and its fruits have been traditionally used as a food and medicine dating back centuries, due to their unique flavors, nutritional properties, and health benefits. More recently, grape fruits have received increased attention among consumers worldwide. Grape fruits are well-known for their nutrition and health-promoting value as a source of phenolic acids, flavonoids, anthocyanins, amino acids, etc. Peel, pulp, and seeds of grape berries play an important role in human nutrition and health because of their nutritional and bioactive principles. In the last decades, compelling evidence has suggested that regular consumption of these products may contribute to reducing the incidence of chronic illnesses, such as cancer, cardiovascular diseases, ischemic stroke, neurodegenerative disorders, and aging [13–20]. Therefore, grape berries have been broadly studied due to their composition in phenolic substances and antioxidant compounds and their potential beneficial effects on human health. Studies have shown that grapes and their derivative products are a rich source of bioactive molecules, including flavonoid compounds (flavonols, monomeric catechins, proanthocyanidins, anthocyanins, anthocyanidins) and non-flavonoid phenolic compounds (resveratrol), as well as their metabolites. Grape cultivars differ from each other based on their phenolic substances and antioxidant capacity. Moreover, different plant parts such as leaves, seeds, berry peel, and berry pulp show differences in biologically active substances. Among grapes, black colored ones and their products contain rich nutritional and phenolic substances. The growing conditions, cultural applications, etc., also affect the biological activity of grape berries [20–29].

Elazığ province is located in eastern Anatolia and viticulture has a long tradition in the province. 'Köhnü' cultivar is one of the most important black grape cultivars grown in Elazığ (particularly in Hoşköy village) and used both for the production of high-quality table grapes and for wine production. Viticulture is carried out on an area of approximately 10.000 decares in Hoşköy, Elazığ in eastern Anatolia and is the main branch of agriculture in this village. Although many different grape cultivars ('Ağın Beyazı',

'Öküzgözü', 'Boğazkere', 'Şilfoni', 'Tahannebi') are grown throughout the village, 'Köhnü' is mostly preferred as a black table grape cultivar. In addition to its high average yield, 'Köhnü' has the characteristic of being the most grown dark-colored cultivar in the region for many years, due to its superior berry quality (balanced sugar-acid ratio, high juice yield, uniform peel color, distinct berry attractiveness, high yield capacity, etc.). It is a dark black-blue colored table cultivar with a long storage and high transportation capacity. The clusters of the cultivar are medium size and, in general, range from 300 to 400 gr with an average yield of 1.2–1.5 tons per decare. The cultivar is sensitive to powder sulphur application. High-dose sulphur applications damage the berries of this cultivar and cause yield loss. 'Köhnü', which is a medium-late maturated cultivar, has a long harvest period between 1st September and 15th November, although it varies according to the years. The cultivar is generally pruned over 9–12 buds. The plantations of this cultivar in Elazığ have a heterogeneous population and show great morphological diversity even in the same vineyard [30]. Therefore, the determination of morphological and bioactive contents of different clones could be important for obtaining clones with better characteristics than the population. The complex selection criteria encompass traits such as grape yield, sugar and organic acid content, and polyphenolic compounds in the grape [31,32].

Considering that there is limited information available on some important plant horticultural characteristics and bioactive content of peel, pulp, and seed extracts of 'Köhnü' clones, providing further data on these morphological and biochemical parameters will be useful for assessing the potential biological effects of these products in the future. In addition, obtained data will be helpful in further exploitation of different clones for food processing and breeding program. In addition, the development of novel products and innovative value chains, particularly in the context of being healthy, to determine bioactive content of different plant parts of this cultivar are promising strategies for the further valorization of grape.

Thus, the aim of this study is to obtain substantial knowledge on morphological traits, bioactive content, and in vitro antioxidant activity of different 'Köhnü' grape clones widely produced in Elazig province in Turkey.

2. Materials and Methods

2.1. Plant Material, Sampling and Location

In this study, six different 'Köhnü' clones were harvested from different vineyards in Hoşköy village of Elazığ province in 2019 growing season. The coordinates of the village are 38.6833° N latitude, 39.3945° E longitude. The sampling vineyards are around 10 years old with trellis wire training system. The cluster samples were picked homogenously, and their laboratory analyses were conducted after the morphological measurements.

2.2. Morphological Traits

Cluster weight, cluster form, berry weight, and berry color were performed in the full ripening period on a representative 10 clusters per clone. Characterization of the berries (weight and skin color) was performed in a representative sample of 40 berries taken randomly from the middle part of clusters [33].

2.3. Extraction

The fresh grape pulp, skin, and seed extracts were carried out according to Contreras-Calderón [34] with slight modifications. Two grams of sample was placed in a capped centrifuge tube and 8 mL of acidic methanol-water (60:40, v/v pH 2) was added, after which the tube was vortexed for 1 min at normal atmosphere in a vortex mixer and shaken at room temperature in a shaker for 1 h. The tube was then centrifuged at 9000 rpm/10 min at 4 °C and the supernatant was recovered. Eight milliliters of acetone-water (70:30) was added to the residue, followed by stirring and sonication for 1 and 15 min, respectively, and centrifuged at 4° C and 9000 rpm/10 min. The last extraction was repeated without sonication. The supernatants were combined and transferred to a 25 mL volumetric flask,

and acetone-water (70:30) was added to reach a final volume of 25 mL. Finally, the extract was stored at −80 °C until analysis.

2.4. Total Phenol Folin-Ciocalteu Assay

The total phenolic content was determined by the Folin–Ciocalteau method [35]. The analysis was conducted with a UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and was estimated as mg gallic acid equivalent (GAE)/100 g fresh weight (FW). Briefly, 9 mL of 80% methanol was added to 1 mL of sample. The mixture was centrifuged at 5500 rpm for 10 min. Fifty microliters of supernatant was added to 250 µL of Folin–Ciocalteu reagent. Then, 750 µL of 20% (*w*/*v*) Na_2CO_3 was supplemented and incubated for 2 h at room temperature. Then, the absorbance was measured at 760 nm against a blank. Quantifications were calculated through a calibration curve prepared daily with known concentrations of gallic acid (GA) standards, and the results are expressed as milligrams of GAE (Gallic acid equivalent) per 100 g of fresh weight (FW).

2.5. Total Antioxidant Capacity Measurement

2.5.1. DPPH Method

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay was carried out according to Brand-Williams et al. [36]. The working DPPH solution was obtained by dissolving DPPH powder in methanol to obtain an absorbance of 0.7 ± 0.02 at 517 nm. Twenty microliters of the test sample, diluted appropriately with water, or Trolox standard or blank (distilled water), was placed in each well of a 96-well polystyrene microplate, after which 280 µL of working DPPH solution was added. After 30 min at 30 °C, the absorbance was measured at 517 nm using a microplate reader. Aqueous solutions of Trolox concentrations (50–500 µM) were used for calibration and the results are expressed as micromoles of Trolox equivalent (TE) per 100 g of fresh weight (µmol of TE/100 g of FW).

2.5.2. FRAP Method

The FRAP (Ferric reducing antioxidant power) assay was performed as previously described by Benzie and Strain [37]. Twenty microliters of test sample, diluted appropriately with water, or Trolox standard, or ferrous sulphate standard or blank (distilled water) was placed in each well of a 96-well polystyrene microplate, after which 280 µL of the FRAP reagent (containing TPTZ, $FeCl_3$ and acetate buffer) freshly prepared and warmed at 37 °C was added. The absorbance values at 595 nm after 30 min were measured using a Fluostart Omega microplate reader thermostatted at 37 °C. The standard curves were constructed using $FeSO_4$ (115–1150 µM) and Trolox solutions (20–400 µM) and the results are expressed as micromoles of Trolox equivalent (TE) per 100 g of fresh weight (µmol of TE/100 g of FW).

2.5.3. TEAC Method

The TEAC (ABTS), Trolox equivalent antioxidant capacity assay was carried out according to Re et al. [38]. TEAC (ABTS) stock solution was prepared from 7 mM ABTS and 2.45 mM potassium persulphate in a volume ratio of 1:1, and then incubated in the dark for 16 h at room temperature. The radical ABTS solution was obtained by diluting ABTS stock solution with phosphate buffer 5 mM at pH = 7.4 to obtain an absorbance of 0.7 ± 0.02 at 730 nm. Thirty microliters of the test sample, diluted appropriately with water, or Trolox standard or blank (distilled water) was placed in each well of a 96-well polystyrene microplate, after which 270 µL of radical ABTS was added. After 30 min at 30 °C, the absorbance was measured at 730 nm using a microplate reader. Aqueous solutions of Trolox concentrations (20–200 µM) were used for calibration and the results are expressed as micromoles of Trolox equivalent (TE)/ 100 g of fresh weight (µmol of TE/100 g of FW).

2.6. Specific Sugars

Sugar (fructose, sucrose, and glucose) analysis was performed with the methods described by Melgarejo et al. [39]. One milliliter of whole berry extract was centrifuged at 10,000 rev per min for 2 min at 4 °C. Supernatants were passed by SEP-PAK C18 cartridge. HPLC readings were made with μbondapak-NH_2 column using 85% acetonitrile as the liquid phase with a refractive index detector (IR). The chromatographic separation in Agilent 1100 series HPLC took place in a DAD detector (Agilent, Waldbronn, Germany). Fructose, glucose, and sucrose standards were used for the calculations of the sugar contents. Results were given as g/100 g fresh weight.

2.7. Organic Acids

The organic acid composition of the berries was determined by Bevilacqua and Califano [40]. Whole berry extracts were obtained by crushing the berries in a cheesecloth. H_2SO_4 (0.009 N) was then homogenized with a shaker for 1 h. The mixture was then centrifuged at 15,000 rpm for 15 min and the supernatants were filtered twice through a 0.45 μm membrane filter with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, Burlington, MA, USA) and passed through an SEP-PAK C18 cartridge. Organic acid readings were performed by HPLC using the Aminex column (HPX-87 H, 300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths in the Agilent package program (Agilent, Santa Clara, CA, USA). Results were given as g/100 g fresh weight.

2.8. Statistical Analysis

The study was planned as four replicates including 10 samples per replicate. In the statistical evaluations, Windows SPSS 20 was used and the differences between the means were evaluated by ANOVA variance analysis and determined with Duncan's multiple comparison test ($p < 0.05$).

3. Results and Discussion

3.1. Morphological Traits

The morphological traits of the six clones of 'Köhnü' grape cultivar grown in same ecology and altitude in Hoşköy village of Elazığ province are shown in Table 1. The clones presented statistically significant differences in cluster and berry weight ($p < 0.05$).

Table 1. Some morphological traits of the six 'Köhnü' clones.

Locations	Clones	Cluster Form	Cluster Weight (g)	Berry Weight (g)	Berry Skin Color	Usage
Hoşköy	Clone 1	Winged conical	388 ± 14 ^{ab}	4.33 ± 0.5 ^{b}	Black-Blue	Table, Juice, Wine
Hoşköy	Clone 2	Conical	341 ± 12 ^{cd}	3.92 ± 0.3 ^{e}	Black-Blue	Table, Juice, Wine
Hoşköy	Clone 3	Irregular winged conical	394 ± 16 ^{a}	4.45 ± 0.3 ^{a}	Black-Blue	Table, Juice, Wine
Hoşköy	Clone 4	Conical	334 ± 11 ^{d}	4.04 ± 0.4 ^{de}	Black-Blue	Table, Juice, Wine
Hoşköy	Clone 5	Conical	357 ± 14 ^{c}	4.12 ± 0.3 ^{d}	Black-Blue	Table, Juice, Wine
Hoşköy	Clone 6	Irregular winged conical	378 ± 13 ^{b}	4.26 ± 0.4 ^{c}	Black-Blue	Table, Juice, Wine

Different letters in the same column indicate significant differences ($p < 0.05$).

Cluster form has been recorded as winged conical in Clone 1, Conical in Clone 2, Clone 4, and Clone 5, and irregular winged conical in Clone 3 and Clone 6 (Table 1, Figure 1). The cluster weight was found between 334–394 g. Among the clones, the highest berry weight was observed in Clone 3 as 4.45 g and followed by, in descending order, Clone 1 (4.33 g) > Clone 6 (4.26 g) > Clone 5 (4.12 g) > Clone 4 (4.04 g) > Clone 2 (3.92 g), respectively (Table 1). All six 'Köhnü' clones had black-blue berry color and all of them are used for table consumption and also processed into juice (Şıra) and wine. The cultivar was grown in a trellis wire system in all vineyards in the village (Figure 2).

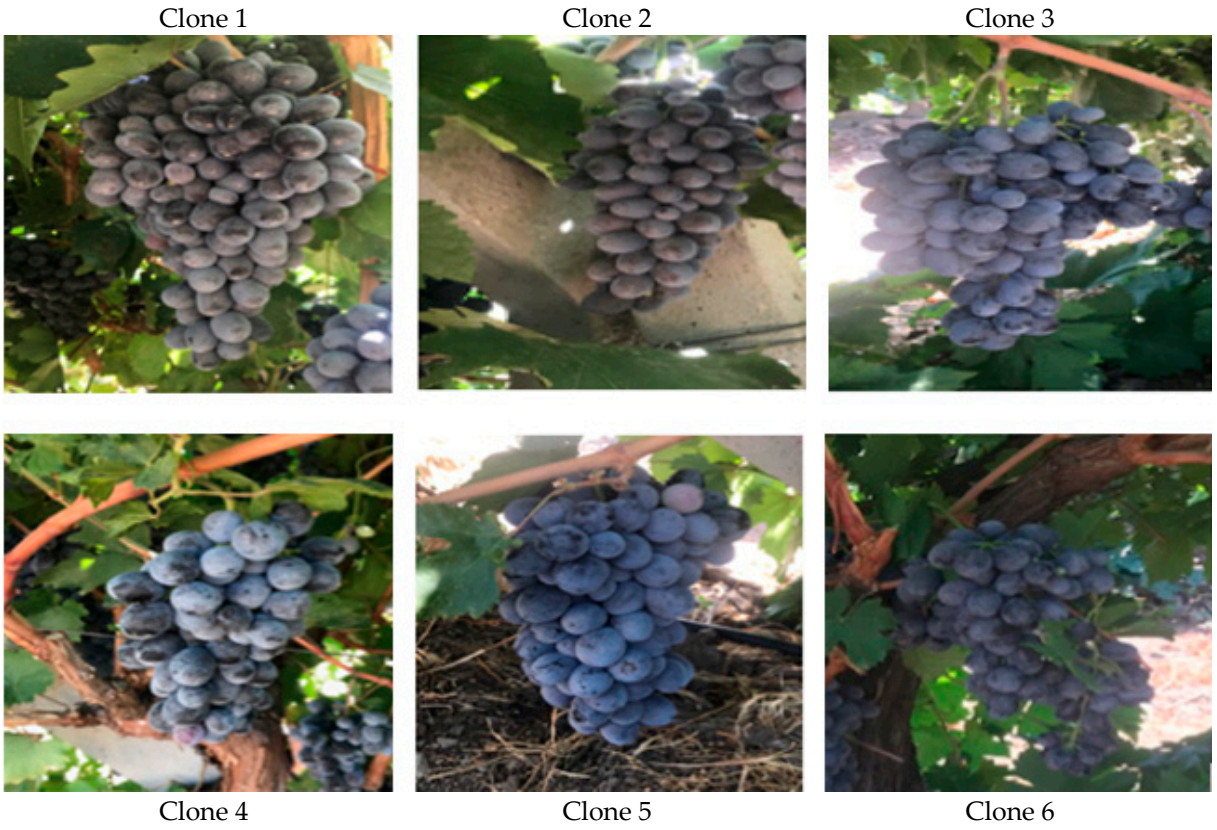

Figure 1. Different cluster forms (conical, irregular winged conical and winged conical) in 'Köhnü' grape clones.

Figure 2. 'Köhnü' grapevines in the trellis wire system in Hoşköy, Elazığ.

In the Elazığ province, the cultivar is preferred by farmers and consumers due to its suitability for fresh consumption and processing. In fact, the cultivar has perfect fresh berry characteristics (bigger berries, attractive color, unique sugar-acid balance, medium-thick peel). Keskin [41] reported the average berry and cluster weight of 'Köhnü' grape cultivar in Elazığ province to be 5.40 g and 338 g. Koc et al. [42] also studied the 'Köhnü' cultivar in Malatya conditions in Turkey and reported the average berry and cluster weight to be 4.16 g and 349 g, respectively. Both studies indicated that the morphological data could be changed according to the growing site. According to OIV [33], the cluster weight of all clones was found to be over 300 g and classified as big. Good quality in table grapes represents a combination of medium-sized clusters of uniformly large, perfect berries with the characteristic color, pleasing flavor, and texture of the cultivar. Uniform color formation and suitability for transportation are also desirable traits for table grapes [43].

In the literature, there are studies that determined morphological characteristics of grape cultivars and these studies indicate a wide variability on cluster weight, cluster form, berry color, and berry weight according to cultivars and treatments [44,45]. We found great diversity within the 'Köhnü' clones, in particular, clusters from even the same vineyard.

3.2. Total Phenolic Content

The total phenolic content of peel, pulp, and seed samples of six clones 'Köhnü' grape cultivar is shown in Table 2. The results clearly indicate statistically significant differences among clones considering peel, pulp, and seed parts ($p < 0.05$) (Table 2)

Table 2. Total phenolic content of six 'Köhnü' grape clones.

Clones	Total Phenolic Content (mg GAE/100 g FW)		
	Peel	**Pulp**	**Seed**
Clone 1	118 ± 6^{c}	30.15 ± 0.6^{c}	224 ± 11^{d}
Clone 2	128 ± 8^{b}	33.67 ± 0.6^{b}	254 ± 14^{a}
Clone 3	139 ± 7^{a}	35.11 ± 0.7^{a}	242 ± 10^{b}
Clone 4	110 ± 5^{cd}	29.38 ± 0.4^{cd}	212 ± 9^{e}
Clone 5	96 ± 4^{d}	27.73 ± 0.3^{d}	193 ± 8^{f}
Clone 6	133 ± 7^{ab}	32.06 ± 0.5^{bc}	233 ± 10^{c}

Different letters in the same column indicate significant differences ($p < 0.05$).

For all used clones, total phenolic content was the highest in seed samples and followed by peel and the lowest values were obtained from pulp samples (Table 2). Overall pulp samples exhibited the lowest total phenolic content (Table 2). Clone 3 showed the highest amount of total phenolic content for peel and pulp samples (139 mg GAE/100 g FW in peel and 35.11 mg GAE/100 g FW in pulp) among the six 'Köhnü' clones. However, the highest total phenolic content in seeds was determined in Clone 2 as 254 mg GAE/100 g FW. Overall, the lowest total phenolic content was obtained from Clone 5 (96 mg GAE/100 g FW for peel, 27.73 mg GAE/100 g FW for pulp, and 193 mg GAE/100 g FW for seed), respectively (Table 2).

The results showed that there was clonal variation for total phenolic content among six clones. In the literature, not many studies have been conducted on the total phenolic content of peel, pulp, and seed samples of different grape cultivars and even a very limited number of studies have been conducted on different clones of a single cultivar.

Yi et al. [46] observed great variability in total phenolic content in whole berries for grape cultivars between 44 and 184 mg GAE/100 g fresh samples which is in accordance with our results. In Spain, Ruiz-Torralba et al. [47] used whole berries of two white and red grape cultivars and reported total phenolic content values between 124–151 mg GAE/g FW. A large number of grape cultivars (whole berries) were used in a total phenol content analysis in Italy and great variation has been observed among cultivars in terms of total

phenolic content 92–468 mg GAE/100 g FW [48]. In China, Liu et al. [49] reported variable total phenolic content ranging from 29 to 140 mg GAE/100 g FW. Total phenolic content was found to be the highest in seeds, followed by skins and pulps indicating similarities with our study [50]. Yilmaz et al. [51] reported total phenolic contents of grape pulp, seed, and peel parts ranged from 9.26 to 62.29, from 162.29 to 326.18, and from 96.61 to 167.42 mg gallic acid equivalents/100 g fresh weight among cultivars, respectively. Our findings coincide with the results of Yilmaz et al. [51]. Previous studies have indicated that total phenolic content varies among plant organs of grape cultivars and clones. Growing location, climate, soil, temperature, cultural practices, ripening stage, training system, etc., affect the total phenolic content [51–55]. Phenolic compounds contribute color and taste characteristics of grape berries and they have also significantly contributed antiradical and antioxidant properties of grape berries [56]. Black and Red grape cultivars in general contain higher amounts of polyphenols than white ones [57]. Karaman et al. [23] reported that the total phenolic content in grape berries cultivar dependent and seeds was found to be richer than peels of all grape cultivars used.

3.3. Total Antioxidant Capacity

3.3.1. DPPH Assay

Table 3 shows the DPPH scavenging against peel, pulp, and seed fraction of six clones of 'Köhnü'. The results revealed significant differences in terms of DPPH scavenging capacity among the samples considering all berry sections (peel, pulp, and seed extracts). ($p < 0.05$). Among the berry sections, seed extracts were found to have a higher DPPH scavenging effect, followed by peel and pulp. Among the clones, the highest activity was obtained from Clone 1 seed, peel, and pulp parts. Berry parts were found in descending order: seed > skin > pulp for DPPH activity (Table 3). The highest DPPH radical scavenging of seed samples was observed in Clone 6 as 1622 µmol Trolox/100 g FW, followed by Clone 3 with 1511 µmol Trolox/100 g FW and Clone 2 with 1478 µmol Trolox/100 g FW, respectively. The lowest values were seen in Clone 5 with 1290 µmol Trolox/100 g FW (Table 3). For peel and pulp samples, the DPPH activity was found between 873–970 µmol Trolox/100 g FW for peel and 210–269 µmol Trolox/100 g FW for pulp, respectively (Table 3). The results strongly indicate that 'Köhnü' grape seed and skin extracts belong to different clones, significantly ($p < 0.05$) inhibited DPPH free radicals' generation.

Table 3. DPPH assay results of six 'Köhnü' grape clones.

Clones	DPPH (µmol Trolox/100 g FW)		
	Peel	**Pulp**	**Seed**
Clone 1	939 ± 14^{b}	229 ± 7^{bc}	1432 ± 10^{c}
Clone 2	958 ± 16^{ab}	244 ± 8^{b}	1478 ± 11^{bc}
Clone 3	987 ± 21^{a}	269 ± 9^{a}	1511 ± 16^{b}
Clone 4	911 ± 9^{c}	221 ± 5^{c}	1378 ± 10^{bcd}
Clone 5	873 ± 8^{d}	210 ± 6^{c}	1290 ± 11^{d}
Clone 6	970 ± 10^{ab}	255 ± 8^{ab}	1622 ± 15^{a}

Different letters in the same column indicate significant differences ($p < 0.05$).

In fact, in the literature, grape seeds were found more effective than peel and pulp for radical scavenging. In a study conducted on Campbell Early seeds, a strong DPPH radical scavenging activity was observed compared to pulps [58]. In another study, it was found that grape cultivars and berry parts exhibited quite different DPPH scavenging effects and concluded that cultivar is the main factor for DPPH scavenging to estimate antioxidant activity in grapes [59]. Six grape cultivars were used in the DPPH activity analysis and seed parts of all cultivars showed stronger DPPH scavenging effects than pulp [60]. Mandić, et al. [61] found that the total phenolic content and antioxidant activity

of grape could explain cultivar differences (genetic background), and also cultivation techniques, maturation stage, and harvest period may affect phenolic biosynthesis and antioxidant capacity in grape seed, skin, and pulp. In Turkey, a number of different colored grape cultivars were analyzed by the DPPH assay and it was found that seeds of grape cultivars are the best source of antioxidants than peel and pulp [51]. Anastasiadi et al. [62] indicated that grape seeds have the best antioxidant activity compared to skin and pulp by using all antioxidant-determining methods. Shen et al. [50] analyzed seeds, pulp, and peel of grape cultivars and found that the DPPH scavenging effects of the seeds were remarkably higher than those of peels and pulps.

3.3.2. FRAP Assay

The FRAP antioxidant activity results for seed, pulp, and seeds of six 'Köhnü' clones are shown in Table 4. As indicated in Table 4 statistically clonal variation in antioxidant activity, determined by the FRAP assay, was evident ($p < 0.05$).

Table 4. FRAP assay results of 'Köhnü' grape clones.

Clones	FRAP (μmol Trolox/100 g FW)		
	Peel	Pulp	Seed
Clone 1	3620 ± 37 ^{c}	76 ± 2.3 ^{bc}	40,200 ± 450 ^{b}
Clone 2	3805 ± 41 ^{b}	90 ± 3.1 ^{ab}	44,310 ± 386 ^{ab}
Clone 3	3911 ± 51 ^{ab}	84 ± 3.3 ^{b}	42,365 ± 515 ^{ab}
Clone 4	3470 ± 48 ^{d}	70 ± 1.3 ^{bc}	38,600 ± 313 ^{bc}
Clone 5	3389 ± 69 ^{de}	65 ± 2.0 ^{c}	36,548 ± 430 ^{c}
Clone 6	4018 ± 60 ^{a}	98 ± 4.3 ^{a}	45,200 ± 538 ^{a}

Different letters in the same column indicate significant differences ($p < 0.05$).

As indicated in Table 4, seed samples of all six grape clones showed the highest FRAP values followed by peel samples. Overall, the pulp samples had the lowest antioxidant activity. Considering seed sample, the antioxidant activity in the FRAP assay was in descending order: 45,200 μmol Trolox/100 g FW (Clone 6) > 44,310 μmol Trolox/100 g F (Clone 2) > 42,365 μmol Trolox/100 g (Clone 3) > 40,200 μmol Trolox/100 g (Clone 1) > 38,600 μmol Trolox/100 g (Clone 4) > 36,548 μmol Trolox/100 g (Clone 5), respectively (Table 4). The second highest antioxidant activity was observed in peel samples which varied from 3389 μmol Trolox/100 g (Clone 5) to 4018 μmol Trolox/100 g (Clone 6) indicating great differences among clones. In terms of pulp samples, the FRAP values were very low and determined in the range of 65 μmol Trolox/100 g (Clone 5) and 98 μmol Trolox/100 g (Clone 6), respectively (Table 4). Liu et al. [49] used pulp samples of 30 common grape cultivars with white, red, and black peel color in China and found FRAP values in the range of 59–612 μmol Trolox/100 g FW, respectively. Our FRAP results showed some similarities to this study.

In a previous study, FRAP values of whole berries of a large number of grape cultivars were found to be quite variable ranging from 173 to 1012 μmol Fe (II)/100 g FW [63]. Sochorova et al. [64] used seeds of different well-known international grape cultivars and found that cultivar shows different FRAP values and seeds had stronger antioxidant activity. In another study [50], seeds of six grape cultivars exhibited higher FRAP values than pulp which is in agreement with our present result. Yilmaz et al. [51] used a large number of grape seed, pulp, and peel extracts and found that FRAP values were the highest in seed samples. The majority of studies showed that grape seed contains higher FRAP values than peels and, in addition, peels had higher FRAP values than pulps [23,65,66].

3.3.3. TEAC Assay

TEAC antioxidant-determining assay results of peel, pulp, and the seed of six clones of 'Köhnü' are given in Table 5. We found statistically significant differences among clones based on peel, pulp, and seed extracts ($p < 0.05$). As obtained in the other antioxidant-determining methods (DPPH and FRAP), the highest antioxidant activity was obtained in seeds, followed by peel and the lowest TEAC values are evident in pulp samples (Table 5). The TEAC values were found between 1195–1468 µmol Trolox/100 g FW for seed samples, 286–365 µmol Trolox/100 g FW for peel samples, and 42–69 µmol Trolox/100 g FW for pulp samples, respectively (Table 5). Previous studies, using the TEAC method, also indicated that among horticultural crops, grape berries were rich in antioxidants [20,47]. Liu et al. [49] used 30 grape cultivars grown in China and determined TEAC values of whole berries in the range of 339–4839 µmol TE/100 g FW, respectively, indicating similarities with our study.

Table 5. TEAC assay results of 'Köhnü' grape clones.

Clones	TEAC (µmol Trolox/100 g FW)		
	Skin	**Pulp**	**Seed**
Clone 1	330 ± 11^{bc}	51 ± 1.4^{bc}	1284 ± 21^{bc}
Clone 2	348 ± 13^{b}	69 ± 1.3^{a}	1468 ± 19^{a}
Clone 3	365 ± 16^{a}	63 ± 1.6^{ab}	1340 ± 16^{b}
Clone 4	302 ± 9^{d}	47 ± 1.1^{bc}	1210 ± 18^{bc}
Clone 5	286 ± 10^{e}	42 ± 1.2^{c}	1195 ± 13^{c}
Clone 6	315 ± 12^{c}	55 ± 1.3^{b}	1260 ± 14^{bc}

Different letters in the same column indicate significant differences ($p < 0.05$).

Weidner et al. [67] studied phenolic compounds isolated from seeds of European and Japanese species of grapevine (*Vitis vinifera* and *Vitis coignetiae*) and found that seeds contained great amounts of tannins and detectable levels of catechins and *p*-coumaric, ferulic, and caffeic acids that have antioxidant effects. Yilmaz et al. [51] used a large number of grape cultivars with different berry peel colors and found great variability both among cultivars and berry parts (peel, pulp, and seeds) in terms of antioxidant activity by using the TEAC assay. They also indicated that DPPH, FRAP, and TEAC assays were useful to determine the antioxidant activity of different plant parts of grapes.

Our results are clearly showed that high DPPH, FRAP, and TEAC values in peel, pulp, and seed samples of 'Köhnü' grape clones corresponded to high total phenolic content, while plants with low antioxidant activity exhibited low total phenolic content. These findings suggest that total phenolic content could be used as an indicator of antioxidant properties for 'Köhnü' grape clones.

3.4. Sugar Content

The sugar content in whole berries of six clones of 'Köhnü' is given in Table 6. As presented in Table 6, six clones showed statistically significant differences from each other for sugar content at $p < 0.05$ level. Glucose was found higher than fructose for all samples and changed from 12.8 (Clone 4) to 14.1% g/100 g FW (Clone 3). Fructose content was in the range of 12.7 (Clone 4) and 13.6 g/100 g FW (Clone 3). Sucrose content was found in negligible amounts in all six 'Köhnü' clones and changed between 0.05–0.08 g/100 g FW.

Table 6. Sugar content (g/100 g FW) in juices of six 'Köhnü' clones.

Clones	Glucose	Fructose	Sucrose
Clone 1	13.3 ± 0.5 ^{b}	12.8 ± 0.4 ^{ab}	0.07 ± 0.0 ^{NS}
Clone 2	13.9 ± 0.4 ^{ab}	13.4 ± 0.4 ^{ab}	0.08 ± 0.0
Clone 3	14.1 ± 0.5 ^{a}	13.6 ± 0.3 ^{a}	0.05 ± 0.0
Clone 4	12.8 ± 0.3 ^{b}	12.7 ± 0.4 ^{b}	0.06 ± 0.0
Clone 5	13.7 ± 0.2 ^{ab}	13.5 ± 0.5 ^{ab}	0.05 ± 0.0
Clone 6	13.0 ± 0.2 ^{bc}	12.8 ± 0.2 ^{b}	0.07 ± 0.0

The 'Köhnü' samples from six clones compared in same columns and different letters shows statistically significant differences ($p < 0.05$) ^{NS}: Non-Significant.

Grape berries are well known for their sugar and organic acid content. In the literature, those phenomena are frequently searched parameters for grape berry quality evaluation. Previous studies have indicated that grape berries are rich in glucose and fructose, and the values of these major sugars, were found to be closer to each other. Studies have also indicated that glucose and fructose content and their ratio greatly changed according to cultivar, growing conditions, etc., in grapes [2,68,69], and both dominant sugars account for 96.00–98.00 of the total sugar content in grape berries. Petrisor and Chirecanu [68] also reported that sucrose content was very low (even nondetectable) in all grape cultivars and they found the ratio of glucose/fructose in grape berries varied in range from 1.0 to 1.06. They also found that some cultivars had slightly higher fructose content than glucose. Sugar in grapes is mainly accumulated in the form of glucose and fructose [70]; during harvest, fructose and glucose are close to each other [71], which is consistent with the results obtained in this study. At the same time, the content of sucrose in grape berry was relatively low because most of the sucrose was hydrolyzed during the transportation from grape leaves to fruits, which was converted into a reducing sugar, resulting in a low sucrose content in grape berries [72]. The content and composition of sugar have great influences on the flavor, color, and other nutritional components of grapes. As an important nutrient in grapes, sugar is also an important sign that shows the ripeness of grapes [68,69].

3.5. Organic Acid Content

Table 7 shows organic acids in juices of six 'Köhnü' grape clones. The results indicate statistically significant differences among clones ($p < 0.05$) for tartaric and malic acid content (Table 7).

Table 7. Organic acid content (g/100 g FW) in juices of six 'Köhnü' clones.

Clones	Tartaric	Malic	Oxalic
Clone 1	10.67 ± 0.3 ^{ab}	5.34 ± 0.2 ^{bc}	0.24 ± 0.04 ^{NS}
Clone 2	11.06 ± 0.4 ^{ab}	5.96 ± 0.3 ^{b}	0.18 ± 0.07
Clone 3	11.25 ± 0.5 ^{a}	6.44 ± 0.2 ^{a}	0.20 ± 0.05
Clone 4	10.04 ± 0.3 ^{ab}	5.24 ± 0.3 ^{bc}	0.16 ± 0.03
Clone 5	10.86 ± 0.2 ^{ab}	6.23 ± 0.2 ^{ab}	0.27 ± 0.08
Clone 6	10.46 ± 0.2 ^{b}	5.10 ± 0.2 ^{c}	0.15 ± 0.04

The 'Köhnü' samples from six clones compared in same columns and different letters shows statistically significant differences ($p < 0.05$) ^{NS}: Non-Significant.

For all 'Köhnü' clones, tartaric acid was the main organic acid, followed by malic acid. Overall, oxalic acid content was lower—even in negligible amounts. The highest tartaric acid content was obtained from Clone 3 and Clone 2 as 11.25 and 11.06 g/100 g FW while the lowest value was observed in juices of Clone 4 as 10.04 g/100 g FW. Malic acid

concentrations were found between 5.10 g/100 g FW (Clone 6) and 6.23 g/100 g FW (Clone 5) (Table 7). Previous studies have also indicated that tartaric acid was the main and dominant organic acid in grape juices followed by malic acid, and both organic acids accounted for 55–60% and 30–39% of the total organic acids in grape, respectively [2,68,69,73]. In Brazil, Couelho et al. [74] found that tartaric acid was the main acid present in wines and juices representing over 50% of the total acids quantified. Zhang et al. [69] showed that among the five cultivars of table grapes in China, tartaric acid and malic acid were the main organic acids. Organic acids are the major metabolites that exist in grapes whose compositions and concentrations are the main parameters related to grape processing and quality evaluation [75,76]. The content can directly affect the taste balance, chemical stability, and pH value. Previous studies have indicated great biochemical differences in horticultural crops [76–83].

4. Conclusions

'Köhnü' grape cultivar is cultivated in Elazig province for centuries and during the long cultivation period new clones are likely to arise due to mutation. This study is the first to report on the total phenolic content, antioxidant activity, specific sugars, and organic acids, which revealed significant differences between the corresponding clones. We found great versatility in particular in the total phenolic content among the studied clones. Clone 3 and Clone 6 differed from the other clones in respect to the highest cluster weight, the highest concentrations of total phenolic content, and antioxidant activity. The findings point to a possible better viticultural potential of these two clones.

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