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Phytochemical Properties of Some Grapevine (*Vitis vinifera* L.) Hybrids

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ABSTRACT

Grapes (*Vitis vinifera* L.) are believed to have health benefits due to their antioxidant activity and polyphenols. Thus scientists have conducted research to explore their positive effects on many chronic diseases. However, the phenolic content is variable depending on grape variety. In this study, we examined the grape hybrids total phenolics and antioxidant activities during the two different harvesting times in 2008 and 2009. Total phenolic content and antioxidant activity of hybrids were significant among hybrids for both years. However, the relationship between different harvest times was not significant. In 2008 KXP-10 hybrid and in 2009 BX2-149 hybrid have shown a higher phenolic and antioxidant activity according to the other varieties. These hybrids have dark berry color and seed. In 2009, hybrids which have seed total phenolic content and antioxidant activity were investigated with seed and without seed. Greater value was obtained hybrids which have analyzed with seed. Before registration 23 different hybrids or variety candidates which were obtained different Institutes were determined total phenolic content and antioxidant content.

Key words: Antioxidant, grapevine, hybrids, phenolics, seedless

INTRODUCTION

Grapevines (*Vitis vinifera* L.) are one of the oldest and most important perennial crops in the world. *Anatolian peninsula* has been the cradle of not only the ancient civilizations but also many agriculturally important crops. It has been linked with the origins of viticulture and wine-making. One of the accepted origins of cultivated and wild grapevines is Anatolia (Gokbayrak and Soylemezoglu, 2010). In addition, the Muslim book the Holy Quran emphasizes the importance of grapes for humankind (Khafagi *et al*., 2006).

In recent years, grapes seem very important crop for human health due to phenolic compounds and antioxidants. The presence of phenolic compounds of grapes and about the effects on human health in recent years, many studies have been done by the researcher. The efficient methods for extracting phenolics from grape seeds have broad range of pharmacological activities, due to the special health promoting and disease preventing effects of polyphenols (Youssef and El-Adawi, 2006). Farbood *et al*. (2009) investigated the effects of Grape Seed Extract (GSE), as a potent antioxidant on spatial memory with Alzhimer's Disease (AD) and found that GSE could be useful agent to prevent neurogenerative disorders such as AD. El-Adawi *et al*. (2006) also found that GSE have obvious hypocholesterolemic effect that has important pharmaceutical applications in the prevention and treatment of cardiovascular disease atherosclerosis.

Turkey is one of main gene centres in the world for grapes. It is believed that cultivated grapes have their origins in Turkey and the surrounding countries. Turkey has a very large amount of wild grapevine populations and grape cultivars which offer to grapevine breeders a valuable gene pool. Turkey has over 1.600 local grape cultivars, among which the majority of them are conserved at the national grape collection vineyard in Tekirdag. They are mostly used as table grapes, dried grapes or for local consumptions. Wild grapes are distributed all over the country territory, mainly in the river basins and forests. Wild grape collection vineyards were established at some universities in Turkey. These grapevines will be screened for the resistance to biotic and abiotic stress factors (Uzun and Bayir, 2010).

Grape breeding programs are conducted in many countries; however, these programs vary in scope and size. Some breeding programs are focused on the production of rootstocks, wine, dessert grapes and raisins (Janick and Moore, 1996). Grape breeding and germplasm conservation is performed by both the public and private sectors in Turkey. Several public institutions are involved in grape breeding. These are Tekirdag Viticulture Research Institute which is germplasm collection center as National Collection of Vitis and Manisa Viticulture Research Institute which is raisin grape production center and Yalova Ataturk Horticultural Central Research Institute which is studying on grape breeding and production practices. In Turkey, breeding studies began in the 1970s and are currently in progress at various institutions. As a result, 12 new table grape cultivars have been registered and several cultivars from these studies have been selected (Kara, 2007).

Polyphenols are found in food (vegetables, fruits, chocolate, tea, coffee, wine, grape juice, vinegar) at different concentrations (Scalbert and Williamson, 2000). Grapes are one of the world's largest and economically important fruit crops. Vitis vinifera fruits show a high concentration and a great variety of phenolic compounds. The phenolic content and composition in grapes are variable depending on cultivar and environmental factors (Thimothe *et al.*, 2007). In general, red grape varieties have higher phenolic content, especially anthocyanins, than their white counterparts. The distribution of phenolics also varies depending on which part of the grape is used for chemical extraction; for example, grape seeds have higher concentrations of catechins and procyanidins than skins and pulp (Pena-Niera *et al.*, 2004; Xia *et al.*, 2010).

Pastrana-Bonilla *et al.* (2003) reported that muscadine grape seeds had 22, 116 and 18 times more antioxidant capacity than skins, pulps and the whole grapes, respectively. Additionally, seeds had, on average, 20% more antioxidant capacity than leaves. The seeds had 6 times more total phenolics than the leaves and the ratio of antioxidant capacity/total phenolics was 6 times higher for the leaves than the seeds. It is presumable that major phenolics in leaves have higher antioxidant capacity than the ones found in seeds or that other antioxidant compounds different from phenolics may be present in higher concentrations in the leaves than in the seeds.

The objectives of the present study were to determine the major phenolic compounds found in grapes, their total phenolic content and the antioxidant capacity of the hybrids. As a result, several phytochemical properties of the candidates were determined prior to registration.

MATERIALS AND METHODS

Twenty-three hybrid grapevines (Table 1) were analyzed to determine their total phenolics and antioxidant activities. Twelve of the 23 hybrids were created at the Atatürk Horticultural Central Research Institution (AHCRI) and 11 hybrids were created at the Tekirdag Viticulture Research Institution (TVRI). The vines were approximately eight years old and were grown under identical conditions.

Preparation of extracts: After collection of the samples, the grape is cut off at the base of the peduncle, washed and dried. Fresh berries were homogenized thoroughly with a homogenizer. Also hybrids with seed analyzed removing seed for total phenolics and antioxidant activity in 2009. Fruit extracts for total phenolics and antioxidant activity measured in methanol extract (AOAM) analysis were prepared using the method of Thaipong *et al.* (2006), with some modifications. Three grams of homogenized grape berry were mixed with 25 mL methanol and homogenized using the Ultra-Turrax homogenizer. The homogenates were kept at 4°C for 12 h and then centrifuged at 9000 rpm for 20 min using a centrifuge (Sigma, 2-16 K, Germany). The supernatants were recovered and stored at -20°C until analysis.

Determination of total phenolics: Total phenolics content was determined by modified Folin-Ciocalteau method which was adapted from Zheng and Wang (2001). The 150 μL of extract, 2400 μL of nanopure water and 150 μL of 0.25 N Folin-Ciocalteu reagent were combined in a plastic vial and then mixed well using a Vortex (Heidolph reax top, Germany). The mixture was allowed to react for 3 min then 300 μL of 1 N Na_2CO_3 solution was added and mixed well. The solution was incubated at room temperature (23°C) in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Shimadzu Biospec-mini, Japan) and the results were expressed in Gallic acid equivalents (GAE; mg/100 g fresh mass) using a Gallic acid (0-0.1 mg mL^{-1}) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Measurement of the antioxidant activity: Antioxidant activity was determined by Benzie and Strain (2000) FRAP method (the ferric reducing ability of plasma FRAP) with some modifications. The method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+} TPTZ). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$) and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. The reagent was added into 150 μL sample extracts. The reduced form of blue color was read at 593 nm after 30 min. Trolox was used as standard and ferric reducing power of the extracts was calculated by standard curve interpolation. The standard curve was linear between 25 and 800 μM Trolox. Results are expressed in μM TE g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Statistical analysis: JMP 5.0.1.2 (2003) statistical program was used for data analysis.

RESULTS AND DISCUSSION

Grape hybrids total phenolics and antioxidant activities were analyzed during the two different harvesting times in 2008 and 2009 (Table 1, Fig. 1, 2). Also hybrids which have seed were analyzed with seed and after removing seed in 2009 (Fig. 3).

Results were processed by using JMP 5.0.1.2 (2003) statistical analysis program. Both phytochemical properties of hybrids were analyzed randomized experimental design analysis of variance with three replicates. Differences at $p<0.05$ were considered to be significant. Results were grouped according to LSD analysis method. According to variance analysis in terms of total phenolics and antioxidant activity in both years were statistically significant differences among

Table 1: The location, parents, fruit colour and seed types of 23 grapevine hybrids

Cultivar candidates	Parents	Colour	Seed	Institute
7/1	İskenderiye Misketi X Beyaz Şam	Yellow	Seed	AHCRI
5/2	Siyah Gemre X Cardinal	Yellow	Seed	AHCRI
70/1	Hafizali X Cardinal	Yellow	Seed	AHCRI
95/3 (Ismetbey)	Siyah Gemre X Royal	Black	Seed	AHCRI
91/3 (Pembe77)	Alphonse Lavallée X Muscat Reine des Vignes	Dark pink	Seed	AHCRI
43/1	Beyaz Şam X Müşküle	Yellow	Seed	AHCRI
CH 1 (Atak77)	Beyaz ÇavuŞ X Hamburg Misketi	Yellow	Seed	AHCRI
130/1	63 (Beyrut Hurmasİ X Perlette) X Siyah Çekirdeksiz	Yellow	Seedless	AHCRI
53/1	Müşküle X Beyaz Şam	Yellow	Seed	AHCRI
83/1	Pembe Gemre X Cardinal	Red	Seed	AHCRI
85/1	Beyaz ÇavuŞ X Perle de Csaba	Yellow	Seed	AHCRI
86/1	Hafizali X Muscat Reine des Vignes	Yellow	Seed	AHCRI
7/S-176	Italia X Superior Seedless	Yellow	Seed	TVRI
26/D-3	Kirmizi Sam X Baris	Pink	Seedless	TVRI
16/A-101	UŞuvi X Sultani Çekirdeksiz	Yellow	Seedless	TVRI
15 /A- 61	İskenderiye Misketi X Sultani Çekirdeksiz	Yellow	Seedless	TVRI
29/C-52	Queen X Beauty Seedless	Yellow	Seed	TVRI
15/B-56	İskenderiye Misketi X Perlette	Yellow	Seedless	TVRI
BX2-149	Italia X Favli	Black	Seed	TVRI
FX1-1	Amasya Beyazİ X 28/259	Yellow	Seed	TVRI
FX1-10	Amasya Beyazİ X 28/259	Yellow	Seed	TVRI
BX1-166	Italia X 28/259	Yellow	Seed	TVRI
KXP-10	Royal X Amasya Siyahİ	Black	Seed	TVRI

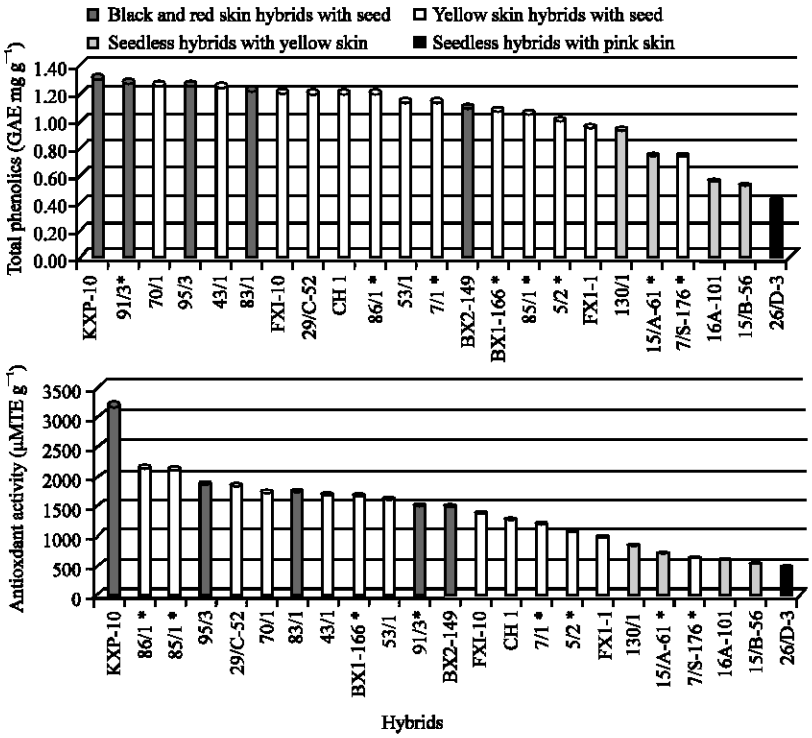

Fig. 1: Total phenolic content and antioxidant activity of hybrids in 2008 (*Mark shows hybrids with muscat flavour)

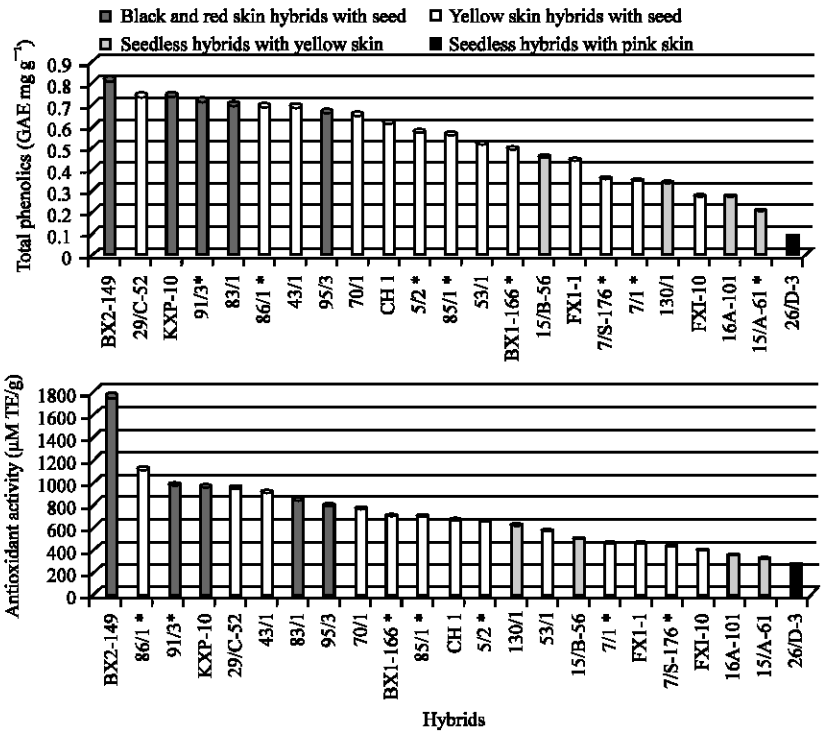

Fig. 2: Total phenolic content and antioxidant activity of hybrids in 2009 (*Mark shows hybrids with muscat flavour)

hybrids (LSMeans differences by student's t-test, $\alpha = 0.05$). On the other hand, different harvest times were not statistically significant. For this reason, evaluation of hybrids is made of the mean repeat itself. Hybrids with seed analyzed removing seed for total phenolics and antioxidant activity in 2009. Differences at $p<0.05$ were considered to be significant hybrids with seed and hybrids removed seed.

Total phenolic activity in terms of 2 different harvest times was not statistically significant. However, differences among hybrids were significant. Antioxidant activity and total phenolic content analysis showed that in the same year largely paralleled.

In 2008, KXP-10 that has black skin with seed had the highest value for total phenolics and antioxidant activity (Fig. 1). BX2-149 that has black skin with seed gave the highest value in 2009 for both contents (Fig. 2). BX2-149 and KXP-10 are available for grape juice industry with high phenolic content and antioxidant activity. It was examined Fig. 1 and 2 seedless hybrids showed lowest phenolic content and antioxidant activity regardless of skin color in both years.

In 2009, also hybrids with seed analyzed removing seed for total phenolics and antioxidant activity. Hybrids removed their seed was observed that a lower antioxidant activity without exception. FX1-10 that has white skin with seed gave the highest value for antioxidant activity. Similar to antioxidant activity results hybrids removed their seed was observed that a lower phenolic content with only one exception. Exception was 53/1 hybrid that has white skin with very small seed gave the higher value with removed seed. BX2-149 that has black skin with seed gave the highest value for phenolic content (Fig. 3).

These results can be related that phenolics and antioxidant activity of compounds take place a large extent in the seed. Seedless varieties come out of these values is very low in both years is seen as the biggest proof of this.

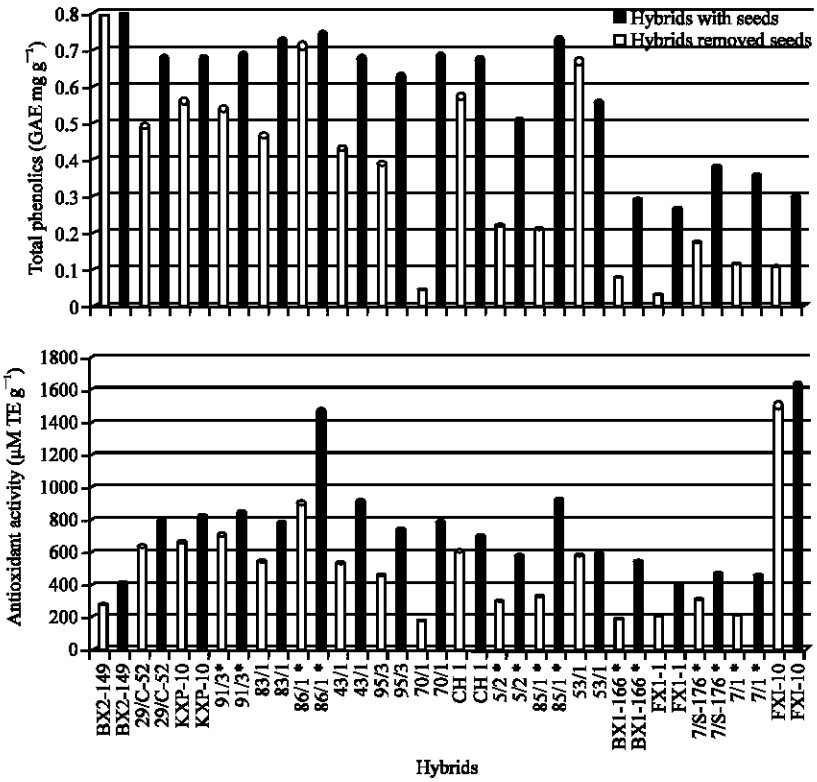

Fig. 3: Total phenolic content and antioxidant activity of hybrids with seed and removed seed in 2009 (*mark shows hybrids with muscat flavour)

Present results were similar with Pastrana-Bonilla *et al.* (2003). In different parts of grape, the highest antioxidant capacity was found in grape seeds, followed by skin and the flesh displayed the lowest antioxidant capacity. Therefore, the extracts from grape seeds are a promising antioxidant for dietary supplement. They found the total phenolics in muscadine grape parts were, on average, 5 times more concentrated in the seed than in the skin and 80 times more than in the pulp. This result may be due to the high concentration of catechins in the seed and the very low presence of major phenolics in pulp. The relatively high value for total phenolics in skins in comparison to the sum of individual phenolics found in them indicates that some other phenolics may be present in the skins but not identified in this study.

As a result of the overall evaluation of these phytochemical properties vary according to years as in similar studies. At the beginning of the main reasons are the changing climatic conditions (Connor *et al.*, 2002; Proteggente *et al.*, 2002).

Total phenolics and antioxidant activity analysis were selected in terms of human health importance increased recent years. Many researchers have used these compounds work by the use of different methods and said the results could be different (Vrhovsek *et al.*, 2001).

Total phenolics and antioxidant activity analysis were found largely related each other in this study. Hybrids with seed and hybrids with colored skin gave higher values in terms of total phenolics and antioxidant activity. In addition, some hybrids which have Muscat flavour gave higher values. Seedless hybrids got involved lowest group even if they have colored skin.

Similar results were obtained by Vicente *et al.* (2011) with sugar cane spirit aged in woody casks. They analyzed phenolic compounds, furfuraldehyde and the antioxidant capacity. The antioxidant capacity and content of phenolic compounds both increase with increasing aging time and it was found that there is significant correlation between them.

According to Orak (2007), total phenolic content and antioxidant activity in grapes may be different such as the variety of compounds, the color of the skin and availability of seed. They mentioned that usually varieties have dark color and seed showed high antioxidant activity and total phenolics. Similar results were obtained as a result of this study.

It was assessed the anthocyanin, total polyphenol and antioxidant activity of 29 common beans from diverse origins and seed coat color by Akond *et al.* (2011). Also they reported that generally bean genotypes with high anthocyanin and polyphenol content exhibit high antioxidant activity. Also Similar results were obtained as a result of this study, hybrids have the higher antioxidant activity was found to be at the same time have higher phenolic content (Fig. 1, 2).

This finding had lead to a similar conclusion reported by Xu *et al.* (2010) with 18 cultivars belonging to five Oriental Vitis species, *Vitis vinifera*, three Euro-Asian Hybrids, one Euro-American Hybrid, and muscadine grape (*Vitis rotundifolia*) grown in different locations of China. They analyzed phenolic compounds and antioxidant properties of these grapes. Distributions of phenolic compounds in seeds and skins varied greatly among them. Additionally, significant correlations among different antioxidant assays in both seeds and skins were observed. Antioxidant properties were also found highly correlated to the main phenolic compounds.

CONCLUSION

Results reported here indicate that total phenolic content and antioxidant activity of hybrids were significant among hybrids for both years. Total phenolic content and antioxidant activity are variable depending on grape variety. Hybrids have dark berry color and seed showed greater value than other hybrids. The information of this study can be used for selecting superior grape hybrids for targeted food and feed purposes and also for breeding program.

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