

Phenolic Compounds and Antioxidant Activities of Skins and Seeds of Foreign and Iranian Grapes

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Abstract: Grape skins and seeds are sources of phenolic compounds that contribute to the sensory characteristics and beneficial bioactivity of wines and other processed foods. Grape seed and skin extracts from foreign, wild and Iranian cultivars were assayed for their antioxidant properties and phenolic compositions. Finally, the results were compared with those of *Vitis vinifera* cv. Muscat of Alexandria and *V. labrusca*. Among the skins of grape cultivars analyzed, those of Lalsiyah contained the highest amount of total phenolics (1067.5 mg 100g⁻¹ gallic acid equivalent of fresh weight) and antiradical activities (0.79 m mol g⁻¹ trolox equivalent of fresh weight). In contrast, Dedeskiramfi contained highest amount of seed total phenolics (2277.3 mg 100 g⁻¹ GAE of fresh weight). The phenolic content of different grapes depends mainly on the grape skin color. The total phenolic content of W8 and W11 with white skins was significantly different from grapes with dark skins. Lalsiyah skin contained the highest amount of total flavonoid, total anthocyanins content, total procyanidin monomers and antiradical activity. Since, total phenolic content is an index of potent antioxidant capability; Lalsiyah will be good resource of antioxidant in food and pharmaceutical industries.

Keywords: *Vitis*, Grape skin, Grape seed, Phenol, Antiradical activity.

1. INTRODUCTION

The genus *Vitis* L. (Vitaceae) includes about 70 woody climber species, spread mostly (but not exclusively) in the temperate hemisphere regions of the northern [1, 2]. Grape is one of the world's largest fruit crops and its annual production amounts to approximately 68 million metric tons (Food and Agriculture Organization (FAO), 2008). Approximately 71% of the world's grapes are used for winemaking; 27%, as fresh fruit; and 2%, as dried fruit [3, 4]. Grapes are well known for possessing many polyphenolic compounds with significant benefits to human nutrition and health [5-7]. Berry skin and seeds are the parts where most phenolic accumulation occurs. These compounds can be classified into two kinds: flavonoids and nonflavonoids [8, 9]. Flavonoids are a large family of over 4000 ubiquitous secondary plant metabolites, which can be further divided into five subclasses including flavonols, flavones, anthocyanins, catechins and flavonones [10]. Grape skins and seeds contain flavonoids (catechin, epicatechin, procyanidins and anthocyanins), phenolic acids (gallic acid and ellagic acid) and stilbenes (resveratrol and piceid). The grape seed and skin constituents have been shown to have health-functional activities [11]. Examples include the observations that anthocyanins possessed the properties of antioxidation and apoptosis induction of tumor cells [12-15]; flavan-3-ols exerted some

beneficial vascular effects to cardiovascular and cerebrovascular diseases [16]; and flavonols were demonstrated to have significant antioxidant effect [6, 17]. The aim of present study was to evaluate and characterize the phenolic compounds and antioxidant activity in varieties of grape with considerable commercial values including Russian, wild and local cultivated grapevines. To our knowledge, no research has been conducted on these varieties. Finally, the results were compared with those of two cultivated grapes *Vitis vinifera* cv. Muscat of Alexandria and *V. labrusca*.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant materials were obtained from the Agricultural Research Centre of West Azerbaijan, Iran (Table 1). A total of 16 grape genotypes were analyzed including four wild grapes (W6, W8, W11 and W16), five Iranian commercial cultivars including LalSiyah, Rasha, GharaShira, KhaliliSiyah and Garmian, five Russian grapes including Dedeskiramfi, Zambil 13- 366, Ramfi TCXA, Uleskibiser and Kibraskiramfi and two cultivated grapes, Muscat of Alexandria and *V. labrusca* were used in this study.

2.2. Sample Preparation

The selected berries were finger pressed to remove juice and pulp. Seeds and skin were separated, washed several times with distilled water, and moisture

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was absorbed on blotting paper. One gram of the samples (skin or seed) in three replicates each was extracted by grinding the sample for 1 min at 24,000 rpm in a blender (Ultra-Turrax T25; Ika-Labortechnik, Germany) with 10 mL of acidified methanol (1:99 v/v, HCl: MeOH). The homogenate was incubated for 12 h at 4 °C in the dark before filtering with Whatman no. 1 filter paper and centrifuging at 3500 rpm for 10 min. The extract was separated and the residual tissue was re-extracted two times following the same procedure in 5 mL of acidified methanol each time. The extracts obtained by extracting the same sample for three times were combined, mixed thoroughly and used for further experiments.

Table 1: Origin and Color Berry Skin

Grape genotypes	Color berry skin	Origin
W6	Red	Iran
W8	White	Iran
W11	White	Iran
W16	Black	Iran
Lalsiyah	Black	Iran
Rasha	Black	Iran
Garmian	Red	Iran
Khalilisiyah	Black	Iran
Ghara sharia	Black	Iran
Dedeskiramfi	Black	Russia
Zanbil 13-366	Red	Russia
Ramfi TCXA	Black	Russia
Uleskibiser	Black	Russia
Kibraskiramfi	Black	Russia
Muscat	White	Africa
<i>V. labrusca</i>	Black	America

2.3. Total Phenol Content (TPC)

Total phenolic was determined using the Folin-Ciocalteu's colorimetric assay [18]. A 0.5 mL aliquot of the prepared extract was diluted five times, of which 100 µL aliquot was taken for further analysis. The 100 µL aliquot was mixed with 1 mL phenol reagent, 1 mL 10% sodium bicarbonate, and 4 mL distilled water. The mixture was allowed to stand for 1 h in the dark. The total phenolic concentration was calculated from a calibration curve ($r^2 = 0.999$) by plotting known solutions of gallic acid ($1-0.0625 \text{ mg mL}^{-1}$) against absorbance at 760 nm. Results were expressed as gallic acid equivalent (GAE) against the fresh weight of the samples (mg g^{-1}).

2.4. Total Flavonoids Content (TFC)

The AlCl_3 method [19] was used for estimation of the total flavonoids content of the extracted samples. An aliquot of 1 mL of each extract was added individually to equal volumes of solution of 2% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2 g in 100 mL methanol). The mixture was vigorously shaken, and after 10 min of incubation, absorbance was taken at 430 nm. The results were expressed as mg quercetin 100 g^{-1} extract.

2.5. Total Monomeric Anthocyanin Content (TAC)

Monomeric anthocyanins were measured using a spectrophotometric pH differential protocol [20], and calculated as cyanidin-3-glucoside equivalents for the samples. The extracts were mixed thoroughly with 0.025 M potassium chloride (pH 1.0) in a known dilution. The absorbance of the mixture was measured at 515 and 700 nm using distilled water to zero the spectrophotometer. The extracts were then combined with 0.4 M sodium acetate buffer (pH 4.5), and the absorbances were measured at the same wavelengths. The absorbance of the diluted sample (A) was as in Eq. (1)

$$A = (A_{515} - A_{700})_{\text{pH } 1.0} - (A_{515} - A_{700})_{\text{pH } 4.5} \quad (1)$$

The anthocyanin content was calculated as the total of monomeric anthocyanin pigment from Eq. (2)

$$\text{Total monomeric anthocyanins (mg } 100 \text{ g}^{-1}) = \frac{\Delta A \times \text{MW} \times 1000}{\epsilon \times 1} \quad (2)$$

where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ϵ in this formula correspond to the predominant anthocyanin in the sample. Since the sample composition was unknown, the pigment content was calculated as cyanidin-3-glucoside (C3G), where MW = 449.2 and $\epsilon = 26,900$.

2.6. Procyanidin Monomers (FLAVAN-3 OLS)

The flavan-3-ols content was determined following the procedure described by [21]. Briefly, a sample (0.2 mL) diluted 1:100 with MeOH was placed in a 1.5 mL Eppendorf tube, and 1 mL of DMACA (0.1% in 1N HCl-MeOH) solution was added. The sample was vortexed and stood for 10 min at room temperature. The absorbance was recorded at 640 nm. The concentration of flavan-3-ols was determined from a calibration curve, constructed by plotting the known concentrations of catechin ($0.26-0.01625 \text{ mg mL}^{-1}$) against absorption at 640 nm ($r^2 = 0.9997$). Results

were expressed as catechine equivalent (CE) against the fresh weight of the sample (mg g^{-1}).

2.7. Measurement of Antiradical Activity

Antiradical activities were determined using the procedure as described by [21]. All samples were diluted 10 times with MeOH. An aliquot of 25 μL of diluted sample was added to 975 μL of DPPH solution (60mM in MeOH) and vortexed for few seconds. The absorbance was read at 0 and 30 min. The antiradical activities (AAR) was determined from the calibration curve ($r^2 \frac{1}{4} 0.99$) by plotting the known concentrations of TroloxTM (20–1.25 mmol g^{-1}) against the absorbance at 515nm and expressed as mmol of Trolox equivalent (TE) against fresh weight (mmol g^{-1}).

2.8. Statistical Analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard error (SE) of the mean or standard deviation (SD) of the mean. Significant differences between means were separated by analysis of variance (ANOVA) followed by Tukey's test at the 5% level. Computations were done by SPSS for windows version 16.0 (SPSS Japan Inc., Tokyo, Japan).

3. RESULTS AND DISCUSSION

3.1. Contents of Total Phenolics and other Metabolites in Grapes Genotypes Berry Skins

Total phenolics, total flavonoid, total anthocyanin, procyanidin monomers and antiradical activity determined in berry skins of 16 grapes analyzed are presented in Table 2. Among 16 grapes analyzed; Lalsiyah skin had the highest total phenolic content ($1067.5 \text{ mg } 100 \text{ g}^{-1} \text{ GAL}$) of fresh weight. The data for the skin from the present study were higher than those reported previously for Cabernet Sauvignon, Merlot and Shiraz red grape skins [22], and those reported in commercial grape skin extracts ($79.20 \text{ g GAE } 100 \text{ g}^{-1}$) [23]. In addition to total phenolics, Lalsiyah skin contained the highest amount of total flavonoid, total anthocyanins content, total procyanidin monomers and antiradical activity. Since, total phenolic content is an index of potent antioxidant capability [24]; Lalsiyah bearing higher total phenolics will be good resources as beneficial health materials. A positive relationship between total phenolics and antioxidant activities has been reported previously [25, 26]. The scavenging effect of extracts on the DPPH radical decreased in the order of Iranian cultivars > Iranian wild grapes > Russian grapes. As usual, the total phenolic of red grape skins is greatly higher than that of white grapes

Table 2: Phenolic Distribution and Antioxidant Properties of Grape Berry Skin

Grape genotypes	Total phenolics ($\text{mgGAE } 100\text{g}^{-1}$)	Total flavonoids ($\text{mgQE } 100\text{g}^{-1}$)	Total anthocyanin ($\text{mg } 100\text{g}^{-1}$)	Procyanidin monomers ($\text{mg g}^{-1} \text{ CE}$)	Antiradical activities ($\text{mmol g}^{-1} \text{ TE}$)
W6	235.0 ± 18.9^c	11.2 ± 0.0^d	29.6 ± 1.4^c	1.5 ± 0.03^c	0.26 ± 0.05^{abcde}
W8	146.4 ± 12.2^b	2.0 ± 0.0^a	2.5 ± 0.03^a	1.3 ± 0.03^c	0.15 ± 0.03^a
W11	94.7 ± 13.1^a	2.5 ± 0.2^a	1.9 ± 0.1^a	0.8 ± 0.01^b	0.20 ± 0.03^{abcd}
W16	577.4 ± 14.0^g	45.3 ± 0.2^n	220.9 ± 3.8^j	4.7 ± 0.15^i	0.45 ± 0.02^f
Lalsiyah	1067.5 ± 6.1^k	74.4 ± 1.0^o	317.8 ± 4.9^k	7.9 ± 0.06^m	0.79 ± 0.2^g
Rasha	508.3 ± 25.3^f	35.8 ± 0.6^k	118.2 ± 1.8^h	3.3 ± 0.20^f	0.30 ± 0.02^{ef}
Garmian	740.1 ± 46.1^i	33.2 ± 0.1^j	116.2 ± 1.7^h	6.5 ± 0.25^k	0.32 ± 0.01^{cde}
Khalilisyah	801.1 ± 14.0^j	40.0 ± 0.2^m	124.7 ± 2.5^i	7.0 ± 0.06^l	0.35 ± 0.07^{bcde}
Gharashira	386.3 ± 7.0^e	21.0 ± 0.2^f	32.5 ± 0.05^c	2.5 ± 0.25^e	0.20 ± 0.02^{abcd}
Dedeskiramfi	396.9 ± 13.7^e	22.9 ± 0.2^g	63.6 ± 0.7^e	2.5 ± 0.08^e	0.27 ± 0.02^{abcde}
Zanbil 13-366	243.1 ± 24.7^c	9.0 ± 0.2^c	15.6 ± 0.2^b	1.9 ± 0.06^d	0.19 ± 0.03^{abc}
Ramfi TCXA	279.7 ± 11.5^d	16 ± 0.0^e	43.5 ± 0.6^d	2.4 ± 0.06^e	0.23 ± 0.01^{abcde}
Uleskibiser	603.9 ± 42.7^g	38.5 ± 0.2^l	82.1 ± 1.1^f	4.1 ± 0.07^h	0.28 ± 0.01^{abcde}
Kibraskiramfi	541.2 ± 7.8^f	28.8 ± 0.0^i	91.4 ± 0.6^g	3.8 ± 0.02^g	0.20 ± 0.01^{abcd}
Muscat	108.9 ± 11.5^a	7.6 ± 0.0^b	1.9 ± 0.3^a	0.5 ± 0.07^a	0.16 ± 0.02^{ab}
<i>V. labrusca</i>	666.7 ± 24.3^h	26.6 ± 0.3^h	89.7 ± 1.4^g	5.5 ± 0.14^j	0.33 ± 0.03^{de}

Values with the same letter(s) within a column are not significantly different at $P < 0.05$ by tukey,s test. Abbreviations were explained in the text.

Table 3: Phenolic Distribution and Antioxidant Properties of Grape Seeds

Grape genotypes	Total phenolics (mgGAE100g ⁻¹)	Total flavonoids (mgQE 100g ⁻¹)	Total anthocyanin (mg 100g ⁻¹)	Procyanidin monomers (mg g ⁻¹ CE)	Antiradical activities (mmol g ⁻¹ TE)
W6	1346.1 ± 81.2 ^c	6.0 ± 0.0 ^d	1.75±0.05 ^{de}	9.5 ± 0.09 ^e	0.74 ± 0.02 ^{cd}
W8	2126.6 ± 106.5 ^g	10.2 ± 0.2 ^f	0.70 ±0.34 ^a	12.9 ± 0.25 ^k	1.47 ± 0.01 ^h
W11	1972.3 ± 83.0 ^f	12.8 ± 0.0 ^h	0.79±0.53 ^{ab}	11.6 ± 0.15 ^j	0.90 ± 0.05 ^{def}
W16	1939.7 ± 61 ^f	6.0 ± 0.0 ^d	1.63 ±0.21 ^{de}	11.0± 0.09 ^h	1.07 ± 0.03 ^{efg}
Lalsiyah	1541.3 ± 133.8 ^d	4.9 ± 0.2 ^c	1.25 ± 0.02 ^c	10.1 ± 0.08 ^g	1.03 ± 0.23 ^{efg}
Rasha	675.0 ± 18.6 ^b	6.1 ± 0.2 ^d	1.22 ± 0.11 ^c	7.8 ± 0.05 ^b	0.48 ± 0.04 ^a
Garmian	1927.6± 99.8 ^f	4.4 ± 0.0 ^b	0.98±0.11 ^{abc}	8.2 ± 0.07 ^c	0.68 ± 0.08 ^{bc}
Khalilisiyah	2126.9 ± 86.5 ^g	4.9 ± 0.2 ^c	1.59 ±0.07 ^d	8.6 ± 0.04 ^d	1.08 ± 0.01 ^g
Ghara sharia	1325.7 ± 7.0 ^c	3.3 ± 0.2 ^a	0.67 ±0.0 ^a	6.7 ± 0.10 ^a	0.59 ± 0.09 ^{abc}
Dedeskiramfi	2277.3 ± 7.0 ^h	10.6 ± 0.2 ^e	1.92± 0.05 ^{de}	13.9 ± 0.10 ^l	0.91 ± 0.07 ^{def}
Zanbil 13-366	1370.5 ± 134.3 ^c	10.1 ± 0.2 ^f	1.90 ±0.03 ^{de}	10.2 ± 0.13 ^g	0.87± 0.24 ^{de}
Ramfi TCXA	1939.8 ± 67.9 ^f	7.3 ± 0.2 ^g	1.92 ±0.18 ^{de}	11.3 ± 0.03 ⁱ	1.1 ± 0.06 ^g
Uleskibiser	406.6 ± 25.3 ^a	4.4 ± 0.0 ^b	1.25 ± 0.05 ^c	7.9 ± 0.05 ^b	0.44 ± 0.13 ^a
Kibraskiramfi	683.2 ± 21.2 ^b	4.8 ± 0.0 ^c	1.1 ± 0.0 ^{bc}	8.3 ± 0.04 ^c	0.56 ± 0.03 ^{abc}
Muscat	1726.7 ± 10.3 ^e	7.6 ± 0.0 ^e	1.94 ± 0.03 ^e	15 ± 0.19 ⁿ	1.1 ± 0.12 ^g
<i>V.labrusca</i>	1364.4 ± 28.2 ^c	7.6 ± 0.0 ^e	1.25 ± 0.07 ^c	9.8 ± 0.02 ^f	0.54 ± 0.02 ^{ab}

Values with the same letter(s) within a column are not significantly different at $P < 0.05$ by tukey,s test. Abbreviations were explained in the text.

due to the loss of the ability to produce anthocyanins in the skins of white grapes. Our results showed that the phenolic content of different grapes depends mainly on the grape skin colour. TPC, TAC and TFC of grapes with red and black skins have significant differences with TPC, TAC and TFC of Muscat with White skin, however significant differences in TPC, TAC and TFC were not found among *V. labrusca* and grapes with similar skin color or among W8, W11 and Muscat with similar skin color.

3.2. Contents of Total Phenolics and other Metabolites in Grapes Genotypes Seeds

Total phenolics, total flavonoid, total anthocyanin, procyanidin monomers and antiradical activity determined in seeds of 16 grapes analyzed are presented in Table 3. Among 16 grapes analyzed, Dedeskiramfi seeds had the highest (2277.3mg 100 g⁻¹ GAE of fresh weight) total phenolic content. The total phenol content (TPC) of the grape seeds used in the study was higher than that in commercial grape seed extract (80.70 g GAE 100 g⁻¹ seed) reported by [23] and in seeds of red grape varieties cultivated in Turkey (7.90-15.46 g GAE 100 g⁻¹ seed) [5]. Pastrana-Bonilla *et al.* (2003) analyzed five bronze and five purple cultivars of muscadine grapes seeds in Georgia and reported that bronze and purple cultivars contained

19.9–32.6 (average 23.8) mg 100 g⁻¹ and 15.4–26.9 (average 19.8) mg g⁻¹ GAE of fresh weight total phenols, respectively [27]. Our findings are also in agreement with these reports. W11, Muscat and W8 had the highest total flavonoid, procyanidin monomers and antiradical activity, respectively. In total, Anthocyanin content of seeds was lower than their berry skin. Guendez *et al.* (2005) found that there is a significant correlation between DPPH scavenging activities of grape seed extracts and total phenolic content ($r = 0.82$, $P < 0.01$) [28]. Our findings are also in agreement with these reports. In conclusion, Lal siyah skin with highest amount of total flavonoid, total anthocyanins content, total procyanidin monomers and antiradical activity could be a good resource as natural antioxidant in food and pharmaceutical industries.

CONCLUSION

The results obtained in the present work denote that grape skins and seeds may constitute a good source of healthy compounds, therefore useful in the prevention of diseases in which free radicals are implicated. Our results showed that the phenolic content of different grapes depends mainly on the grape skin colour. The total phenolic content of W8 and W11 with white skins was significantly different from grapes with dark skins. However, significant differences in total phenolic

content were not found among Muscat, W8 and W11 that each three had white skins. The *V. labrusca* and other grapes with dark skin color also did not appear to show significant difference in phenolic compounds. Among all grapes analysed, Lal siyah berry skin had the highest total phenolic, total flavonoid, total anthocyanin content and antiradical activity. The higher antiradical activity of Lal siyah could be probably due to the higher amount of phenolics and anthocyanins.

We also propose hybridization of wild grapes with cultivated grapes which might change or enhance the characters of bioactive components in grape skin.

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