



## Evaluation of Phenolic Compounds, Antioxidant Activities and Antioxidant Enzymes of Wild Grape Peel and Pulp

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### Abstract

Peel and pulp of grape (*Vitis vinifera* L.) berries contain a wide range of bioactive compounds that may be responsible for their biological activities. Phenolic compounds, antioxidant capacity and antioxidant enzymes of wild grape accessions from the West Azerbaijan province were investigated in different fractions of berries, i.e., peel and pulp fractions. The total phenol (TP) content ranged from 139.29-843.10 and 151.67-416.91 mg Gallic acid equivalent (GAE) per 100 g in the peel and pulp, respectively. The highest level of antioxidant capacity and antioxidant enzymes activities (Catalase (CAT) and Superoxide dismutase (SOD)) in all the native accessions was observed in peel fraction. Also, principal component analysis (PCA) demonstrated that genotypic effect is more pronounced toward peel antioxidant activity based on 2,2-Diphenyl-1-picrylhy (DPPH) assay and pulp total anthocyanin (TA) of grape berries. The present study displays the potential of native grape accessions studied for improvement of nutritional value through germplasm enhancement programs.

**Keywords:** Catalase enzyme, Iran, Principal component analysis, Total phenol

### Introduction

Grapes (*Vitis* sp.) are one of the most important fruit crops worldwide. They are used in different forms such as fresh, raisin, juice, wine and etc. The increasing interest regarding the nutraceuticals has led researchers to start the election of plants with greater than normal antioxidant activities, including sea buckthorns [8], plums and peaches [5] and apples and strawberries [24].

The previous studies revealed that fruit consumption was significant positively correlated with reduced risk of certain chronic diseases [2, 14]. Also, it has been determined that a wide range of phytochemical and antioxidant compound exist within small fruits [21]. Grapes because of many polyphenolic and antioxidant compounds are substantial for human nutrition and health [16]. So, the consumption of grape plays an important role in disease prohibition including cardiovascular diseases, inflammation, cancer and ageing-related disorders [12]. It has been believed that the effect of

phytochemical compounds is achieved through direct reacting with free radicals for scavenging them, reducing of peroxides, chelating of metals and inducing the antioxidative defense systems [17]. These results have led researchers to determine various crops concerning to polyphenolic compounds and antioxidant activity [15].

It is well-known that the peel and pulp of grape berries contain polyphenolic compounds, mainly resveratrol, catechin and (-)-epicatechin, hydroxycinnamic acids, flavonols, stilbenes, anthocyanins and gallic acid, that are able to scavenge various free radicals [27]. However, the phytochemical composition of grape varies greatly among different cultivars, genotypes and accessions [25].

The concentration of phenolic compounds, antioxidant compounds and antioxidant enzymes of grapes are different with regard to the genotype and cultivar of grapevine and is affected by part of the fruit, method of juice extraction, viticultural and environmental factors [14]. Many efforts have been done to determine the compositions of polyphenolic and antioxidant compounds in grape variety and wild genotypes because of the effect

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of antioxidant compounds in recognizing the grape quality and its products [19].

Many uncultivated native species exist within the *Vitis* genus. These species are extensively found in many regions of the world such as East Asia, South Europe and North and Central America [19]. These native species are main germplasm sources for breeding of grapes because of the hybridization of them with various varieties of grape. Recently, the phenolic and anthocyanin contents of some wild species *Vitis vinifera* subsp. *sylvestris* (C.C.Gmel.) Hegi was evaluated by Revilla *et al.* [22] in Spain and observed considerable differences among the studied accessions. Since, no data are available about the phenolic compounds, antioxidant activities, and antioxidant enzyme activities in the peel and pulp fractions of wild grape berries. Therefore, in this research, we report the antioxidant activity, total phenol, total flavonoid, total anthocyanin and antioxidant enzyme activities of peel and pulp for 20 selected native grape accessions for the first time in Iran.

## Material and Methods

### Chemicals

Folin-Ciocalteu phenol reagent, sodium ascorbate, sodium carbonate, dithiothreitol (DTT), H<sub>2</sub>O<sub>2</sub>, ferric chloride, FeSO<sub>4</sub>·7H<sub>2</sub>O, and hydrogen peroxide were bought from Merck Co. (Darmstadt, Germany). Guaiacol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-Tris (2-pyridyl)-1, 3, 5-triazine (TPTZ), disodium ethylene diamine tetra acetate acid (EDTA) and malvidin-3-glucoside were bought from Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

### Collection and preparation of wild grape berry samples

Samples (Twenty wild grapes accessions) were obtained from West Azerbaijan provinces (Sardasht and Piranshahr) in Iran (Table 1). The color of berries in all of accessions was red. Approximately 500 g of ripe grape berries per accession was harvested manually in September 2014 and afterward instantly was taken to the laboratory. Thus, the peel and pulp of berries were separated and kept at -80 °C for future analysis.

### Determination of Total Phenol

One g of samples was homogenized with 10 ml of methanol for 1 h for the determination of TP content. Then, TP content was measured using the Folin-Ciocalteu reaction [26]. The result was expressed as mg Gallic acid (GAE) per 100 g FW.

### Determination of Total Anthocyanin and Total Flavonoid

The extract for the TA and TF content was obtained by homogenizing of 0.5 g of berries with 5 ml of methanol containing 1% HCl (v/v) and kept at 0 °C for 15 min and

then was centrifuged at 10000 g for 5 min at 4 °C and afterward the supernatant was utilized. The pH differential method was used for measuring of TA content [10]. In brief, the sample was read photospectrometrically at 520 and 700 nm in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). TA content was calculated using a molar extinction coefficient of 28000 (malvidin-3-glucoside) [23].

$$\text{Absorbance (A)} = (A_{520 \text{ pH } 1} - A_{700 \text{ pH } 1}) - (A_{520 \text{ pH } 4.5} - A_{700 \text{ pH } 4.5})$$

The results were expressed as mg malvidin-3-glucoside equivalents per 100 g FW basis.

The content of TF was measured by a colorimetric method [20]. One milliliter of the extract was mixed to 300 µl of 5% NaNO<sub>2</sub>, then after 5 min 600 µl of 10% AlCl<sub>3</sub>·6H<sub>2</sub>O was added to this mixture. Afterward, 2 ml of 1 mol L<sup>-1</sup> NaOH was added after 6 min and the final volume was reached to 10 ml using deionized water. The absorbance of the sample was read at 510 nm. The obtained results are expressed as catechin (CAT) equivalents per 100 g FW basis.

### Antioxidant Capacity

#### DPPH Radical Scavenging Activity

For the assessment of antioxidant activity based on DPPH method, after extraction of samples with methanol, then obtain extract were centrifuged at 10000 g for 15 min. The supernatants were used for measurement of free radical scavenging activity [4].

One hundred microliters of extracts were mixed with 1000 µl of 6 × 10<sup>-5</sup> mol L<sup>-1</sup> DPPH. After shaking of the mixture and then samples kept at room temperature for 30 min, afterward the absorbance of samples was read spectrophotometrically at 515 nm.

In this assay, experimental control was methanol. The percent of inhibition of DPPH free radical was obtained based on the following equation:

$$\% \text{ inhibition of DPPH} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$$

Abs control is the absorbance of DPPH solution without the extract.

#### Ferric-reducing Antioxidant Power (FRAP)

For the assessment of antioxidant activity based on FRAP method was used Benzie & Strain [1] method. For preparation of FRAP reagent, 100 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM ferric chloride was mixed in a ratio 10:1:1 (by volume). One hundred microliters of samples were mixed with 4.9 ml of FRAP reagent and then incubated at 35 °C for 15 min. The absorbance of samples was read at 593 [1]. The FRAP-value was determined based on the standard curve of FeSO<sub>4</sub>·7H<sub>2</sub>O and the results were expressed as µmol Fe<sup>2+</sup>/100g FW.

**Table 1** The altitude, latitude, longitude and climatic conditions where the accessions were collected.

A	Species	Collected site	Altitude (m)	Latitude (utm)	Longitude (utm)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Average of relative humidity (%)
P1	<i>Vitis vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1690	4036989	0528386	14.2	671	49
P2	<i>V. vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1675	4036991	0528389	14.2	671	49
P3	<i>V. vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1680	4036891	0528399	14.2	671	49
P4	<i>V.vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1765	4036825	0528395	14.2	671	49
P5	<i>V. vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1730	4036829	0528638	14.2	671	49
P6	<i>V.vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1745	4036822	0528530	14.2	671	49
P7	<i>V. vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1720	4036772	0528595	14.2	671	49
P8	<i>V.vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1595	4036883	0527701	14.2	671	49
P9	<i>V. vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1739	4036979	0528293	14.2	671	49
P10	<i>V.vinifera</i> spp. <i>sylvestris</i>	Piranshahr	1662	4037118	0527772	14.2	671	49
S1	<i>V. vinifera</i> spp. <i>sylvestris</i>	Sardasht	1600	4017095	0553580	15.3	858.2	47
S2	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1560	4021495	0543759	15.3	858.2	47
S3	<i>V. vinifera</i> spp. <i>sylvestris</i>	Sardasht	1610	4021076	0550258	15.3	858.2	47
S4	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1641	4116432	0545032	15.3	858.2	47
S5	<i>V. vinifera</i> spp. <i>sylvestris</i>	Sardasht	1620	4013398	0545124	15.3	858.2	47
S6	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1590	4003888	0540240	15.3	858.2	47
S7	<i>V. vinifera</i> spp. <i>sylvestris</i>	Sardasht	1575	4004867	0546885	15.3	858.2	47
S8	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1565	4006527	0559155	15.3	858.2	47
S9	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1635	4047666	0528841	15.3	858.2	47
S10	<i>V.vinifera</i> spp. <i>sylvestris</i>	Sardasht	1609	4047092	0550232	15.3	858.2	47

## Antioxidant Enzyme Measurements

### Guaiacol Peroxidase (G-POD)

The activity of G-POD was measured based on Erkan *et al.* [9] method. Sample (2 g) was ground in a cold mortar and pestle with 2 ml potassium-phosphate buffer (0.1 mol L<sup>-1</sup>, pH 7.3) including 1 mmol L<sup>-1</sup> EDTA and 2 mmol L<sup>-1</sup> DTT. Then, the obtained homogenate was centrifuged at 10,000 × g for 15 min at 4 °C. The resulting supernatant was applied for assessment of G-POD activity. The G-POD assay mixture including 4 mmol L<sup>-1</sup> guaiacol as donor, 3 mmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> as substrate, 0.1 mol L<sup>-1</sup> phosphate buffer (pH 6.1) and 1.0 ml crude enzyme extract (400–800 µg protein). The volume of final reaction was 3.0 ml. The value of variation in absorbance at 420 nm was determined, and afterward the activity of the enzyme was expressed based on the difference in absorbance (OD) [9].

### Superoxide Dismutase (SOD)

Sample (2 g) was ground in a cold mortar and pestle with 2 ml potassium phosphate buffer (0.1 mol L<sup>-1</sup>, pH 7.4) including 1 mmol L<sup>-1</sup> EDTA and 2 mmol L<sup>-1</sup> DTT. The resulting homogenate was filtered and centrifuged at 10,000 × g for 15 min at 4 °C. Afterward the obtained supernatant was applied for assessment of the SOD activity [28]. The SOD assay mixture including 60-70 µl of enzyme extract (24–56 µg protein). The volume of final reaction was 3.0 ml. One unit of SOD enzyme was expressed as the value of the enzyme, which produced a 50% inhibition of nitro blue tetrazolium (NBT) reduction under the assay conditions.

### Catalase (CAT)

Fruit sample (0.5 g) was ground in a cold mortar and pestle with 5 ml potassium-phosphate buffer (100 mM, pH 6.8) at 4 °C. Afterward, the resulting homogenate was centrifuged at 10,000 × g for 10 min at 4 °C. Then, the obtained supernatant was applied for assessment of CAT activity [6] via investigating the disappearance of H<sub>2</sub>O<sub>2</sub> by reading the reduction in absorbance at 240 nm. The reaction mixture including 12.5 mmol/L H<sub>2</sub>O<sub>2</sub>, 50 mmol/L sodium phosphate buffer (pH 7.0) and 1 ml of enzyme extract (400-800 µg protein). One unit of CAT enzyme was expressed as the value of enzyme that decomposes 1 mmol of H<sub>2</sub>O<sub>2</sub> per minute per milligram of protein under the conditions of the assay.

### Statistical Analysis

This study was carried out as a completely randomized design with three replications. Analysis of variance was performed for obtained data and comparison of means was carried out using Duncan's multiple range test in SAS (software Version 9.1 SAS). The SPSS (software

Version 16 SPSS) was used for correlation analysis between measured parameters and the statistical graphics plus 5.1 was used for principal components analysis (PCA) and cluster analysis.

## Results

The differences in TP, TA and TF contents of the pulp and peel fractions among wild grape accessions were statistically significant ( $p < 0.01$ , Table 2). According to obtained results, the TP content ranged within 139.29-843.10 mg GAE per 100 g FW in peel and 151.67-416.91 mg GAE per 100 g FW in pulp. The TA content of wild grape accessions ranged from 3.75 to 36.44 in peel and 0.74-11.89 mg in pulp, expressed as malvidin-3-glucoside equivalents per 100 g FW basis.

The results of TF content in the peel and pulp fractions of wild grape berries are presented in Table 2. The TF content of wild grape accessions was in the range of 96.83–415.83 and 138.5–415.5 mg CAT per 100 g FW basis in the peel and pulp, respectively.

The differences in antioxidant capacity using DPPH and FRAP assays and antioxidant enzymes activity (SOD and CAT) of the pulp and peel fractions among wild grape accessions were statistically significant ( $p < 0.01$ , Table 3). According to obtained results, accession P9 had the highest peel antioxidant values (76.91%) based on DPPH assay and the highest peel antioxidant values (757.22 µmol Fe<sup>+2</sup>/100g) based on FRAP assay was observed in accession S1. The lowest antioxidant values were seen in accessions S2 and P4 based on DPPH and FRAP assays, respectively in peel. Also, the highest pulp antioxidant values were observed in accessions S1 and P4 (56.62% and 570.56 µmol Fe<sup>+2</sup>/100g for DPPH and FRAP assays, respectively).

Based on the obtained results, the G-POD activity ranged from 1.07 to 2.11 in peel and 1.21 to 2.21 DA mg<sup>-1</sup> protein in pulp. Also, the SOD activity of wild grape accessions ranging from 11.63 to 42.28 in peel and 12.25 to 29.04 U mg<sup>-1</sup> protein in pulp. The highest SOD activity of peel and pulp was observed in S8 and S1 accessions at 42.25 and 29.34 U mg<sup>-1</sup> protein, respectively. Also, The CAT activity of wild grape accessions was in the range of 1.37–6.30 and 1.32–5.24 U mg<sup>-1</sup> protein in the peel and pulp fractions, respectively.

The results of correlation analysis were observed in Table 4. In this study, the Significant correlations was seen between two antioxidant assays based on DPPH and FRAP assays in the both of peel and pulp. The results of PCA showed that the seventh PC displayed 18.7, 16.4, 15.9, 10.5, 7.9, 6.9 and 6.4% of the variance, respectively; in total, these PCs explained 82.74% of the variation. The biplot was plotted based on the first two

PCs on the reduced space (Fig. 1). The results of hierarchical cluster analysis were observed in Fig 2.

## Discussion

Our results indicated that TP content of wild grape berries was in the order of peel>pulp. According to previous studies, a high variation in terms of TP content was seen in berries of grape of 1.404 mg GAE per g FW basis [18] and 3.8–20.2 mg GAE per g FW basis [19].

The geographical and environmental factors, including light intensity and temperature may be varied content of polyphenolic compounds in berries of grape [29].

The content of TA in the native grape berries may be affected by accession and genotype. Our data show the all of wild grape accessions have elevated levels of TA content in peel than the pulp. In earlier studies, it has been reported that TA content was affected by genetic and environmental factors. The TA content in cultivated species of *V. vinifera* Were 0.946 mg per g of FW [18], in grape cultivars grown in southern Serbia Vineyard were 44–120 mg per 100 g [20], and were 2.54–9.07 mg per g of FW in peel of red grape cultivars grown in different locations of China [29].

The considerable amounts of flavonoids were revealed in the berries of wild grape accessions. The composition of the fruits could be affected by geographical, environmental and genetic factors, such as soil condition, degree of fruit maturity at harvest time and etc [12]. Also, our results indicated that the climate status affects the polyphenolic compound in all studied accessions. However, the TF, TP and TA variations had the different patterns in studied accessions.

The accessions grown in areas with higher mean annual temperature and low relative humidity had the highest total flavonoid content. Therefore, although part of the variation in total flavonoid, total phenol and total anthocyanin contents could be due to climate status, but the variation depends on genetic factors and soil conditions was observed in different growth regions. Previously, it has been revealed that the polyphenolic compounds in fruits could be varied by the genetic factors more than climatic conditions [18]. In this study, we observed that the peel of wild grape berries contains highest antioxidant activity using DPPH assay than pulp. Also, our data indicated, the antioxidant activity based on FRAP assay was in the order of peel>pulp, which is similar to the study of Guo *et al.* [11]). The results revealed that the antioxidant activity in this study higher than the other results [23,29]. The climate status, genotypes, cultivars and harvest date could be affected the antioxidant activity [7].

The antioxidant defense systems of plant cells, such as G-POD, CAT and SOD antioxidant enzymes can scavenge

the reactive oxygen species (ROS) for reducing of oxidative stress [30]. For example, Superoxide radical can be dismutated into H<sub>2</sub>O<sub>2</sub> by SOD [3] in mitochondria, chloroplasts, and peroxisomes. The studied grape accessions in this research showed higher antioxidant enzyme activity. In general, the CAT, SOD, and G-POD antioxidant enzyme were found to be higher in berries peel for all the wild grape accessions. The results revealed that the both of peel and pulp parts of grape berries are the main resources of antioxidant enzymes, that apply as free radical suppressors and therefore, they are the primitive antioxidants that scavenge the free radicals [7].

Based on correlation results, the antioxidant assays based on DPPH correlated with FRAP assay in the both of peel and pulp. So, it could be concluded that the both DPPH and FRAP assays in characterizing antioxidant activity of wild grape accessions are approximately comparable and substitutable. The obtained results coincide with previous researches [17, 29]. The TP and TF content of grape peel and pulp were correlated with DPPH and FRAP antioxidant assays with a reducing ordering of TP > TF. Besides, the significant correlation was seen among the TP, TA and TF in both peel and pulp. It has been previously reported that the antioxidant capacity was correlated with TP content in red grape cultivars [13] and fruits of cornelian cherry genotypes [12]. According to the results, no significant correlation was seen among the antioxidant enzymes (G-POD, SOD and CAT) in both peel and pulp. Also, our results showed that TA wasn't correlated with DPPH and FRAP methods of antioxidant assays in pulp. Perhaps, this result is because the anthocyanins are not the principal compound in grape pulp.

The principle component analysis was carried out to find how studied parameters of grape peel and pulp assist with diversity among the wild grape accessions. Based on PC analysis, the first PC is positively correlated with G-POD, and negatively and highly correlated with DPPHP, FRAP, TA, G-PODP and SODP. While, the DPPH, CAT, TFP, TPP, TF, TP, TAP and CATP attributes were highly and positively correlated with the second PC (Fig. 1).

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among wild grape accessions. Based on analyzing examined properties on grouping wild grape accessions, it can be concluded that the antioxidant activity of peel grape based on DPPH assay and TA of pulp grape had the biggest influence.

**Table 2** Total phenolic (TP), total anthocyanin (TA) and total flavonoid (TF) contents of wild grape berries

A	TP (mg GAE/100 g FW)		TA (mg/100g FW)		TF (mg CAT/100 g FW)	
	Peel	pulp	Peel	pulp	peel	Pulp
P1	273.33±0.47 def	202.62±0.31 fgh	28.93±0.31 abc	0.94±01 e	115.00±0.46 ij	297.5±0.16 de
P2	203.10±0.71 ghi	207.38±0.47 fgh	20.24±0.30 de	4.71±0.02 b-e	114.00±0.54 ij	166.33±0.04 jk
P3	297.86±0.22 de	215.95±0.35 fgh	25.84±0.65 bcd	1.81±0.05 e	113.33±0.60 j	248.33±0.47 gh
P4	270.72±0.26 def	342.62±0.49 abc	9.18±0.47f g	6.75±0.46 bcd	323.67±0.55 b	293.17±0.54 de
P5	218.33±1.34 e-i	354.53±1.16 ab	29.95±0.46 ab	3.75±0.53 bc	169.83±0.53 fg	138.5±0.56 k
P6	249.76±0.68 e-h	416.91±0.56 a	6.20±0.95 g	2.67±0.03 e	225.33±0.42 c	274.5±0.48 efg
P7	191.19±0.88 ghi	404.53±0.42 a	7.14±0.52 fg	0.74±0.53 e	216.33±0.35 d	144.33±0.41 k
P8	843.10±0.56 a	316.90±0.83 bcd	8.33±0.29 fg	11.89±0.55 a	178.67±0.52 fg	283.33±0.41 ef
P9	422.62±0.42 c	374.52±0.49 ab	5.79±0.05 g	1.93±0.37 e	198.83±0.60 de	244.67±0.47 h
P10	756.43±0.82 b	342.14±0.89 bc	3.75±0.53 g	3.97±0.24 bc	193.83±0.54 ef	161.0±0.49 k
S1	218.33±1.05 f-i	206.90±0.71 fgh	24.41±0.47 bcd	6.75±0.16 bcd	114.5±0.32 ij	314.33±0.10 d
S2	391.19±1.22 c	265.00±0.50 def	20.82±0.36 cde	3.02±0.36 de	135.33±0.51 hi	358.5±0.54 c
S3	139.29±0.24 i	151.67±0.31 h	17.21±0.35 e	2.04±0.46 e	226.83±0.29 c	222.0±0.35 i
S4	166.43±0.35 hi	161.66±0.43 gh	17.59±0.49 de	7.75±0.47 b	116.5±0.10 ij	287.5±0.38 de
S5	193.10±0.24 f-i	242.62±0.53 efg	14.67±0.53 f	5.38±0.23 bc	118±0.53 ij	375.0±0.29 bc
S6	338.33±0.52 cd	171.67±0.87 gh	25.38±0.41 bcd	7.31±0.10 bc	415.83±0.32 a	413.0±0.26 a
S7	388.81±1.16 c	360.24±1.05 ab	25.26±0.49 bcd	3.53±0.18 cde	96.83±0.53 j	415.5±0.06 a
S8	410.71±1.17 c	295.95±0.41 cde	22.17±0.47 cde	4.71±0.46 bc	155±0.06 gh	402.17±0.39 ab
S9	263.10±0.64 d-g	214.05±0.41 fgh	36.44±0.31 a	3.64±0.31 bc	107±0.37 j	172.83±0.47 jk
S10	176.43±0.82 ghi	224.05±0.71 fgh	22.42±0.56 b-e	1.27±0.46 e	136.5±0.097 hi	202.33±0.08 ij

Values in the same column with different lower-case letters are significantly different at <0.01. Data were expressed as mean±SE (n = 3)

**Table 3** Antioxidant capacities based on DPPH and FRAP assays and Guaiacol peroxidase (G-POD), Polyphenol oxidase (PPO) and Catalase (CAT) activities of wild grape berries

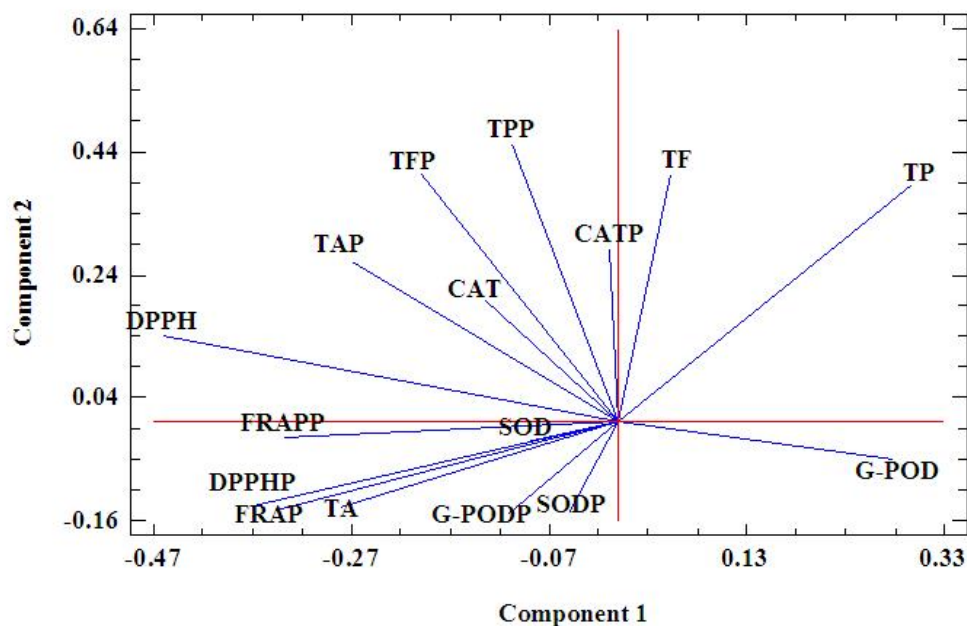
A	DPPH (%)		FRAP ( $\mu\text{mol Fe}^{+2}/100 \text{ g FW}$ )		G-POD ( $\text{DAmg}^{-1} \text{ protein}$ )		SOD ( $\text{Umg}^{-1} \text{ protein}$ )		CAT ( $\text{Umg}^{-1} \text{ protein}$ )	
	Peel	pulp	Peel	pulp	peel	pulp	peel	Pulp	Peel	Pulp
P1	47.90±0.10 de	49.02±0.24 abc	547.22±0.51 c	298.71±0.54 i	1.12±0.05 a	1.35±0.04 a	22.04±0.08 b-e	20.29±0.53 abc	4.72±0.56 c-g	4.46±0.05 ab
P2	49.73±0.53 cd	43.13±0.50 c-f	394.63±0.41 fg	303.52±0.52 hi	1.14±0.04 a	2.21±0.05 a	25.48±0.53 bc	23.07±0.05 abc	5.64±0.05 a-d	4.33±0.03 abc
P3	31.52±0.52 g	28.23±0.10 hi	392.78±0.69 g	350.93±0.60 fgh	1.12±0.02 a	1.29±0.01 a	27.33±0.01 b	19.24±0.03 abc	5.16±0.04 a-e	4.54±0.01 ab
P4	32.9±0.41 fg	32.54±0.13 ghi	383.52±0.58 g	570.56±0.63 a	1.25±0.30 a	1.21±0.04 a	26.54±0.50 b	12.25±0.03 c	6.18±0.32 ab	3.71±0.02 cde
P5	42.13±0.10 def	21.96±0.04 i	417.22±0.53 efg	475.74±0.41 b	1.32±0.01 a	1.25±0.01 a	25.48±0.46 bc	19.20±0.02 abc	4.99±0.06 b-f	3.32±0.04 c-f
P6	61.32±0.30 bc	34.19±0.05 e-i	403.52±0.53 fg	192.41±0.41 j	1.32±0.42 a	1.27±0.02 a	23.36±0.10 bcd	24.67±0.49 ab	2.50±0.10 j	3.12±0.00 ef
P7	38.96±0.41 def	28.15±0.27 ghi	457.96±1.16 de	359.81±0.47 fg	2.11±0.30 a	1.37±0.56 a	24.9±0.04 bc	23.64±0.01 ab	4.44±0.31 e-h	4.30±0.02 bcd
P8	45.59±0.29 def	39.29±0.16 c-g	452.41±1.75 e	360.92±0.58 efg	1.15±0.13 a	1.35±0.01 a	27.76±0.12 b	26.77±0.52 ab	5.95±0.09 abc	3.28±0.12 def
P9	76.91±0.19 a	33.64±0.34 f-i	423.52±0.53 efg	389.81±0.49 ef	1.26±0.12 a	1.35±0.02 a	22.69±0.15 bcd	19.98±0.26 abc	4.04±0.47 f-i	5.24±0.04 a
P10	38.52±0.41 ef	52±0.41 ab	542.78±0.46 c	440.93±0.53 bcd	1.26±0.09 a	1.30±0.02 a	17.14±0.10 ef	16.88±0.19 bc	3.10±0.18 ij	4.03±0.03 b-e
S1	17.02±0.35 h	56.62±0.13 a	735.37±2.56 a	410.93±0.68 cde	1.29±0.10 a	1.35±0.52 a	27.24±0.21 b	29.04±0.18 a	3.58±0.06 ghi	2.45±0.01 f
S2	16.73±0.41 h	34.74±0.34 d-h	592.41±1.59 b	287.22±0.06 i	1.36±0.08 a	1.37±0.06 a	16.39±0.10 ef	14.79±0.06 bc	3.42±0.06 hij	3.33±0.52 c-f
S3	33.33±0.45 fg	38.19±0.45 d-g	534.26±0.93 c	382.41±0.54 ef	1.28±0.43 a	1.25±0.10 a	11.63±0.21 f	15.21±0.19 bc	3.77±0.19 ghi	1.32±0.09 g
S4	40.11±0.54 def	24.31±0.22 hi	499.81±1.35 d	392.41±0.43 def	1.23±0.19 a	1.38±0.06 a	24.40±0.05 bc	23.44±0.14 ab	6.20±0.16 a	4.21±0.13 bcd
S5	49.35±0.22 cd	45.88±0.19 bcd	535.37±1.11 c	375.74±0.41 ef	1.38±0.16 a	1.38±0.08 a	27.06±0.18 b	17.34±0.09 bc	3.16±0.28 ij	3.86±0.19 b-e
S6	73.11±0.1 ab	24.47±0.06 hi	543.15±0.64 c	400.56±0.57 c-f	1.15±0.07 a	1.39±0.10 a	26.22±0.22 b	24.38±0.31 ab	4.20±0.08 e-i	4.22±0.12 bcd
S7	48.77±0.09 cde	44.70±0.13 b-e	529.45±0.63 c	352.04±0.51 fgh	1.07±0.22 a	1.37±0.26 a	23.96±0.28 bcd	21.82±0.09 abc	4.85±0.04 c-g	3.80±0.31 b-e
S8	31.60±0.06 g	24.07±0.37 i	435.0±0.38 ef	447.22±0.45 bc	1.17±0.16 a	1.33±0.04 a	42.28±0.17 a	20.84±0.10 abc	4.78±0.04 c-g	3.77±0.27 b-e
S9	48.77±0.26 cde	28.78±0.13 ghi	757.22±1.01 a	314.26±0.47 gh	1.25±0.06 a	1.31±0.01 a	23.41±0.04 bcd	19.87±0.19 abc	6.30±0.02 a	3.86±0.12 b-e
S10	34.34±0.19 fg	27.05±0.39 ghi	509.07±0.72 c	360.19±0.45 efg	1.19±0.08 a	1.36±0.04 a	27.56±0.24 b	21.32±0.46 abc	1.37±0.01 k	1.58±0.02 g

Values in the same column with different lower-case letters are significantly different at  $p < 0.01$ . Data were expressed as mean  $\pm$  SE ( $n = 3$ )

**Table 4** Pearson's correlation coefficients of antioxidant capacity (DPPH, FRAP), antioxidant enzymes (G-POD, SOD, CAT), total phenols (TP), total flavonoids (TF) and total anthocyanins (TA) in grape

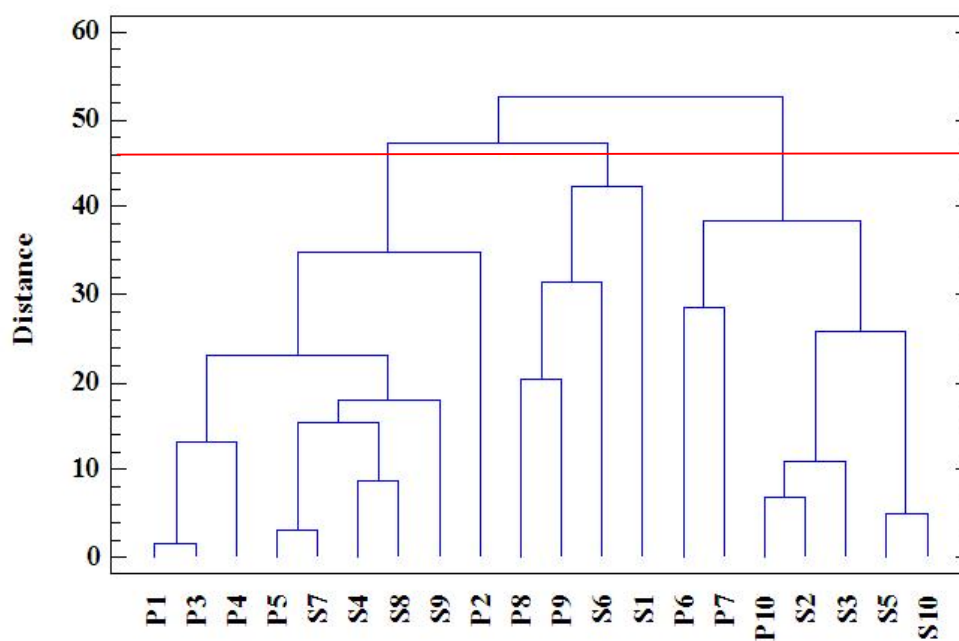
peel								
variables	TP	TA	TF	DPPH	FRAP	G-POD	SOD	CAT
TP	1	0.468**	0.596**	0.584**	0.621**	0.303*	0.093 <sup>ns</sup>	0.197 <sup>ns</sup>
TA	-	1	0.324*	0.348*	0.314*	-0.026 <sup>ns</sup>	-0.47 <sup>ns</sup>	-0.060 <sup>ns</sup>
TF	-	-	1	0.556**	0.546**	-0.086 <sup>ns</sup>	-0.097 <sup>ns</sup>	0.06 <sup>ns</sup>
DPPH	-	-	-	1	0.781**	0.119 <sup>ns</sup>	-0.301*	0.319*
FRAP	-	-	-	-	1	0.197 <sup>ns</sup>	0.286*	0.313*
G-POD	-	-	-	-	-	1	0.134 <sup>ns</sup>	0.175 <sup>ns</sup>
SOD	-	-	-	-	-	-	1	-0.200 <sup>ns</sup>
CAT	-	-	-	-	-	-	-	1
pulp								
TP	1	0.495**	0.673**	0.604**	0.550**	0.081 <sup>ns</sup>	0.087 <sup>ns</sup>	0.168 <sup>ns</sup>
TA	-	1	0.345*	0.128 <sup>ns</sup>	0.124 <sup>ns</sup>	-0.031 <sup>ns</sup>	-0.207 <sup>ns</sup>	-0.054 <sup>ns</sup>
TF	-	-	1	0.537**	0.527**	-0.074 <sup>ns</sup>	-0.078 <sup>ns</sup>	0.11 <sup>ns</sup>
DPPH	-	-	-	1	0.756**	0.302*	-0.203 <sup>ns</sup>	0.305*
FRAP	-	-	-	-	1	0.097 <sup>ns</sup>	0.266*	0.324*
G-POD	-	-	-	-	-	1	0.131 <sup>ns</sup>	0.143 <sup>ns</sup>
SOD	-	-	-	-	-	-	1	-0.198 <sup>ns</sup>
CAT	-	-	-	-	-	-	-	1

95% confidence interval. <sup>ns</sup>, no significant; \*, significant at  $p < 0.05$ ; \*\*, Significant at  $p < 0.001$



**Fig. 1** Principal component analysis (PCA) projection of two first principal components, Parameters: G-POD (Peel guaiacol peroxidase), TP (Peel total phenol), TF (Peel total flavonoid), CATP (Pulp catalase); TPP (Pulp total phenol); TFP (Pulp total flavonoid); CAT (Peel catalase); TAP (Pulp total anthocyanin); DPPH (Peel DPPH assay); FRAPP (Pulp FRAP assay); SOD (Peel superoxide dismutase); DPPHP (Pulp DPPH assay); FRAP (Peel FRAP assay), TA (Peel total anthocyanin); G-PODP (Pulp guaiacol peroxidase); SODP (Pulp superoxide dismutase).





**Fig. 2** Dendrogram of grouping 20 wild grape accessions based on 7 main factors and Ward's method.

The first cluster includes 9 accessions, which had medium antioxidant activity of peel grape based on DPPH assay and TA of pulp grape. The second cluster includes 4 accessions with higher antioxidant activity of peel grape based on DPPH assay and TA of pulp grape. The third cluster includes 7 accessions, which had low antioxidant activity of peel grape based on DPPH assay and TA of pulp grape (Fig. 2). As a conclusion, the present study reveals the potential value of wild grape germplasm. The berries of wild grape accessions are rich in polyphenolic compounds, flavonoids and anthocyanins.

The Antioxidant enzymes and antioxidant capacity values were higher in the both peel and pulp of berries and varied greatly among the accessions. The most of the antioxidant and phenolic compounds were higher in the peel compared to pulp, which the reason of reduced antioxidant activity may be due to high water in the pulp. Therefore, it could be concluded that studied wild grape accessions were rich in natural antioxidants. Also, they can possibly be utilized in food formulations and nutraceutical supplement. Besides, these studied accessions can be used as breeding materials in future breeding programs.

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