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*Original Article*

# Evaluation of the Antioxidant Potential and Antioxidant Enzymes of Some Yemeni Grape Cultivars

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## ABSTRACT

**Objective:** The evaluation of antioxidant activity of grape from different cultivars was conducted by several methods. **Materials and Methods:** Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, scavenging of 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) and formation of phosphate/molybdenum complex. **Results:** The phenolic content of peel and flesh from different cultivars of grapes showed concentration dependent scavenging of DPPH and ABTS radicals and formation of phosphate/molybdenum complex. The results appeared high values of correlation coefficient ($R^2$) between phenolic content of grapes and antioxidant activity indicating the strong correlation. The antioxidant assay $IC_{50}$ value was variable among different grape cultivars. The activities of antioxidant enzymes were screened in the crude extracts of the peel and flesh of the grape cultivars tested, which had high, moderate and low levels of polyphenol oxidase, peroxidase and catalase, respectively. **Conclusion:** The results indicated that the imported grapes retained the most antioxidant activities. Finally, this study will be supply information on the antioxidant activity of grapes that benefits grapes producers, nutritionists and consumers.

**Key words:** Antioxidants, Yemeni grape, Flavonoids, Antioxidant enzymes.

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## INTRODUCTION

The global production of grape juices is estimated to be around 11–12 million hectoliters and the main producer and consumer countries are the United States of America, Brazil and Spain.[1] in Yemen there are about 27 grape varieties reported. The major varieties include Aasmi (Red), Raziqi, Aswad (Black). Grape is an important fruit crop in Yemen. Grape consumption has been increasing since the 1970s as a consequence of rapid urban population growth and a sharp increase in demand. In a 1998 Food and Agriculture Organization (FAO) report, Yemen ranked sixth among Arab countries in grape production.[1] In the past few years there has been increasing interest in the determination of suitable dietary sources of antioxidant phenolic compounds. Grapes (Vitis) are among the fruits consumed in greatest quantities around the world, whether processed or in their natural form, and they also have one of the highest phenolic compound contents.[2] Grape juice has also been reported to improve cognitive and motor function.[3,4] Some of these effects are related to protection against oxidative stress,[5] and the antioxidant activity of grape juices may be an indicator of the relative level of health benefit they offer. Phenolic compounds are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables and other plants.[6] These compounds and their antioxidant activity have long been associated with the beneficial effects of grapes and wines.[7,39] Catechin and gallic acid act as free radical scavengers, and epicatechin has also demonstrated antibacterial activity and protective effects against membrane oxidation.[8] Grape juices are rich in phenolic compounds and different studies have demonstrated that these substances possess biological activity related to health benefits for the consumers.[9,10] The phenolic compounds in grape juices, mainly the flavonoids flavanols, flavonols and anthocyanins, are associated with improved health, along with other compounds which are not flavonoids, such as phenolic acids and the stilbene resveratrol. [11-14] The flavonols are represented mainly by *kaempferol*, quercetin and myricetin and simple orthomethylated derivatives such as isorhamnetin, which have received considerable interest due to their antioxidant properties.[15] Among the flavanols, (+)-catechin, (-)-epicatechin and procyanidins have gained attention due to their antioxidant, antimicrobial and bactericidal activity. [14] The principal anthocyanins found in juices are malvidin, cyanidin, delphinidin, petunidin, peonidin and pelargonidin. The consumption of these anthocyanins is associated to biological activities, such as antioxidant capacity and prevention of cardiovascular diseases.[14] Phenolic acids, such as gallic, caffeic and chlorogenic, have been studied for their antioxidant capacity and for acting as venous dilators.[15] The aim of of the present study is (1) to explore the potential antioxidant capacity, using the DPPH test, (2) to systematically evaluate the antioxidant capacity, total phenolic and flavenoid contents and to evaluate the changes in the activities of some antioxidant enzymes catalase, peroxidase and polyphenol oxidase in three locally growing grapes. This information will be useful for nutritionists and consumers.

## MATERIALS AND METHODS

### Grape samples

The study included three types of grape (Aasmi, Raziqi and Aswad) were obtained from three production areas/governorates (Sanaa, Amran and Saadah). Each sample was individually analyzed by triplicate.

### Chemicals

The solvent used in the present work were purchased from Riedel-de-Haen (Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis

(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), ammonium molybdate were obtained from Fluka (Germany). Hydrogen peroxide, guaiacol and catechol were purchased from Sigma (USA).

## Preparation of solvent extracts

Two g of gape peel or fleshwere extracted by shaking at 150 rpm and 25°C for 24 h with 10 ml of 80% methanol and filtered through filter paper No. 1. The filtrate designated as methanol extract.

## Determination of the total phenolic contents

Total phenolic content was measured according to available literature.[16] Fifty µL of the methanol extract was mixed with 100 µL Folin-Ciocalteu reagent, 850 µL of distilled water and allowed to stand for 5 min at ambient temperature. A 500 µL of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid (Figure 1). The results expressed as mg gallic acid equivalent/g tissues.

## Determination of the total flavenoid contents

The total flavonoid content of the samples was determined using a modified colorimetric method described previously by[17] and used catechin as a standard. Methanol extract or standard solution (250 µL) was mixed with distilled water (1.25 mL) and 5% $NaNO_2$ solution (75 µL). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 µL). 1 MNaOH (0.5 mL) and distilled water (275 µL) was added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured. Total flavenoids were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catchin acid (Figure 2). The results expressed as mg catechin equivalent/g tissues.

## Antioxidant assays

## DPPH radical scavenging activity

Free radical scavenging activity of crude methanol extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method**.** A methanol solution (100 µL) containing methanol extracts was added to 900 µL of freshly prepared DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a control. After incubation for 30 min at room temperature in the dark, the absorbance was measured at 517 nm using a spectrophotometer. Activity of scavenging (%) was calculated using the following formula:

DPPH radical scavenging%=[(OD control–OD sample)/OD control] × 100.

The results were plotted as the % of scavenging activity against concentration of the sample. The inhibition concentration (IC50) was defined as the amount of crude methanol extract required for 50% of free radical scavenging activity. The IC50 value was calculated from the plots as the antioxidant concentration required for providing 50% free radical scavenging activity.

## ABTS radical cation decolorization assay

ABTS (2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) also forms a relatively stable free radical, which decolorizes in its non-radical form. The spectrophotometric analysis of ABTS•+scavenging activity was determined according to the method of[18] ABTS•+ was produced by reacting 7 mM ABTS in H2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), store in the dark at room temperature for 16 h. The ABTS•+ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer (pH 7.4). Then, 1 mL of ABTS•+ solution was added to crude methanol extract. The absorbance was recorded 1 min after mixing and the percentage of radical scavenging was calculated relative to a blank containing no scavenger. The extent of decolorization was calculated as percentage reduction of absorbance. The scavenging capability of test compounds was calculated using the following equation:

ABTS·+scavenging(%)=[(ODcontrol–ODsample)/ODcontrol] × 100

The results were plotted as the % of scavenging activity against concentration of the sample. The inhibition concentration (IC50) was defined as the amount of crude methanol extract required for 50% of free radical scavenging activity. The IC50 value was calculated from the plots as the antioxidant concentration required for providing 50% free radical scavenging activity (Figure 2).

## Phosphomolybdenum complex assay

Spectrophotometric evaluation of antioxidant activity through the formation of a phosphomolybdenum complex was carried out according to Prieto *et al.*[19] Sample solutions (50 µL) were combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of aqueous solutions of each was measured at 820 nm against a blank. The antioxidant activity was expressed as the absorbance of the sample (Figure 3).

## Determination of antioxidant enzymes

## Preparation of crude extract

One g of grape was homogenized with 20 mM Tris–HCl buffer, pH 7.2 contained 0.1 M NaCl and 2% triton-x100 using homogenizer. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C. The

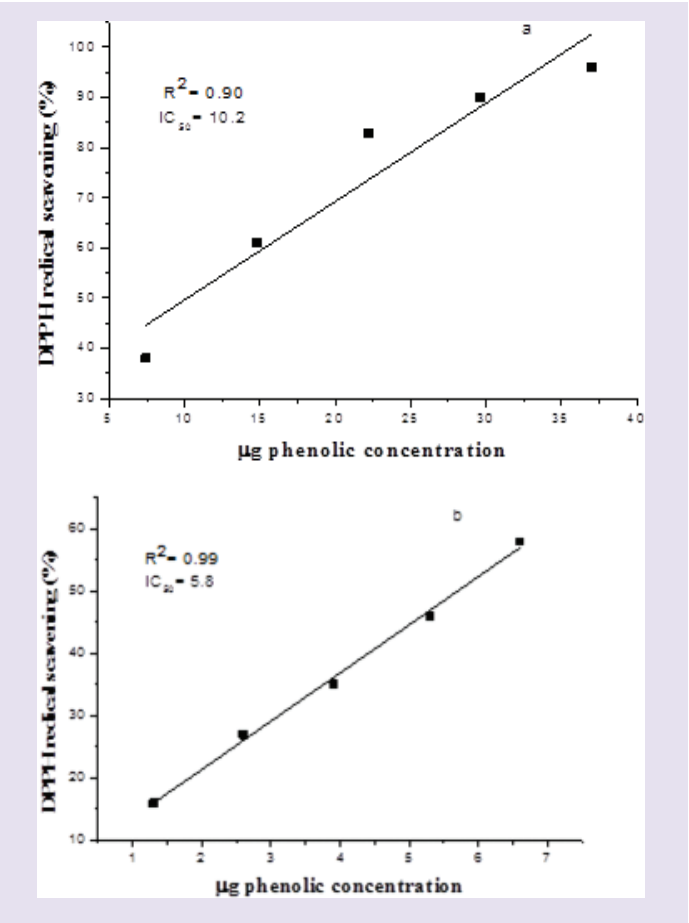


**Figure 1:** Correlation between concentrations of phenolic compounds of Aasmi grape peel (a) and flesh (b), and their antioxidant activity as determined by DPPH assay.

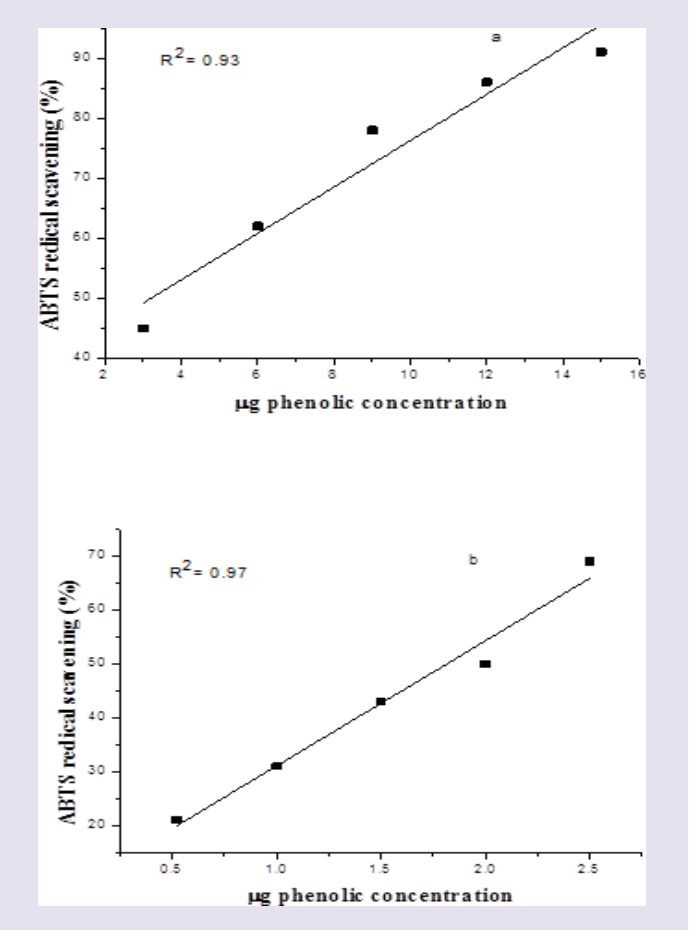


**Figure 2:** Correlation between concentrations of phenolic compounds of Aasmi peel (a) and flesh (b), and their antioxidant activity as determined by ABTS assay.

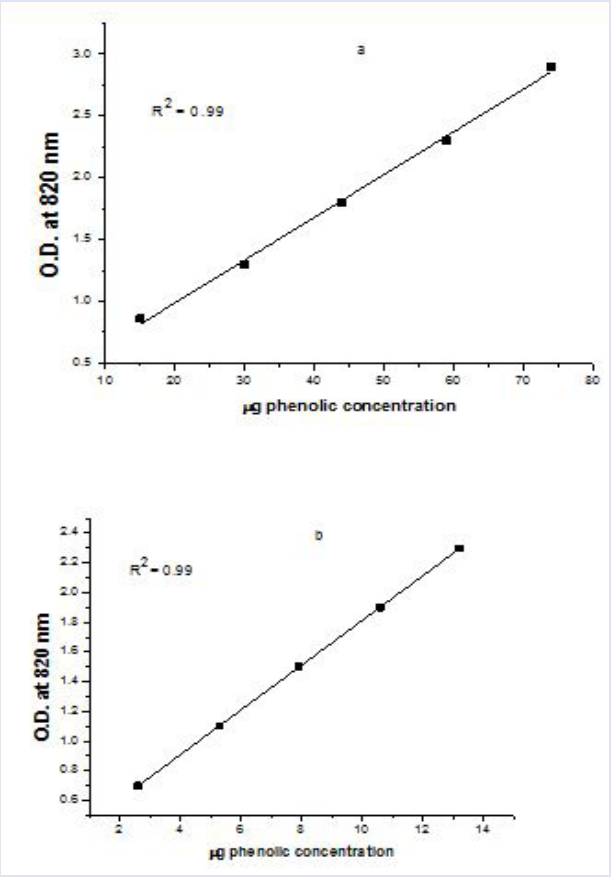


**Figure 2:** Correlation between concentrations of phenolic compounds of Aasmi peel (a) and flesh (b), and their antioxidant activity as determined by ABTS assay.

supernatant was designed as crude extract and stored at −20°C for further analysis.

## Peroxidase assay

Peroxidase activity was carried out according to Yuan and Jiang.[20-21] The reaction mixture contained in one ml: 8 mM H2O2, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5 and least amount of crude extract. The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of peroxidase activity was defined as the amount of enzyme which increased the O.D. 1.0 per min under standard assay conditions.

## Catalase assay

Catalase activity was determined according to Bergmeyer.[22] Two and half ml of substrate solution was made up of 25 mM H2O2 in a 75 mM sodium phosphate buffer pH 7.0 and crude extract. The decrease in absorbance at 240 nm and 25°C was recorded for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min under standard assay conditions.

## Polyphenoloxidase assay

Polyphenoloxidase activity was assayed with catechol as a substrate according to the spectrophotometric procedure of Jiang *et al.*[23] The enzyme solution (100 µL) was rapidly added to 900 µL of 40 mM catechol solution prepared in 0.01 M sodium phosphate buffer, pH 6.8. The increase in absorbance at 400 nm and 25°C was recorded for 3 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min.

## Statistical analysis

The statistical analyses were performed by a one-way ANOVA and the Student's *t*-test. The results were expressed as means ± S.E. to show variations in the various experimental. Difference are considered significant when $P < 0.05$.

# RESULTS

## Estimation of the total content of phenols

The results for the total content of phenolics of the grapes from 3 types (Aasmi, Aswad, Raziqi) are giving in Table 1. A great variation in terms of total phenolic content was observed among the grape cultivars (2.5-7.4 mg GAE/g peel; 0.33-1.32 mg GAE/g flesh). The highest amount of total phenolics in peel was observed for Aasmi (7.4 mg GAE/g peel), followed by Aswad (5.3 mg GAE/g peel), while lowest was in Raziqi (2.5 mg GAE/g peel). For flesh, the highest amount of total phenolics was also observed for Aasmi (1.32 mg GAE/g flesh), followed by Raziqi (0.72 mg GAE/g flesh), while lowest was in Aswad (0.33 mg GAE/g flesh).According to the literature, the total phenolic compounds concentrations in commercial grape juices in Brazil varies from 270 to 3433 mg/ L, with mean values of between 1430 and 1915 mg/ L, these differences being mainly due to the production techniques and to the particular

**Table 1: The phenolic contents in grape from different cultivars**

| Grape cultivar | Tissue | mg GAE/g tissues |
|---|---|---|
| Aasmi | Peel | 7.4 ± 0.056 |
| | flesh | 1.32 ± 0.005 |
| Raziqi | Peel | 2.5 ± 0.023 |
| | flesh | 0.72 ± 0.003 |
| Aswad | Peel | 5.34 ± 0.027 |
| | flesh | 0.33 ± 0.004 |

Values are presented as means ± SE (n=3).
GAE, gallic acid equivalent.

**Table 2: Flavenoid contents in different grape cultivars**

| Grape cultivar | Tissue | mg CE/g tissues |
|---|---|---|
| Aasmi | Peel | 1.94 ± 0.014 |
| | flesh | 0.46 ± 0.008 |
| Raziqi | Peel | 0.97 ± 0.007 |
| | flesh | 0.31 ± 0.005 |
| Aswad | Peel | 1.38 ± 0.025 |
| | flesh | 0.08 ± 0.002 |

Values are presented as means ± SE (n=3).
CE, catchin equivalent.

**Table 3: Antioxidant effect of phenolic concentrations of different grape cultivars on reduction of DPPH radical scavenging**

| Grape cultivar | Tissue | $IC_{50}$: in µg phenolic contents |
|---|---|---|
| Aasmi | Peel | 10.2 |
| | flesh | 5.8 |
| Raziqi | Peel | 5.7 |
| | flesh | 3.9 |
| Aswad | Peel | 6 |
| | flesh | 1.7 |

$IC_{50}$ (µg phenolic content) is the inhibition concentration of the test sample that decrease 50% initial radical.

characteristics of the region.[24-26] In commercial Spanish juices[27] reported values of 705 to 1177 mg/ L, while the values for juices produced from the varieties Noble (*V. rotundifolia*) and Concord (*V. labrusca*) varied from 1280 to 2880 mg/ L, respectively.[28,29]

## Estimation of the total content of flavonoids

The total content of flavenoids in grape from 3 types (Aasmi, Aswad, Raziqi) ranged from 0.97 to 1.94 mg CE/g peel and 0.08 to 0.46 mg CE/g flesh) (Table 2). The highest amount of total flavenoids in peel was

**Table 4: Antioxidant effect of phenolic concentration of different grape cultivars on reduction of ABTS radical scavenging**

| Apple cultivar | Tissue | $IC_{50}$: in µg phenolic content |
|---|---|---|
| Aasmi | Peel | 3.2 |
| | flesh | 1.8 |
| Raziqi | Peel | 6 |
| | flesh | 1.7 |
| Aswad | Peel | 1.37 |
| | flesh | 1.7 |

$IC_{50}$ (µg phenolic content) is the inhibition concentration of the test sample that decrease 50% initial radical.

**Table 5: Antioxidant effect of phenolic concentration of different apple cultivars on formation of phospho-molybdenumcomplex**

| grape cultivar | Tissue | $EC_{50}$ (µg phenolic compounds) |
|---|---|---|
| Aasmi | Peel | 9.8 |
| | flesh | 1.37 |
| Raziqi | Peel | 2.9 |
| | flesh | 0.79 |
| Aswad | Peel | 5.9 |
| | flesh | 0.76 |

$EC_{50}$ (µg phenolic content) is the efficient concentration of the test sample that increases O.D 0.5.

observed for Aasmi (1.94 mg CE/g peel), followed by Aswad (1.38 mg CE/g peel), while lowest was in Raziqi (0.97 mg CE/g peel). For flesh, the highest amount of total flavenoids was also observed for Aasmi (0.46 mg CE/g flesh), followed by Raziqi (0.31 mg CE/g flesh), while lowest was in Aswad (0.08 mg GAE/g flesh). The concentration of catechin in the red grape juices was within the range of previous reports in this type of juice (0.38–86.4 mg/L).[31-33] Epicatechin concentrations in the grape juices were also within the range of previous findings (from 0.92 to 22.13 mg/L) in grape juices,[31,33] whereas the concentrations in the white and red wines were lower than previous reports (from 0.68 to 150 mg/L).[34-36] Catechin acts against free radicals and chelating metals,[37] and epicatechin has demonstrated vasodilator activity in human beings.[38]

# DISCUSSION

The phenolics content of grape from different cultivars showed concentration dependent scavenging of DPPH radical, which may be attributed to its hydrogen donating ability (Figure 1). For all grape cultivars tested, a linear increase of antioxidant activity was detected with increasing the

**Table 6: The antioxidant enzyme activities of different grape cultivars**

| grape cultivar | Tissue | Units of polyphenol oxidase/g tissues | Units of peroxidase/g tissues | Units of catalase/g tissues |
|---|---|---|---|---|
| Aasmi | Peel | 148 ± 0.05 | 44 ± 0.05 | 18 ± 0.03 |
| | Flesh | 65 ± 0.03 | - | 9 ± 0.007 |
| Raziqi | Peel | 87 ± 0.07 | 38 ± 0.012 | 14 ± 0.003 |
| | Flesh | 20 ± 0.02 | - | 7 ± 0.06 |
| Aswad | Peel | 32 ± 0.06 | 34 ± 0.08 | 9 ± 0.017 |
| | Flesh | 15 ± 0.008 | - | 4 ± 0.006 |

phenolic concentrations. In grape cultivar peels, the correlation coefficient ($R^2$) between phenolic concentratios and DPPH scavenging activity was found to be 0.99 (Raziqi), 0.97 (Aswad), 0.90 (Aasmi). In flesh, the correlation coefficient ($R^2$) between phenolic concentrationsts and DPPH scavenging activity was found to be 0.99 (Aasmi), 0.97 (Raziqi), 0.96 (Aswad). These high values of correlation coefficient ($R^2$) indicating the strong correlation. The DPPH assay $IC_{50}$value (the inhibition concentration as µg phenolic content of the test sample that decrease 50% initial radical) was ranged from 5.7 to 10.2 µg phenolic concentrations/ml grape cultivar peels (Table 3). The highest $IC_{50}$ was observed for grape peels of Raziqi (5.7 µg phenolic concentration), followed Aswad (6 µg phenolic concentration), while the lowest was in Aasmi(10.2 µg phenolic concentration). For grape fleshes, the highest $IC_{50}$ was also observed for Aasmi(1.7 µg phenolic concentration), followed Raziqi (3.9 µg phenolic concentration), while the lowest was in Aasmi 5.8 µg phenolic concentration).

The phenolics content of grape from different cultivars showed concentration dependent scavenging of ABTS radical, which may be attributed to its hydrogen donating ability (Figure 2). For all grape cultivar tested, a linear increase of antioxidant activity was detected with increasing the phenolic concentrations. In grape cultivar peels, the correlation coefficient ($R^2$) between phenolic concentrations and ABTS scavenging activity was found to be 0.97 (Aswad), 0.94 (Raziqi), 0.93 (Aasmi). In flesh, the correlation coefficient ($R^2$) between phenolic concentrations and ABTS scavenging activity was found to be 0.99 (Raziqi), 0.97 (Aasmi), 0.96 (Aswad). These high values of correlation coefficient ($R^2$) indicating the strong correlation. The ABTS assay $IC_{50}$value was ranged from 1.7 to 6 µg phenolic concentration/ml grape cultivar peels (Table 4). The highest $IC_{50}$ was observed for grape peels of Aswad (6 µg phenolic concentration), followed Aasmi (3.2 µg phenolic concentration), while the lowest was in Raziqi (1.7 µg phenolic concentration). For grape fleshes, the highest $IC_{50}$ was also observed for Aasmi (1.8 µg phenolic concentration), followed Aswad (1.7 µg phenolic concentration), while the lowest was in Rqziqi (1.37 µg phenolic concentration).

Spectrophotometric quantitation of antioxidant capacity through the formation of a phosophomolydenum complex showed an increase of activity with increasing the phenolic concentrations of grape cultivars (Figure 2). In grape cultivar peels, the correlation coefficient ($R^2$) between phenolic concentrations and the formation of a phosophomolyddenum complex was found to be 0.99 (Aasmi), 0.98 (Aswad), 0.98 (Raziqi). In flesh, the correlation coefficient ($R^2$) between phenolic concentrations and the formation of a phosophomolyddenum complex was found to be 0.99 (Aasmi), 0.98 (Raziqi), 0.90 (Aswad). These high values of correlation coefficient ($R^2$) indicating the strong correlation. The formation of a phosophomolyddenum complex $EC_{50}$value (is the efficient concentration as µg phenolic content of the test sample that increases O.D. 0.5) was ranged from 0.76 to 9.8 µg phenolic concentration/ml grape cultivar peels (Table 5). The highest $EC_{50}$ was observed for grape peels of Raziqi (2.9 µg phenolic concentration), followed Aswad (5.9 µg phenolic concentration), while the lowest was in Aasmi Raziqi (9.8 µg phenolic concentration). For grape fleshes, the highest $EC_{50}$ was observed for Aswad (0.76 µg phenolic concentration), followed Raziqi (0.79 µg phenolic concentration), while the lowest was in Aasmi (1.37 µg phenolic concentration).

Table 6 shows the activities antioxidant enzymes, polyphenoloxidase, peroxidase and catalase in the crude extracts of the peel and flesh of the grape cultivars (Aasmi, Raziqi and Aswad). The highest level of polyphenoloxidase activity was recorded in the peel of Aasmi, Raziqi and Aswad with 148, 84 and 32 units/ g tissues, respectively. Moderate level of peroxidase activity was observed for all grape cultivars tested (34 to 44 units/g tissues). All apple cultivars tested exhibited lowest level of catalase activity (9 to 18 units/g tissues).

## CONCLUSION

The results indicated that the imported grapes retained the most antioxidant activities. The present manuscript precisely explores the beneficial effect of yemeni grapes which are very rich source of antioxidants. These grapes contains high amount of free radicals scavengers which are intact and thus are helpful in the delaying aging process, cancer, and other related pathophysiological diseases which are so common in today's stressed lifestyle. This manuscript reveals the enzymes present in yemeni grapes that are responsible to combat and prevent the diseases. This manuscript in current light of research promotes the cultivation and consumption of yemeni grapes for the general good to consumers, nutritionist thus boosting the economy of grapes cultivators. Finally, this study will be supply information on the antioxidant activity of grapes that benefits grapes producers, nutritionists and consumers.

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## CONFLICT OF INTEREST

The authors report no conflict of interest.

## ABBREVIATION USED

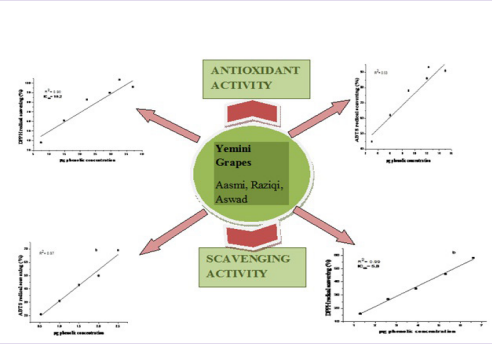
**DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 3-ethylbenzo-thiazoline-6-sulfonic acid; **FAO:** Food and Agriculture Organization; **IC:** Inhibition concentration; **$R^2$:** Correlation coefficient.

## REFERENCES

1. Organisation Internationale de la Vigneet du Vin. 2013. Vine and wine outlook 2008–2009. 2013. OIV – 18.Belgique: Peters SA;ISBN 979-10-91799-08-9. Available from: <http://www.oiv.int/oiv/info/esplubicationoiv> Accessed 11.2013.
2. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, Am. J Clin Nutr. 2005;81(1):230-42.
3. Joseph JA, Shukitt-Hale B, Willis LM. Grape juice, berries, and walnuts affect brain aging and behavior. Journal of Nutrition. 2009;139(9):1813-7. http://dx.doi.org/10.3945/jn.109.108266 ; PMid:19640963.
4. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD. Concord grape juice supplementation and neurocognitive function in human aging. Journal of Agricultural and Food Chemistry. 2012;60(23):5736-42. http://dx.doi.org/10.1021/jf300277g ; PMid:22468945.
5. Yuan LH, Meng LP, Ma WW, Xiao ZX, Zhu X, Feng JF, *et al*. Impact of apple and grape juice consumption on the antioxidant status in healthy subjects. International Journal of Food Sciences and Nutrition. 2011;62(8):844-50. http://dx.doi.org/10.3109/09637486.2011.587399 ; PMid:21639820.
6. Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chemistry. 2011;126(4):1821-35. http://dx.doi.org/10.1016/j.foodchem.2010.12.026 . PMid:25213963.
7. Balasundram N, Sundram K, Samman S. Phenolic compounds and agriindustrial by-products: antioxidant activity, occurrence, and potential uses. Food Chemistry. 2006;99:191-203. http://dx.doi.org/10.1016/j.foodchem.2005.07.042.
8. Xia E, Deng G, Guo Y, Li H. Biological activities of polyphenols from grapes. International Journal of Molecular Sciences. 2010;11(2):62-646. http://dx.doi.org/10.3390/ijms11020622 ; PMid:20386657 PMCid:PMC2852857.
9. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD. Concord grape juice supplementation and neurocognitive function in human aging. Journal of Agricultural and Food Chemistry. 2012;60(23);5736-42. http://dx.doi.org/10.1021/jf300277g ; PMid:22468945.
10. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JPE. Polyphenols and human health: Prevention of disease and mechanisms of action. Nutrients. 2010;2:1106-31. http://dx.doi.org/10.3390/nu2111106; PMid:22254000 PMCid:PMC3257622.
11. Ali K, Maltese F, Choi Y, Verpote R. Metabolic constituents of grapevine and grape – derived products. Phytochemistry Reviews. 2010;9(3):357-78. http://dx.doi.org/10.1007/s11101-009-9158-0; PMid:20835385 PMCid:PMC2928446.

12. Krikorian R, Boespflug EL, Fleck DE, Stein AL, Wightman JD, Shidler MD *et al*. Concord grape juice supplementation and neurocognitive function in human aging. Journal of Agricultural and Food Chemistry. 2012;60(23):5736-42. http://dx.doi.org/10.1021/jf300277g ; PMid:22468945.
13. Sautter C K, Denardin S, Alves AO, Mallmann CA, Penna NG, Heckttheuer LH. Determinacao de resveratrol emsucos de uva no Brazil.Ciência e Tecnologia de Alimentos. 2005;25:437-42. http://dx.doi.org/10.1590/S0101-20612005000300008.
14. Xia EQ, Deng GF, Guo YJ, Li HB. Biological activities of polyphenols from grapes. International Journal of Molecular Sciences. 2010;11(2):622-46. http://dx.doi.org/10.3390/ijms11020622 ; PMid:20386657 PMCid:PMC2852857.
15. Mudnic I, Modun D, Rastija V, Vukovic J, Brizic I, Katalinic V *et al*. Antioxidative and vasodilatory effects of phenolic acids in wine. Food Chemistry. 2010; 119(3):1205–10. http://dx.doi.org/10.1016/j.foodchem.2009.08.038.
16. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agriculture Food Chemistry. 1998;46(10):4113-7. http://dx.doi.org/10.1021/jf9801973 .
17. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64(4):555-9. http://dx.doi.org/10.1016/S0308-8146(98)00102-2.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Rad Biol Med. 1999;26(9):1231-7. http://dx.doi.org/10.1016/S0891-5849(98)00315-3.
19. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337-41. http://dx.doi.org/10.1006/abio.1999.4019 ; PMid:10222007.
20. Yuan ZY, Jiang TJ. Horseradish peroxidase. In Handbook of Food Enzymology. Edited by Whitaker JR,Voragen A, Wong DWS. New York: Marcel Dekker Inc; 2003:403–411. PMid:12881723.
21. Jiang YM, Zhang ZQ, Joyce DC, Ketsa S. Postharvest biology and handling of longan fruit (Dimocarpuslongan Lour.). Postharvest Biol Technol. 2002;26(3):241-52. http://dx.doi.org/10.1016/S0925-5214(02)00047-9 .
22. Bergmeyer HU: In Methods of Enzymatic Analysis, Volume Volume 1. 2nd edition. Edited by Bergmeyer HU. New York: Academic press; 1974:438.
23. Jiang YM, Zhang ZQ, Joyce DC, Ketsa S. Postharvest biology and handling of longan fruit (Dimocarpuslongan Lour.). Postharvest Biol Technol. 2002;26(3):241-52. http://dx.doi.org/10.1016/S0925-5214(02)00047-9.
24. Burin VM, Falcao LD, Gonzaga LV, Fett R, Rosier JP, Bordignon-Luiz MT. Colour, phenolic content and antioxidant activity of grape juice. Ciência eTecnologia de Alimentos. 2010;30(4):1027-32. http://dx.doi.org/10.1590/S0101-20612010000400030.
25. Malacrida CR, Motta S. Compostosphenolics total e antocianinasem suco de uva. Ciência e Tecnologia de Alimentos. 2005;25:659-64. http://dx.doi.org/10.1590/S0101-20612005000400006.
26. Sautter CK, Denardin S, Alves AO, Mallmann CA, Penna NG, Heckttheuer LH. Determinacao de resveratrol emsucos de uva no Brazil. Ciência e Tecnologia de Alimentos. 2005;25:437-42. http://dx.doi.org/10.1590/S0101-20612005000300008.
27. Davalos A, Bartolome B, Gomez-Cordove SC. Antioxidant properties of commercial grape juices and vinegars. Food Chemistry. 2005;93(2):325-30. http://dx.doi.org/10.1016/j.foodchem.2004.09.030.
28. Iyer MM, Sacks GL, Padilla-Zakour OI. Impact of harvesting and processing conditions on green leaf volatile development and phenolics in concord grape juice. Journal of Food Science. 2010;75(3);297-304. http://dx.doi.org/10.1111/j.1750-3841.2010.01559.x ; PMid:20492283.
29. Talcott ST, Lee JH. Ellagic acid and flavonoid antioxidant content of Muscadine wine and juice. Journal of Agricultural and Food Chemistry. 2002;50(11):3186-92. http://dx.doi.org/10.1021/jf011500u.
30. Garcia BL, Mitjans M, Rivas MC, Verde SJ, Oranday CA, Vinardell MP. Antioxidant comparative effects of two grape pomace Mexican extracts from vineyards on erythrocytes. Food Chemistry. 2016; 194:1081-8.
31. Stalmach A, Edwards CA, Wightman JD, Crozier A. Identification of (Poly)phenolic compounds in Concord grape juice and their metabolites in human plasma and urine after juice consumption. Journal of Agricultural and Food Chemistry. 2011;59(17):9512–22. http://dx.doi.org/10.1021/jf2015039; PMid:21812481.
32. Lutz M, Jorquera K, Cancino B, Ruby R, Henriquez C. Phenolics and antioxidant capacity of table grape (Vitisvinifera L.) cultivars grown in Chile. Journal of Food Science. 2011;76(7):1088-93. http://dx.doi.org/10.1111/j.1750-3841.2011.02298.x; PMid:21819404.
33. Dani C, Oliboni LS, Vanderlinde R, Bonatto D, Salvador M, Henriques JA. Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food and Chemical Toxicology. 2007;45(12):2574-80. http://dx.doi.org/10.1016/j.fct.2007.06.022; PMid:17683842.
34. Hernanz D, Gallo V, Recamales AF, Mele´ndez-Martı´nez AJ, Gonza´ lez-Miret ML, Heredia FJ. Effect of storage on the phenolic content, volatilecomposition and colour of white wines from the varieties Zalema and Colom-bard. Food Chemistry. 2009;113(2):530-7. http://dx.doi.org/10.1016/j.foodchem.2008.07.096
35. Garcı´a-Falco´ n, MS, Pe´ rez-Lamela C, Martı´nez-Carballo E, Simal-Ga´ ndara J. Determination of phenolic compounds in wines: influence of bottle storage of young red wines on their evolution. Food Chemistry. 2007;105(1):248-59. http://dx.doi.org/10.1016/j.foodchem.2006.11.006.
36. Kallithraka S. Tsoutsouras E, Tzourou E, Lanaridis P. Principal phenolic compounds in Greek red wines. Food Chemistry. 2006;99(4):784-93. http://dx.doi.org/10.1016/j.foodchem.2005.07.059.
37. Modun D, Music I, Vukovic J, Brizic I, Katalinic V, Obad A, Palada I, Dujic Z, Boban M. The increase in human plasma antioxidant capacity after red wine consumption is due to both plasma urate and wine polyphenols. Atherosclerosis. 2008;197:250-6. http://dx.doi.org/10.1016/j.atherosclerosis.2007.04.002; PMid:17498718.
38. Padilla E, Ruiz E, Redondo S, Gordillo-Moscoso A, Slowing K, Tejerina T. Relationship between vasodilation capacity and phenolic content of Spanish wines. European Journal of Pharmacology. 2005;517(1): 84-9. http://dx.doi.org/10.1016/j.ejphar.2005.04.044 ; PMid:15967426.
39. Abhilasha S, Sanghpriya G, Kamal RA, Rama KS. Comparative Study of Phytochemicals, Antioxidative Potential & Activity of Enzymatic Antioxidants of Eclipta alba and Plumbago zeylanica by *in vitro* Assays. Free Radicals and Antioxidants. 2016;6(2):139-44. http://dx.doi.org/10.5530/fra.2016.2.2.


## PICTORIAL ABSTRACT




## SUMMARY

- The phenolic content of peel and flesh from different cultivars of grapes showed concentration dependent scavenging of DPPH and ABTS radicals.
- The phenolic content of grapes revealed the formation of phosphate/molybdenum complex through which extract perform its scavenging activity.
- High correlation coefficient ($R^2$) was observed between yemini grapes and its antioxidant activity.
- The phenolic extract revealed the high amount of polyphenoloxidase, peroxidase and catalase activity.
- The extract revealed the presence of total flavonoids caliberated with catechin acid.

## ABOUT AUTHORS

**Ruqaiyah Khan:** Obtained her PhD degree in the field of Pharmacology focused on liver cancer and cardiac remodeling. She have excellent skill in paper and project writing with significant novel ideas. Moreover, she has many international publications on her name and has command over experimental pharmacology concerned with liver cancer. She is currently appointed as associate professor at Siddhartha Institute of Pharmacy and an active member of Institutional Animal Ethical committee.

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