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Research Article

Red Grapes Conferring an Excellent Array of Phytonutrients to Consumers

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ABSTRACT

Red grapes are grown from the ancient times for culinary, religious and traditional purposes. In current study red grapes were screened using antioxidant analysis through the determination of Total Phenolic Content (TPC), total flavonoids, free radical scavenging ability (DPPH assay), Ferric Reducing Antioxidant Power (FRAP) and β-carotene antioxidant activity, which was 1876.62 ± 92.02 mg GAE/100 g, 489.12 ± 15.45 mg/100 g, 75.09 ± 6.44%, 72.32 ± 3.61% and 43.24 ± 2.15 mg TE/g, respectively. The obtained data for each parameter were subjected to descriptive statistical analysis.

Keywords: Red grapes; Culinary; Antioxidant screening; Statistical analysis

INTRODUCTION

Grapes are widely known for their nutritional as well as medicinal and therapeutic characteristics. Chemically grapes contain sugars, phenolics, organic acids, nitrogenous compounds, aromatic substances, pectin and minerals, along with water. In grapes fructose and glucose are the primary sugars and sugar content ranges from 150 to 250 g/L. Phenolic compounds are the most abundant entity present in the grapes after sugars and acids [1]. They primarily present in the skin and seeds of the berry so the juice of grapes contain less amount of phenolics as compared to the puree or pulp of whole fruits. Two main polyphenolic groups present in grapes are anthocyanins and tannins. Anthocyanins are colored pigments and impart red and purple colors to various grape varieties as red grapes but they also exist in colorless grapes as well. Among various colored and colorless grape varieties red grapes are one of the most attractive and esteemed variety.

Red grapes contain an appreciable amount of sugars and it was estimated that at the time of harvest it contains about 24% sugar by weight. Furthermore, the varieties used for the purpose of raisin manufacturing are harvested at 15% sugar by weight [2]. Fructose is the most abundant sugar which is present in the raisins along with antioxidants. Red grapes mainly contain water and carbohydrates as 82% and 12%-18%, respectively, whilst, small amounts of proteins, fat, minerals and vitamins are also present in red grapes. There are many therapeutic characteristics of red grapes which include laxative and diuretic action, anti-dyspepsia, anti-hemorrhoids along with ability to prevent stones formation in the urinary tract and bile ducts. Phytochemicals present in red grape seed and skin are used in the production of nutritional supplements like resveratrol, gallic acid, catechin and epicatechin. Flavan-3-ols principally contribute to the body of the juice, concentrate etc. Red grapes skin have Total Anthocyanins (TAs), total flavonols, dihydroflavonols (TFOs) and Total Cinnamic Acids (TCA) contents ranging from 1065.63 to 16840.99 μg Malvidin-3-Glucoside (MGE)/g Dry Weight (DM), 67.08 to 1892.53 μg Quercetin Equivalence (QE)/g DW, trace amounts to 230 μg Caffeic Acid Equivalence (CAE)/g DW, respectively.

Conclusively, scientific investigations and clinical trials have revealed the health improving potential of red grapes. By keeping in view, the above mentioned facts the current research project was designed to explore the nutritional and phytochemical potential of red grapes with clear objective of elucidating the phytochemical potential of red grapes [3].

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MATERIALS AND METHODS

Extraction

Bioactive components were extracted through the prescribed protocol of Orak by using aqueous methanol (50:50, water:methanol). Dried red grapes powder was taken in conical flask and then aqueous methanol was added in 6:1 (solvent:powder) ratio. It was then shaken in orbital shaker at 280 rpm for 50 min and at 50°C. Afterwards, the extracts were filtered using fine muslin cloth and then rotary evaporated to remove the excess solvent from the sample.

Total Phenolic Contents (TPC)

Total Phenolic Contents (TPC) in red grapes extracts were measured using folin-ciocalteu method based on the reduction of phosphotungstic acid to phosphotungstic blue and as result absorbance increased due to rise in number of aromatic phenolic groups [4]. For the purpose, 50 µL of red grapes extract was separately added to test tube containing 250 µL of folin-ciocalteu reagent, 750 µL of 20% sodium carbonate solution and volume was made up to 5 mL with distilled water. After two hours, absorbance was measured at 765 nm using UV/visible light spectrophotometer against control that has all reaction reagents except sample extract. Total polyphenols was estimated and values were articulated as gallic acid equivalent (mg gallic acid/100 g).

Total phenolic compounds of each extract in Gallic Acid Equivalents (GAE) was calculated by following formula.

$C = c \times V/M$

C=Total phenolic contents (mg/g plant extract, in GAE)

c=Concentration of gallic acid (mg/mL)

V=Volume of extract (mL)

M=Weight of red grapes extract (g)

Total flavonoids

Total flavonoid content was ascertained spectrophotometrically using method that based on the development of a flavonoid-aluminum complex. Quercetin was used as a standard to measure total flavonoids in red grape extracts. Purposely, 1 mL extract was added to a 10 mL volumetric flask and volume was made up to 5 mL with distilled water followed by addition of 0.3 mL of 5% (w/v) sodium nitrite. After time duration of 5 minutes, 0.6 mL of 10% (w/v) $AlCl_3$ was added and then at 6 min 2 mL of 1 M NaOH was mixed, followed by the addition of 2.1 mL distilled water. Absorbance was measured immediately at 510 nm on UV/visible light spectrophotometer. Data was expressed as quercetin equivalents in mg per 100 g of extract [5].

Free radical scavenging activity (DPPH assay)

DPPH (1,1-Diphenyl-1-Picrylhydrazyl) is a stable and highly colored oxidizing radical that result in formation of a yellow colored hydrazine (DPPH-H) associated with abstraction of free hydrogen atoms from phenolic antioxidants. Protocol of Santos, et al., was followed to determine DPPH (1,1-Diphenyl-2-Picrylhydrazyl) free radical scavenging activity of red grapes extract. Sample solution was prepared by dissolving 0.025 mL of sample extract in 10 mL of respective solvent. 3 mL of freshly prepared DPPH solution in respective solvent (6×10^{-5} M) was mixed with 77 µL sample extract. Each sample was kept in dark place for about 15 minutes at room temperature and decrease in absorbance was measured at 517 nm on UV/visible light spectrophotometer. Similarly, blank sample absorbance having the same amount of solvent and DPPH solution except extract was prepared and absorbance was estimated at same wavelength on UV/visible light spectrophotometer. The free radical-scavenging activity of each red grapes extract can be presented as percentage reduction in DPPH due to given amount of each extract [6].

Reduction of absorbance (%)=((AB-AA)/AB) × 100

AB=Absorbance of blank sample at t=0 minute

AA=Absorbance of tested extract solution at t=15 minutes

Antioxidant Activity (AA)

Antioxidant activity of sample extracts was based on coupled oxidation of β-carotene as well as linoleic acid. It was calculated according to the guidelines of Orak. According to which, 2 mg of β-carotene was dissolved in 20 mL of chloroform. A 3 mL of aliquot was taken in flask containing 40 mg linoleic acid along with 400 mg tween 20 and the mixture was then evaporated at 40°C for 10 minutes using rotary evaporator to remove chloroform. This mixture was diluted with 100 mL distilled water and was mixed properly by vortex mixer to prepare emulsion. Then, 3 mL of β-carotene emulsion as well as 0.12 mL phenolic extracts were taken in test tubes and were thoroughly mixed. Afterward, test tubes were incubated at 50°C in a water bath for time duration of 30 minutes. Absorbance of each sample was measured at 470 nm on UV/visible light spectrophotometer [7]. The degradation rate of the extracts was also calculated according to the first order kinetic reaction using following expression.

Sample degradation rate=ln (a/b) × 1/t

ln=Natural log

a=Initial absorbance on 470 nm at time zero

b=Absorbance on 470 nm after 30 min

t=Time in minutes

The antioxidant activity was expressed as percentage inhibition (%) relative to the control by following equation.

AA (%)=Degradation rate of control-Degradation rate of sample

Ferric Reducing Antioxidant Power (FRAP) assay

The reducing power of sample extracts was determined by measuring capability of extracts to reduce ferric tripyridyltriazine into blue colored ferrous that can be detected at 593 nm as described by Yardpiroon, et al. FRAP reagent was prepared by mixing 25 mL acetate buffer (0.1 M at pH 3.6), 2.5 mL TPTZ

(10 mM) and 2.5 mL ferric chloride (20 mM) and was incubated at 30°C for 10 minutes. To determine reducing power of red grapes extract immediately 1.5 mL of FRAP reagent was mixed with 100 µL of red grapes extract or standard and 100 µL of distilled water. Then absorbance was taken at 593 nm on UV/visible light spectrophotometer. A calibration curve was drawn using ferrous sulphate (0-500 µmol/mL) and was expressed as µM Fe^{2+} equivalents per g of sample [8].

Data analysis

Data secured for each parameter was subjected to analysis using MS Excel and means along with standard deviation were determined.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Health promoting effects of various phenolics have necessitated their inclusion in various food products. It is evident from that the recorded value of total phenolic contents of red grapes is 1876.62 ± 92.02 mg GAE/100 g. The present research illustrate that red grapes are rich source of phenolics having remarkable antioxidant properties. The results of TPC are in harmony with the earlier findings of Porto et al., they reported the phenolic contents in red grapes as 2813.2 mg GAE/100 g dry matter. The maximum extraction of phenolic content depends on the extraction method and conditions. In the study, results suggest that pressure of 8 MPa was the most suitable pressure of supercritical carbon dioxide extraction and the highest values of phenols extraction was obtained as 2600 mg GAE/100 g dry matter. Likewise, Gris, et al., evaluated the grape juice samples for total phenols and found them in a range of 2287.6-2813.7 GAE mg/L. The grape polyphenols are present in ample amounts in the seed and peel portion. However, the flesh contains slightly lower level. Mostly these are present in the grape skin and seed portion. Similarly, Bunea, et al., noted the TPC content in organic and conventional grapes in a range from 163.23-1341.37 and 148.47-1231.38 mg GAE/kg fresh weight. Recently, Breksa et al., evaluated the total phenolic contents in red grapes ranging from 316.3 to 1141.3 with an average value as 459.3 ± 192.2 mg GAE/100 g. Similarly, Jariyapamornkoon et al., revealed highest phenolic content present in red grapes peel and found as 246.3 ± 0.9 mg gallic acid equivalent/g dried extract. Results derived from the study of Luna-Vazquez et al., indicate that grape fruit contain phenolic compounds which elicit significant value as 160.9 mg of GAE/100 g of fresh weight. One of their peers, Panceri et al., measured the phenolic content and calculated as 119.62 mg gallic acid/100 g berries. It is mainly influenced by the phenolic compounds concentration which increased because of water loss and reduced due to oxidative enzymes. Earlier, Burin et al., concluded TPC in a range from 56.6 mg-102.2 mg of gallic acid/100 g of grapes. Total polyphenols reported in the literature of red grape varieties (*V. vinifera*) ranged from 731 to 3486 mg of gallic acid per kg of grape (fresh weight). Furthermore, Rockenbach et al., conducted a study on grape pomace and seeds. Results deduced from the study revealed that overall TPC ranged from 32.62 to 74.75 mg (GAE)/g while TPC in pomace and seeds were separately recorded as 78.5 and 50.41 mg (GAE)/g, respectively. Whereas, Doshi et al., noticed that the concentration of phenolic content in grapes is highly dependent on the extraction process that was used, genetic makeup, environmental factors and cultural practices. Among the grape varieties, cabernet sauvignon extract showed higher total phenolic content than pinot noir (73.66 mg/g) and regente (49.73 mg/g) grapes variety. Cabernet sauvignon extract contains more phenolics as compare to Isabel variety. Similarly, Santos et al., studied the total phenolics of grapes which ranged between 0.04 and 122.35 mg GAE/g. This study showed that seeds are a good source of phenolic contents as ranged between 89.83 and 122.35 mg GAE/g as compare to other parts of fruit, mainly in isabel varieties of grapes. These values are also in line with the results deduced by Bozan et al. They studied the seeds of 11 grape varieties and concluded that polyphenol contents in seeds range between 79.2 and 154.6 mg GAE/g. Where as, in peel portion the phenolic compounds were in range of 1.43 to 2.46 mg GAE/g. In a scientific exploration, Soares et al., also estimated the amount of phenolic concentration in peels that was quite similar to previous findings. It was mentioned as 1.93 mg GAE/g in Isabel grapes and 1.83 mg GAE/g in Niagara grapes. Phenolic content decreased with high moisture present in the pulp portion. Yang et al., elucidated a range of TPC of different varieties of red grapes from 228 mg/100 mL-424 mg/100 mL. All grape varieties that were analyzed, cabernet franc and pinot noir showed highest levels of total phenolic content as 424.6 ± 3.8 and 396.8 ± 12.4 mg of gallic acid equivalents/100 g of grape, respectively, which were followed by concord, sheridan, chancellor and marechal foch varieties. It was found that the red grape varieties contain high content of total phenolics. Comparatively, the green grapes have lesser values of phenolic compounds. However, the distribution of phenolics in various portions like juice, pulp, skins and seeds were estimated as approximately 5%, 1%, 30% and 64%, respectively. While, the average value of phenolic content especially for seeded white *V. vinifera* varieties was calculated about 4000 mg Gallic Acid Equivalents (GAE)/kg and for red grapes was about 5500 mg GAE/kg. Similarly, the total phenolic compounds in red grape skins are higher than the white grapes due to less quantity of anthocyanins in the white grapes (Table 1) [9].

Table 1: Phytochemical screening of red grape.

Parameters	Concentration
Total phenolic contents	1876.62 ± 92.02 mg GAE/100 g

Total flavonoids	489.12 ± 15.45 mg/100 g
DPPH assay	75.09 ± 6.44%
Antioxidant activity	72.32 ± 3.61%
FRAP assay	43.24 ± 2.15 mg TE/g

Total flavonoids

Total flavonoids in red grapes were measured spectrophotometrically. The method is based on the development of a flavonoid-aluminum complex. It is evident from Table 1 that red grapes contain significant amount of flavonoids *i.e.,* 489.12 ± 15.45 mg/100 g dry weight of red grapes. Recently, Yardpiroon, et al. investigated different grapes for their Total Flavonoid Contents (TFC) by colorimetric aluminum chloride assays. They observed that the extracts of red grape fruit exhibit high TFC content ranging from 5.245 ± 0.013-19.902 ± 0.48 mg CE/gFW which are in harmony with the results of the present study. Grape fruits of different colors have different types of phytochemicals and are directly associated with biological activities. In this study, TFC were in greater amount in red fruits. This may be due to the presence of anthocyanins in red colored fruit. Moreover, the method of extraction also affects the quantity of obtained phytochemical moieties. Previously, total flavonoids of the 14 grape extracts were measured by Yang et al., in which the highest flavonoid content (301.8 ± 6.2 mg of catechin equivalents/100 g of fresh grapes) was observed in the pinot noir variety. Afterwards, Luna-Vazquez et al. also estimated flavonoid content as 121.2 ± 4.9 mg of CE/100 g of fresh weight. A method based on Pressurized Solvent Extraction (PSE) was used by Alvarez-Casas, et al. to determine main polyphenolic compounds in the grape marc obtained as a byproduct of the white winemaking process. This study showed the total flavonoid contents as 28.47 mg CTE/g dry weight. Indeed, Makris et al. examined the variation of total flavonoid content of white grape pomace from *V. vinifera* variety roditis as 35.22 mg CTE/g dry weight. Grape seeds are a promising source of polyphenolic constituents having significant quantity of total flavonoid contents of seeds are dependent on the extraction, variety of grapes and maturation level. In an investigation, Selcuk et al. found flavonoid contents as 49.20 mg CE pergram flavonoids of grape seeds. Lako et al., observed the variation among flavonoid content in grapes and ascribed it as a function of cultivars, maturity, colors, part of fruits [10]. However, genetic and agronomic factors play role in the composition and nutritional value of phytochemicals.

Free radical scavenging ability (DPPH assay)

DPPH (1,1-Diphenyl-1-Picrylhydrazyl) is a stable and highly colored oxidizing radical that results in formation of a yellow colored hydrazine (DPPH-H) associated with abstraction of free hydrogen atoms from phenolic antioxidants. Mean values in Table 1 depicted that redgrapes possess remarkable free radical scavenging ability *i.e.,* 75.09 ± 6.44%. The results are in accordance with the conclusions of earlier findings Darra et al. They studied the free radical scavenging activity of red grape extracts ranging from 1 µg/mL-50 µg/mL by DPPH assay and found in a range of 52%-75%. In red grapes, cabernet sauvignon and merlot grapes extract have the greatest activity toward DPPH radical [11]. Formerly, Rockenbach, et al. determined the antioxidant activity of the extracts by using the DPPH method. The DPPH value for grapes was estimated to about 505.52 µMol TEAC/g. In a previous study on red grape pomace from regente and pinot noir varieties, mean values were obtained as 479 and 480 µMol TEAC/g through the DPPH method, respectively. Afterwards, Selcuk et al. concluded that grape seeds of calkarasi variety of red grapes obtained from two different origins exhibited DPPH radical scavenging activity of about 41 µmol TE/g dry matter and this was significantly higher than the seeds from winery. Seeds of red grapes such as merlot, cabernet, okuzgozu, bogazkere and kalecik karasi revealed considerable DPPH radical scavenging capacity. In another investigation, Genova, et al. assessed the red grape extracts and observed that they are more active towards free radical scavenging activity. The antioxidant activity of red grape extract recorded as three times higher than the white grape extract. Red grape extract required around 10 min to reduce 50% the radical concentration whereas, white grape extracts acquired about 40 min to obtain the same results.

Antioxidant Activity (AA)

Red grapes exhibited significant antioxidant activity as shown in the Table 1. The antioxidant activity of red grapes is about 72.32 ± 3.61% based on coupled oxidation of β-carotene as well as linoleic acid and absorbance was taken at 470 nm using UV/visible spectrophotometer. Previously, Porto et al., determined the results of total antioxidant activity for different extraction methods. The results indicated the antioxidant activity for grape marc extracts obtained by SC-CO_2 as 2358.4 mg α tocopherol/100 g DM, 2649.6 mg α tocopherol/100 g DM and 698.6 mg α tocopherol/100 g DM. While, the methanolic extracts showed antioxidant activity of 677.9 mg α tocopherol/100 g DM. It is interesting to note that the highest value of antioxidant activity do not correspond to the highest content of phenols. This suggests that different conditions of supercritical fluid extraction technique allowed the extraction of different phenol fractions responsible for the high antioxidant activity of the extracts. The Antioxidant Activity (AA) of the Pressurized Solvent Extraction (PSE) grape marc extracts vary between 2.43 and 4.14 mM TRE/g dry weight as explicated by Alvarez-Casas et al. The obtained average value for the AA of Albarino extracts was 2.93 ± 0.19, slightly greater than of white grape pomace from roditis variety, 2.22 ± 0.17 and indeed, greater than reported data on the AA of Albarino wines *i.e.,* 0.77

mM-2.01 mM TRE. It is well known that grapes contain significantly lower amounts of total polyphenols compared to red wines. However, for the bagasse, the situation is the opposite a sit can be deduced comparing these data with the available AA data of PSE red grape pomace extracts (mainly composed of anthocyanins), which vary from about 1.0 to less than 2.5 mM TRE/g dry weight. Thus, it is expected that less polyphenols are transferred to the juice during white wine making, producing a bagasse keeping high antioxidant activity and opening the way for the reutilization of this wineries byproduct. But for the fresh grapes this relation is totally opposite as explored by Yang et al. in study which revealed a strong correlation between total antioxidant activity and total phenolics. The varieties containing high total phenolic contents had higher antioxidant activities. Total antioxidant activities of the 14 grape varieties were analyzed and found that phytochemical extracts of Cabernet Franc had the highest antioxidant activity (149.0 ± 10.0 µmol/g, $p<0.05$), followed by Pinot noir (122.4 ± 5.7), Concord (106.0 ± 6.0), Sheridan (106.6 ± 3.6), Chancellor (102.8 ± 6.0), Marechal foch (100.2 ± 6.0), Catawba (98.0 ± 4.6), de Chaunac (96.3 ± 6.1), Riesling (79.8 ± 4.3), Niagara (65.3 ± 3.8), Vidal blanc (64.7 ± 2.5), Baco noir (63.4 ± 4.6), Cayuga white (63.3 ± 4.6) and Chardonnay (61.9 ± 6.1). The total antioxidant activities of pinot noir and concord were similar but lower than that of cabernet franc which are in line with the findings of Makarova et al.

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power of red grapes was determined by measuring capability of extracts to reduce ferric tripyridyltriazine into blue colored ferrous that is detected at 593 nm. It is evident from Table 1 that red grapes exhibit noteworthy ferric reducing antioxidant power and its value is 43.24 ± 2.15 mg TE/g. The determination of the antioxidant activity of the extracts was carried out using the reducing power through the FRAP method by Rockenbach et al. Among the red grape varieties widely cultivated in Brazil, the cabernet sauvignon had greater antioxidant activity (249.46 µMol TEAC/g by FRAP method) than the other varieties evaluated. Similarly, Perez-Jimenez et al. evaluated the reducing power of red grapes by conducting FRAP analysis as 273.9 µMol TEAC/g. Earlier, Selcuk et al. estimated the Ferric Reducing Antioxidant Power (FRAP) of the grape seeds from winery byproduct that was about 20 µmol TE/g dry matter which was lower than the seeds of molasses (pekmez) production. Determining antioxidant activities of 28 fruits, Guo et al. found that redrose grape seeds had the highest FRAP value (55 mmol $FeSO_4$ equivalent/100 g wet weight) among the other seed fractions. Storage temperature and water activity can influence antioxidant activity of grape obtained from a red wine vinification process. The outcomes of Yardpiroon et al. showed the ferric reducing power as 207.290 ± 7.75, 140.370 ± 5.38 and 138.790 ± 7.73 mM $FeSO_4$/g fresh weight for green, red and black color of wild grapes, respectively. The methanolic and ethanolic extracts of red and green grapes revealed higher reducing ability as 239.010 ± 10.291, 560.610 ± 9.370 mM $FeSO_4$/g fresh weight, correspondingly, than that of water extract. Furthermore, the FRAP values of the methanolic extract from green wild grape fruits (560.610 ± 9.370 mM $FeSO_4$/100 g FW) showed the highest value as compared to the ethanolic extracts (361.750 ± 6.507 mM $FeSO_4$/100 g FW). Red grape skin extract had FRAP value about 114.24 ± 0.03 mM $FeSO_4$/g dried extract as examined by Jariyapamornkoon et al. Likewise, Luna-Vazquez et al. also reported the FRAP value of grapes as 593.8 ± 46.9 µmol TE/100 g of fresh weight [12].

CONCLUSION

Novel nutritional therapies have gained immense consumer attention owing to their prophylactic role against several lifestyle related disorders. The healthy properties of these dietary regimes are accredited to the presence of polyphenolic constituents. In this milieu, red grapes are in limelight due to their potential to ameliorate various infirmities owing to its distinct composition with special reference to antioxidants and phytochemicals. The current research investigation was carried out to assess the nutritional and antioxidant potential of red grapes which proved excellent nutritional profile of red grapes.

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