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ORIGINAL ARTICLE

In vitro Antioxidant Activities and Polyphenol Contents of Seven Commercially Available Fruits

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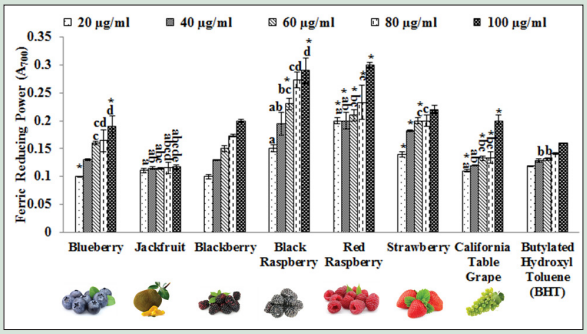
ABSTRACT

Background: Fruits are considered one of the richest sources of natural antioxidants. Their consumption has been linked to the prevention of oxidative stress-induced diseases. **Objective:** In this study, *in vitro* antioxidant activities of blueberry, jackfruit, blackberry, black raspberry, red raspberry, strawberry, and California table grape extracts were evaluated. **Materials and Methods:** Antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant potential (FRAP), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), nitric oxide (NO), superoxide anion (O_2^-) scavenging assays, and ferric reducing power. **Results:** Black raspberry extract had the highest phenolic (965.6 ± 2.9 mg gallic acid equivalents [GAE]/g), flavonoid (186.4 ± 1.7 mg quercetin equivalents/g), and proanthocyanidin (2677 ± 71.1 mg GAE/g) contents. All fruit extracts exhibited increasing radical scavenging activities with increased concentrations. At 100 μg/ml, red raspberry extract showed the highest ferric reducing power (A_{700} = 0.3 ± 0.0052) and FRAP activity (A_{593} = 11.43 mM Fe^{2+}/g). Black raspberry extract (100 μg/ml) exhibited the highest DPPH activity (A_{517} = 89.03 ± 0.0471). Jackfruit extract (100 μg/ml) had the highest ABTS (A_{734} = 35.6 ± 0.613), NO (A_{540} = 81.7 ± 0.2), and O_2^- radical scavenging (A_{230} = 55.5 ± 0.2) activities. Positive correlations were observed between IC_{50} values for different radical scavenging activities and different polyphenolics. Red raspberry extract had the highest Pearson's coefficient values (0.952–1) between total phenolics, flavonoids, and proanthocyanidins and DPPH and superoxide radical scavenging activities. **Conclusions:** The antioxidant rich fruits in this study are good source of functional food and nutraceuticals that have the potential to improve human health.

Key words: Antioxidant activity, flavonoids, IC_{50}, phenols, proanthocyanidins, radical scavenging

SUMMARY

- All fruit extracts exhibited increasing radical scavenging activities with increased concentrations
- Black raspberry extract is enriched in total phenols, flavonoids, and proanthocyanidins and showed the highest 2,2-diphenyl-1-picrylhydrazyl scavenging activity and red raspberry extract showed the highest ferric reducing power and ferric reducing antioxidant potential activity
- Jackfruit extract exhibited the highest 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, nitric oxide, O_2^- scavenging activities
- Positive correlations were observed between IC_{50} values for different radical scavenging activities and different polyphenolics.



Abbreviations Used: Abs: Absorbance, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, BHT: Butylated hydroxytoluene, DPPH: 2,2-diphenyl-1-picrylhydrazyl, DW: Dry weight, FRAP: Ferric reducing antioxidant potential, FW: Fresh weight, GAE: Gallic acid equivalents, NADH: β-nicotinamide adenine dinucleotide hydrate, NFL: The National Food Laboratories, NO: Nitric oxide, ONPG: *ortho*-nitrophenyl-β-galactoside, PBS: Phosphate buffered saline, PMS: Phenazine methosulfate, QE: Quercetin equivalents, ROS: Reactive oxygen species, SD: Standard deviation, SOD: Superoxide dismutase, TCA: Trichloroacetic acid, TPTZ: 2,4,6-tris(2-pyridyl)-s-triazine, Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

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INTRODUCTION

Free radicals are generated in all living cells as part of normal cellular function. However, generation of excess free radical either from exogenous or endogenous sources is responsible for many diseases. Ageing, immunosuppression, and many chronic and degenerative diseases, including cancer, atherosclerosis, diabetes mellitus, and neurodegenerative diseases are examples of free radical mediated oxidative stress.[1] To protect themselves from free radical-mediated oxidative stress, cells employ different cellular antioxidant systems, such as low molecular mass antioxidants (glutathione, tocopherols, ascorbic acid); enzymes interacting with reactive oxygen species (ROS) such as superoxide dismutase, peroxidases, catalases; and other enzymes generating reduced forms of antioxidants.[2] Epidemiological studies have established a positive correlation between the consumption of fruits and vegetables and prevention of diseases associated with oxidative stress.[1,3] Plant tissues contain different antioxidants such as flavonoids, tannins, and

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lignin precursors, which act as ROS-scavenging compounds.[2] Berries, small fleshy fruits consumed fresh or processed, are rich in phenolic compounds such as phenolic acids, stillbenoids, proanthocyanidins (condensed tannins), ellagitannins and gallotannins (hydrolysable tannins), and flavonoids (flavonols, flavanols, anthocyanins).[3] In North America, the commonly consumed berries are blackberry, black raspberry, blueberry, cranberry (*Vaccinium macrocarpon, Ericaceae*), red raspberry, strawberry, and grape.[3] Jackfruit, mostly consumed in Asia, is another rich source of phenolic compounds providing health benefits.[4]

In this study, *in vitro* antioxidant activities of seven fruit extracts of blueberry (*Vaccinium corymbosum*, *Ericaceae*), jackfruit (*Artocarpus heterophyllus*, *Moraceae*), blackberry (*Rubus fruticosus, Rosaceae*), black raspberry (*Rubus occidentalis, Rosaceae*), red raspberry (*Rubus idaeus, Rosaceae*), strawberry (*Fragaria x ananassa, Rosaceae*), and California table grape (*Vitis vinifera*, *Vitaceae*) were determined. The aim of this study was to evaluate and compare the polyphenolic contents and antioxidant activities of commercially available fruits and selected freeze-dried fruit powders used in shakes and smoothies. All fruit extracts under study exhibited strong radical scavenging activities.

MATERIALS AND METHODS

Chemicals

Ethyl alcohol (95%), ortho-nitrophenyl-β-galactoside, Folin–Ciocalteu's phenol reagent, Griess' reagent for nitrite, aluminum chloride, β-nicotinamide adenine dinucleotide hydrate (NADH), phenazine methosulfate (PMS), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), sodium acetate, L-ascorbic acid, quercetin, gallic acid, ferrous chloride, ferrous sulfate heptahydrate, trichloroacetic acid (TCA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), potassium persulfate, sodium nitroprusside, butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation of fruit extracts

Fresh blueberry, blackberry, and strawberry fruits (all packed by Driscoll's) were purchased at a local market in Denton, Texas. Jackfruit was purchased at a Vietnamese market in Carrollton, Texas. Freeze-dried powders of red raspberry, black raspberry, and California table grape were obtained from The National Food Laboratories and California table grape from California Table Grape Commission. Fruit extracts were arranged alphabetically according to their respective plant families in tables and figures. Fresh fruits were frozen and milled in an SPEX Sample Prep 6870 Freezer/Mill (Metuchen, NJ, USA) before extraction. Fruit powders were extracted in 95% ethanol (1:4 w/v) at room temperature for 2 days and then centrifuged at 3500 rpm for 20 min. Supernatants were filtered through Whatman #54 filter paper and stored at −20°C until further analysis.

Determination of total phenolics, flavonoids, and proanthocyanidins

Total phenolic content of fruit extracts was determined by Folin–Ciocalteu method. Four hundred microliter of fruit extracts (20 μg/ml–100 μg/ml), 1.6 ml of sodium carbonate (7.5% dissolved in deionized water), and 2 ml of Folin–Ciocalteu reagent (diluted 10 times in deionized water) were added. The reaction mixtures were incubated at room temperature for 1 h, and absorbances were measured at 765 nm.[5] Gallic acid was used as a standard and the total phenolic content in fruit extracts was expressed as mg gallic acid equivalents (GAE)/g of fruit fresh weight (FW) or dry weight (DW) (powder). Total flavonoids were determined by the method of Ordonez *et al*.[6] Five hundred microliters of 2% aluminum chloride prepared in ethanol were added to 500 μl of each fruit extract (20 μg/ml–100 μg/ml). The reaction mixtures were incubated at room temperature for 1 h, and the absorbances were measured at 430 nm. Quercetin was used as a standard, and total flavonoid content of fruit extracts was expressed as mg quercetin equivalents (QE)/g of fruit FW or DW. Total proanthocyanidins were determined by the method of Sun *et al*.[7] Three milliliters of vanillin-methanol (4% v/v) and 1.5 ml of hydrochloric acid were added to 0.5 ml of each fruit extract (20 μg/ml–100 μg/ml) and vortexed thoroughly. The resulting mixtures were allowed to stand at the room temperature for 15 min, and absorbance was measured at 500 nm. Total proanthocyanidin content in fruit extracts was expressed as mg GAE/g of fruit FW or DW.

Ferric reducing power

Ferric reducing power was determined by the method of Oyaizu.[8] Each reaction mixture contained 2.5 ml of 0.2 M phosphate buffer (pH 6.6), 2.5 ml of $K_3Fe(CN)_6$ (1% w/v), and 1 ml of each fruit extract (20 μg/ml–100 μg/ml). The resulting mixtures were incubated at 50°C for 20 min. After addition of 2.5 ml TCA (10% w/v), the mixtures were centrifuged at 3000 rpm for 10 min. Supernatants (2.5 ml) were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%, w/v), and absorbance was measured at 700 nm.

Ferric reducing antioxidant potential assays

Ferric reducing antioxidant potential (FRAP) reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM hydrochloric acid, and 20 mM ferric chloride in a ratio of 10:1:1. The assays were performed by the method of Othman *et al*.[9] Reaction mixtures consisted of 16 μl of each fruit extract (20 μg/ml–100 μg/ml), 500 μl FRAP reagent, and 50 μl distilled water. Mixtures were incubated at room temperature for 4 min and absorbances were measured at 593 nm. A $FeSO_4 \times 7H_2O$ standard curve was used to estimate FRAP activity of fruit extracts, which was expressed as μmol Fe^{2+}/g.

In vitro free radical scavenging activities of fruit extracts

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH radical scavenging activity of fruit extracts was determined by the modified method of Gülçin *et al*.[10] Reaction mixtures contained 50 μl of fruit extract (20 μg/ml–100 μg/ml) and 2.95 ml of 0.1 mM DPPH in methanol. Mixtures were vortexed thoroughly and kept in the dark at room temperature for 30 min. After 30 min of incubation, the absorbances were measured at 517 nm. Ascorbic acid was used as a standard. The percentage of DPPH* radical scavenging was calculated according to equation 1, in which Abs = absorbance at 517 nm.

% of free radical scavenging activity =

$$([Abs_{control} - Abs_{sample}]/Abs_{control}) \times 100 \quad (1)$$

2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity

The ABTS radical scavenging activity was determined by the method of Re *et al*.[11] The ABTS working solution was prepared by mixing 7 mM ABTS and 2.4 mM potassium persulfate in equal amounts and allowed to react in the dark at room temperature for 12 h. One ml of $ABTS^+$ solution was mixed with 60 ml of methanol to obtain an absorbance of 0.76 ± 0.001 at 734 nm. About 1 ml of each fruit extract (20 μg/ml–100 μg/ml) was allowed to react with 1 ml $ABTS^+$ solution,

and absorbances were measured at 734 nm after 7 min. Ascorbic acid was used as a standard. The percentage of ABTS* radical scavenging was calculated according to Eq. 1.

Nitric oxide scavenging activity

Nitric oxide (NO) scavenging activity was determined by the modified method of Balakrishnan *et al.*[12] About 2 ml of sodium nitroprusside in phosphate buffered saline was mixed with 1 ml of each fruit extract (20 μg/ml–100 μg/ml). Mixtures were incubated at 25°C for 150 min, after which 0.5 ml of incubation solution was mixed with Griess reagent. Mixtures were allowed to stand at the room temperature for 30 min, and absorbances were measured at 540 nm. Quercetin was used as a standard. The percentage of NO* radical scavenging was calculated according to Eq. 1 at Abs_{540}.

Superoxide anion scavenging activity

The superoxide anion scavenging activity was determined by the method of Yen and Chen.[13] The reaction mixtures contained 1 ml of each fruit extract (20 μg/ml–100 μg/ml), 1 ml PMS (60 μM) prepared in 0.1 M (pH 7.4) phosphate buffer, and 1 ml NADH prepared in phosphate buffer. Mixtures were incubated at 25°C for 5 min, and absorbances were measured at 230 nm against phosphate buffer blank. Quercetin was used as a standard. The percentage of superoxide anion radical scavenging was calculated according to Eq. 1 at Abs_{230}.

IC_{50}

IC_{50} values (concentrations of samples required to scavenge 50% of free radicals) were calculated by linear regression analysis.

Statistical analysis

Means and standard deviations of three experiments were calculated. One-way ANOVA was performed and significance of differences among means was determined by Tukey's test ($P \leq 0.01$ or $P \leq 0.05$). Pearson's correlation was performed to indicate the relationship between total phenolics, flavonoids, proanthocyanidins, and radical scavenging activities of fruit extracts.

RESULTS AND DISCUSSION

Total phenolics, flavonoids, and proanthocyanidins of fruit extracts

Total phenolics, flavonoids, and proanthocyanidins were estimated [Table 1] to identify what phytochemicals correlated with specific *in vitro* free radical scavenging activities of fruit extracts. Berries are known to contain high levels of diverse phytochemicals, most of which are the products of phenylpropanoid pathway, such as anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, and phenolic acids.[3] Black raspberry extract contained the highest phenolics (965.6 ± 2.9 mg GAE/g), flavonoids (186.4 ± 1.7 mg QE/g), and proanthocyanidins (2677 ± 71.1 mg GAE/g) [Table 1]. Study by Wu *et al.*[14] reported protocatechuic acid as the major phenolic acid in black raspberry followed by p-coumaric, caffeic, ferulic, and 3-hydroxybenzoic acids. In another study, Tulio *et al.*[15] reported two anthocyanins, cyanidin 3-rutinoside, and cyanidin 3-xylosylrutinoside to significantly contribute to the antioxidant activity of black raspberry.

Table 1: Total phenolics, flavonoids, and proanthocyanidins contents of fruit extracts

Fruit extracts	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Total proanthocyanidins (mg GAE/g)
Blueberry	443.6±17^{a}	151.7±1.1	1589.6±24.3
Jackfruit	411.5±11.2^{ab}	0.24±0.02	39±2.3
Blackberry	269.5±16^{c}	56.7±0.2	763.2±2.8
Black raspberry	965.6±2.9	186.4±2	2677±71.1
Red raspberry	434.3±6.3^{ab}	114.5±2	946.9±32.3
Strawberry	250.1±17.1^{c}	22±1^{f}	488.9±14.2*
California table grape	398.9±22.2^{b}	25±0.2^{f}	378.9±6.8*

Results represent means±SD of three experiments. In each column, mean values with no superscript letters are significantly different from each other at $P \leq 0.01$; mean values with * are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different (Tukey's test). GAE: Gallic acid equivalents; QE: Quercetin equivalents; SD: Standard deviation

Total phenolic contents of fruit extracts ranged from 250.1 ± 17.12 to 965.6 ± 2.9 mg GAE/g of fruit FW or DW [Table 1]. The total phenolic content of fruit extracts in our study ranked as follows: Black raspberry >blueberry, jackfruit, and red raspberry >California table grape >blackberry >strawberry [Table 1]. Pantelidis *et al.*[16] reported higher phenolic content in two varieties (Autumn Bliss and Heritage) of red raspberry ranging from 1052 ± 75 to 2494 ± 77 mg GAE 100/g DW, whereas the present study showed the phenolic content of red raspberry as 434.3 ± 6.31 mg GAE/g DW. Pantelidis *et al.*[16] also reported higher phenolic content in four varieties (Choctaw, Thornless Evergreen, Chester Thornless, Hull Thornless) of blackberry extracts ranging from 1703 ± 71 to 2349 ± 1531 mg GAE/g DW compared to the present study where the total phenolic content of blackberry extract was 269.51 ± 16 mg GAE/g FW. Total phenolic contents of strawberry and white grape extracts reported by Park *et al.*[17] were 7.8 ± 0.7 g/kg FW and 5.5 ± 0.5 g/kg FW, respectively. Study by Shrikanta *et al.*[18] showed low phenolic (1.04 mg GAE/g FW) content of grape pulp. In the present study, total phenolic contents of strawberry and California table grape extracts were 250.1 ± 17.12 mg GAE/g FW and 398.93 ± 22.2 mg GAE/g DW, respectively. Studies by Jagtap *et al.*[4] and Shrikanta *et al.*[18] reported low phenol content (0.21 ± 0.012 mg GAE/g FW and 1.27 ± 0.33 mg GAE/g, respectively), in jackfruit pulp extracts. The present study however reported statistically significant higher amount of total phenol (411.5 ± 11.23 mg GAE/g FW) as compared to the aforementioned studies. These differences in phenolic contents of fruit extracts among different studies may be due to different quantification methods (including use of different standards) as well as other factors such as the genus, species, cultivar of the plants used, and environmental differences at the plant growing location such as climate, soil composition, light, temperature, pest exposure, ripening stage, and handling and storage of fruit.[19,20]

Total flavonoid contents of fruit extracts ranged from 0.24 ± 0.02 to 186.4 ± 1.7 mg GAE/g of fruit FW or DW ranking as follows: Black raspberry >blueberry >red raspberry >blackberry >strawberry and California grape >jackfruit [Table 1]. In our study, jackfruit has the lowest total flavonoid content, 0.24 ± 0.02 mg QE/g FW [Table 1]. This result is consistent with results of previous studies by Jagtap *et al.*[4] and Shrikanta *et al.*,[18] which reported that jackfruit pulp extracts contained low level of flavonoids (1.20 mg of rutin equivalents/g FW and 0.11 ± 0.03 mg catechin equivalents [CE]/g FW, respectively). Sharma *et al.*[21] reported higher amount of flavonoid content (10.5 ± 0.21 mg CE/g FW) in jackfruit shell powder. Study by Shrikanta *et al.*[18] showed low flavonoid (0.23 ± 0.02 mg CE/g FW) contents of grape pulp, whereas the present study reported a higher flavonoid content of 25 ± 0.2 mg QE/g DW in California table grape extract. The differences in flavonoid content among studies may be the result of different grapevine varieties used, as well as the use of different standards (catechin vs. quercetin) to estimate total flavonoids in fruit extracts and way of reporting flavonoid content, FW versus DW.

Total proanthocyanidins contents of fruit extracts in this study ranged from 39 ± 0.23 to 2677 ± 71.1 mg GAE/g of fruit FW or DW and ranked as follows: Black raspberry >blueberry >red raspberry >blackberry

>strawberry >California table grape >jackfruit [Table 1]. Huang *et al.*[22] using high-performance liquid chromatography showed high levels of proanthocyanidins in blueberry extracts obtained from fruit cultivated in Nanjing, which contributed to high DPPH and ABTS radical scavenging activities. Hwang *et al.*[23] reported lower (11.8 ± 5.0 mg CE/g FW) total proanthocyanidin content of blueberry extract cultivated in Korea as compared to the present study (1589.6 ± 24.3 mg GAE/g FW). The difference may attribute to the different cultivars used for plant extracts as well as the use of different standards, catechin versus gallic acid, in estimating the proanthocyanidin content.

Determination of ferric reducing power

Ferric reducing power assay is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidant compounds visible in changing the yellow color of the test solution to various shades of green and blue, depending on the reducing power of antioxidants. Increased absorbance at 700 nm indicates high ferric reductive ability. In general, except for jackfruit extract, all fruit extracts showed increased ferric reducing power with increasing concentrations [Figure 1]. All fruit extracts at 100 μg/ml, except for jackfruit extract, showed higher ferric reducing power than BHT [Figure 1]. At 100 μg/ml, black and red raspberry extracts exhibited the highest reducing power of 0.27 and 0.29, respectively. The range of reducing power for other fruit extracts at 100 μg/ml was 0.11–0.29. Hyun *et al.*[24] reported that Korean black raspberry extracts (100–200 μg/ml) obtained from fruit at different ripening stages exhibited a range of 0.13–0.28 reducing power. Weidner *et al.*[25] reported a range of 0.553–0.931 ferric reducing power of extracts from California grape germinating seed, after seeds were exposed to three temperature conditions: Chill stress, optimal condition, and postchill recovery. The above results show that ripening stages and stressful condition for germination affect ferric reducing power of fruit extracts. Fruits have different polyphenolic content at different developmental stages.[26] It is known that secondary metabolites in plants including antioxidants are intensively synthesized under stress.[27]

Ferric inducing antioxidant potential assays

FRAP assay is based on the reduction at low pH of a colorless ferric complex (Fe^{3+}-tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donors. Red raspberry exhibited the highest FRAP activity (103.9 ± 0.9 μM Fe^{2+}/g). The activities of other extracts ranked as follows: Strawberry >blackberry >black raspberry and blueberry >jackfruit >California table grape [Figure 2]. Borges *et al.*[28] reported the FRAP activity of blueberry extract as 30 ± 1.9 μM of Fe^{2+}/g, whereas present study reported it as 22.5 ± 0.6 μM Fe^{2+}/g. Jagtap *et al.*[4] studied the FRAP activity of jackfruit pulp extracts in different solvents (acetone, ethanol, methanol, water) and found it to increase with increased concentrations of fruit per volume of solvent (1–5 mg/ml). Different studies reported FRAP activity in different ways. At 5 mg/ml, jackfruit pulp extract in one study showed a FRAP activity of 1.38 mM TEAC/g,[4] whereas our study reports the FRAP activity of jackfruit extract as 12.63 ± 0.1 μM Fe^{2+}/g. Pantelidis *et al.*[16] reported FRAP activities of two red raspberry and two blackberry cultivars ranging from 77.7 to 145.4 μM ascorbic acid per g DW and 113.6–169 μM ascorbic acid per g DW, respectively. Another study by Koca and Karadeniz[29] reported the FRAP activities of different varieties of blackberries and blueberries ranging from 35.05 to 70.41 μM/g and 7.41 to 57.92 μM/g, respectively. Therefore, no comparison can be made between our results and those reported in other studies due to use of different standards ($FeSO_4$, ascorbic acid, Trolox) and reporting FRAP results.

In vitro free radical scavenging activities of fruit extracts

In vitro antioxidant activities of fruit extracts were determined by DPPH, ABTS, NO, and superoxide anion scavenging activities [Table 2]. Blueberry, black and red raspberry extracts (20–100 μg/ml) show the highest percentage of DPPH radical inhibition ranging from 38.5% to 87.9%, 64.2% to 89%, and 37.6% to 87%, respectively [Table 2]. Hwang *et al.*[23] reported the DPPH radical scavenging activities of Korean blueberry extracts at different concentrations (10, 50 and 500 μg/ml) to be 29.4%, 29.6%, and 40.6%, respectively, which are lower than that obtained in the present study where 100 μg/ml of blueberry extract show 87.94% of DPPH scavenging activity. The DPPH radical scavenging activities of other fruit extracts at 100 μg/ml ranked as follows: Blackberry >strawberry >California table grape >jackfruit [Table 2]. In the present study, the DPPH scavenging activity of jackfruit extract (100 μg/ml) was 4.5% similar to that reported by Jagtap *et al.*[4] Except for jackfruit, all other fruit extracts exhibited higher DPPH radical scavenging activities than that of ascorbic acid standard.

ABTS scavenging activity measures the reduction of the blue–green chromophore $ABTS^+$ (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to colorless ABTS by an antioxidant. Jackfruit extract (20–100 μg/ml) showed the highest ABTS radical scavenging activity of 11.7–35.6% [Table 2]. The percentage of ABTS radical inhibition for other fruit extracts (100 μg/ml) are as follows: Red raspberry

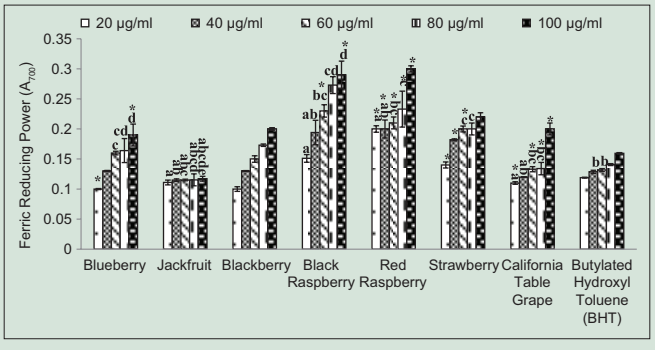

Figure 1: Ferric reducing power of fruit extracts. Butylated hydroxyl toluene was used as a standard. Results represent means ± standard deviation of three experiments. Bars for each plant extract with no superscript letters are significantly different from each other at $P \leq 0.01$. Bars for each plant extract with * are significantly different from each other at $P \leq 0.05$ (Tukey's test)

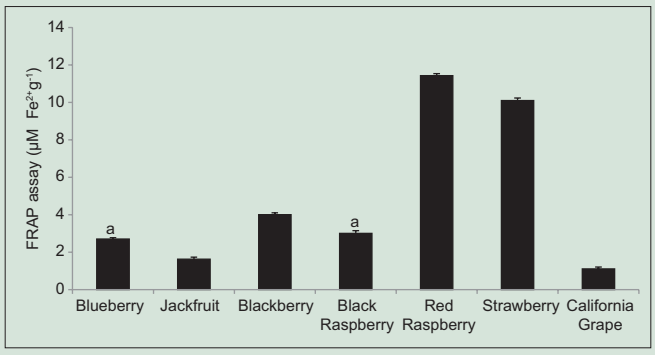

Figure 2: Ferric reducing antioxidant potential of fruit extracts. Results represent means ± standard deviation of three experiments. Bars with no superscript letters are significantly different from each other at $P \leq 0.01$ (Tukey's test). Bars with same superscript letters are not significantly different

Table 2: Radical scavenging capacity of fruit extracts

	Inhibition (%) (μg/ml)				
	20	40	60	80	100
DPPH radical					
Blueberry	38.5±0.3	63.1±2.3	71.6±1.4^{c}	75.5±0.6^{c}	87.9±0.2
Jackfruit	2.3±0.1^{a}	3.23±0.6^{ab*}	3.9±0.1^{bc}	4.24±0.1^{bcd}	4.5±0.2^{cd*}
Blackberry	40.5±2	61.2±0.8	73.8±0.7^{c}	75±0.9^{cd}	77.8±2^{cd}
Black raspberry	64.2±1.4	86.2±0.3^{b*}	87.1±0.08^{bc}	88.1±0.3^{bcd}	89±0.1^{cd*}
Red raspberry	37.6±1.1	61.2±1.8	78.7±1.1^{c}	81.5±1^{c*}	87±1.2*
Strawberry	32.8±0.1	44.9±0.8^{b}	46.2±1.1^{b}	53.6±0.8	70.2±1
California table grape	15.6±0.5	26.4±1.3	35.9±0.6^{c}	38.4±0.3^{c}	44±2
Ascorbic acid	5.2±0.3	10.7±1	16.8±0.1	20.5±0.4	23±0.2
ABTS radical					
Blueberry	11.1±0.6	15.6±0.6*	18.2±0.6*	22.2±0.6^{d}	23.1±0.6^{d}
Jackfruit	11.7±0.4*	13.8±0.7^{b*}	15.1±0.6^{b}	21.8±0.7	35.6±0.6
Blackberry	18.2±0.6	22.2±0.6^{b*}	22.2±0.6^{bc*}	23.1±0.6^{bcd}	25.3±1.1^{d*}
Black raspberry	20.1±0.1^{a}	20.4±0.6^{ab}	20.4±0.6^{abc}	20.4±0.6^{abcd}	21.3±1^{abcd}
Red raspberry	20.9±0.6^{a}	21.8±1.3^{ab}	21.8±0.7^{abc}	23.1±0.6^{abc}	31.1±0.6
Strawberry	8.4±0.6	15.6±0.6	22.2±0.7*	24.3±0.4*	26.2±0.7*
California table grape	11.1±0.6^{a}	13±0.6^{ab}	14.2±0.7^{b*}	16.1±0.2*	19.1±0.6
Ascorbic acid	12.7±0.5	23.4±1.5^{b}	23.8±1^{bc}	24.2±1.1^{bcd}	27.4±2.6^{bcd}
NO radical					
Blueberry	44.2±0.5	46.3±0.5	55.9±0.3	62.1±0.2	67±0.6
Jackfruit	75.3±0.2	76.8±0.1	79.7±0.5^{c}	80.5±0.05^{c}	81.7±0.2
Blackberry	61.8±0.1	68.2±0.7	71.2±0.4	74±0.14	77.2±0.2
Black raspberry	13.1±0.5	16.3±0.2	30.6±0.2	47.5±0.2	64.4±0.2
Red raspberry	37±0.2	47±0.12	57.2±0.4	61.9±0.2	70.1±0.6
Strawberry	75.3±0.4^{a*}	75.6±0.1^{ab}	75.6±0.1^{abc}	75.6±0.3^{abcd}	76.2±0.1^{bcd*}
California table grape	66.9±0.1*	67.7±0.5*	71.5±0.1	74.8±0.14	76.6±0.1
Quercetin	37±1	46.3±0.5	68±0.5	76±0.2	86±0.1
O_2^-radical					
Blueberry	10±0.4	36.5±0.7	45.9±0.1	49.2±0.3	54.6±0.4
Jackfruit	46±0.7	48.6±0.2	52.4±0.4^{c}	52.8±0.05^{c}	55.5±0.2
Blackberry	35±0.2	38.3±0.4	42.9±0.3*	44±0.3*	51±0.3
Black raspberry	8.7±0.7	42.4±0.2	48.5±0.4	52±0.05	54.4±0.2
Red raspberry	43±0.3	46.7±0.3	49.9±0.2	52.2±0.1	54.4±0.2
Strawberry	44±0.5	46.6±0.4	50.5±0.4^{c}	50.4±0.3^{c}	53.2±0.2
California table grape	43±0.3	45.3±0.7	48.9±0.3*	50.7±0.4*	53.3±0.2
Quercetin	5.4±0.2	10.2±0.5	18±1	23.4±0.5	28.3±0.5

Results represent means±SD of three experiments. For each row: Mean values with no superscript letters are significantly different from each other at $P \leq 0.01$; mean values with * are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different (Tukey's test). SD: Standard deviation; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; NO: Nitric oxide; O_2^-: Superoxide anion

>blueberry, blackberry, black raspberry, and strawberry >California table grape. Blackberry, red and black raspberry extracts have the same or higher ABTS radical inhibition as ascorbic acid at all concentrations used. Jackfruit extract showed the highest ABTS radical scavenging activity of 35.6% at 100 μg/ml, similar to reports by Shanmugapriya *et al.*,[30] where jackfruit pulp extract (100 μg/ml) inhibited ABTS radical by 31.26%. Hwang *et al.*[23] showed that ABTS radical scavenging of Korean blueberry extract at concentrations of 10, 50 and 500 μg/ml were 2.3%, 4.2% and 8.6%, respectively, whereas the present study reports higher inhibition of ABTS radical by blueberry extracts (11.1–23.1%, 20–100 μg/ml).

NO is generated in biological tissues when specific NO synthases metabolize arginine to citrulline via a five electron oxidative reaction. Jackfruit extract (20–100 μg/ml) showed the highest inhibition of NO radical ranging from 75.3% to 81.7% [Table 2]. The percentage of inhibition for other fruit extracts (100 μg/ml) were as follows: Blackberry, strawberry, and California table grape >red raspberry >blueberry. Jagtap *et al.*[31] reported the NO inhibition by jackfruit wine (100–500 μl) ranging from 50% to 60%.

Superoxide anion radical (O_2^-) is one of the strongest ROS, which gets converted to other harmful ROS as well as free radicals such as hydrogen peroxide and hydroxyl radical in the cells. In this study, fruit extracts showed superoxide scavenging activity ranging from 51% to 55.5% at 100 μg/ml which were higher than those of quercetin standard [Table 2]. Wang and Jiao[32] reported inhibition of superoxide radicals by 100 μl of unknown concentration of blueberry, blackberry, black raspberry, red raspberry, and strawberry extracts as follows: Blueberry (52.5–69.3%), blackberry (43.3–72%), raspberries (40.8–66.9%), and strawberry (57.9–73.6%).

The differences between the radical scavenging activities in this study and previous studies could be attributed to different methods of extraction, usage of various concentrations, methods of reporting results, conditions of climate, soil composition, varieties, cultivar as well as different modes of processing. In some cases, no comparison can be made between our results and others mainly due to unknown extract concentration reported.[26]

IC_{50}

A lower IC_{50} value indicates greater antioxidant activity. In the present study, the lowest IC_{50} value (1.3 ± 0.1 μg/ml) required to quench at least 50% DPPH radicals was that of blackberry extract [Table 3]. Ivanovic *et al.*[33] reported that blackberry extract of "Čačanska Bestrna"

cultivar showed lower IC_{50} values (96–118.1 μg/ml) for DPPH radical with increasing sonication time and/or temperature. In comparison to the Ivanovic *et al.*[33] study, the present study shows much lower IC_{50} value for DPPH radical (1.3 μg/ml). The differences in results can be attributable to the methods of extraction and different plant cultivars. Among the fruit extracts tested, jackfruit extract showed the lowest IC_{50} values for ABTS (8.5 ± 0.22 μg/ml) and O_2^- radicals (2.6 ± 0.1 μg/ml). The IC_{50} value for O_2^- radical (2.6 ± 0.1 μg/ml) in the present study is much lower than that reported by Ruikar *et al.*[34] for the jackfruit extract (24.3 ± 2.09 μg/ml). Blueberry extract showed the lowest IC_{50} value (2.23 ± 0.1 μg/ml) for NO radical. The IC_{50} value (100 μg/ml) of Korean blueberry extract for NO radical[35] was much lower than that reported in the present study (2.23 μg/ml) indicating differences in fruit varieties, methods of extraction, and other factors. These results indicate that aforementioned fruit extracts are the powerful radical scavengers at very low concentrations.

The antioxidant capacity of the fruit extracts was evaluated and correlated to chemical classes of polyphenols content. Table 4 shows the correlation between IC_{50} values of radical scavenging activities and total phenolics, flavonoids, and proanthocyanidins of fruit extracts. Jackfruit, blackberry, red raspberry, and California table grape fruit extracts show high correlation of total phenolics with DPPH radical scavenging activity, whereas jackfruit, black and red raspberry, strawberry show high correlations of total proanthocyanidins with DPPH radical scavenging activity. Except for black and red raspberry extracts, all fruit extracts show low correlations of total flavonoids with DPPH radical scavenging activity. These results indicate that total phenolics and proanthocyanidins resulted in a stronger DPPH scavenging activities of the fruit extracts than total flavonoids.

Blueberry, jackfruit, blackberry, and California table grape extracts show high correlations of ABTS scavenging activities with total flavonoids. In addition, California table grape extract has a high correlation between total proanthocyanidins content and ABTS scavenging activities. Black and red raspberry extracts show high correlation of ABTS scavenging activities with total phenolics. These results indicate that total flavonoids rather than total phenolics and proanthocyanidins in most fruit extracts resulted in stronger ABTS scavenging activities. Total flavonoids of blueberry, jackfruit, and California table grape extracts and total proanthocyanidins of blackberry, strawberry, and California table grape show high correlation with NO radical scavenging activity.

Red raspberry and strawberry extracts show high correlations of total phenolics, flavonoids, and proanthocyanidins with O_2^- radical scavenging activity. Furthermore, for O_2^- radical scavenging activity high correlations were observed with total proanthocyanidins in blackberry extract and total flavonoids and proanthocyanidins in California table grape extract. These results indicate that total phenolics, flavonoids, and proanthocyanidins could contribute to the NO radical scavenging activity [Table 4].

Table 3: IC_{50} values of radical scavenging activities of fruit extracts

Fruit extracts	IC_{50} values (μg/ml)			
	DPPH radical	ABTS radical	NO radical	O_2^- radical
Blueberry	1.4±0.1[a]	14±0.5[a]	2.2±0.1[a]	4.1±0.1*
Jackfruit	90±12.3	8.5±0.2[ab]	15±0.7[ab]	2.6±0.1
Blackberry	1.3±0.1[ac]	23±5[abc]	2.6±0.2[abc]	5.1±0.1
Black raspberry	3.4±0.4[acd]	79±18.7	4.2±0.1[abcd]	3.9±0.1[d*]
Red raspberry	1.4±0.1[acde]	15±0.9[abce]	2.4±0.1[abcde]	3.3±0.1*
Strawberry	3.1±0.02[acdef]	9.9±0.4[abcef]	118±45.2	3.5±0.1[f*]
California table grape	5.6±0.2[acdef]	21±0.7[abcef]	5.1±0.2[abcde]	3.7±0.1[df]

Results represent means±SD of three experiments. In each column: Mean values with no superscript letters are significantly different from each other at $P \leq 0.01$; mean values with * are significantly different from each other at $P \leq 0.05$; mean values with same superscript letters are not significantly different (Tukey's test). SD: Standard deviation; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; NO: Nitric oxide; O_2^-: Superoxide anion

Table 4: Correlation between IC_{50} of radical scavenging activities and phenolics, flavonoids, proanthocyanidins of fruit extracts

	Correlation (*R*)		
	Phenolics	Flavonoids	Proanthocyanidins
DPPH radical			
Blueberry	0.532	0.628	0.488
Jackfruit	0.967	0.300	0.902
Blackberry	0.995	0.5	0.5
Black raspberry	0.111	0.802	0.802
Red raspberry	0.952	1	0.999
Strawberry	0.300	0.188	0.827
California table grape	0.969	0.277	0.683
ABTS radical			
Blueberry	0.133	0.894	0.082
Jackfruit	0.621	0.994	0.755
Blackberry	0.627	0.969	0.270
Black raspberry	0.799	0.106	0.106
Red raspberry	0.671	0.414	0.400
Strawberry	0.092	0.392	0.689
California table grape	0.025	0.998	0.862
Nitric oxide radical			
Blueberry	0.467	0.987	0.511
Jackfruit	0.380	0.685	0.201
Blackberry	0.580	0.5	1
Black raspberry	0.292	0.5	0.5
Red raspberry	0.739	0.5	0.486
Strawberry	0.454	0.023	0.909
California table grape	0.777	0.654	0.926
Superoxide anion radical			
Blueberry	0.532	0.628	0.488
Jackfruit	0.537	0.802	0.371
Blackberry	0.281	0.755	0.944
Black raspberry	0.292	0.5	0.5
Red raspberry	0.952	1	0.999
Strawberry	0.976	0.755	0.900
California table grape	0.033	1	0.890

DPPH:2,2-diphenyl-1-picrylhydrazyl;ABTS:2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

CONCLUSIONS

In the present study, we investigated the total phenolic, flavonoid, proanthocyanidin contents, and *in vitro* antioxidant activities of seven fruit extracts by employing different *in vitro* antioxidant assays with relevance to oxidative stress in human chronic diseases. Black raspberry among all fruit studied contains the highest amounts of phenols, flavonoids, and proanthocyanidins. The highest DPPH scavenging activity was exhibited by black raspberry extract and jackfruit extract showed the highest ABTS, NO, and O_2^- radical scavenging activities.

These antioxidant-rich aforementioned fruits are good candidates for functional food and nutraceuticals to help in the prevention of oxidative stress induced diseases. In spite of discrepancies in reported results among studies, attributed mostly to cultivar and methodology differences, our data add valuable information to current knowledge of nutritional properties of commercially available berries used fresh or processed. In conclusion red and black raspberry and California table grape powder in shakes, smoothies, and ice cream formulations will provide high levels of polyphenols and antioxidant properties.

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Conflicts of interest

There are no conflicts of interest.

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