

Natural Purple foods offer Numerous Health Benefits

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Abstract

Fruit and vegetable have been the source of many medicines in the pharmacopoeia. So much so that an industry has arisen selling plant extracts as health foods. The present study was carried out to evaluate the bioactive compounds in red and purple fruit and vegetables (*Rubus fruticosus*, *Vitis*^{HYPERLINK "http://en.wikipedia.org/wiki/Vitis_labrusca"} *labrusca*, *Ficus carica*, *Olea europaea*, *Punica granatum*, *Daucus*^{HYPERLINK "http://en.wikipedia.org/wiki/Daucus_carota"} *carota*, *Prunus Americana*, *Beta vulgaris*, *Brassica oleracea*). Extraction of nine different foods in methanol yielded highest extraction in *Ficus carica* (fig) 44.95 %. This research studies showed high content of total Phenol in *Brassica oleracea* (purple cabbage) 122.355 mg /g GAE determined by the Folin-Ciocalteu reagent and total flavonoid content in *Olea europaea* (black olive) 60.097 mg /g QE. The antioxidant activities determined by the DPPH and FRAP methods, very high values of antioxidant activity were found in all intensely colored vegetables especially in red cabbage 84.96 % and 37.085 respectively. Values were very low in black olive 28.03% and 7.916.

Keywords: Pharmacopodia, Bioactive compound, *Ficus carica*, *Brassica oleracea*, Folin-ciocalteu, *Olea europaea*, Antioxidant activity, DPPH, FRAP

Introduction

Consumption of fruits and vegetables has been associated with lower incidence and lower mortality rates due to cancer in several human cohort and case-control studies for all common cancer types (1,2,3) There is a highly significant negative association between intake of total fruits and vegetables and cardio- and cerebrovascular disease mortality (4,5,6,7). Vegetarians and non-vegetarians with a high intake of fruits and vegetables also have reduced blood pressure (8,9).

The protection that fruits and vegetables provide against diseases, including cancer and cardio- and cerebrovascular diseases has been attributed to the various antioxidants, especially antioxidant like vitamins, including ascorbic acid and α -tocopherol, contained in these fruits and vegetables (10,11). However, the majority of the antioxidant activity of a fruit or vegetable may be from compounds other than vitamin C, vitamin E, or β -carotene. Our research group has already reported that some common plants (parts) have high antioxidant activities which cannot be accounted for by their vitamin C content alone. There are clear cut indications that polyphenols, flavonoids and other phytochemicals attribute to this activity. The objective of this study was to determine the antioxidant capacities of red/ purple fruits and vegetable.

The new report has highlighted that the color of fruits and vegetables eaten can be as important as the quantity. But by eating fruits and vegetables of a variety of different colors, one can get the best all-around health benefits. Each differently colored fruit and vegetables contain unique health components that are essential to our health. Red/purple fruits and vegetables are colored by natural plant pigments called "anthocyanins." Anthocyanins in blueberries,

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grapes and raisins act as powerful antioxidants that protect cells from damage. They may help reduce risk of cancer, stroke and heart disease. Other studies have shown that eating more blueberries is linked with improved memory function and healthy aging.

Pomegranate juice has become more popular because of the attribution of important biological actions (12). Thus, the antioxidant and antitumoral activity of pomegranate bark tannins (punicalcortin) (13,14) and the antioxidant activity of the fermented pomegranate juice (15) have been reported. These reports are only indicative, however, detailed investigations of the phenolic compounds and the antioxidant activity of the juice have not yet been carried out.

While cooking purple cabbage, the compound (*anthocyanin*) that gives the cabbage that beautiful color will also turn it blue when it is cooked along with any alkaline substance (tap water) and acidic agents (lemon juice, vinegar, or wine) can bring back the original color.

Berries such as blueberries, cranberries, strawberries, mulberry and raspberries are rich in disease-fighting

phytochemicals that prevent and even reverse serious diseases like cancer, diabetes, heart disease, stomach ulcers and even help lower cholesterol levels. The key is to be eating these berries in their raw, whole fruit form rather than trying to eat processed berries or drink fruit drinks made from berries.

Eating fruits and vegetables of different colors gives your body a wide range of valuable nutrients, like fiber, folate, potassium, and vitamins A and C. Some examples include green spinach, orange, sweet potatoes, black beans, yellow corn, purple plums, red watermelon, and white onions.

Materials and Methods

Plant material

The different red/purple fruits and vegetable were either purchased from a market place or collected from gardens (Figure 1). Air-drying was carried out at +24 °C in the dark, and materials were stored in a freezer at -10 °C and lyophilized before analysis. All samples were analyzed within the same month of collection.



Figure 1. Fruit and vegetable used for the study

Chemicals

Reagents like-Folin–Ciocalteu reagent, Sodium carbonate, Gallic acid, Quercetin, Aluminium Chloride, Potassium acetate, Potassium chloride, Sodium acetate, Phosphate buffer, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride were purchased from SD fine, Methanol, Ethanol and Distil water were used as solvent.

Methods

Determination of extract yield- Organic solvent extraction

OSE is the most common and economically important technique for extracting aromatic compounds. For this purpose Methanol (MeOH) has been used as a polar solvent. Raw materials are submerged and agitated in a solvent that can dissolve the desired aromatic compounds. The solvent is then removed by a low temperature distillation process so as to yield the compound. Extraction yield (%w/w) and the extraction efficiency were calculated using equation:

$$\text{Extraction yield} = \frac{\text{Mass of extract}}{\text{Mass of raw material}} \times 100$$

Determination of bioactive compounds

Bioactive compounds like Polyphenols, Flavonoids, Anthocyanins etc. in the purple vegetable and fruits extracts were determined by colorimetric assays.

Determination of Total phenol content

The phenolic content of the different vegetables was determined by Folin–Ciocalteu (FC) reagent (16). Each sample (0.1 mL at proper dilution) was added to 4.2 mL of deionized water and 0.5 mL of FC reagent. After 1 min of mixing, 1 mL of an 80% solution of sodium carbonate and 4.2 mL of deionized water were added. The mixture was left for 2 h at room temperature in the dark and the absorbance at 760 nm was measured. The concentration of the total phenolic content was determined by a comparison with the values obtained with a standard solution of Gallic acid.

Determination of Total flavonoid content

Aluminum chloride colorimetric method was used for the determination of flavonoids (17). Each plant extracts (0.5 ml of 1:10 g/ ml) in methanol were separately mixed with 1.5 ml of MeOH, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Helios α UV/Visible spectrophotometer. The calibration curve was prepared by preparing Quercetin solutions at concentrations 50 - 250 mg/L.

Total anthocyanin measurement using pH differential method

Total anthocyanins were measured according to a modification of the method described earlier (18,19). Two dilutions of the sample were prepared, one for pH 1.0 using potassium chloride buffer (0.03 M, 1.9 g KCl into 980 mL distilled water) and the other for pH 4.5 using sodium acetate buffer (0.4 M, 54.4 g $\text{CH}_3\text{CO}_2\text{Na} \cdot 3\text{H}_2\text{O}$ in 960 mL distilled water). Samples were diluted 10 times to a final volume of 2 mL. The absorbance of each sample was measured at 520 nm against distilled water as blank. Since the samples had no haze or sediment and thus correction at 700 nm was omitted. The concentration (mg/L) of each anthocyanin was calculated according to the following formula and expressed as Cy-3-glc equivalents:

$$\frac{A \times MW \times DF \times 10^3}{\epsilon \times l}$$

Where A is the absorbance = $(A_{\lambda_{\text{vis-max}}})_{\text{pH } 1.0} - (A_{\lambda_{\text{vis-max}}})_{\text{pH } 4.5}$, MW is the molecular weight (g/mol) = 449.2 g/mol for Cy-3-glc, DF is the dilution factor (0.2 ml sample is diluted to 2 ml, DF = 10), and ϵ is the extinction coefficient ($\text{L} \times \text{cm}^{-1} \times \text{mol}^{-1}$) = 26,900 for Cy-3-glc, where L (path length in cm) = 1. For comparison, the same extinction coefficient was used for other standards to calculate the concentration of each anthocyanin and thus results has been expressed as Cy-3-glc equivalents.

Antioxidant activity test by DPPH methods

This was determined according to the Blois (20) method with a slight modification, 1 ml of a 0.781 mM solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical in methanol was mixed with 3 ml of extract solution in methanol (containing 50 – 400 μg of dried extract), and after 30 min the absorbance was measured at 517 nm. This activity is given as % DPPH radical scavenging calculated according to the following equation:

$$\% \text{DPPH}_{\text{sc}}^* = (A_{\text{cont}} - A_{\text{samp}}) \times 100 / A_{\text{cont}},$$

Where A_{cont} is the absorbance of the control, and A_{samp} the absorbance of the sample

Ferric-reducing antioxidant power (FRAP)

This method was proposed by scientist (21) which involves the presence of antioxidants in extract to reduce the ferricyanide complex to the ferrous form. Extracts (50–500 μg) in 1 mL of appropriate solvents were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide $[\text{K}_3\text{Fe}(\text{CN})_6]$ (1%), and then the mixture was incubated at 50 $^\circ\text{C}$ for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL

of distilled water and 0.5 mL of Ferric Chloride (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. Triplicate tubes were prepared for each extract. The FRAP values, expressed in mg GAE/g, were derived from a standard curve.

Results and Discussion

People who eat more generous amounts of fruits and vegetables as part of a healthy diet are likely to have

reduced risk of chronic diseases, including strokes, diabetes, some types of cancer, and perhaps heart disease and high blood pressure.

Extract yield

The yield of methanolic extract of plants is shown in (Table 1). Fig (Anjeer) shows the highest extraction yields (44.95%) and lowest yield was evaluated in black olive (Jaitoon) (2.98 %).

Table 1. Yield determination of extracts

Common name	Botanical name	Code	Yield in %
Mulberry	<i>Rubus fruticosus</i>	MB	7.86
Concord grapes	<i>Vitis labrusca</i>	BG	10.92
Fig	<i>Ficus carica</i>	BF	44.95
Black olive	<i>Olea europaea</i>	BO	2.98
Pomegranate	<i>Punica granatum</i>	PG	10.89
Purple carrot	<i>Daucus carota</i>	PC	12.90
Plum	<i>Prunus Americana</i>	RP	17.87
Purple beet root	<i>Beta vulgaris</i>	BR	14.56
Purple cabbage	<i>Brassica oleracea</i>	RC	10.09

Total Phenolic content

The amount of total phenolics in red/purple fruit and vegetables, values ranged from 122.355 mg GAE/g – 17.362 mg GAE/g. The highest total phenolic levels were detected

in red cabbage and lowest in fig (figure 2). Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (22). Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants.

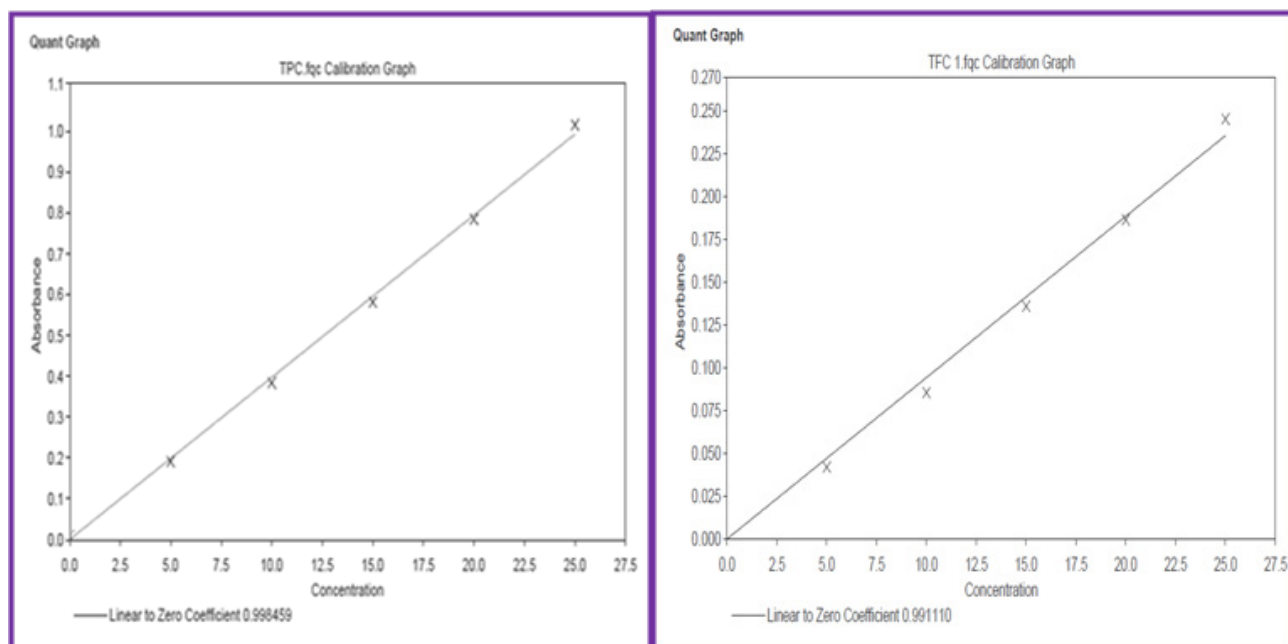


Figure 2. Calibration graph-Total phenol and total flavonoid content

Total Flavonoid content

Anthocyanins are flavonoid category of poly-phenolic compounds is found in many vegetables featuring blue or deep purple color. Eating fruits rich in blue pigments offer many health benefits. These compounds have potent

antioxidant properties, remove free radicals from the body and thus offer protection against cancers, aging, infections etc. The total flavonoid contents were usually higher in black olive (60.097 mg QE/g of extract by reference to standard curve ($y = 0.0067x + 0.0132, r^2 = 0.999$), (Figure 3).

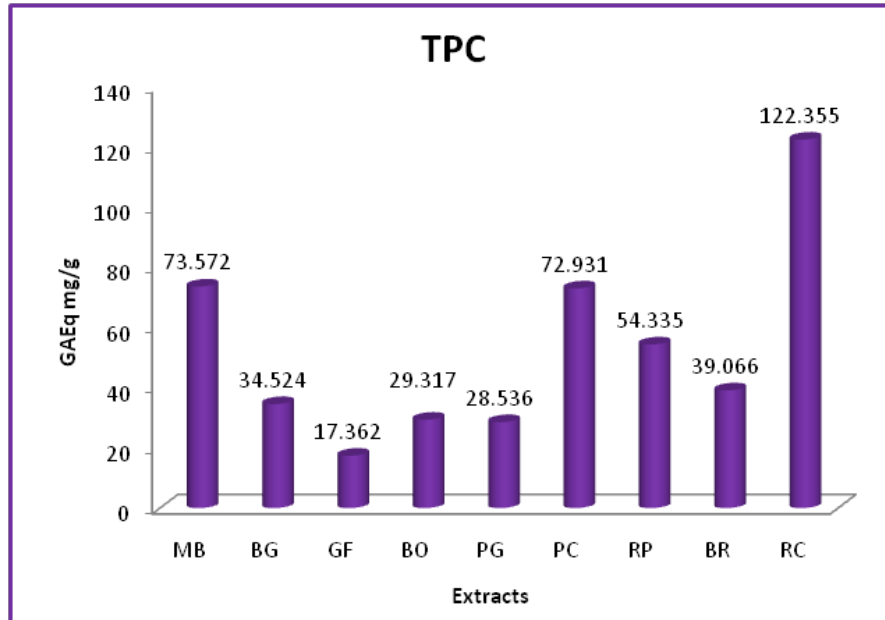


Figure 3. Determination of Total phenol content in extracts

Total Anthocyanin content using pH differential method

Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The oxonium (orange-purple) form predominates at pH 1.0 and the hemiketal (colorless) form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and

rapid measurements for the total amount of anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds. Since Cy-3-glc was the most abundant anthocyanin in fruits and vegetables, the extinction coefficient of Cy-3-glc standard was used to measure the total anthocyanins (Cy-3-glc equivalent) (figure 4).

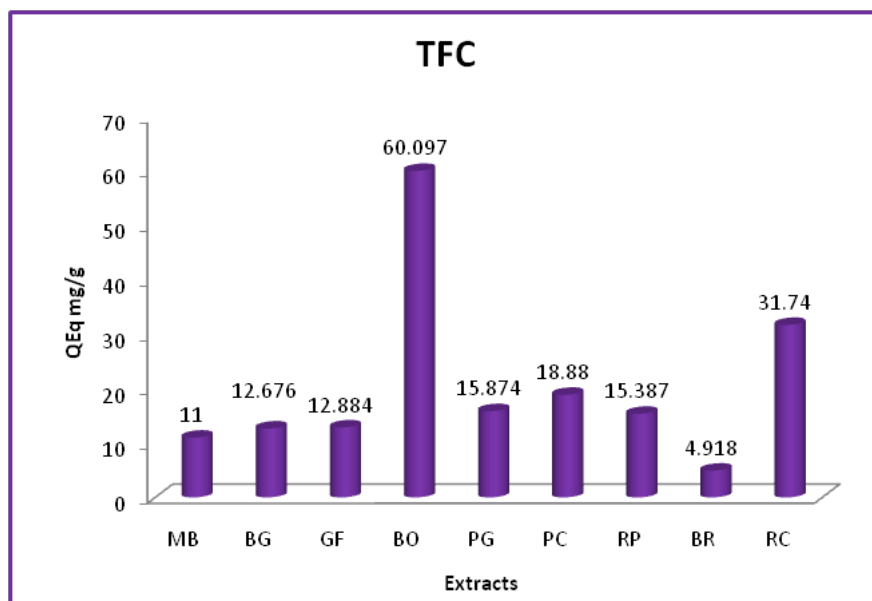


Figure 4. Determination of Total Flavonoid contents in extracts

Antioxidant Activity Rank Order of the Vegetables

Fruits and vegetables contains many anti-oxidants like poly-phenolic flavonoids, vitamin-C, anthocyanins. The comparative study of antioxidant activity is desirable not only from an academic point of view but also in the interest of vegetable producers and consumers. Consequently, there are plenty of papers attempting to rank antioxidant capacities of different plant extracts, including fruits and vegetables.

Measure of Antioxidative Ability by the DPPH Method

Being a stable free radical, DPPH is frequently used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH. Thus, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution (Figure 5).

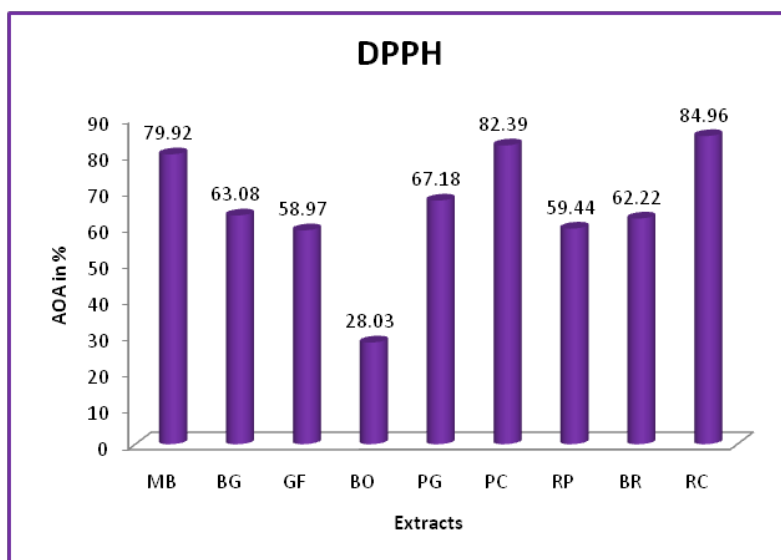


Figure 5. DPPH free radical scavenging effects on extracts

FRAP assay

The reducing power of a compound is related to its electron transfer

ability and may serve as a significant indicator of its

potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power. Figure 6 presents the reductive capabilities of the methanolic extracts of raw fruits and vegetables.

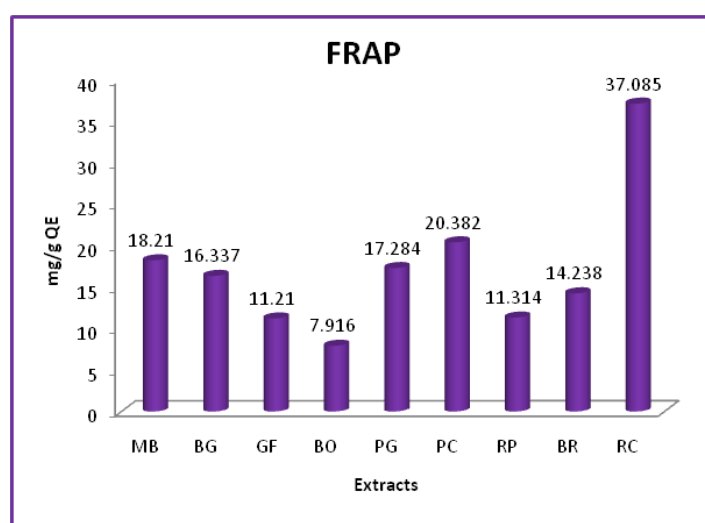


Figure 6. Antioxidant activity in extracts

Conclusions

The phrase “eating a rainbow” of fruits and vegetables is

a simple way of remembering to get as much color variety in your diet as possible, so that you can maximize your intake of a broad range of nutrients. Nutrients should come

primarily from foods. Foods such as fruits and vegetables contain not only the vitamins and minerals that are often found in supplements, but also other naturally occurring substances that may help protect you from chronic diseases.

Qualitative and quantitative anthocyanin compositions of colored foods are important factors in determining the functionality and health benefits of foods enriched with anthocyanins. It is obvious that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quantity or quality of the phenolic constituents in the extracts. Similarly it must be noted that the efficiency of antioxidants depends strongly on the oxidation conditions and method used in this screening study. It gives only one approximation of the possibilities of an extract to act as an antioxidant. Yet the results gained by these methods provide simple data that make it possible to classify extracts with respect to their antioxidant potential. As can be observed also from the present data, antioxidant activity does not necessarily correlate with high amounts of phenolics alone, and that is why both phenolic content and antioxidant activity information has been discussed elaborately.

Conflict of Interest: None

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