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Relationship between Bioactive Components and Antioxidant Capacity of Some Commonly Consumed Vegetables in Punjab

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A B S T R A C T

Many potential compounds such as flavonoids, phenolics and carotenoids possess anti-oxidant activity. Various categories of vegetables like green leafy, roots and tubers and other vegetables have these bioactive compounds and potent antioxidant activity. Total anti-oxidant activity and its correlation with total phenolics and flavonoids among various vegetables commonly consumed in Punjab (India) have not been much reported. The aim of this research was to determine the antioxidant capacity from methanol extracts of various vegetables and its correlation with the total flavonoid and phenolic content. Among all the vegetables, bathua leaves (*Chenopodium album*) were found to have highest phenolic and flavonoid content i.e. 247.20 mg GAE/100g and 225.10 mg RE/100g, respectively. The maximum total antioxidant capacity by FRAP was observed in fenugreek leaves (*Trigonella foenum-graecum L.*) i.e. 171.91 mg TE/100g. Bathua leaves (*Chenopodium album*) were also found to have maximum antioxidant activity as measured by DPPH radical scavenging activity i.e. 126.80 mg TE/100g. Total antioxidant activity correlated significantly with the total phenolic content of all vegetables ($p<0.01$). However, non-significant correlation was observed between flavonoid content and total antioxidant activity.

Introduction

Fruits and vegetables have been gaining great attention among consumers because they play an important role in the human health and diet. Being considered as wholesome foods, the nutritional value of vegetables and fruits is attributed to the optimal mix of phytochemicals which are mainly constituted by fibres, natural antioxidants and bioactive components. Anti-oxidants are the substances which are capable of inhibiting the oxidative stress in the body caused by production of free radicals during metabolic reactions in the body. These free radicals are the agents which are involved in the pathogenesis of various non-metabolic disorders and degenerative diseases such as diabetes, Parkinson's and Alzheimer's diseases, cancers, asthma and atherosclerosis (Podsedek, 2007; Dasgupta and De, 2007; Turkmen *et al.,* 2005 and Cai *et al.,* 2004).

Regular consumption of plant foods rich in anti-oxidant compounds inhibits the oxidative stress leading to degenerative diseases. These beneficial effects have been reported due to the presence of phytochemicals present in plants such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones, lignans, stilbenes, coumarins, tannins), nitrogenous compounds (alkaloids, amines, betalains), endogenous metabolites, vitamins (C and E), and terpenoids (including carotenoids) (Cai *et al.,* 2004). The protective effect of bioactive components in fruits and vegetables is primarily due to the reduction in oxidative stress, which is caused when the formation of reactive oxidants (most importantly reactive oxygen, nitrogen and iron species) is higher in the antioxidant defence system. Due to the possession of high antioxidant activity by these bioactive compounds, great interest has risen towards the relationship between phytochemical compounds and incidence of metabolic diseases (Nilsson and Stegmark 2004).

It has been reported that with increase in every serving of fruit and vegetable, the risk of various cancers, cardiovascular diseases and mortality is reduced by 15%, 30% and 20%, respectively (Steimez and Potter 1996; Rimm *et al.,* 1996), which can be credited to the presence of different antioxidant compounds in fruits and vegetables such as vitamin C and E, carotenoids, lycopenes, polyphenols and other phytochemicals (Prior and Cao 2000).

According to recent studies, different phytochemicals in common fruits and vegetables have multiple benefits including scavenging of oxidative radicals, strengthening of the defence system, regulating gene expression for the process of cell proliferation, maintaining hormone metabolism, and antibacterial and antiviral properties (Halvorsen *et al.,* 2006). The present study was conducted to analyze the bioactive compounds and total antioxidant capacity of commonly consumed vegetables in Northern India especially Punjab and to derive associated correlations, which can prove to be of enormous benefit to human health.

Materials and Methods

Plant materials

A total of 15 commonly consumed vegetables (other vegetables, roots and tubers and green leafy vegetables) were collected from five locations of the local market of Ludhiana, Punjab which were further combined to withdraw a uniform sample.

All the vegetables were thoroughly cleaned and washed with distilled water to remove the dirt and other impurities. The samples of edible portions of all the vegetables were homogenized with the help of pestle and mortar and immediately processed for the extraction of bioactive components using standard procedures.

Extraction of bioactive components - phenolic and antioxidant compounds from sample

Samples (5 g) were homogenized in 80% aqueous methanol, acidified to pH 2.0 with 6N Hydrochloric acid by shaking at room temperature for 30 minutes and the supernatant was saved.

The residue was re-extracted twice with 80% ethanol for complete removal of phenolic and antioxidant compounds. The procedure was repeated two times. Pooled supernatants were centrifuged at 6000 rpm for 15 minutes and then filtered through Whatman No.1 filter paper. Finally the volume was made upto to 50 ml with the solvent, transferred to micro centrifuge tubes and stored at -20°C for analysis.

Determination of total phenol (Folin-Ciocalteu method)

Total phenolic compounds were determined using a modified Folin-Ciocalteau colorimetric method (Singleton *et al.,* 1999).

Briefly, 0.5 ml of sample aliquot of sample was taken and volume was made up to 1.5 ml with distilled water. Further, 0.5 ml of Folin-Ciocalteau reagent was added followed by 10 ml of 7.5% Na_2CO_3.

It was incubated at 37 $^{\circ}$C for 60 minutes. The absorbance of blue-colored complex solution was read at 750 nm using UV-spectrophotometer. Different concentrations of gallic acid (5-20 µg/mL) were used to construct a calibration curve. The results were expressed as miligram of gallic acid equivalent per hundred gram (mg GAE/100 g).

Determination of Flavonoid content (Zhishen *et al.,* 1999)

Two ml of sample aliquot was taken in a test tube and volume was made up to 5 ml with distilled water. Further, 0.3 ml of 5 % sodium nitrite was added to it. After 5 min, 0.6 ml of 10 % aluminium chloride was also added and mixed thoroughly. After keeping the test tubes again for 6 min, 2 ml of 1 N sodium hydroxide was added and mixed.

In the end, the volume was made up to 10 ml by adding 2.1 ml distilled water. The absorbance of resulting pink colour was read at 510 nm against blank.

Standard series of known concentration of Rutin (50-200 µg) were also made by taking different concentrations i.e. 0.5, 1.0, 1.5, 2.0 ml rutin aliquots and treated same as sample. The results were expressed as mg RE/ 100 g of flavonoids.

Determination of Total antioxidant capacity by Ferric Reducing Antioxidant Power (FRAP) assay

Ferric reducing antioxidant power (FRAP) was determined in sample extracts according to Benzie and Strain (1999) and modified by Tadhani *et al.,* (2007). This method is based on the ability of the sample to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. In the presence of TPTZ (2,4,6–Tris (2-pyridyl)–s–triazine) the Fe^{2+}–TPTZ complex exhibits blue colour which can be read at 593 nm.

Sample aliquots (0.1 ml) were taken and the volume was made up to 0.3 ml with distilled water. Then, 1.8 ml of FRAP working reagent was added to it After incubating for 10 min at room temperature, the absorbance was measured at 593 nm against blank. Similarly, the standard series with known concentration of Trolox (0.5-2 µg) was also developed with same procedure as sample. The results were expressed as mg TE per 100 g sample.

Determination of total antioxidant capacity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

This method is based on the ability of the antioxidant to scavenge the DPPH cation radical (Brand-Williams *et al.,* 1995). Sample aliquots i.e. 0.3 ml was taken in a test tube. The volume was made up to 1 ml with methanol.

After this, 3 ml of DPPH reagent was added to the test tubes, mixed properly and incubated for 20 min at 37°C. After the incubation, the absorbance of the resulting oxidized solution was read at 517 nm against methanol as blank. Standard series of known concentration of 10mg% Trolox (5-20 µg) by taking concentrations as 0.05, 0.1, 0.15, 0.2 ml aliquot was made and treated same as sample. The results were expressed as mg TE/ 100 g.

Statistical analysis

Assays were performed in triplicate for each sample. Results were expressed in terms of mean values ± standard deviation (SD). To determine whether the bioactive compounds contributed to the antioxidant capacity, pearson's correlation coefficients were calculated at 1%.

Results and Discussion

Total Phenolic (TPC) and Flavonoid (TFC) Content of vegetables

Phenolic compounds are natural antioxidants present in plant foods and they have been reported to have beneficial effects on human health as they act as reducing agents, hydrogen donators and single oxygen quenchers (Chang *et al.,* 2001), thereby reducing the incidence of various degenerative diseases.

Total phenolic content (TPC) of the various green leafy vegetables under study varied from 60.12 to 247.05 mg GAE/100g (Table 1). TPC among various green leafy vegetables was found in the order of Bathua>Spinach>Fenugreek leaves>Green amaranth>Mustard. Total polyphenol content in Kale (green leafy vegetable) has been reported as 171.27 µg GAE/mg of sample (Sikora *et al.,* 2008). In the present study, the TPC in mustard leaves was found to be 60.12 mg GAE/100 g which is similar to the literature i.e. 62.62 mg GAE/100g (Bembem 2014). The phenols in spinach and bathua leaves were found to be 128.04 and 247.05 mg GAE/100g, respectively. These values are approximately equivalent with the findings to the values of 196.3 mg/100g and 253.5 mg/100g in spinach and bathua leaves, respectively (Kaur and Kapoor, 2002). Among various other vegetables analysed for their total phenolic content, lady's finger was found to have highest content (151.7 mg GAE/100g) followed by bottle gourd, pumpkin, French beans and bitter gourd, respectively. Total Flavonoid content of above said vegetables was found to be in descending order of pumpkin>lady's finger>bitter gourd>French beans>bottle gourd. Findings from another study revealed a higher value of TPC in bitter gourd procured from thailand as 224 mg GAE/100g dry sample (Kubola and Siriamornpun 2008). TPC of 35.2 mg GAE/100g in French beans reported in present study was quite lower as compared to that reported in other study which was 97.0 mg/100g (Kaur and Kapoor, 2002).

Among another category of vegetables i.e. Roots and tubers, five vegetables namely colocasia, radish, carrot, turnip and sweet poattao were analysed for their TPC and TFC. For TPC, the value in ascending order were carrot> sweet potato> radish> colocasia> turnip i.e. turnip was found to have highest TPC as 117.1 mg GAE/100g. A previous study has also reported TPC in turnip as 127.0 mg/100g, which is comparable to the values in present study (Kaur and Kapoor 2002). However, in carrots, TPC was found to be 31.6 mg GAE/100g, whereas in literature, higher values have been reported as 55 mg/ 100g has been reported (Kaur and Kapoor 2002). Furthermore, another study conducted by Koley *et al.,* 2014 reported that the total phenols in sixteen Indian carrot varieties ranged from 7.98 to 291.48 mg/100g. TPC in radish reported in the present study and in the literature (Sreeramulu and Raghunath, 2010) are found to be comparable. The values being 61.73 and 66.73 mg GAE/100g, respectively.

Flavonoids are a group of secondary plant metabolites naturally found in fruits and vegetables, which helps to maintain the body's health and provides protection against various ailments. They also exhibit anti-nutritional properties due to their metal chelating

properties. As depicted in Table 2, the highest content of total flavanoids among green leafy vegetables was found in the bathua leaves, followed by mustard, spinach, fenugreek and green amaranth, respectively. A previous study in the literature (Bembem, 2014) reported a value of 23.09 QE/100 g, TFC in mustard leaves. However, in the present study, the value was found to be 129.09 mg RE/100g, the difference might be due to the method used for the estimation. The total flavonoid content in spinach was found to be 148.14 mg RE/100g which is slightly higher as compared to the value of 108.7 mg QE/100 g reported in another study (Singh *et al.*, 2016).

The flavonoid content among other vegetables ranged from 20.12 mg RE/100g in bottle gourd to 65.21 mg RE/100 g in pumpkin. The value for the flavonoid content of bitter gourd has been reported to be 80.5 mg QE/100 g in the previous study by Singh *et al.*, 2016, which is comparatively higher to the value reported in the present study i.e. 50.21 mg RE/100g. In contrast to the present study, the authors of the previous study analyzed flavonoid content in 62 Malaysian edible plants and reported the flavonoid content in pumpkin, lady's finger and french beans as 371.0, 260 and 172.5 mg/kg of dry weight, respectively (Miean and Mohamed, 2001).

The flavonoid content among roots and tubers was found to be low as compared to green leafy vegetables and other vegetables. Sweet potato was found to have the highest flavonoid content i.e. 48.1 mg RE/100g and carrot was found to have the lowest value (6.12 mg RE/100g) among roots and tubers.

Table.1 Total Phenolic (TPC) and Total Flavonoid Content (TFC) of the vegetables

S.No.	Samples	Scientific name	Total Phenols (mg GAE/100g)	Total Flavonoid (mg RE/100g)
	Leafy vegetables			
1.	Mustard	*Brassica campestris*	60.12±0.06	129.09±0.07
2.	Bathua	*Chenopodium album*	247.20±1.17	225.10±1.64
3.	Spinach	*Spinacia oleracea*	128.13±0.30	148.14±0.24
4.	Fenugreek	*Trigonella foenum-graecum L.*	93.66±1.58	136.24±0.83
5.	Green amaranth	*Amaranthus viridis*	86.50±2.21	106.90±0.29
	Other vegetables			
1.	Pumpkin	*Cucurbita maxima*	39.40±1.23	65.21±0.68
2.	Bottle gourd	*Lagenaria vulgaris*	42.64±0.87	20.12±0.21
3.	Bitter gourd	*Momordica charantia*	34.74±0.58	50.21±0.67
4.	French beans	*Phaseolus vulgaris*	35.24±2.31	42.97±0.74
5.	Lady's finger	*Abelmoschus esculentus*	151.71±1.12	64.80±0.98
	Roots and tubers			
1.	Colocassia	*Colacasia antiquorum*	74.57±0.58	38.51±1.24
2.	Radish	*Raphanus sativus*	61.73±2.04	35.42±0.53
3.	Carrot	*Daucus carota*	31.60±0.25	6.12±0.29
4.	Turnip	*Brassica rapa var. Rapa*	117.10±2.41	21.90±0.25
5.	Sweet potato	*Ipomoes batatas*	61.27±1.36	48.10±1.07

Values are Mean (±SD); GAE- Gallic Acid Equivalent; RE- Rutin Equivalent

Table.2 Total antioxidant capacity of vegetables as measured by FRAP and DPPH

Sl no.	Samples	Scientific name	Ferric Reducing Antioxidant Power (FRAP) assay (mg TE/100g)	DPPH Radical Scavenging Activity (mg TE/100g)
	Leafy vegetables			
1.	Mustard	*Brassica campestris*	104.30±0.24	65.91±0.06
2.	Bathua	*Chenopodium album*	76.20±1.08	126.80±0.14
3.	Spinach	*Spinacia oleracea*	54.34±0.14	56.78±0.06
4.	Fenugreek	*Trigonella foenum-graecum L.*	171.91±0.14	29.7±0.51
5.	Green amaranth	*Amaranthus viridis*	158.27±0.45	78.10±0.52
	Other vegetables			
1.	Pumpkin	*Cucurbita maxima*	42.80±0.47	31.51±1.15
2.	Bottle gourd	*Lagenaria vulgaris*	31.90±0.34	40.02±0.34
3.	Bitter gourd	*Momordica charantia*	65.20±0.87	24.25±2.05
4.	French beans	*Phaseolus vulgaris*	22.60±0.67	39.70±0.21
5.	Lady's finger	*Abelmoschus esculentus*	31.50±0.51	86.60±0.67
	Roots and tubers			
1.	Colocassia	*Colacasia antiquorum*	19.80±0.33	75.03±1.84
2.	Radish	*Raphanus sativus*	61.70±2.04	24.02±1.02
3.	Carrot	*Daucus carota*	57.75±0.24	11.45±0.47
4.	Turnip	*Brassica rapa var. rapa*	117.10±2.41	14.90±0.27
5.	Sweet potato	*Ipomoes batatas*	61.30±1.36	19.20±1.01

Values are mean ± SD
TE- Trolox Equivalent

Table.3 Correlation coefficient between total phenolic, flavonoid content and total anti-oxidant activity

	r^2 (Pearson Correlation coefficient)
TPC vs TAC by FRAP	0.542 ^{NS}
TPC vs TAC by DPPH	0.001*
TFC vs TAC by FRAP	0.142 ^{NS}
TFC vs TAC by DPPH	0.004*

*Correlation is significant at the 0.01 level (2-tailed).
NS- Non-significant

A wide range of total flavonoids i.e. 3.00 to 111.70 mg/100g in carrot has been provided in the literature (Koley *et al.*, 2014). A previous study has reported a significantly higher TFC in carrot i.e. 232.5 mg/kg of dry weight (Miean and Mohamed, 2001), while another study reported a value of 26.9 and 43.5mg CE/100 g of flavonoids in carrot and radish respectively (Srivastava *et al.*, 2013).

A wide range of values of phenols and flavonoids in various vegetables has been reported in the literature, which may be attributed to genetic variety or cultivar,

season, soil condition, water availability, degree of maturity, method of estimation and standards used play an important role in determining the various bioactive components.

Anti-oxidant capacity of vegetables

Anti-oxidant capacity of various vegetables was studied by two methods i.e. by Ferric Reducing Assay Power (FRAP) and DPPH Radical Scavenging Activity. The results of anti-oxidant activity in various vegetables by both the methods have been presented in Table 2.

Total anti-oxidant activity as measured by FRAP assay was found to be highest (171.9 mg TE/100g) in fenugreek leaves, when measured by DPPH Radical Scavenging Activity, it was found to be lowest in fenugreek leaves (29.7 mg TE/100g). The TAC by FRAP assay among various leafy vegetables was in order of fenugreek> green amaranth> mustard> bathua> spinach. So, the lowest value of TAA by FRAP assay was in spinach i.e. 54.34 mg TE/100g.

TAC by DPPH Radical Scavenging Activity in mustard leaves (65.91 mg TE/100g) was comparable to that 69.44 per cent reported in the literature (Bembem, 2014). TAC by DPPH in kale leaves was 82.80 per cent which can be considered as comparable to those reported among various leafy vegetables in the present study.

On contrary to this, quite low values of TAC by DPPH in fenugreek (25.7%) and spinach leaves (20.4%) was reported in a previous study by Gacche *et al.*, (2010).

Among other vegetables, TAC by FRAP assay was found to be higher (65.2 mg TE/100g) in bitter gourd, while it was lowest in lady's finger.

However, TAC as DPPH (%) free radical scavenging activity was found to be higher in lady's finger (86.6 mg TE/100g), followed by bottle gourd (40.02 mg TE/100g), French beans (39.7 mg TE/100g), pumpkin (31.5 mg TE/100g) and bottle gourd (24.2 mg TE/100g), respectively. The authors of a study have analyzed TAC by DPPH in some of the Asian vegetables and reported the comparable values of 43.8 % and 40.7 % in lady's finger and bottle gourd, respectively (Gacche *et al.*, 2010). Kamath *et al.*, (2015) had reported TAA as 15.05 mg AAE/g in ridge gourd and 12.39 mg AAE/g in cabbage, which are also categorized under other vegetables.

Among various roots and tubers analysed in the present study, radish and turnip were found to have almost similar values of TAC (by FRAP) i.e. 67.7 and 61.3 mg TE/100g, respectively, while another study in the literature have reported very high values of 1294.36 and 422.56 mg/100g in radish and sweet potato, respectively, estimated by similar method i.e. FRAP assay (Sreeramulu and Raghunath, 2010).

The differences might be credited to the varietal or agronomical differences which may vary from place to place. In addition to this, methods used to extract hydrophilic/lipophilic phases from vegetables also contribute significantly to the differences in the estimated values of antioxidant activity. TAC as measured by DPPH Radical Scavenging Activity was found to be highest in colocasia (75.03 mg TE/100g), followed by radish (24.02 TE/100g), sweet potato (19.20 TE/100g), turnip (14.90 TE/100g) and carrot (11.45 TE/100g), respectively. The value for TAA by DPPH in the literature (Sreeramulu and Raghunath, 2010) has been reported to be 29.02 mg/100g in radish and 25.03 mg/100g in sweet potato which are comparable to those reported in the present study.

Correlation among bioactive components and total antioxidant capacity (TAC)

Pearson correlation coefficient was calculated to test the significance of relationship between the bioactive components (Total phenols and flavonoids) and total antioxidant activity. The relationship between the Total phenolic and flavonoid content with the Total Antioxidant capacity by DPPH was found to be significant ($p<0.01$). Similar findings have been observed in another study (Kaur and Kapoor, 2002). Another study by Sreeramulu and Raghunath (2010) also depicted a significant correlation ($p < 0.01$) between TPC and AOA both in roots and tubers (r values being 0.76 and 0.85 respectively with DPPH and FRAP) and other vegetables ($r = 0.79$ and 0.85 with DPPH and FRAP). The associations between the bioactive components and TAC suggest that various phytochemicals found in the vegetables are capable of scavenging free radicals. A non-significant correlation was found in the bioactive components with the Total antioxidant capacity by FRAP assay. Findings from a study have also concluded that the phenolic contents and the antioxidant activities of vegetables correlated very well with the methods used for analysis (Stratil *et al.,* 2006) (Table 3).

The antioxidant activity can be attributed to a number of molecules in the matrix under investigation, for instance ascorbate, GSH etc. The phenolic content in plant foods comprises flavonoids, phenolic acids, simple phenols, condensed hydrolysable tannins and lignans etc., which fluctuates widely during different seasons and different stages of the life cycle of a plant. The potential and presumptive antioxidant capacity of each of these components depends on the kind of test system employed.

The most commonly used methods FRAP and DPPH assess the potential of these compounds, which consists of their capacity to release hydroxyl ions responsible for neutralizing free radicals. Normally, the correlation between phenolics and antioxidant activity is high, it is interesting to analyze each class or compound and relate to the antioxidant potential.

Further, researches are needed to evaluate the specificity of phenolic compounds responsible for the antioxidant activity and the possible mechanisms responsible for their actions. Besides this, *in vivo* antioxidant assays are required for the confirmation of role of these species in prevention of various diseases.

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