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Research Article

Quantification of Phytochemicals Imparting Antioxidant Activities in Commonly Used Vegetables

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Abstract

Plant derived phytochemicals have recently became of great importance in the protection of various diseases, like heart disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataract and age related functional disorders caused by free radicals. The present study was carried out to explore the commonly used vegetables having higher content of antioxidant imparting phytochemicals such as ascorbic acid, carotenoids, total phenolic content, carbohydrate and protein content in commonly used vegetables. The results revealed that the concentration among tested samples ranged from 7.07 mg/100g of FW (*Momardica charantia* leaf) to 174.15 mg/100g of FW (*Allium sativum* leaf) for ascorbic acid; 1.31 µg/g of FW (*Chenopodium album* leaf) to 14.00 µg/g of FW (*Allium sativum* leaf) for carotenoid content; 8.72 mg of GAE/g of DW (*Cucurbita maxima* fruit) to 67.20 mg/g of DW (*Colocasia esculentum* leaf) for total phenolic content; 27.15 mg/g (*Laginaria vulgaris* leaf) to 901.00 mg/g (*Cucurbita maxima* fruit) for carbohydrate content and 35.96 mg/g (*Amarphophyllus* fruit) to 589.23 mg/g (*Beta vulg*aris fruit) for protein content. Results also showed that these bioactive phytochemicals are widely distributed in the vegetables and their concentrations are variable in different vegetables as well as vegetable part's itself. Hence, vegetable rich diet having higher content of phytochemicals can be used to cure or in the prevention of various chronic diseases such as hepatotoxicity, diabetes, cardiovascular diseases, cancer, oxidative stress etc and may serve as a good source of nutraceuticals which have potential for use in health care formulations.

Keywords: Phytochemicals; Antioxidant; Free radicals; Lipid peroxidation; DNA damage

Introduction

Vegetables are rich source of important nutrients such as vitamins, minerals and other important components such as phytochemicals. The whole part of vegetables or its parts such as root, stem, leaf, flower, fruit, and even seed can be used for cooking or consumption. There are several studies that indicate, high consumption of vegetable had an inverse relationship with the incidence of degenerative diseases including heart diseases (Hertog *et al*., 1993), cancer (Block *et al*., 1992; Byers and Perry, 1992), inflammation (Giugliano *et al*., 2006; Rahman *et al*., 2006) and brain dysfunction (Trewavas and Stewart, 2003). Free radicals (having unpaired electron) can cause oxidative damage to naturally occurring biomolecules, resulting in various type

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of chronic diseases such as liver diseases, cataracts, diabetes etc (Singh *et al*., 2016a; Singh *et al*., 2016b). These oxidative damage can be repaired by different type of antioxidant such as antioxidant nutrients (Vitamin A, C, E), antioxidant enzymes (SOD, CAT, GPx), phytochemicals (phenoics and flavonoids) and metal chelating proteins (transferrin, ceruloplasmin, ferritin) (Singh *et al*., 2013). The role of antioxidants is to protect all biomolecules from the oxidative damage caused by free radicals. Exogenous intake of antioxidants can help the body by scavenge free radicals effectively. Furthermore, many studies have shown that increased dietary intake of natural phenolics correlates with reduced coronary heart disease, cancer mortality with longer life expectancy (Halliwell, 2007; Singh *et al*., 2014). On the other hand, commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) have led to increased interest on natural antioxidants which occur in plants as secondary metabolites (Ghafar *et al*., 2010). In vegetables, there are some naturally occurring phytochemicals which has powerful antioxidants properties due to their ability to quench oxygen-derived free radicals (FRs) by donating electron to FRs, by chelating metal ions or by terminate oxidation process. Phenolic compound are important natural antioxidant due to their health benefits for human, decreasing the risk of cardiovascular and degenerative disease by reduction of oxidative stress and counteraction of macromolecular oxidation (Singh *et al*., 2010). In view of increasing awareness about health and side effect of synthetic medicine, there is need to find out some safe alternative drug/ nutraceuticals which are used in our day today life in one or the other form. In the proposed investigation an attempt will be made to evaluate the vegetables having high phytochemical content which play a major role as an antioxidant.

Materials and Methods

Vegetable Samples

Fourteen vegetables sample were purchased from markets of Ayodhya and Naveen mandi, Faizabad, UP. The common and scientific names of these vegetables are given in Table 1. Edible parts of vegetable were chopped, dried, powdered and stored in polythene bags at 4°C till further analysis. The Ascorbic acid and carotenoids content were estimated in fresh weight (FW) whereas total phenolic, protein and carbohydrate content were estimated in dry weight (DW).

Chemicals and Reagents

Gallic acid and Bovine serum albumin (BSA) were procured from Sigma-Aldrich, St. Louis, USA. β-Carotene, Ascorbic acid, Folin phenol reagents were the product of E. Merk, Mumbai, India. All Other reagents and chemicals used were of analytical grade.

Estimation of Phytochemicals

Ascorbic acid

Ascorbic acid content of vegetables was estimated by the method of Arlington (1984). One gram of fresh plants sample were crushed with 20 ml of 0.4% oxalic acid and filtered. The volume was made up to 20 ml with 0.4% oxalic acid. The supernatant was analyzed for ascorbic acid content by titrating with 2, 6-dichlorophenol sodium salt dye. Ascorbic acid content was reported as mg/100g of fresh weight (FW).

Table 1: Vegetables used in the quantification of phytochemicals

S.N.	Vegetables common name	Botanical name with short name	Parts used
1.	Garlic	*Allium sativum* (AS)	Leaf, Fruit
2.	Suran	*Amarphophyllus campanulatus* (AC)	Leaf, Fruit
3.	Bitter gourd	*Momardica charantia* (MC)	Leaf, Fruit
4.	Arvi	*Colocasia esculentum* (CE)	Leaf, Fruit
5.	Pumpkin	*Cucurbita maxima* (CM)	Leaf, Fruit
6.	Lauki	*Laginaria vulgaris* (LV)	Leaf, Fruit
7.	Lady's finger	*Abelnoschus esculentus* (AE)	Leaf, Fruit
8.	Chukander	*Beta vulgaris* (BV)	Leaf, Fruit
9.	Pig weed	*Chenopodium album* (CA)	Leaf
10.	Soya	*Anethum graveolens* (AG)	Leaf
11.	Palak	*Rumex dentatus* (RD)	Leaf
12.	Tomato	*Lycopersican esculentum* (LE)	Fruit
13.	Cauliflower	*Brassica oleracea* (BO)	Fruit
14.	Potato	*Solenum tuberosum* (ST)	Fruit

Carotenoids

Carotenoids content of vegetables was estimated by the method of Jensen (1978). One gram fresh sample was crushed with 10ml of 80% methanol and kept over night at room temperature. Residue was discarded and supernatant was concentrated to dryness on boiling water bath. The dried extract was dissolved in 5ml ether and 5ml of 10% KOH and the mixture was kept for 1 hour at room temperature in dark. The ether layer was washed with 1ml of 3% NaCl for 3 times to remove alkaline methanol and dried over sodium sulphate for one hour. The absorbance of ether extract was measured at 450 nm. Carotenoids content was reported as µg/g of fresh weight.

Total phenolic content (TPC)

TPC was measured with the method of Ragazzi and Veronese (1973). Twenty five mg of powdered plant material was extracted with 50%methanol+1%HCl, filtered and made up to 10 ml with distilled water. Take 0.1 ml plant extract and made up to 1ml with distilled water. 0.5 ml of Folin's reagent (1N) and 1.0 ml of sodium carbonate was added subsequently. The test mixture was mixed properly and kept at room temperature for 30 minutes and made up to 12.5 ml with distilled water. The absorbance was measured at 720 nm wavelengths. The TPC was reported as mg of gallic acid equivalent (GAE) /g of dry weight.

Protein

Protein content was estimated by the method of Lowry *et al*. (1951). Twenty five mg of dried and powdered plant sample was extracted with 2 ml of 1N NaOH for one hour on boiling water bath. The volume was made up to 5 ml with distilled water and supernatant was used for estimation. 100 µl of test solution was diluted with distilled water to make 1 ml, followed by addition of 5 ml of Lowry's reagent, mixed and incubated for 10 minutes at room temperature. Folin's phenol reagent (0.5 ml of 1N) was added and the solution was kept for 30 min. at room temperature. Absorbance was measured at 660 nm. Protein content was reported as mg/g of dry weight.

Carbohydrate

Carbohydrate content was estimated by Anthrone method (Thomas *et al*., 1956). Twenty five mg of dried and powdered sample was extracted with 10 ml distilled water on boiling water bath for 15 minutes. The extract was filtered and volume was made up to10ml with distilled water. 100µl of test solution was diluted with distilled water to maintain 1ml; 4ml of Anthrone reagent was added to it and boiled for 10 min and kept for cooling at room temperature. Absorbance was measured at 620 nm. Carbohydrate was reported as mg/g of dry weight basis.

Statistical Analysis

Values obtained from the estimation of phytochemicals shown in Table 2 are the mean ±standard deviation (SD) of at least three independent determinations. The statistical analysis was done by software Prism.

Results and Discussion

The ascorbic acid content in different tested vegetables ranged between 7.07 to 174.15 mg/100g of fresh weight (FW) (Table 2). The higher value of ascorbic acid content was present in *Allium sativum* leaf, *Momardica charantia* fruit and *Colocasia esculentum* leaf to the extent of 174.15, 165.99 and 117.01 mg/100g of fresh weight, respectively. The moderate ascorbic acid content present in *Brassica oleracea* fruit, *Laginaria vulgaris* leaf, *Anethum graveolens* leaf, *Amarphophyllus* leaf, *Cucurbita maxima leaf, Beta vulgaris* leaf, *Rumex dentatus* leaf, *Chenopodium album* leaf, *Laginaria* v*u*il*g*ar*i*s fruit, *Solanum tuberosum* fruit, *Allium sativum* fruit, *Beta vulgaris* fruit, *Lycopersican esculentum* fruit and *Cucurbita maxima* fruit are in the range of 69.38 to17.69mg/100g of fresh weight. The lower value of ascorbic acid content present in *Amarphophyllus* fruit and *Momardica charantia* leaf was 7.62, 7.07mg/100g, respectively. Okiei *et al*. (2009) reported that the ascorbic acid content in *Cucurbita maxima*, *Allium sativum* and *Lycopercican esculentum* were 12.00, 35.24 and 27.70 mg/100g, respectively. In a similar study, reported by the Gacche *et al*. (2010), ascorbic acid content in *Brassica oleracea, Anethum graveolens, Cucurbita maxima and Ablenoschus esculentus* were 12.28, 10.24, 15.36 and 25.60 mg/100g, respectively. Abushita *et al*. (1997) showed that vitamin C content in tomato was 48 mg/100g. In present study, results are showing similar finding as compared to previous study. Literature showed that ascorbic acid acts as antioxidant and role of ascorbic acid is to neutralize the free radicals. The possible anticarcinogenic effect of vitamin C appears to be related to its ability to detoxify carcinogens or block carcinogenic processes through its action as an antioxidant or as a free radical scavenger (Rock *et al*., 1996). Another benefit of vitamin C in bone healing may be through its antioxidant effect. Vitamin C may reduce the risk of chronic diseases such as cancer, cardiovascular disease and cataracts probably through antioxidant mechanism (Carr *et al*., 1999).

The carotenoid content in different vegetable parts ranged between 1.31 to 14.00 µg/g of fresh weight (Table 2). The higher values of carotenoid content were present in *Allium sativum* leaf and *Cucurbita maxima* leaf. The moderate values of carotenoid content were shown by *Beta vulgaris* fruit, *Colocasia esculentum* leaf, *Beta vulgaris* leaf, *Ablenoschus esculentus* fruit, *Laginaria vulgaris* leaf and fruit, *Momardica charantia* leaf and fruit, *Rumex dentatus* leaf, *Cucurbita maxima* fruit, *Amarphophyllus* fruit, *Solanum tuberosum* fruit *and Anethum graveolens* leaf in the range of 6.29 to 3.59 µg/g of fresh weight. The lower values of carotenoid content were present in *Amarphophyllus* leaf, *Allium sativum* fruit, *Brassica oleracea* fruit, *Lycopersican esculentum* fruit and

Chenopodium album leaf in the range of 2.83 to 1.31µg/g of fresh weight. In previous study, carotenoids content of *Beta vulgaris, Chenopodium album, Cucurbita maxima* and *Brassica oleracea* were reported as 52.40, 449.90, 53.27, 10.32 mg/100g of dry weight, respectively which is much higher than present study (Raju *et al*., 2007). These differences may be related to climatic, geographic conditions and cultivars differences from where samples were collected for analysis (Rajyalakshmi *et al*., 2001). Carotenoids protect the body by decreasing risk of heart disease, stroke, blindness, and certain types of cancer. They may also help to slow the aging process, reduce complications associated with diabetes and improve lung function. Many of the researches proved that both the carotenoids and the xanthophylls or even the apocarotenoids, function as antioxidants. Diet rich in carotenoids have an effect on normalization of antioxidant enzymatic activity. In addition to the body's endogenous defenses against oxidative stress, diet-derived antioxidants including carotenoids, ascorbic acid (Vitamin C) and alpha-tocopherol (Vitamin E) may be important in protecting against disease (Halliwell, 1996).

The amount of total phenolic content varied in different vegetables samples between 8.72 to 67.20 mg of GAE/g of dry weight (Table 2). The higher values of total phenolic content were present in *Colocasia esculentum* leaf, *Anethum graveolens* leaf, *Allium sativum* leaf, *Chenopodium album* leaf, *Amarphophyllus* fruit, *Rumex dentatus* leaf and *Brassica oleracea* fruit and content was 67.20, 40.61, 39.52, 35.89, 35.78, 37.13 and 31.37 mg of GAE/g of dry weight, respectively. The moderate total phenolic were shown by *Amarphophyllus* leaf, *Momardica charantia* leaf and fruit and *Allium sativum* leaf and the value was 21.67, 18.09, 16.82 and 13.08 mg of GAE /g of dry weight, respectively. The lower values of total phenolic content present in *Cucurbita maxima* fruit, and *Laginaria vulgaris* fruit and leaf were 8.44, 8.43 and 6.52 mg of GAE /g of dry weight, respectively. Data obtained from experiments demonstrate that polyphenols are widely distributed in vegetable samples and their quantities vary in different types of vegetables. According to Bravo (1998), polyphenols that present in these vegetables is largely affected by genetic factors, environmental conditions, variety, etc. Total phenolic content reported in previous studies showed some variation as compared to our study. Gacche *et al*. (2010) reported the values of total phenolics in *Anethum graveolens, Brassica oleracea, Cucurbita maxima* and *Ablenoschus esculentus* as 8.70, 3.00, 13.30 and 5.35 mg/g, respectively. Melo *et al*. (2006) showed that total phenolic content in *Lycopersican esculentum* was 30.83mg/g which is very much similar to the present study. Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenolic compound is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Nagulendran *et al*., 2007).

Table 2. Phytochemical content in different tested vegetable samples

Name of vegetables	Parts of vegetable	Ascorbic Acid (mg/100 g of FW)	Carotenoids (µg/g of FW)	TPC (mg of GAE/g of DW)	Carbohydrates (mg/g of DW)	Protein (mg/g of DW)
Allium sativum	Leaf	174.15±2.46	14.00±0.01	39.52±0.11	86.35±0.01	178.60±0.02
	Fruit	23.166±2.30	2.66±0.55	17.54±0.01	520.99±0.01	125.27±0.01
Amarphophyllus campanulatus	Leaf	57.14±4.08	2.83±0.01	23.58±0.01	59.25±0.01	295.36±0.09
	Fruit	7.62±0.23	3.68±0.08	35.78±0.03	165.17±0.02	35.96±0.03
Beta vulgaris	Leaf	32.65±8.16	6.29±0.71	29.30±0.50	62.23±0.10	163.20±0.08
	Fruit	23.13±4.71	8.25±0.59	27.93±1.20	803.20±3.50	589.23±3.10
Momardica charantia	Leaf	7.07±1.02	5.49±0.01	18.09±0.02	71.60±0.05	50.76±0.05
	Fruit	165.99±6.23	5.88±0.05	13.97±0.02	40.36±0.01	166.86±0.01
Cucurbita maxima	Leaf	53.80±2.20	8.30±0.08	23.50±0.10	149.80±0.05	137.50±0.04
	Fruit	17.69±2.35	5.40±0.03	8.72±0.01	901.00±0.03	186.66±0.01
Laginaria vulgaris	Leaf	61.22±4.08	5.98±0.03	29.35±0.05	27.15±0.04	274.00±0.05
	Fruit	24.99±4.06	4.79±0.01	19.38±0.08	266.00±0.01	66.90±0.01
C. album	Leaf	25.84±0.15	1.31±0.24	35.89±0.05	64.19±0.02	251.23±0.01
A. graveolens	Leaf	59.86±2.35	3.59±0.65	40.61±0.05	91.35±0.05	211.40±0.02
Rumex dentatus	Leaf	28.56±0.10	5.78±0.19	37.13±0.04	101.23±0.01	250.86±0.02
L. esculentum	Fruit	21.76±0.05	1.34±0.25	26.02±0.01	184.34±0.02	170.23±0.03
B. oleracea	Fruit	69.38±4.67	1.79±1.21	31.37±2.78	61.72±4.27	177.17±14.18
S. tuberosum	Fruit	24.48±0.20	3.62±0.18	21.30±1.10	273.00±3.20	73.20±2.10
A. esculentus	Fruit	83.90±0.50	6.12±0.12	27.80±1.10	401.90±2.20	180.30±1.15
C. esculentum	Leaf	117.01±6.19	7.31±0.04	67.20±0.03	150.36±0.02	259.00±0.02

The amount of total protein content varied in different vegetable samples between 35.96 to 589.23 mg/g of dry weight (Table 2). The higher values of total protein content were present in *Beta vulgaris* leaf, *Amarphophyllus* leaf, *Laginaria vulgaris* leaf**,** *Colocasia esculentum* leaf, *Chenopodium album* leaf, *Rumex dentatus* and *Anethum graveolens* leaf and content were 589.23, 295.36, 274.00, 259.00, 251.00, 250.00 and 211.40 mg/g of DW, respectively. The moderate values of protein content were present in *Cucurbita maxima* fruit *Ablenoschus esculentus* fruit, *Allium sativum leaf*, *Brassica oleracea* fruit, *Lycopersican esculentum*, *Momardica charantia* fruit, *Beta vulgaris* leaf, *Cucurbita maxima* leaf and *Allium sativum* fruit as 186.66, 180.30, 178.60, 177.17, 170.23, 166.86, 163.20, 137.50 and 125.27 mg/g, respectively. The lower values of protein content present in *Solanum tuberosum* fruit, *Laginaria vulgaris* fruit, *Momardica charantia* leaf and *Amarphophyllus* fruit were 73.20, 66.90, 50.76 and 35.96mg/g, respectively. Protein is good source as antioxidant additive in food because they can inhibit lipid peroxidation through multiple pathways. The protein and peptides also have excellent potential to inhibit allergencity as well as their ability to alter food texture (Elias *et al*., 2008). Experimental result suggested that wheat germ protein hydrolysate is a suitable natural antioxidant rich in nutrient and is nontoxic. The antioxidant activity of wheat germ protein hydrolysate increases with the increase of its concentration (Hui *et al*., 2006). The protein isolated from chick pea and white bean showed the good potential as thermostable natural food antioxidants (Arcan and Yemenicioglu, 2007).

The carbohydrate content varied in different vegetables samples between 27.15 to 901.00 mg/g of dry weight (Table 2). The higher amount of carbohydrate were present in *Cucurbita maxima* fruit, *Beta vulgaris* fruit, *Allium sativum* fruit, *Ablenoschus esculentus* fruit, *Solanum tuberosum* fruit and *Laginaria vulgaris* fruit and values were 901.00, 803.20, 520.99, 401.90, 273.00 and 266.00 mg/g, of dry weight, respectively. The moderate carbohydrate content were present in *Lycopersican esculentum* fruit, *Amarphophyllus fruit*, *Colocasia esculentum* leaf *Cucurbita maxima* leaf and *Rumex dentatus* leaf as 184.34, 165.17, 150.36, 149.80 and 101.23 mg/g of dry weight, respectively. The lower values of carbohydrate content were detected in *Anethum graveolens* leaf, *Allium sativum* leaf, *Momardica charantia* leaf, *Chenopodium album* leaf, *Beta vulgaris* leaf, *Brassica oleracea* fruit, *Amarphophyllus* leaf, *Momardica charantia* fruit and *Laginaria vulgaris* leaf and content were 91.35, 86.35, 71.60, 64.19, 62.23, 61.72, 59.25, 40.36 and 27.15 mg/g of dry weight, respectively. The extracellular polysaccharide isolated from probiotic bacteria showed antioxidant and free radicals scavenging activity (Kodali and Sen, 2008). Consumption of complex carbohydrates in combination with different antioxidant micronutrients may enhance the antioxidant defenses (Roberts *et al*., 2008). So, *Cucurbita maxima* and *Laginaria vulgaris* show better antioxidant activity than other plant samples.

Conclusion

Presence of phytochemicals such as vitamin C, carotenoids, total phenolic content, protein, carbohydrate content in plant and its parts is widely varied. Among the tested samples, leaf of *Allium sativum* showed highest value of vitamin C and carotenoids content whereas fruit of *Beta bulgaris* showed highest value of protein content. Leaf of *Colocasia esculentum* had highest TPC value whereas fruit of *Cucurbita maxima* had highest carbohydrate content. Therefore, it can be stated that vegetable's part having higher content of tested phytochemicals may be good and easily accessible source for nutraceuticals compounds for various herbal formulation and health care product. Indian Ayurvedic literature had also shown that *Allium sativum* and *Beta bulgaris* have antihepatotoxicity, anti-HIV-1, antimalarial, antimutagenic and antifungal activities which may be due to presence these phytochemicals.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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