

Antioxidant Capacity and Total Phenol Content of Commonly Consumed Selected Vegetables of Bangladesh

Sharmin H, Nazma S*, Md. Mohiduzzaman & Cadi PB

Institute of Nutrition and Food Science, University of Dhaka, Dhaka – 1000, Bangladesh

ABSTRACT

Introduction: The purpose of the study was to determine the antioxidant capacity (AC) and total phenolic content (TPC) of selected commonly consumed Bangladeshi vegetables and herbs. **Methods:** Hydrophilic extracts from edible portions of ten vegetables and two herbs were analysed. The total phenolic amount ranged from 27.65 ± 1.45 mg to 1.08 ± 0.15 Gallic Acid Equivalent (GAE)/g on a fresh weight (FW) basis. Contents of total phenol were determined spectrophotometrically according to the Folin-Ciocalteu method and the antioxidant capacity was determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA). **Results:** Antioxidant capacity varied from 8328.80 ± 29.15 to 0.61 ± 0.19 μ mol Trolox Equivalent (TE)/g of FW. Ipomoea leaves showed the highest AC (8328.80 ± 29.15 μ mol TE/g), while the lowest AC (0.61 ± 0.19 μ mol TE/g) was seen in radish. A linear relationship was observed between Trolox Equivalent Antioxidant Capacity (TEAC) values and total phenol. Antioxidant capacity of the assayed samples correlated significantly and positively with total phenolic content ($R^2 = 0.814$, $p < 0.01$). Vegetables with high polyphenol like Ipomoea leaves and mint showed high AC with the exception of raw banana which demonstrated moderate AC though it contained high TPC. Moderate TPC vegetables like amaranths and coriander leaves did not show substantial AC. **Conclusion:** The data indicates that indigenous vegetables containing high polyphenols may be a potential source of dietary antioxidants.

Keywords: Anti-oxidant capacity, DPPH radical scavenging activity, total phenol, vegetables

INTRODUCTION

Epidemiological and scientific studies have shown that nutritional factors play an important role in the prevention of consequences of free radical activity in an organism. An association between consumption of a diet high in fruits and vegetables and decreased risk of chronic degenerative diseases is well documented (Steinmeitz & Potter, 1996; Cox, Whichelow

& Prevost, 2003; Knekt *et al.*, 1997). Fruits and vegetables contain a wide variety of biologically active non-nutritive compounds known as phytochemicals. The protective effect of these foods is attributed to the presence of phytochemical components such as carotenoids, tocopherols and polyphenols that have antioxidant properties (Kalt & Kushad, 2000; Prior & Cao, 2000). Scientific studies suggest that polyphenols perform much more important

* Correspondence author: Professor Nazma Shaheen; Email: nzmshaheen58@gmail.com

roles than providing basic nutrition in disease prevention and control. The basic structure of polyphenols is composed of one or more phenolic rings that are substituted with several hydroxyl groups and these are related to their strong antioxidant activity. This antioxidant activity of polyphenols is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans, Miller & Paganga, 1997).

Dietary polyphenols are prominent sources of antioxidants for humans. These are derived from plants and are consumed in the forms of fruits, vegetables, spices and herbs (Graf, Milbury & Blumberg, 2005). There is a limited amount of information on the content of phenolic compounds in common Bangladeshi food stuff of plant origin. This work aimed to screen a selected number of vegetables and herbs consumed in the local diet with respect to their total phenolic content and antioxidant capacity. Further, the relationship between total phenolic content and antioxidant capacity was determined.

METHODS

The research materials comprised 10 vegetable species (leafy and non-leafy) and 2 herbs. The vegetables included two different varieties of brinjal (green and purple) (*Solanum melongena*), cabbage (*Brassica oleracea-capitata*), cauliflower (*Brassica oleracea-botrytis*), radish (*Raphanus sativus*), raw banana (*Musa paradisiaca*), lal shak (*Amaranth gangeticus*), data shak (*Amaranth viridis*), ipomoea leaves (*Ipomoea aquatica*) and spinach (*Spinacia oleracea*). The herbs included coriander leaves (*Coriandrum sativum*) and mint (*Mentha viridis*). Samples were purchased fresh from a local market, washed with distilled water and air dried.

Sample preparation

The collected food samples were processed in the food analysis laboratory of the Institute

of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Edible portions of the fresh vegetables were cut into small pieces (<0.25 cm) and dried at 55°C in an oven. The dried matter was weighed, ground in a grinder and stored in desiccators prior to extraction.

Chemicals and reagents

Hexane (MERCK, Germany), dichloromethane (MERCK, Germany), acetone (MERCK, Germany) and acetic acid (MERCK, Germany) were used for solvent extraction. Gallic acid (TIC, Japan), sodium carbonate and Folin-Ciocalteu reagent (MERCK, Germany) were used for estimation of total phenol. MES buffer (DojinDo), DPPH (2,2-diphenyl-1-picrylhydrazyl radical) (Wako, Japan), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a vitamin E analogue) (ALDRICH, Denmark) and ethanol (MERCK, Germany) were used for estimation of antioxidant capacity. All chemicals used for the analysis were of analytical grade.

Instruments

A shaker (Controlled Environmental Incubator Shaker, New Brunswick Scientific Co INC), a centrifuge machine (Hettich Universal II), and a sonication bath (Cole-Parmer) were used for sample extraction. An UV-VIS spectrophotometer (Schimadzu UV-1601) was used to record absorbance at specific wavelengths (520 nm and 750 nm).

Sample extraction

Ground dry plant material (500mg) was put into a screw-capped glass tube. A total of 12.5 ml of Hexane: Dichloromethane (1:1) solution was added, and the suspension was stirred slightly. It was then allowed to shake overnight in a shaker at a rate of 100 rpm at room temperature. On completion of shaking, the mixture was centrifuged for 15 min. The supernatant was discarded and the precipitate was dried for 5 min at 60°C to evaporate the left-over solvent. To the

precipitate, 12.5 ml of AWA (acetone: water: acetic acid: 70: 29.5: 0.05) was added and then sonicated for 15 min using the sonication bath. After sonication, the sample was again centrifuged for 15 min. The supernatant was then separated from the precipitate into a 25 ml volumetric flask and the volume was adjusted with extraction solvent to 25 ml. Subsequently, aliquots of the extracts were transferred to 3 screw-capped glass tubes and stored at 4°C and -20°C for further analysis.

Determination of total phenol content in plant extracts

Total phenol content of the selected sample extracts was estimated colorimetrically according to the Folin-Ciocalteu method (Singleton & Rossi, 1965). For each sample, 10 µl of extracts were put into 3 test tubes. Then, 60 µl of water was added to each of the test tubes. Then, 15 µl of two times diluted Folin-Ciocalteu reagent (by water) were added and allowed to stand for 5 mins at room temperature. This was followed by the addition of 75 µl of 2% (w/v) sodium carbonate solution to the mixture which was allowed to stand for 15 min at room temperature. The absorbance was measured at 75 nm with Shimadzu UV-VIS spectrophotometer. A control sample was prepared containing the same volume of reference gallic acid. A gallic acid standard curve of varying concentrations was constructed by plotting gallic acid concentrations on abscissa and absorbance on ordinates for quantification of total polyphenol. The total polyphenol content was expressed as gallic acid equivalent per fresh weight (mgGAE/g FW).

Estimation of antioxidant capacity (DPPH-RSA) of plant extracts

Estimation of the antioxidant capacity of the samples was performed by DPPH radical scavenging assay of Brand-Williams, Cuvelier & Berset (1995). Sample extracts were mixed with the same volume of 30%

acetone. Amounts of 200, 400 and 800 µL of the samples were put into test tubes and added with 800, 600 & 200 µL of 50% acetone. The next step was the addition of 500 µL of 200 mM MES buffer to all the tubes. Then, 500 µL of 400 µM DPPH solution in EtOH was added to all these tubes (one by one at a similar interval rate). After 20 min (room temperature) absorbance was measured at 520 nm. For the sample blank, three tubes with 200, 400 and 800 µL each were added with 50 % acetone in similar volumes as the samples - 800, 600 and 200 µL. Then, 500 µL of 200 mM MES buffer was added to all the tubes. Instead of DPPH solution, 500 µL of ethanol was added to all the blanks and absorbance was measured to subtract the sample colour. A standard curve was constructed by plotting varying Trolox concentrations on abscissa and absorbance on ordinates. The antioxidant capacity of the assayed samples was calculated from the standard curve, in terms of Trolox Equivalent Antioxidant Capacity (TEAC) and was expressed as µmol TE/g FW.

Statistical analysis

The assays were run in triplicate for each sample and the results expressed as mean values ± standard deviation (SD). Pearson's correlation coefficient test was applied to test the association between the polyphenol content and the antioxidant capacity of the samples analysed.

RESULTS

The results of the total phenolic content (TPC) and their antioxidant capacity (AC) of twelve commonly consumed Bangladeshi vegetables and herbs are given in Table 1. Total phenolic content of the vegetables assayed varied from 27.65 ± 1.45 to 1.08 ± 0.15 mg GAE/g of fresh weight. The highest concentration of polyphenols was found in Ipomoea leaves and the lowest in radish. The wet weight of the phenol contents of

Table 1. Total phenolic contents and antioxidant activity in selected vegetables and herbs

Common Name	Scientific Name	Moisture (%)	Total polyphenols (mg GAE/g FW)	TEAC ($\mu\text{mol TE/g FW}$)
<i>Amaranth gangeticus</i> (AG)	<i>Amaranth gangeticus</i>	89.0	7.65 \pm 0.47	131.10 \pm 47.7
<i>Amaranth viridis</i> (AV)	<i>Amaranth viridis</i>	88.4	7.22 \pm 0.31	94.40 \pm 21.0
Banana (raw)(BA)	<i>Musa paradisiaca</i>	86.0	21.45 \pm 2.5	2294.0 \pm 170.2
Brinjal (green)(BG)	<i>Solanum melongena</i>	93.2	2.71 \pm 0.05	27.91 \pm 5.79
Brinjal (pink)(BP)	<i>Solanum melongena</i>	93.0	3.94 \pm 0.19	1.72 \pm 0.05
Cabbage(CA)	<i>Brassica oleracea-capitata</i>	91.1	1.87 \pm 0.19	4.70 \pm 1.32
Cauliflower(CU)	<i>Brassica oleracea-botrytis</i>	92.0	3.5 \pm 0.26	3.63 \pm 3.2
Coriander leaves(CO)	<i>Coriandum sativum</i>	86.0	8.35 \pm 0.15	169.20 \pm 19.0
Mint(MI)	<i>Mentha viridis</i>	89.0	17.55 \pm 1.65	3847.50 \pm 336.5
Ipomoea leaves(IP)	<i>Ipomoea aquatica</i>	85.0	27.65 \pm 1.45	8328.80 \pm 29.15
Radish(RA)	<i>Raphanus sativus</i>	96.0	1.08 \pm 0.15	0.61 \pm 0.19
Spinach(SP)	<i>Spinacia oleracea</i>	95.0	2.58 \pm 1.81	1.71 \pm 1.72

vegetables was measured because they are purchased and eaten fresh. The concentration difference in the polyphenolic content was noted between the leafy and non-leafy vegetables, being higher in the leafy vegetables than in the non-leafy vegetables with the exception of raw banana. Raw banana, which is commonly used as a vegetable in cooking, contains high amounts of polyphenols (21.45 \pm 2.5 mg GAE/g). Moisture content of the studied vegetables varied from 85 to 96% of the total mass. A higher concentration was observed in non-leafy vegetables (91-96%) and a lower concentration (85-89%) in leafy vegetables with the exceptions of spinach (95%) and raw banana (86%). Low phenol content in cabbage, cauliflower and radish has also been reported by Kaur & Kapoor (2002).

A large variation in the antioxidant capacity was observed in the assayed sample, ranging from 8328.80 \pm 29.15 $\mu\text{mol TE/g}$ in ipomoea leaves to as low as 0.61 \pm 0.19 $\mu\text{mol TE/g}$ in radish. The total antioxidant capacity assayed by DPPH-RSA method varied considerably from one vegetable to the other. A similar wide range on antioxidant capacity has been reported earlier (Wang, Cao & Prior, 1996; Gazzani *et al.*, 1998). On the basis of fresh weight of the

vegetables, the values determined for individual species in descending order are ipomoea > mint > coriander > amaranth g. > amaranth v. > brinjal (green) > raw banana > cabbage > cauliflower > brinjal (purple) > spinach > radish. Interestingly all vegetables, except raw banana, which had a high phenolic content also had a high antioxidant capacity and those which had low phenolic contents, also had low antioxidant capacity (Figure 1). Banana (raw) though showing high content of total phenol is comparatively low in anti-oxidant capacity.

The relationship between TPC and AC are presented in Table 2. Pearson correlation coefficient showed that among the 12 studied samples, 8 showed positive correlation while the remaining 4 showed negative correlation between TPC and AC ($p < 0.01$). A linear relationship was observed between antioxidant capacity and total polyphenol content (Figure 2). The statistical analysis showed a high and significant correlation between total phenol content and antioxidant capacity ($R^2 = 0.814$) indicating a good agreement with selective foods, as also reported by others (Kaur & Kapoor, 2002; Sellappan, Akoh & Krewer, 2002; Deighton *et al.*, 2000).

Table 2. Relationship between total phenolic content and antioxidant capacity

Samples	TPC(mg GAE/g FW)	TEAC(μ mol TE/g FW)	R ²
<i>Amaranth gangeticus</i>	7.65 ± 0.47	131.10± 47.7	0.505
<i>Amaranth viridis</i>	7.22 ± 0.31	94.40± 21.0	-1.00
Banana (raw)	21.45 ± 2.5	2294.0 ± 170.2	0.271
Brinjal (green)	2.71 ± 0.05	27.91± 5.79	0.969
Brinjal (purple)	3.94 ± 0.19	1.72± 0.05	-1.00
Cabbage	1.87 ± 0.19	4.70± 1.32	1.00
Cauliflower	3.50 ± 0.26	3.63± 3.2	-1.00
Coriander leaves	8.35 ± 0.15	169.20± 19.0	0.568
Ipomoea leaves	27.65 ± 1.45	8328.80±29.15	0.934
Mint	17.55 ± 1.65	3847.50±336.5	0.840
Radish	1.08 ± 0.15	0.61± 0.19	0.566
Spinach	2.58 ± 1.81	1.71± 1.72	-1.00

Correlation is significant at the level of $p < 0.01$

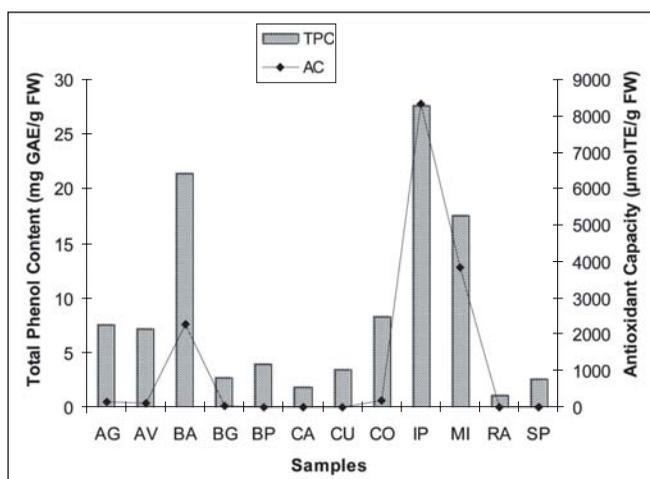


Figure 1. Total phenolic content and hydrophilic antioxidant capacity of vegetables

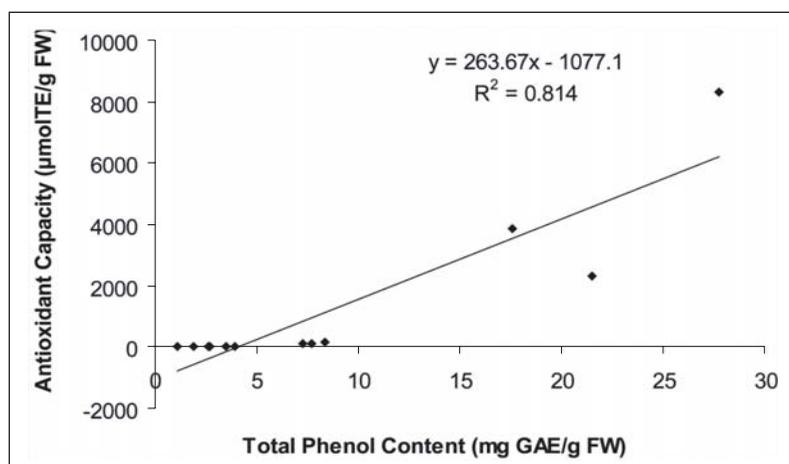


Figure 2. Correlation between total phenolic content and antioxidant capacity

DISCUSSION

The present study findings initiate a new dimension in the field of food science research in Bangladesh. Based on the frequency of consumption, the food items chosen for this study showed differences in their total polyphenol contents and corresponding antioxidant capacities. The correlation between total polyphenol content and antioxidant activity has been widely studied in different foodstuffs such as fruits and vegetables (Klimczak *et al.* 2007; Jayaprakasha, Girenavar & Patil, 2008). The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al.*, 2003). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (van Acker *et al.* 1996). It is known that only flavonoids with a certain structure and a particular hydroxyl position in the molecule can function to donate protons and show radical scavenging activity (Mensor *et al.*, 2001; Hou *et al.*, 2003).

Total phenol content and radical scavenging activity of 12 hydrophilic (or acidic aqueous acetone) extracts from commonly consumed local vegetables and herbs were analysed. Ipomoea leaves, a commonly eaten leafy vegetable showed high total phenols and also high antioxidant capacity, followed by mint and raw banana. Coriander leaves and amaranth (both types), though exhibiting moderate presence of phenolic content, did not show significant antioxidant capacity. It is reported that the AC of an extract cannot be predicted on the basis of its phenolic content, but varies remarkably, depending on its chemical structure, and thus requires proper characterisation of individual phenolic compounds (Statue-Garcia, Heionen & Frankel, 1997). In the present study, great variations in the TPC and AC of

the studied vegetables were observed. The variation may be due to several reasons. The content of phenolic compounds and the antioxidant capacity are partly dependent on the colour of the variety of the vegetables and the water content (Pavel, Borivoj & Vlastimil, 2006). Dark green leafy samples like ipomoea and mint exhibited high values of antioxidant capacity, while very low values were found in watery samples such as radish, brinjal, cauliflower and cabbage. The probable factor for very poor AC of spinach could be its high moisture content (95%). On the other hand raw banana though poorly colored showed high AC which could be attributed to low moisture content (86%). Furthermore, properties of polyphenols are also greatly affected by their interactions with other constituents of the food matrix and likely to interfere with the metabolism of polyphenol activity (Cheynier, 2005).

The results of the present study reveal that ipomoea leaves, mint and raw banana with their high levels of polyphenols and antioxidant activity could be important sources of functional food in the local diet.

REFERENCES

- Brand-Williams W, Cuvelier ME & Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm-Wiss u-Technol* 28: 25-30.
- Cheynier V (2005). Polyphenols in food are more complex than often thought. *Am J Clin Nutr* 81(suppl.): 223S-239S.
- Cox BD, Whichelow MJ & Prevost AT (2000). Seasonal consumption of salad vegetables and fresh fruits in relation to development of cardiovascular disease and cancer. *Publ Health Nutr* 3(1): 19-29.
- Deighton N, Brennan R, Finn C & Davies HV (2000). Antioxidant properties of domesticated and wild *Rubus* Species. *J Sc Food Agri* 80: 1307-1313.
- Gazzani G, Papetti A, Massolini G & Daglia M (1998). Antioxidative and pro-

- oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *J Food Chem* 6: 4118-4122.
- Graf BA, Milbury PE & Blumberg JB (2005). Flavonols, flavones, flavonones and human health: epidemiologic evidence. *J Med Food* 8(3): 281-290.
- Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY & Lee MH (2003). Free radical scavenging activity of Taiwanese native plants. *Phytomedicine* 10: 170-175.
- Jayaprakasha GK, Girenavar B & Patil BS (2008). Radical scavenging activities of Rio Red grapefruits and sour orange fruit extracts in different *in vitro* model systems. *Bioresour Techno* 99(10): 4484-4494.
- Kalt W & Kushad MM (2000). The role of oxidative stress and antioxidants in plant and human health: introduction to the colloquim. *Hort Sci* 35: 572-574.
- Kaur C & Kapoor HC (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sc Tech* 37: 153-161.
- Klimczak I, Malecka M, Szlachta M & Gliszczynska-Swiglo A (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *J Food Compost Anal* 20: 313-322.
- Knekt P, Jarvinen R, Seppanen R, Heliövaara M, Teppo L & Aromaa A. (1997). Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am J Epid* 46: 223-230.
- Lee SE, Hwang HJ, Ha JS, Jeong HS & Kim JH (2003). Screening of medicinal plant extracts for antioxidant activity. *Life Sci* 73: 167-179.
- Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS & Leitao SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res* 15: 127-130.
- Pavel S, Borivoj K & Vlastimil K (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables – Evaluation of spectrophotometric methods. *J Agri Food Chem* 54: 607-616.
- Prior RL & Cao G (2000). Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Hort Sci* 35: 588-592.
- Rice-Evans CA, Miller NJ & Paganga C (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science* 4: 304-309.
- Sellappan S, Akoh CC & Krewer G (2002). Phenolic compounds and anti-oxidant capacity of Georgia-grown blueberries and blackberries. *J Agric Food Chem* 50: 2432-2438.
- Singleton VL & Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16: 144-158.
- Statue-Garcia MT, Heionen M & Frankel EN (1997). Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. *J Agric Food Chem* 5: 362-367.
- Steinmeitz KA & Potter JD (1996). Vegetables, fruits and cancer prevention: a review. *J Am Diet Assoc* 96: 1027-1039.
- van Acker SA, van Den Berg DJ, Tromp MN, Griffioen DH, van Bennekom WP, van der Vijgh WJ & Bast A (1996). Structural aspects of antioxidant activity of flavanoids. *Free Radic Biol Med* 20(3): 331-342.
- Wang H, Cao GH & Prior RL (1996). Total antioxidant capacity of fruits. *J Agric Food Chem* 44: 701-705.