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Hydrophilic Antioxidant Capacities and Total Phenol Content of Seasonal Fruits of Bangladesh

Mamun S[1], Shaheen N[1*], Basak Tukun A[1], Md Mohiduzzaman[1], Banu CP[1] & Takano-Ishikawa Y[2]

[1] *Institute of Nutrition and Food Science, University of Dhaka, Dhaka – 1000, Bangladesh*
[2] *National Food Research Institute (NFRI), Kannondai, Tsukuba, Japan*

ABSTRACT

Introduction: Consumption of fruits and vegetables helps to scavenge free radicals owing to the presence of antioxidant nutrients and secondary metabolites, especially polyphenolic compounds. This may lead to a reduction in the risk of diet-related chronic diseases. The purpose of the study was to determine the antioxidant capacity (AC) and total phenolic content (TPC) of selected seasonal fruits of Bangladesh. **Methods:** Hydrophilic extracts from edible portions of fifteen fruits available in the summer season were analysed. Total phenol content was determined spectrophotometrically according to the Folin-Ciocalteau method while antioxidant capacity was determined by 2, 2-diphenyl-1-1-picrylhydrazyl radical scavenging activity (DPPH-RSA). **Results:** TPC ranged from 0.6 ± 0.01 to 0.01 ± 0 mg Gallic acid equivalent (GAE)/g of fresh weight (FW). Antioxidant capacity varied from 4.882 ± 0 to 0.113 ± 0.03 μmol Torolox equivalents (TE)/g of FW. *Carissa carandas* showed the highest AC (4.882 ± 0 μmol TE/g), while the lowest (0.113 ± 0.3 μmol TE/g) was seen in *Baccaura ramiflora*. A positive and significant correlation (R^2 = 0.957) between antioxidant capacity and total phenolic content of the analysed samples was identified. A significant and positive correlation ($p<0.05$) between AC and TPC was found in *Manikara zapota*, *Artocarpus heterophyllus*, *Litchi chinensis* and *Articarpus lakoocha*. **Conclusion**: The data indicates that some indigenous seasonal fruits of Bangladesh contain high polyphenols that may serve as a potential source of dietary antioxidants.

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

INTRODUCTION

There is much evidence that supports the hypothesis that food can modulate various body functions in addition to supplying the basic nutrients. The popular term of 'functional foods' introduces a new dimension to food science, that is, medicine and food have a common origin. Bioactive compounds such as secondary metabolites of plant origin have been reported for their potential beneficial anti-carcinogenic roles (Manthey & Grohmann 2001), protection against heart disease (Vita, 2005), anti-inflammatory and anti-allergic roles (Liu, 2003; Yammamoto *et al*., 2004).

Polyphenolic compounds of plant food origin drew the attention of researchers as a

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

strong anti-oxidant, particularly in helping to scavenge free radicals in the human body thus contributing towards cell damage and ageing. Several studies have established the association between oxidative stress and increased risk of diseases such as arterial sclerosis, high blood pressure, myocardial infarction, cerebral apoplexy, dementia, diabetes and cataract (Steinmeitz & Potter, 1996; Devasagayam *et al.* 2004; Florence, 1991). Dietary antioxidants could have a role in protecting humans against diseases associated with free radical damage to cellular DNA, lipids and proteins.

Many research findings suggest that protection against oxidative damage and related disease is best served by the variety of antioxidant substances found in fruits and vegetables (Jacob & Burri, 1996). Epidemiological studies report that fruits and vegetables and also some processed foods such as chocolate, tea, coffee or wine are good sources of phenolics, which can reduce the risk of diet related degenerative diseases (Aherne & O'Brien, 2002). Studies have shown that some plant phenolics appear in plasma and body tissues and thus may be important nutritional antioxidants (Duthie & Crozier, 2000).

Limited numbers of research findings are available from commonly consumed Bangladeshi vegetables, fruits and other plant sources (Hossain *et al*., 2011; Alam *et al.*, 2011; Hossaina & Rahman, 2011). The present study has focused on evaluating the TPC and AC of tropical fruits in order to highlight the higher phenolic contents and their correlation with corresponding antioxidant capacity.

METHODS

A total of 15 fruits was collected from the local markets and home gardens in Dhaka city, capital of Bangladesh for the estimation of total phenol content and anti-oxidant capacity. The study samples were *Mangifera indica* (Mango), *Artocarpus heterophyllus* (Jackfruit), *Litchi chinensis* (Litchi), *Syzgium cumini* (Black berry), *Ananus comosus* (Pineapple), *Averrhoea carambola* (Carambola), *Borassus flabellifer* (Palmyra fruit), *Manikara zapota* (Sapota), *Syzgium samarangense*(Rose apple), *Syzgium aqueum* (Wax-apple), *Baccaura ramiflora* (Burmese grape), *Carissa carandas* (Karonda), *Cucumis melo* (Honeydew melon), *Articarpus lakoocha* (Lakuch) *and Carissa carandas* (Hog plum). All the samples were collected as fresh as possible.

The collected food samples were processed in the food analysis laboratory of the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Samples were washed with distilled water immediately after collection and then air dried. The samples were peeled and seeds removed. The samples were then cut into small pieces to increase the surface area and facilitate freeze drying. Then the samples were weighed again and freeze dried at -180°C until the samples were completely free of moisture. Subsequently, samples were weighed again and grinded in a grinder. The ground samples were stored at -4°C in a refrigerator until analysis.

The following reagents were used for solvent extraction: n-Hexane, dichloromethane, acetone, and acetic acid (all MERCK, Germany). Gallic acid (TIC, Japan), sodium carbonate and Folin-Ciocalteau 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-tramethylchromane-2-carboxylic acid, a vitamin E analogue) (Aldrich, Denmark) and ethanol (Merck, Germany) were used for estimation of antioxidant capacity. All the chemicals used for the analysis were of analytical grade.

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).

Ground sample of 500 mg was measured into a screw cap tube. Then 12.5 ml of hexane:dichlromethane (1:1) solvent was added and allowed to shake overnight in a shaker at the rate of 100 rpm. On completion of shaking, the mixture was centrifuged at 1000g for 15 min. Then the supernatant was discarded and the precipitate was dried at 60°C until evaporation of the remaining solvents. To the precipitate, 12.5 ml of acetone: water: acetic acid (AWA) in the ratio of 70: 29.5: 0.5 was added and sonicated for 15 min to disrupt the cell matrix of the sample for maximum extraction. After sonication, the samples were again centrifuged at 1000g for 15 min. The supernatant was then separated and stored in a freezer at -20°C until further analysis.

Determination of total phenol content in plant extracts

One gram of gallic acid was dissolved in 10 ml of ethanol and the volume made up to 100 ml with distilled water in a 100 ml volumetric flask. This was stock-I. An aliquot of 5 ml stock solution was added to 5 ml of AWA to make stock-II. One ml of stock-II was mixed with 9 ml of AWA. This constituted the working standard solution. Finally, from the working standard solution, 100 μl, 200μl, 300 μl, 500 μl and 1 ml were taken and the final volume of each was made up to 1 ml with AWA. From each of these 1 ml standard solutions, 10 μl was taken for the preparation of the standard curve.

For each sample, three test tubes were filled with 10 μl of sample extracts instead of 10 μl standard solution. Then, 60 μl of water was added to the test tubes, followed by the addition of 15 μl of FCR diluted two times. The test tubes were let to stand for 5 min at room temperature. Then, 75 μl of 2% sodium carbonate solution was added to the mixture and test tubes were allowed to stand for 15 min at room temperature. Finally absorbance was measured at 750 nm in UV-VIS Spectrophotometer.

Radical-scavenging activity

The radical-scavenging activity of the extracts of study samples were evaluated according to the DPPH radical scavenging activity analysis method of Brand-Williams, Covelier & Berset (1995). The extracts and Torolox at different concentrations (200, 400 & 800) were filled into different test tubes and made into1.0 ml by addition of an appropriate volume of 50% acetone. Then, 500 μL of 200 mM MES buffer was added to all the tubes followed by 500 μL of 400 μM DPPH solutions in EtOH (one by one at similar intervals). After 20 min (27°C), absorbance was measured at 520 nm. The sample blank was prepared as above without any extracts; three tubes with 200, 400 and 800 μL eac were taken. Fifty percent acetone was added to the tubes in similar volumes as the samples - 800,600 and 200 μL. This was followed by the addition of 500 μL of 200 mM MES buffer. 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 ordinate. 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 total phenol content and the anti-oxidant activity of the fruits analysed.

RESULTS

Total polyphenols and Antioxidant activity

The results of the TPC and their antioxidant capacity (AC) of fifteen Bangladeshi fruits are given with their scientific name, English name and local name in Table 1. TPC of the fruits assayed varied from 0.01 to 0.6 mg GAE / g of fresh weight. The highest concentration of polyphenols was found in *Syzgium samarangense* and the lowest in *Manikara zapota.* Moisture content of the analysed fruits varied from 72 to 93.5% of the total mass. The highest content was observed in *Averrhoea carambola* (93.5%) and the lowest (72%) in *Carissa carandas.* Among the selected fruits, *Syzgium samarangense* contained the highest amount of TPC (0.6mg GAE / g) whereas *Manikara zapota* contained the lowest amount (0.01 mg GAE / g). The hierarchy of TPC of analysed fruits was *Syzgium samarangense> Carissa carandas > Litchi chinensis > Averrhoea carambola> Borassus flabellifer > Cucumis melo > Syzgium cumini > Ananus comosus > Carissa carandas > Mangifera indica > Artocarpus heterophyllus > Articarpus lakoocha > Baccaura ramiflora > Syzgium aqueum > Manikara zapota.*

Variations in the radical scavenging activity was observed in the analysed fruits, ranging from 4.882 ± 0 µmol TE / g in *Carissa carandas* to as low as 0.113 ± 0.03 µmol TE / g in *Baccaura ramiflora.* The radical scavenging activity, as assessed by DPPH-RSA method, varied considerably from one fruit to another. On the basis of fresh weight of the fruits, the AC determined in descending order was *Carissa carandas > Averrhoea carambola > Syzgium samarangense > Artocarpus heterophyllus >Articarpus lokoocha > Manikara zapota > Spondias pinnata > Mangifera indica > Cucumis melo > Borassus flabellifera > Syzgium cumini > Litchi chinensis > Ananus comosus > Syzgium aqueum > Baccaura ramiflora.*

According to the results of the DPPH assay obtained for free radical scavenging property, *Syzgium samarangense* and *Syzgium aqueum* had higher values (94.4µg / ml & 94.1µg / ml) and *Averrhoea bilimbi, Carissa carandas* and *Baccaura ramiflora* ranged from 74µg / ml to 86µg / ml (Figure 2).

Table 1. Total phenolic contents and antioxidant capacity of selected fruits

English Name	*Local name*	*Scientific Name*	*Moisture (%)*	*TPC (mg GAE/g FW)*	*TEAC (mol TE/g FW)*
Blackberry	Kalojaam	*Syzgium cumini*	85.4	0.05 ± 0.023	0.246 ± 0.04
Burmese grape	Latkan	*Baccaura ramiflora*	90.4	0.01 ± 0.01	0.113 ± 0.03
Carambola	Kamranga	*Averrhoea carambola*	93.5	0.05 ± 0.01	1.533 ± 0.4
Hog Plum	Amra	*Carissa carandas*	72.0	0.03 ± 0.1	0.435 ± 0.07
Honeydew Melon	Chinar	*Cucumis melo*	91.9	0.03 ± 0.001	0.334 ± 0.04
Jackfruit	Kathal	*Artocarpus heterophyllus*	79.2	0.03 ± 0.04	0.824 ± 0.0
Karonda	Karamcha	*Carissa carandas*	88.5	0.1 ± 0.01	4.882 ± 0.0
Lakuch	Dewa	*Articarpus lakoocha*	72.0	0.03 ± 0.01	0.801 ± 0.08
Lichi	Litchu	*Litchi chinensis*	84.1	0.1 ± 0.0	0.236 ± 0.0
Mango	Aam	*Mangifera indica*	84.2	0.03 ± 0.01	0.429 ± 0.0
Palmyra fruit	Tal shash	*Borassus flabellifer*	91.6	0.03 ± 0.0	0.302 ± 0.0
Pineapple	Anarosh	*Ananus comosus*	87.3	0.03 ± 0.04	0.207 ± 0.2
Rose Apple	Golapjam	*Syzgium samarangense*	89.9	0.6 ± 0.01	1.53 ± 0.3
Sapota	Safeda	*Manikara zapota*	76.5	0.01 ± 0.0	0.712 ± 0.1
Wax Apple	Jamrul	*Syzgium aqueum*	91.4	0.01 ± 0.01	0.198 ± 0.7

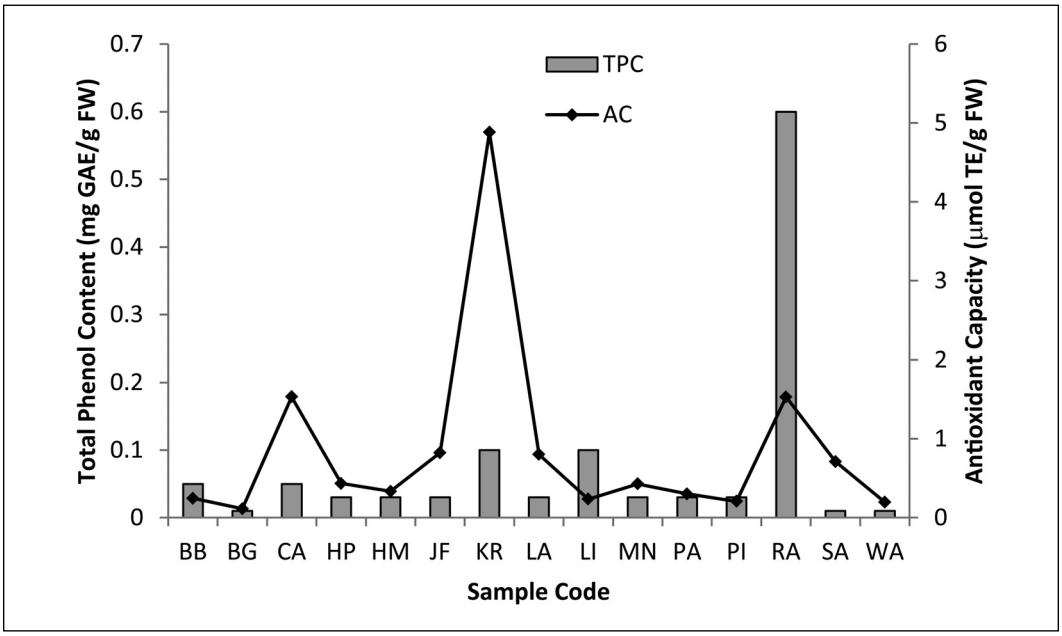

Figure 1. Correlation of total phenolic content and antioxidant capacity of fruits
Note: BB, Blackberry; BG, Burmese grape; CA, Carambola; HP, Hog Plum; HM, Honey Melon; JF, Jackfruit; KR, Karonda; LA, Lakuch; LI, Litchi; MN, Mango; PA, Palmyra fruit; PI, Pineapple; RA, Rose Apple; SA, Sapota; WA, Wax Apple.

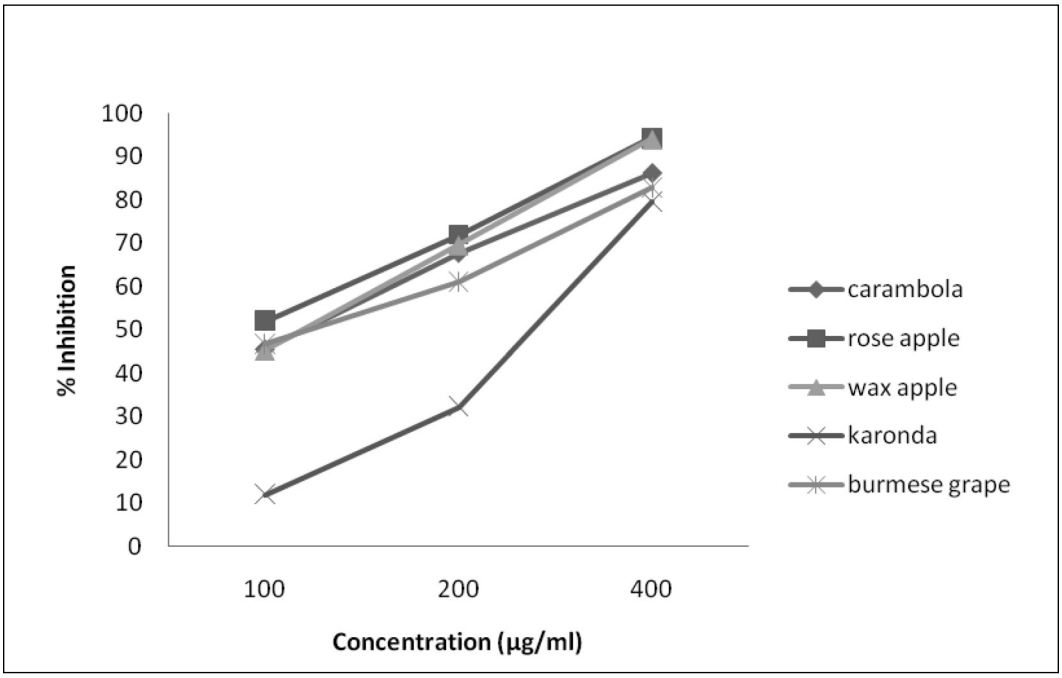

Figure 2. DPPH radical scavenging activity of selected seasonal fruits of Bangladesh

Table 2. Relationship between antioxidant capacity and total phenolic content of seasonal fruits of Bangladesh

Samples	*TPC(mg GAE/g FW)*	*TEAC(mol TE/g FW)*	R^2
Blackberry	0.05 ± 0.023	0.246± 0.04	0.778
Burmese grape	0.01 ± 0.01	0.113± 0.03	0.822
Carambola	0.05 ± 0.01	1.533± 0.4	1.00*
Hog Plum	0.03 ± 0.1	0.435±0.07	0.5
Honeydew Melon	0.03 ± 0.001	0.334±0.04	0.961
Jackfruit	0.03 ± 0.04	0.824±0.0	1.00*
Karonda	0.1 ± 0.01	4.882± 0.0	0.5
Lakuch	0.03 ± 0.01	0.801±0.08	1.00*
Lichi	0.1 ± 0.0	0.236± 0.0	1.00*
Mango	0.03 ± 0.01	0.429±0.0	1.00*
Palmyra fruit	0.03 ± 0.0	0.302± 0.0	0.5
Pineapple	0.03 ± 0.04	0.207±0.2	-1.00*
Rose Apple	0.6 ± 0.01	1.53±0.3	0.5
Sapota	0.01 ± 0.0	0.712± 0.1	0.404
Wax Apple	0.01 ± 0.01	0.198±0.7	1.00*

* Correlation is significant at $p<0.01$ level

The co-relationship between TPC and AC is presented in Table 2. Pearson's correlation coefficient showed that among the fifteen samples studied, all had a positive correlation while only one had a negative correlation. A linear relationship was observed between antioxidant capacity and total polyphenol content. The statistical analysis indicated a high and significant correlation between TPC and AC (R^2=0.9571) among four of the fruits namely *Manikara zapota*, *Artocarpus heterophyllus*, *Litchi chinensis* and *Articarpus lakoocha*.

DISCUSSION

Bangladesh abounds with a large variety of tropical and subtropical fruits. Almost all the major and minor fruits usually mature during summer (May-July), and are available in the market. A few fruits are available throughout the year. Fruits and vegetables are important for the daily diet as a source of micronutrients (vitamins and minerals), fibre and polyphenolic compounds which function as antioxidants (Jahan *et al.*, 2011).

The present study was undertaken to evaluate the hydrophilic antioxidant capacity, that is, radical scavenging activity and total phenolic content (TPC) of fifteen commonly consumed Bangladeshi fruits of summer season. The findings of the present study are a significant addition to the field of food science research in Bangladesh. Based on the frequency of consumption, the food items chosen for this study have shown differences in their total polyphenol contents and corresponding antioxidant capacities. The correlation between total polyphenol contents and antioxidant capacity has been widely studied in different foodstuffs such as fruit and vegetables (Klimczak *et al.*, 2007; Jayaprakasha, Girennavar & 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). It is known that only flavonoids with a certain structure and particular hydroxyl position in the molecule can donate proton and show radical scavenging activity (Mensor *et al.*, 2001; Hou *et al.*, 2003).

Total phenol content and radical scavenging activity of fifteen hydrophilic (or acidic aqueous acetone) extracts from commonly consumed local fruits were analysed in this study. *Syzgium samarangense*, a tropical fruit available in the summer season showed the highest total phenols and also high antioxidant capacity by DPPH, followed by *Litchi chinensis* and *Syzgium cumini*. Meanwhile, *Manikara zapota* showed much higher Trolox Equivalent Antioxidant Capacity (TEAC) with comparatively low TPC (0.01 mg GAE / g FW) compared to *Carissa carandas* (0.1 mg GAE / g FW). It is reported that the TEAC of an extract cannot be predicted on the basis of its phenolic content only, as it varies remarkably depending on its chemical structure, and thus requires proper characterisation of individual phenolic compounds (Statue-Garcia, Heionen & Frankel, 1997). This study found considerable variations in the TPC and TEAC of the analysed fruits. The variation may be due to several reasons. Content of phenolic compounds and the antioxidant capacity are partly dependent on the colour of the variety of the vegetables and on the water content (Pavel, Borivoj & Vlastimil, 2006). In fruits, TPC is higher in green and immature fruits and gradually decreases with the ripening process. Dark green leafy vegetables like ipomoea and mint exhibit high values of TEAC, and very low values are found in watery samples such as radish, brinjal, cauliflower and cabbage (Hossain *et. al.*, 2011). 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 polyophenol activity (Cheynier, 2005).

Compared to commonly consumed local vegetables (Hossain *et al.*, 2011), locally grown tropical fruits contain much lower amounts of TPC. The most recent study suggests commonly consumed local vegetables and herbs contain higher amounts of polyphenols and corresponding TEAC which could serve as important sources of antioxidants that prevent cellular and tissue damage by scavenging of free radicals (Hossain *et al.*, 2011). On the other hand, lack of information regarding cooking effects on TPC and ultimate TEAC of commonly consumed local vegetables does not reflect bioavailability of TPC and antioxidant effects. Although, the tropical fruits analysed in the present study contained low amounts of TPC, fruits are usually consumed raw and could be a greater source of available polyphenols and antioxidants.

However, the findings of the present study revealed that the hydrophilic fraction of *Averrhoea carambola, Syzgium samarangense* and *Syzgium aqueum* contained sufficient amounts of TPC which can function as antioxidants in the local diet through their radical scavenging activity.

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