International Food Research Journal 23(6): 2399-2404 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant activity and total phenolic content of some indigenous fruits of Bangladesh

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Article history

Received: 11 January 2015
Received in revised form: 18 March 2016
Accepted: 24 March 2016

Keywords

Citrus fruits
Antioxidant activity
Phenolic content
Dietary intake of phenolics

Abstract

The extracts of five species of citrus fruits namely tamarind, star fruit, Indian gooseberry, ambarella and satkara were examined for their potential sources of natural polyphenols and antioxidant activities. Total phenolic contents were determined using the Folin-Ciocalteau reagent method and antioxidant activity was determined according to the DPPH method. Tamarind had the highest total phenolic content (52.23±0.91 mg of Gallic acid/g dry weight) followed by satkara (45.62±.33 mg GAE/ml), Indian gooseberry (39.38±2.15 mg GAE/ g), star fruit (31.76±1.45 mg GAE/ g) and ambarella (27.08±1.66 mg GAE/ g). The daily intake of total phenolics in the Bangladeshi diet was estimated of those fruits and tamarind contributed the highest amount of total phenolics (629.37 mg GAE g^{-1} fresh product) followed by Satkara (549.72 mg GAE/g fresh product), Indian gooseberry (474.53 mg GAE/g fresh product), star fruit (326 mg GAE/ g fresh product) and Ambarella (326.31 mg GAE/g fresh product). Tamarind (concentration 5000 µg/ml) exhibited highest scavenging effects (95.05±1.40%) followed by Satkara (94.21±.12%), Indian gooseberry (93.35±2.10%), star fruit (81.26±1.10%) and ambarella (74.12±1.44%). A linear positive relationship existed between the antioxidant activity and total phenolic content of the citrus fruit extracts (R^2 =0.82). The extracts of citrus fruits represent as a significant source of phenolic content with potential prophylactic properties for the human body.

Introduction

The importance of dietary factors on health status has long been recognized but it has recently become more important when epidemiological and clinical studies provided a clearer insight on the chemical and physiological mechanisms of the effects of bioactive foods on human health (Shahidi, 2009). Phytophenolics play a crucial function in health promotion and disease prevention by mechanisms related to cell differentiation, maintenance of DNA repair, and inhibition of N-nitrosamine formation and change of estrogen metabolism (Shahidi, 2004). Free radical scavenging and metal chelation activities are the major mechanism of the anti-oxidative effect of phenolics in functional foods. Reactive oxygen species (ROS), such as the superoxide radical, hydrogen peroxide, hypochlorous acid and the hydroxyl radical are natural byproducts of the normal metabolism of oxygen in living organisms with important roles in cell signaling activity (Aruoma and Cuppette, 1997; Cavas and Yurdakoc, 2005). However, Diseases such as cancer, stroke, diabetes and degenerative processes associated with ageing are propagated by significant damage of cell structure, break down of membrane protein and DNA mutation, which is caused by bio molecular oxidation directly or indirectly (Ames 1983; Wiseman, 1996). Thus, antioxidants act not only as inhibitors of lipid peroxidation for food protection, but also as a defense mechanism of living cells against oxidative damage (Halliwell, 1991). Moreover, Antioxidants play vital role in preventing the destruction of cells (Slonim *et al*., 1983; Murthy *et al*., 1992) and also prevent or inhibit oxidation processes in the human body and food products (Diaz *et al*., 1997).

Citrus fruits, besides providing an ample supply of vitamin C, folic acid, potassium and pectin, contain a host of active phytochemicals that can protect health. Polyphenols including flavonoids and phenolic acids are secondary metabolites that constitute one of the most widely occurring groups of phytochemicals. These compounds have considerable physiological and morphological roles in plants (Scalbert, 1993). Various origins of Citrus species have been considered for their phenolic constituents, antioxidant activities and antimicrobial properties (Proteggente *et al*., 2003; Gorinstein *et al*., 2004; Anagnostopoulou *et*

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al., 2006; Guimarães *et al*., 2009; Rahman *et al*., 2015a). Promising biological properties including antiatherogenic, anti-inflammatory and antitumor activity, inhibition of blood clots and strong antioxidant activity were exhibited by Citrus fruits (Middleton and Kandaswami, 1994; Samman *et al*., 1996; Montanari *et al*., 1998). Citrus is consumed mostly as fresh produce or juice product. Thus, citrus varieties could be important sources of dietary polyphenolic antioxidant compounds that may have potential benefits on health and disease management. After conducting frequent studies over fruits and vegetable intake and cancer development, there is an unanimity that consuming these citrus fruits has a protective effect (Lampe, 1999). The objectives of this study are to investigate the radical scavenging activity of four local available citrus fruits, to estimate the content of total phenolics and dietary intake by this foodstuff and also to explore relationship between phenolic content and antioxidant activity.

Materials and Methods

Plant material

Citrus fruits namely tamarind *(Tamarindas indica)*, star fruit *(Averrhoa carambola*), Indian gooseberry *(Phyllanthus emblica*), ambarella *(Spondias dulcis)*, satkora *(Citrus macroptera)* were collected from local market of Sylhet region in Bangladesh. All of the fruits were washed under running tap water and remained in open air for several hours for further processing. At least 10 pieces of each fruits were taken while the peels were carefully removed with a manual peeler and cut into small pieces. All of these fruits except satkara were kept in Oven (drier) at 40ºC for 14 days.

Extraction

The extraction procedure was carried out based on a method in the literature (Kosar *et al.*, 2007). About 10 g ground samples were extracted in 70% aqueous methanol for 1h using a shaker. The residues, separated by filtering through Whatman filter paper, were re-extracted twice with the fresh solvent. The three extracts were pooled and then methanol was distilled off at 40ºC using a rotary vacuum evaporator. For the extraction of satkara, edible portion of the fruits, after removal of peel, was separated carefully where Juice was extracted by a Juicer and was centrifuged at 15000 RPM for 20 min at 40ºC. The supernatant was collected and preserved at 20ºC until further use. All analyses were carried out repeatedly, at least three times.

Estimation of total phenolic intake

According to a study (Chun *et al*., 2005), the total phenolic intake obtained from daily food consumption was estimated from food consumption and total phenolic content as:

Total phenolic intake of vegetables and fruits (mg GAE person^{-1} day^{-1}) = $\sum C_i P_i$

C_i = Food consumption of the selected fruit or vegetable (g food person^{-1} day^{-1}) and

P_i = Total phenolics for the selected commodity (mg GAE g food^{-1}).

Total phenolic content

Total phenolic content was assayed by Folin–Ciocaltue method (Velioglu *et al*., 1998). In this method, 1.5ml of deionized water was added to 1.0 ml aliquots of samples in a tube, followed by adding 0.5 ml of 0.1 M Folin-Ciocaltue reagent and the content was mixed thoroughly. A blank was prepared using 1.0 ml of 70% methanol without addition of sample. After 1 min, 1mL of 20% sodium carbonate was added to the mixture of the sample. The tube contents were mixed by vortex mixer before keeping it in incubation for 30 min in a water- bath set at 37ºC. The absorbance of the blue coloration formed was measured at 750 NM against the blank standard. Total phenolics were calculated in respect of Gallic acid standard. Results are expressed in mg of Gallic acid/g fresh weight of plant material.

Determination of antioxidant activity

The DPPH free radical scavenging activity of fruits was determined using Spectrophotometer according to the method described by Yen and Duh (1995). A 0.1 mm Solution of DPPH was prepared in methanol. The initial observance of the DPPH was measured at 515 nm. About 2.0 ml of methanol solution of each extract at different concentration (5, 0.5, 0.05, and 0.005 mg ml^{-1} were mixed with 2.0 ml of 0.16 ml DPPH methanol solution. The mixture was kept in a vortex for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 517 nm. All samples were assayed in triplicate. Various concentrations of 1, 1 diphenyl-2- picryl hydroxyl (DPPH), Gallic acid in 70% methanol were used as standards, and 70% methanol was used as the control. The ability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = [1 - ($A_{sample} - A_{sample\ blank}$) / $A_{control}$] × 100

Table 1. Antioxidant activity of methanolic extracts at different concentration

Samples	Scavenging effect (%)			
	5000(µg/ml)	500 (µg/ml)	50 (µg/ml)	5 (µg/ml)
Ambarella	74.12±1.44	65.64±2.03	62.71±0.12	61.11±0.05
Tamarind	95.05±1.40	67.24±2.54	55.13±1.23	47.51±2.01
Indian gooseberry	93.35±2.10	63.74±1.95	57.27±1.02	52.13±0.35
Star fruit	81.26±1.10	64.44±3.21	63.13±1.01	61.06±1.15
Satkara	94.21±.12	92.56±.89	56.85±1.24	63.42±18

*Mean ± standard deviation

Where, $A_{control}$ is the absorbance of the control (DPPH solution without sample); A_{sample} is the absorbance of the test sample (DPPH solution plus test sample) and A_{sample} blank is the absorbance of the sample only (sample without DPPH solution). Gallic acid and ascorbic acid were used as positive controls.

Statistical analysis

Data obtained from experiments, subjected to analysis of variance and means, were compared and significant difference was determined at $P<0.05$.

Result and Discussion

Scavenging effect of selected fruits

Antioxidant activities of the methanolic extract of five different local fruits are evaluated by free radical scavenging assay along with galic and ascorbic acid shown in the Table 1. Highest scavenging effects were shown in tamarind extracts (5000 µg/ml) which ranges from 95% to 97%. In satkara, range of scavenging effect was 94-95%, whereas in case of Indian gooseberry (5000 µg/ml), the range was close to satkara (93 to 96%). Ambarella and star fruit (concentration of 5000 µg/ml) showed lower scavenging effect which was in the range of 76-83%. With a view to comparing the values, Scavenging effect of star fruit was 75.00% that slightly differs from another study (Lim *et al*., 2013). Added to the Citrus fruits, another study conducted in Bangladesh showed that methanolic extract of fresh green mango at 50mg/ml has shown scavenging activity 98.72 ± 0.88% (Rahman *et al.*, 2015b). The result of the present study was slightly different from other due to the difference in the determination method of antioxidant activity and some other environmental conditions related to the sample. The stable free radical DPPH has been widely used to test the free radical scavenging ability of various dietary antioxidants (Brand-Williams *et al.*, 1995). The anti-oxidative as well as the scavenging potential of the extract is directly proportional to the DPPH reduction. The more antioxidants are found in the extract, the more DPPH reduction will occur. Higher DPPH reduction is associated with greater scavenging potential. Since all extracts showed dose dependent DPPH scavenging activity, these extracts exert pronounced and significant free radical scavenging activity. The result of our study indicates a strong relationship between phenolic content and DPPH scavenging as well as antioxidant activities, suggesting that the phenolic compounds are probably responsible for the antioxidant activity.

Total phenolic content and dietary intake

A standard curve was used for the measurement of total phenolic content. A standard curve was made by using Gallic acid at different concentration (0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, 1 ppm). The results from this study showed that the total phenolic content of selected fruits varied and ranged from 52 to 27 mg GAE/ g DW). The choice of extracting solvents mainly depends on the polarity of the compounds of interest. In the present study, methanol was used, which resulted in higher extraction yields of phenolic compounds due to high polarity. The highest total phenolic levels were detected in tamarind was 50.23 ±. 91 which was higher than those reported in Malaysia (2.14 ± 0.05 mg GAE) /100g) (Khairunnuur *et al*., 2009) but lower than those reported in Brasil (361. 42 mg GAE)/100g) (Reis *et al*., 2013). TPC in Indian gooseberry was 39.38± 2.15 which was higher than those reported in India (1285.63±0.71 µg GAE) /100g) (Ali *et al*., 2010) but lower than those reported in Thailand (130.8 ±.34 mg GAE/ g DW) (Mayachiew and Devahastin, 2007). In star fruit TPC was detected 31.76 ± 1.45 mg GAE/ g DW which was higher than those reported in Malaysia (16.18 ± 1.40 mg GAE/ g DW) (Lim and Lee, 2013) but lower than those reported in India (112±20 16.18 ± 1.40 mg GAE/ g DW) (Das, 2012). In ambarella TPC was 27.08 ± 1.66 which was lower than reported in Bangladesh (659.74 ± 0.97 mg GAE/ g DW) (Islam *et al*., 2013). Total phenolic content of satkara was 45.28± 0.33. There was no study about satkara for which we compare our present study. Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kahkonen *et al.*, 1999). Phenolic acids have repeatedly been implicated as natural

Table 2. Total Phenolic content and dietary intake

Sample	Total Phenolic content (ppm)	Food Consumption Kg year^{-1}	Per capita g day^{-1}	Total phenol Mg GAE g^{-}fresh product
Indian gooseberry	39.38±2.15			474.53
Kambarellanga	31.76±1.45	4.4	12.05	382.71
Tamarind	52.23±0.91			629.37
Ambarella	27.08±1.66			326.31
Satkara	45.62±.33			549.72

*Data expressed in mg GAE/ g on dry weight basis (Food Consumption Per capita, Calculated as Chun *et al.* 2005)

antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, Ferulic acid, and vanillic acid are widely distributed in the plant kingdom (Larson, 1988). Again, per capita consumption of citrus fruit is 4.4 kg (Joshi *et al*., 2007). Table 2 shows per capita consumption and the contents of total phenolics in five indigenous fruits. Higher dietary intake was 629 mg GAE g^{-1} fresh product through tamarind and lowest was 326.31 through ambarella. Contribution of citrus fruits to provide polyphenols by dietary intake were tamarind > satkara > Indian gooseberry >star fruit > ambarella. The total intake of polyphenol was substantial. For evaluation of the intake of total polyphenols, no comparable data from other studies are available. According to the study by Saura-Calixto *et al*., 2007, where polyphenols were analyzed using unspecific spectrophotometric methods, the mean daily intake of total polyphenols ranged from 2600 to 3000 mg. These figures are much higher than those obtained here, probably due to analytical differences. A Study (Manach *et al*., 2004), estimated that the total polyphenol intake probably reaches 1 g/d in people who eat several servings of fruits and vegetables daily. This estimation was lower to the intake obtained in our present study.

Correlation

With a view to rationalizing the antioxidant potential of the citrus fruit extracts in terms of their phytophenolic constituents, linear regression plots were generated and the Pearson correlation co-efficient were calculated (Figure 1). A striking correlation between total phenolics and antioxidant capacity of citrus fruit extracts was noted (DPPH r^2 = 0.82). Antioxidant activity (free radical scavenging activity) was significantly positively correlated ($p < 0.05$) with TPC. Extracts with the highest phenolic contents had the highest antioxidant potential in all assayed system whilst extracts characterized by lower total phenolic levels exhibited a poor antioxidant activity. For instance, a tamarind which had highest phenolic content (52.23 mg GAE / g of dry weight) showed high scavenging activity (95.05%) and in Ambarella which had lowest

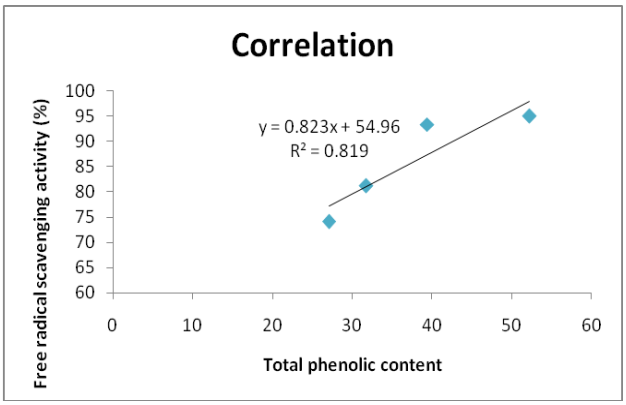

Figure 1. Correlation between free radical scavenging activity and total phenolic compounds

phenolic content (27.08 mg GAE/g of dry weight) showed the lowest scavenging effect (74.12%). This results are consistent with a previous study which revealed that the content of phenolics in the medicinal and aromatic plant extracts correlates (r^2 = 0.84) significantly with their anti-radical activity as measured by a 2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) assay (Miliauskas *et. al*., 2004). Another study shows that the phenolic compounds contribute greatly towards antioxidant activity than that of ascorbic acid or carotenoids (Lim *et al*., 2007; Shofian and Hamid, 2011). According to the report of Prior *et al*. 2005, antioxidant activity in DPPH assay may be exerted through either hydrogen atom transfer or single electron transfer mechanism.

Conclusion

The present study showed that the fruits of tamarind, star fruit, Indian gooseberry, ambarella and satkara are strong radical scavengers and can be considered as good sources of natural antioxidants. In Bangladesh, average consumption of fruit contributes a large amount of antioxidants in the diet. However, due to the diversity and complexity of the natural mixtures of phenolic compounds in the citrus fruit extracts it is quite difficult to characterize every compound and compare their antioxidant activities. Each fruit generally contains various phenolic compounds and each of these compounds possesses

differing amounts of antioxidant activity. The present study reveals that consuming high amount of citrus fruits can provide considerable amount of antioxidant that acts as anti-carcinogenic agent diminishing free radicals which are produced in our body.

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