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Original Article

Antioxidant Activity of Some Common Seasonal Fruits of Bangladesh

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ABSTRACT: Natural antioxidants are beneficial for health promoting properties due to its ability to neutralize oxygen contain harmful, DNA-damaging radicals. Fruits and vegetables are good sources of antioxidants. In this study, Bangladeshi seasonal fruits wood apple, jujube, hog plum and elephant apple juice were analyzed for total phenolic content (TPC), total flavonoid content (TFC) and DPPH assay, which are the parameters for determining antioxidant capacity. The study suggests wood apple is a rich source of antioxidants whereas hog plum is a poor source. In addition, jujube and elephant apple were found to have the antioxidant amount equivalent to apple. Moreover, wood apple can be used commercially as a stored antioxidant source.

KEYWORDS: Antioxidant, wood apple, jujube, elephant apple, hog plum, total phenolic content (TPC), total flavonoid content (TFC), DPPH assay, IC_{50}.

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INTRODUCTION

The local fruits of Bangladesh can serve as an ideal source of dietary antioxidants. Due to seasonal availability and low cost, these fruits are always within the reach of the normal populace. Consumption of antioxidant rich fruits can play a protective role against a number of seasonal diseases as well as improve general well being.

Wood apple (*Aegle marmelos*) belongs to the Rutaceae family, a native fruit of south-east Asia, namely India, Sri Lanka, Bangladesh and Malaysia. The fruit is well known for its medicinal use and aromatic oil production in Ayurveda. Wood apple is rich in macronutrients such as carbohydrate, protein and fibre as well as micronutrients such as Vitamin C.[1] In Ayurveda, Wood Apple juice is used to treat diarrhea, dysentery, peptic ulcer and diabetics. Wood apple has also been reported to contain tannins, which work as an antibacterial agent.[2]

Elephant apple (*Dillenia indica*) belongs to the Dilleniaceae family and is a tropical fruit native of South Asia, especially Bangladesh. Elephant apple is rich in carbohydrates, tannins and flavonoids, which make it medicinally important.[3] Generally, this fruit cannot be consumed directly rather it is more popular to general people in the form of pickle and jams.

Chinese date or jujube is the common name for *Ziziphus jujube* which belongs to the Rhamnaceae family. Jujube is rich in carbohydrate, Vitamin B and C, as well as minerals such as calcium, magnesium, phosphorus and potassium. Its high flavonoids content makes it ideal to fight against reactive oxygen species. The jujube is reported to have antifungal, antibacterial, immunosuppressant and neuro-protective properties, which makes it one of the most important Bangladeshi seasonal fruits for research.[4]

Hog plum (*Spondias mombin*) belongs to the Anacardiaceae family, native to the South America, Africa, Indonesia, Sri Lanka, India and Bangladesh as a summer fruit. Hog plum has high concentration of carbohydrate, vitamins and minerals, as well as micronutrients such as vitamin C, flavonoids, tannins and saponins providing it antifungal, antibacterial and analgesic properties.[5]

A number of researches have been done regarding the determination of nutritional and medicinal properties of the four above mentioned fruits. Some of these fruits are analyzed for their antioxidant content by international research groups. The objective of the present study was to relate the content of total phenolics and flavonoids to the antioxidant potential of the four fresh and stored seasonal fruits of Bangladeshi variants.

MATERIALS AND METHODS

Plant materials

Four seasonal fruits; Wood Apple, Elephant Apple, Jujube, and Hog plum were purchased from 3 different vendors. The fruits were kept in zip lock bags and stored at 20° C prior to extractions.

Preparation of fruit extracts

Elephant Apple, Jujube and Hog Plum peels were cut off with a sharp knife to ensure minimum loss of pulp. In case of Wood Apple the coarse peel was cracked and the pulp was scraped out with the help of a metal spatula. Seeds from all fruit samples were disposed. The fruit extracts were prepared by adding equal weight of fruit pulp in 100ml of water followed by mixing in thoroughly in a kitchen grade blender. For each fruit samples 100 ml of fiber free liquid extract was collected by passing the blended mixture through a muslin sieve. Photochemical analyses were done immediately with 50 ml of fiber free fruit extracts and rest of it was stored at 4°C for one week to determine the impact of long time refrigeration in different photochemical parameters.

Estimation of total phenolic content

Folin-Ciocalteu (FC) method was implied to estimate the phenolic content in the fruit juice samples. 100µl of each fruit juice sample was mixed with 900µl of methanol. To this, 5ml of FC reagent was added followed by 4ml of 20% Na_2CO_3 and incubated for 30 minutes[6]. The absorbance of the reaction mixture was read at 765nm[7]. A standard curve was plotted using different concentrations of Gallic acid (Standard, 0-100µg/ml). Total phenolic content was estimated as microgram Gallic acid equivalents (GAE)/ml of fruit juice.

Estimation of total flavonoid content

A modified colorimetric method of Wiosky and Salatino[8] was used to measure the total flavonoid contents of fruit juice samples. A 100µl of each fruit juice sample was mixed with 900µl of methanol. To these 10ml of distilled water was added to dilute the samples. At 0, 5, and 6 minutes, 0.3ml 5% $NaNO_2$, 0.3ml of 10% NH_4Cl, and 2 ml of 1M NaOH were added to the mixture respectively. Immediately the mixtures were diluted to volume with the addition of 2.4ml distilled water per sample mixture and thoroughly mixed. The reaction mixtures were left to stand at room temperature for 1 hr.[9] The absorbance of the reaction mixtures were measured at 415nm. A standard curve was plotted using different concentrations of Quercetin hydrate: standard for bioflavonoid (Standard, 0-250µg/ml). Total flavonoid content was estimated as mg of Quercetin hydrate equivalents (QHE)/ml of fruit juice.

Antioxidant activity of fruit juice samples by DPPH free radical scavenging assay

The radical scavenging ability of fruit extract samples was carried out on the basis of the radical scavenging affect on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The fruit extract samples (1.5 to 100µl/ml) were prepared in methanol. In clean and labeled test tubes, 2ml of DPPH solution (0.004% in methanol) was mixed with 1ml different concentrations of fruit extract samples separately. The tubes were incubated at room temperature in dark for 30 minutes and the OD was measured at 517 nm using a spectrophotometer. The absorbance of the DPPH control (1ml of methanol was added instead of fruit extract) was also measured. The scavenging activity of the fruit extract samples were calculated using the formula; Scavenging activity (%) = [(A-B)/A] × 100, where A is the absorbance of DPPH (control), and B is the absorbance of DPPH and fruit juice concentration.[10] Ascorbic acid was used as positive control. Inhibiting concentration 50 (IC_{50}) value is the concentration of sample required to scavenge 50% of DPPH free radical and was calculated from the plotted graph of radical scavenging activity against the concentration of fruit sample extract.

RESULTS AND DISCUSSION

Determination of total phenolic contents (TPC)

A linear calibration curve of gallic acid in the range of 0 to 50 µg/ml with coefficient determination (r^2) value of 0.977 was obtained (Figure 1). TPC of the four different fruit pulp extracts – wood apple, jujube, hog plum and elephant apple for both stored and fresh samples is demonstrated in Table 1. The TPC amount of fresh samples has been observed to be higher than the stored samples. Moreover, among the four fruit extracts studied wood apple has the highest amount of TPC in both fresh and stored samples.

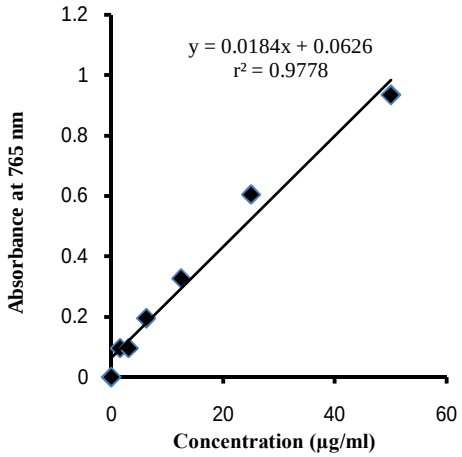

Figure 1. Calibration curve of standard gallic acid for determination of the TPC

The candidate phenolic antioxidants in foods include flavonoids, anthrocyanins, catechins, chalcones, hydroxy-benzoic and hydroxy-cinammic acids. Many of these are found to be present in fruit juices.[11] The total phenolic contents of the fruit extracts was conducted by Folin-Ciocalteu method which is used to determine all the phenolic acids both bound and free

phenols of plant extracts.[12] The TPC experiment was optimized by using apple (*Malus domestica*) which showed the value of 0.65 mg GAE/ml equivalent of fruit pulp extract, which is in line with the value found by Savatovic *et al.*[13] Previously Gaffar *et al.*[14] has shown a direct relation between antioxidant activity and TPC contents of fruits. Therefore, fruit juices containing high phenolic contents have been found to exert high antioxidant potential.

Wood apple has been found to contain a significantly higher amount of TPC compared to other fruits analyzed including apple, which indicates it as a rich source of antioxidant. The similar amount of TPC in wood apple was also reported by Sonawane *et al.*[15] Moreover, Jujube has approximately 45% more TPC than apple suggesting that jujube is also a rich source of antioxidant, whereas Elephant apple has similar level of antioxidant activity as apple. Comparison between fresh and stored juice of analyzed fruits revealed that the decrease in TPC is lowest for Wood apple (19.5%) followed by Hog plum, Jujube, and Elephant apple indicating stored wood apple and hog plum juice can be used as an antioxidant source.

Table 1. Total phenolic content of extracts from seasonal fruit samples.

Sample Name	TPC (mg of GAE/ml of fruit pulp extract)		
	Fresh	Stored at Low Temp	TPC decrease (%)
Wood Apple	2.00 ± 0.06	1.61 ± 0.03	19.5
Jujube	0.94 ± 0.04	0.40 ± 0.02	57.4
Hog plum	0.18 ± 0.02	0.11 ± 0.01	38.9
Elephant apple	0.64 ± 0.04	0.21 ± 0.05	67.0

Table 2. Total flavonoid content of samples.

Sample Name	TFC (mg of QE/ ml of fruit pulp extract)	
	Fresh	Stored
Wood Apple	6.44 ± 0.06	3.56 ± 0.04
Jujube	0.52 ± 0.03	0.44 ± 0.05
Hog plum	0.27 ± 0.01	0.12 ± 0.03
Elephant apple	0.40 ± 0.03	0.36 ± 0.05

Determination of total flavonoid content

A linear calibration curve of Quercetin hydrate in the range of 0 to 250 µg/ml with coefficient determination (r^2) value of 0.974 was obtained (Figure 2). TFC of four different fruit juice for both stored and fresh samples is provided in Table 2. Like TPC the TFC of fresh samples are higher than the stored samples. Flavonoids are one of the major plant phenolics with potent antioxidant activities. Generally phenolic acids and flavonoids are the major contributors of antioxidant activity. Increase in TFC level in fruit juice samples also contributes to increase the TPC level in respective samples.[16] The TFC of four fruit juice samples have also showed similar relationship with TPC content (TPC). Total flavonoid content of four fresh and stored fruit extracts have been compiled in table 2. Similar to the TPC content wood apple fresh and stored extracts have higher TFC content.

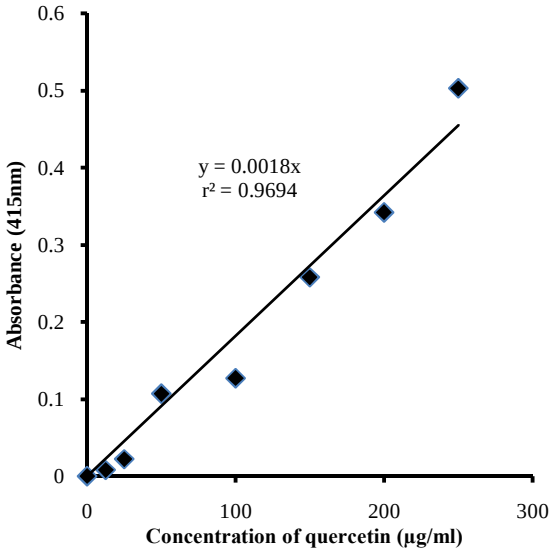

Figure 2. Calibration curve of Quercetin hydrate for total flavonoid content estimation.

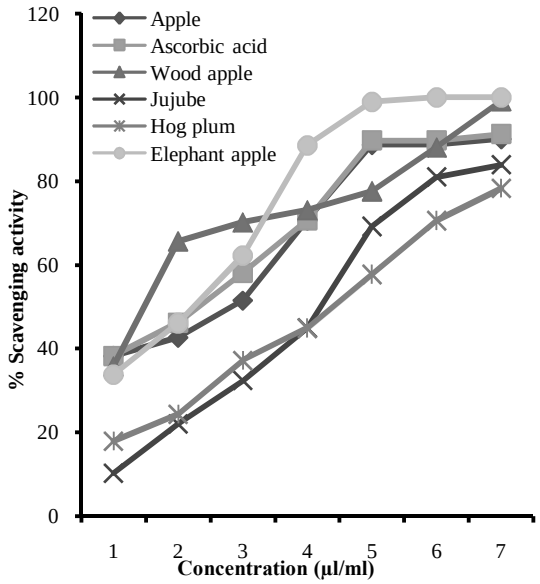

Figure 3. The free radical scavenging activity of the different methanol extracts with DPPH.

Scavenging activity on 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical

Activity on scavenging DPPH radical is a well-established and relatively quick method. Stable DPPH free radical shows strong absorbance at 517nm which is effectively scavenged by antioxidants. DPPH radical accepts an electron or hydrogen radical from antioxidants present in the samples to be analyzed and become a stable diamagnetic molecule which can be visualized as a noticeable discoloration from purple to yellow.[17]

The percentage scavenging activity of the analyzed fruit extracts were determined using different concentrations (2-100 µl/ml) which have been taken to compare with the standard ascorbic acid (Figure 3). The DPPH radical scavenging capacity in this study has been reported after 30 minutes of incubation for all samples analyzed. Wood apple methanolic extract is

the only sample which showed observable antioxidant activity in all different concentrations analyzed. Wood apple has been observed to contain the highest scavenging activity both in lower and higher concentration.

Extent of DPPH radical scavenged was determined by increase in intensity of violet color in the form of IC_{50} values, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in specified time period. IC_{50} of the four methanolic extracts along with standards ascorbic acid and positive control (apple) has been shown in Table 3. Elephant apple showed the lowest and hog plum showed the highest IC_{50} value.

Table 3. Half inhibitory concentrations (IC_{50}) of the standard and different samples.

Sample Name	IC50 (µl/ml extract in methanol)
Ascorbic acid	8.27
Apple	10.74
Wood Apple	3.72
Elephant Apple	2.15
Jujube	33.88
Hog plum	38.08

From the present study we can conclude that, wood apple is a great source of antioxidant based on both TPC and TFC values along with DPPH scavenging activity and IC_{50} value. In comparison to apple, wood apple can be considered a better source of antioxidant (2X higher). Moreover wood apple has fewer effects on antioxidant quantity on storage which makes it commercially important fruit in jam, pickle and juice production.

Jujube and elephant apple have shown antioxidant level closer to apple. Jujube showed lower scavenging activity then elephant apple. Both of these fruits should be consumed freshly due to loss of noticeable antioxidant activity on storage. On the other hand hog plum showed the lowest source of antioxidant among the four seasonal fruits tested, suggesting to be a poor source of antioxidant which may have other nutritional values.

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