

*Original Research*

# Antioxidant Activity and Bioactive Compounds of Fruits Available in Bangladesh

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## ABSTRACT

**Background:** The complex problems of different chronic degenerative diseases are spreading in human health across the globe. To combat this problem, fruits are the effective gear proved by many epidemiological studies. The aim of the study was to determine the antioxidant activity and three bioactive compounds like total flavonoids, total polyphenols and total anthocyanins in 23 species of fruits available in Bangladeshi market.

**Methods:** Antioxidant activity (AOA) and bioactive compounds of fruits were determined by UV-visible spectroscopy. Antioxidant activity was undertaken by DPPH scavenging assay, total polyphenol content (TPC) was measured by folin-ciocalteu method and total flavonoid content (TFC) content was measured by aluminium chloride colorimetric method.

**Results:** Among 23 species of fruits, AOA was higher in Katajamin (303 mg/100 g) followed by Dragon Fruits (171 mg/100 g), Guava (131 mg/100 g), Bael (130 mg/100 g) and average value was 125 mg/100 g of extract. TPC was varied from 10 to 305 mg GAE/100 g and average value was 87 mg GAE/100 g. Mango (305 mg GAE/100 g) showed higher TPC, followed by Pome Granate (222 mg GAE/100 g), Apple (207 mg GAE/100 g), Katajamin (141 mg GAE/100 g) and Bael (130 mg GAE/100 g). Again, TFC was ranged from Ashari (06±3.94 mg QE/g) to Tomato (84±13.69 mg QE/g) and the average TFC was 37 mg QE/g. In addition, total anthocyanin content (TAC) was highest in Mango (142±3.46 mg TA/100 g) and lower in Ashari (02±0.74 mg TA/100 g). There was a significant correlation ( $p \leq 0.05$ ) between antioxidant activity and bioactive compounds and among the bioactive compounds.

**Conclusion:** Important messages from this study are, majority of the rare fruits are the important sources of antioxidant and bioactive compounds which helps to reduce the risk of chronic degenerative diseases and can be used in food processing industry for nutraceutical product development.

**Keywords:** Antioxidants; flavonoid; polyphenol; anthocyanin; degenerative diseases

## 1. INTRODUCTION

Nowadays, doctors and dietitian recommend people to consume fruits due to their attractive colors, flavors,

energy, vitamins, minerals, antioxidants and rich sources of bioactive compounds.<sup>(1,2)</sup> So, consumption of fruits has gained an increasing amount of attention to the maintenance of good health.<sup>(3,4)</sup> Current life style, globalization and enormous changes in food habit causes over production of free radicals and reactive oxygen species in human body which damage biomolecules and involves in the onset of degenerative diseases like aging, Parkinson's and Alzheimer's diseases that cause death and disability to millions of people.<sup>(5)</sup> Consumption of fruits have protective effects against degenerative and aforesaid diseases due to the presence of several functional components like vitamins, antioxidants, flavonoids, anthocyanins and their phenolics compounds.<sup>(6a)</sup> These phytochemicals consumed through fresh fruits or their derived products have been suggested to have a wide variety of biological functions including antioxidant, antiinflammation, antimutagenicity, anti-carcinogenicity and anti-aging to human health. Therefore, fruits play an important role both economically and nutritionally through their consumption.<sup>(7,8)</sup>

Fruits are the pioneer source of natural antioxidant and more than 170 antioxidants have been reported in the current literature.<sup>(9)</sup> Metabolic processes of the body spontaneously generate free radicals and reactive oxygen species (ROS) by enzymatic and non-enzymatic reactions which damage living tissues even genetic materials like- DNA, RNA, other proteins and induced age-related diseases.<sup>(10,11)</sup> Fruits contain different antioxidant compounds like- carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione and minerals which scavenge these free radicals and ROS and prevent oxidative damage of the body.<sup>(12,13)</sup> Fruits have attracted interest most and consumption is increasing in domestic and international markets due to high potentially, safety and therapeutic effects.<sup>(14)</sup> A comprehensive investigation on antioxidant activity of fruits was systematically reviewed in the current study.

Bioactive compounds are phytochemicals produced from plants as secondary metabolites, have pharmacological and toxicological effects in body and resulting in the promotion of better health.<sup>(15)</sup> Fruits are the major source of bioactive compounds like polyphenols, carotenoids, anthocyanins, glucosinolates, vitamin C and tocopherols.<sup>(16,17)</sup> They are accumulated in skin and pulp of the fruits and varied widely by species, environment factors, maturity, genetic factors, pre- and

post-harvest treatment of the fruits.<sup>(18)</sup> These compounds act an antioxidant, anticarcinogenic, anti-inflammatory and antimicrobial properties and provide health benefits beyond the basic nutritional value.<sup>(19)</sup> Fruits and vegetables are the rich source of polyphenols and flavonoids, and their possible role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases.<sup>(6b)</sup>

Polyphenols are the important group of natural antioxidants comprised of benzene ring with one or more hydroxyl groups. They are the secondary metabolites of plant kingdom with vast structure and functions. They exhibit dramatic functions including hypolipidemic, antioxidative, anti-carcinogenic, antiproliferative, and anti-inflammatory effects and protect cell from oxidative damage in human body.<sup>(20,21)</sup> Again, flavonoids are another important group of bioactive compounds possess the basic flavan skeleton- a 15-carbon phenylpropanoid and subdivided into 6 groups like isoflavonoids, flavanones, flavanols, flavonols, flavones and anthocyanidins. They are widely distributed in fruits and have the both antioxidative and pro-oxidative functions in human body. Flavonoids are also possessed anticancer effects, anti-inflammatory and suppress cancer cell proliferation in body.<sup>(22)</sup> Anthocyanins are the water soluble pigments accumulated in vacuoles and protecting plants and fruits from UV-radiation. They play role as antioxidants and protecting body from cold stress, effects of high UV-radiation, arthritis and cancer.<sup>(23)</sup> A special attention has paid towards fruits due to the presence of these bioactive phytochemicals.

In addition, dietary intake of fruits help displaces fatty and sugar rich foods which cause chronic disease in human body. So, we should increase the consumption of fruits with direct or processed form. Over the last decade, a number of studies have investigated on nutritional composition, microbial quality and phytochemicals of fruits in Bangladesh. But, a clear summary on available fruits of Bangladesh is lacking. So, the present study was carried out to investigate bioactive compounds and antioxidant activity of fruits available in Bangladesh.

## 2. METHODS

### 2.1 Chemicals and reagents

2,2-Diphenyl-1-picryl hydrazyl (DPPH), gallic acid, folin ciocatea (FC) reagent, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and quercetin were obtained from Sigma, USA. Absolute ethanol, methanol, aluminum chloride, sodium carbonate and potassium acetate and mili-Q quality water were collected from local sources.

### 2.2 Sample collection and extract preparation

A total 23 variety of matured, unripe and damaged free fruits (three fruits of each variety) were randomly collected from six districts of Bangladesh and transported to the laboratory. Collected fruits were cleaned and washed with distilled water, edible portions were cut into small pieces with sharp knives. Then, juice containing fruits were squeezed and solid fruits were blended by domestic grinder before mixing with solvent. About 2-5g prepared sample were mixed with acidified aqueous methanol and ethanol separately and left for 72 h at 25 °C temperature with straining. Then the filtrates were collected and residues were re-extracted with fresh solvent. After extraction, the filtrates were centrifuged at 5000 rpm for 10 min and supernatant was stored amber flask at 5 °C until analysis.

### 2.3 Quantification of antioxidant activity by DPPH assay

Antioxidant activity was determined using DPPH assay with slight modifications.(24) The DPPH was generated by dissolving of 6 mg DPPH in 100 mL of absolute methanol. Then, 100 µL of methanoic extract or standard and 2 mL DPPH reagent was added and vortexed vigorously. The mixture was stand for 30 min in dark place at room temperature and discoloration of DPPH was measured against blank (absolute methanol) at 517 nm and scavenging activity are expressed as trolox equivalent mg/100g using the following formula:

$$\% \text{ inhibition} = \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

### 2.4 Quantification of total phenolic content (TPC)

TPC of the fruits were measured by Folin-Ciocalteu method slight modifications.(25) Briefly, ethanoic extract (1 mL) was mixed with 1.5 ml of Folin-Ciocalteu (FC) reagent (10%) and left for three minutes. Then 1.5 mL Na<sub>2</sub>CO<sub>3</sub> (7.5%) was added with the mixture

and left for 60 minutes in dark place at room temperature. Finally, absorbance was read at 765 nm against blank (ethanol). Aqueous gallic acid solution was used for calibration and the results are expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

### 2.5 Quantification of total flavonoid content (TFC)

Aluminum chloride colorimetric method with slight modifications.(26) Ethanoic extract (0.5 mL) of each fruit was separately mixed with 1.5 mL methanol, 0.1 mL of 10% aluminum chloride, 0.1 M potassium acetate and 2.8 mL of distilled water and left for 30 min at room temperature. Finally, absorbance of the reaction mixture was measures at 415 nm against blank (distilled water). TFC content was estimated by comparing the absorbance of the sample extracts with a quercetin standard curve and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

### 2.6 Quantification of total anthocyanin contentment (TAC)

TAC was determined using calorimetrically.(27) Briefly, ethanoic extract (3 mL) was pipetted into a cuvette and intensity of color was measured at wavelength 520 nm against blank (ethanol). TAC was calculated and expressed as milligrams per 100 g (mg/100 g) using the following equation-

$$\text{TAC} = \frac{\text{Absorbance of sample} \times \text{DF} \times 100}{m \times E}$$

Where, DF = dilution factor, m = means the weight of sample and E = refers to extinction coefficient (55.9).

### 2.7 Statistical analyses

Statistical analysis was performed by storing data in Microsoft Excel 2013 and then R Statistical Software (version 3.4.1; R Foundation for Statistical Computing, Vienna, Austria).

## 3. RESULTS AND DISCUSSION

Fruits are the manifested source of antioxidants and bioactive compounds which fight against oxidative stress and regenerative diseases in human body. In this study, 23 different fruits available in Bangladeshi markets both local and imported respectably were analysed.

### 3.1 Total antioxidant activity of fruits by DPPH assay

Since, DPPH, ABST and FRAP radical scavenging assays, DPPH was used to quantify fruits antioxidant activity due to its accuracy, simplicity and stability. Antioxidant activity of fruits ranged from 55 to 303 mg/100 g and average was 125 mg/100 g. High activities being found in Katajamin (303 mg/100 g), Dragon Fruits (171 mg/100 g) and Guava (131mg/100 g) while low activity was observed for Pani Fol (55 mg/100 g), Ashari (94 mg/100 g) and Cow Fol (94 mg/100 g) (Table 1). Higher DPPH scavenging activity may due to the higher contents of vitamin C, vitamin E, carotenoids, particularly b-carotene and lycopene as well as flavonoids and other polyphenols.<sup>(28,29,30)</sup> The obtained results are in agreement with those reported earlier in Bangladesh and some Brazilian exotic fruits

reported.<sup>(31,32)</sup> The results are also discordance with the reported data.<sup>(33,34)</sup> In this study, antioxidant activity of the fruits from same species were varied which might be the influence of several factors such as the time of harvest, maturity, variety, weather and soil conditions, sun exposure, location of fruit on the plant and post-harvest handling.<sup>(35)</sup> Fruits have higher antioxidant capacity that prevent many disorders in human health like arthritis, diabetes, arteriosclerosis, age-related macular degeneration, certain types of cancer, inflammation, genotoxicity, and Alzheimer disease.<sup>(36,37)</sup> Because antioxidant compounds exert their effects through different mechanisms such as inhibiting hydrogen abstraction, binding transition metal ions and disintegrating peroxides and reactive oxygen species (ROS), and reactive nitrogen species (RON).<sup>(38)</sup>

**Table 1.** Antioxidant activity and bioactive compounds of fruits available in Bangladesh

Common Name	Scientific Name	Antioxidant activity DPPH (trolox equ.) mg/100 g	Total phenolic content (TPC)(mg GAE/g)	Total flavonoid content (TFC) (mg QE/g extract)	Total anthocyanin content (TAC) (mg TA/100 g)
Orange	<i>Citrus sinensis L.</i>	110±8.94	76±3.37	42±8.52	14±0.26
Malta	<i>Cheirolophus crassifolius L.</i>	120±12.29	47±7.28	29±4.74	14±3.11
Pineapple	<i>Ananas cosnosus L.</i>	122±2.67	54±10.16	26±5.67	54±2.63
Apple	<i>Malus pumila L.</i>	122±4.90	207±5.43	52±11.08	13±1.31
Pear Small	<i>Opuntia aita L.</i>	101±15.54	71±22.04	22±2.29	06±0.80
Guava	<i>Psidium guajava L.</i>	131±3.10	73±19.68	26±4.55	30±5.42
Mango	<i>Magnifera indica L.</i>	130±1.57	305±3.20	18±7,11	142±0.78
Lichi	<i>Litchi chinensisl L.</i>	128±5.03	28±0.63	15±6.88	03±1.05
Lemone	<i>Citrus limon L.</i>	107±4.93	46±0.23	51±11.00	02±0.55
Tomato	<i>Solanum lycopersicum L.</i>	118±15.93	32±0.55	84±13.69	128±5.26
Grape	<i>Vitis vinifera L.</i>	120±14.54	116±2.84	33±19.54	39±0.87
Pome Granate	<i>Punica granatum L.</i>	130±1.94	222±0.25	27±7.01	04±0.39
Dragon Fruits	<i>Selenicereus undatus L.</i>	171±9.53	114±2.43	12±1.89	52±16.04
Bael	<i>Aegle Mermelos L.</i>	130±3.80	130±7.85	68±7.86	76±10.58
Jamrul	<i>Syzygium samarangense L.</i>	113±3.29	36±0.37	46±1.99	03±0.11
Pani Fol	<i>Flacourtia jangomas L.</i>	55±4.23	48±3.72	29±4.07	07±1.83
Belombo	<i>Averrhoa bilimbi L.</i>	115±20.76	32±5.60	52±5.01	08±5.16
Cow Fol	<i>Garcinia cowa L.</i>	94±13.20	29±3.01	27±5.50	20±1.91
Safeda	<i>Manilkara zapota L.</i>	125±1.29	86±11.40	31±8.55	52±9.91
Katajamin	<i>Citrus limon L.</i>	303±8.87	141±34.98	76±6.67	30±0.46
Ashari	<i>Sabal palmetto L.</i>	94±30.19	10±2.19	06±3.94	02±0.74
Bet Fol	<i>Calamus tenuis L.</i>	129±0.25	27±12.77	33±10.01	102±3.46
Dumur	<i>Ficus carica L.</i>	118±0.52	65±9.93	54±18.83	04±0.45
Maximum	-	303	305	84	142
Minimum	-	55	10	06	02
Average	-	125	87	37	35

Values are mean ± SD; n=3

### 3.2 Total Polyphenol Contents (TPC)

Total polyphenol content of the fresh fruit extracts was analyzed and results varied from 10 to 305 mg GAE/100 g and average value was 87 mg GAE/100 g. Mango (mg GAE/100 g) showed higher TPC, followed by Pome Granate (222 mg GAE/100 g), Apple (207 mg GAE/100 g), Katajain (141 mg GAE/100 g) and Bael (130 mg GAE/100 g). Again, Ashari (10 mg GAE/100 g) showed lower TPC, followed by Lichi (28 mg GAE/100 g), Cow Fol (39 mg GAE/100 g), Belombo (32 mg GAE/100 g) and Tomato (32 mg GAE/100 g) presented in Table 1. The amount of phenolic contents is classified as- low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (>500 mg GAE/100 g).<sup>(39)</sup> So, Mango, Pome Granate, Apple, Katajain and Bael, Grape and Dragon Fruits have medium and others have low polyphenol contents. These findings on the total phenolic contents are scarce in the available literature and the present findings were comparable with the following studies.<sup>(40,41,42)</sup> Phenolic compounds are the secondary metabolites which varied with color, water content, food matrix, oxidative stability of fruits and ripening stages of fruits.<sup>(43)</sup> Moreover, polyphenol synthesis in plants is induced under conditions of biotic and abiotic stress like disease, injury, UV-radiation, water stress, ozone, salinity and heat.<sup>(44)</sup> Dietary requirement of polyphenols from fruits and vegetables are roughly the same which come from mango, banana, apple, orange and from many of the vegetables. Furthermore, the intake is similar for each gender, except for apple, which is consumed slightly more by women. So, available fruits of Bangladesh can fulfil the demand of polyphenol and especially for rural people.

### 3.3 Total flavonoid content (TFC)

These bioactive secondary metabolites present in almost plant species and their efficiency depends on the number of hydroxyl groups and positions in the molecules. Most common family members include flavonol, flavanol, flavones, flavanes, flavonols and catechins.<sup>(45)</sup> Result shows (Table 1) that TFC content is ranged from Ashari (06±3.94 mg QE/g) to Tomato (84±13.69 mg QE/g) and the average TFC was 37 mg QE/g which are also similar with other studies.<sup>(46,47)</sup> We intake 126 mg flavonoid from vegetables and herbs, 45 mg from cereals and 44 mg from grains and nuts.<sup>(48)</sup> So, the consumption of these available fruits from Bangladesh can bring off the demands of flavonoid to

protect lipids and vital cells from oxidative damage of lipid and vital cells, prevention of coronary heart diseases and exhibit antiproliferative or anticancer activities.<sup>(49)</sup>

### 3.4 Total anthocyanin content (TAC)

Anthocyanins, water-soluble antioxidant pigments, are responsible for most of the red, blue, and purple colors of fruits. In this current study, the TAC of the fruits varied from 2 to 142 mg/100 g and average content was 35 mg/100 g. Total anthocyanin content being seen in Mango (142±0.78 mg/100 g) and lower in Ashari (02±0.74 mg/100 g). These results are highly compatible with others research on fruits.<sup>(50)</sup> So, available fruits of Bangladesh are the good sources of anthocyanin and their consumption helps to reduce the risk of cardiovascular disease, diabetes, arthritis and cancer.<sup>(51)</sup> Anthocyanins are unstable, easily oxidized and sensitive to temperature, UV radiation, ascorbic acid and metal ions and their content may vary from fruit to fruit, season to season, species to species and region to region even from the same source due to genetic and agronomic factors, intensity and type of light, temperature, postharvest treatments, processing and storage.<sup>(52)</sup>

### 3.5 Correlation coefficient between antioxidant activity and bioactive compounds

There was a statistically significant correlation ( $P \leq 0.05$ ) between antioxidant activity and bioactive compounds are expressed. There was a moderate positive correlated between AOC vs. TPC ( $r = 0.29$  and  $r^2 \% = 8.81$ ) and AOC vs. TFC ( $r = 0.28$  and  $r^2 \% = 7.84$ ) but very weak correlation between AOC vs. TIC with  $r$  and  $r^2 \%$  values being 0.12 and 1.44 respectively (Table 2 and Table 3).

Table 2. Correlations between antioxidant activity and bioactive compounds of rare fruits

Correlations	r	r <sup>2</sup> (%)	p
AOA vs. TPC	0.29	8.41	$P \leq 0.05$
AOA vs. TFC	0.28	7.84	$P \leq 0.05$
AOA vs. TAC	0.12	1.44	$P \leq 0.05$

Table 3. Correlations coefficient among three bioactive compounds of rare fruits

Correlations	r (%)	r <sup>2</sup> (%)	p
TPC vs. TFC	0.007	0.005	$P \leq 0.05$
TPC vs. TAC	0.32	10.24	$P \leq 0.05$
TFC vs. TAC	0.18	03	$P \leq 0.05$

These results indicate that higher the bioactive compounds of fruits higher the antioxidant capacity. These observations are in line with some other reported studies in fruits.<sup>(54,55)</sup> The present study proposed that bioactive compounds of fruits could be the good contributors of antioxidant activity. Statistically significant correlations ( $P \leq 0.05$ ) were also observed among three bioactive compounds.

#### 4. CONCLUSION

In this study, there were 23 available fruits were analyzed to determine the antioxidant activity and bioactive compounds. The outcomes of the study reveal that, most of the available fruits of Bangladesh are rich source of antioxidant and bioactive compounds. Antioxidant activity was measured by DPPH method and it was observed that, Katajamine, Dragon Fruits, Guava, Mango and Bet Fol and has good antioxidant activity. Again, Mango, Katajamine, Tomato, Bet Fol and Dragon Fruits have good bioactive compounds. Many previous studies also reported that, these fruits are also rich sources of vitamins, minerals and energy. So, they could play a dramatic role to alleviating the malnutrition of the country. Consumption of these fruits may help us to make a healthy life and in the long run may cause a good potential on human health.

#### Conflict of Interest

The authors declare no conflicts of interest

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