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BIOACTIVE COMPOUNDS AND BIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF SELECTED MINOR INDIGENOUS FRUITS IN BANGLADESH

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ABSTRACT
Minor fruits are a potential source of antinutrients, but there is no complete primary data source in the Bangladeshi context. Therefore, the present study was undertaken to acquire documentation for a database of the composition of selected minor fruits. The total phenolic (TPH), vitamin C, total carotene, and ß-carotene contents and antioxidant activity of selected minor fruits were determined by 1,1-diphenyl-2picryl hydrazyl (DPPH) scavenging and reducing power assays (RPA). Phenolic compounds were assessed using high-performance liquid chromatography coupled with a photodiode array detector and autosampler. Results revealed that minor fruits contain different phytochemicals, particularly TPH, ascorbic acid, total flavonoid (TF), ß-carotene, total carotenoid (TC), and total anthocyanin content (TAC); values ranged, respectively, 0.23-176.50 mg GAE/g, 16.67-664.92 mg/100 g, 2.26-150.02 mg QE/100 g, 1.41-6897.57 µg/100 g, 1.26-98.24 mg/100 g and 1.15-47.46 mg/100 g. In the parameters antioxidant activity, total antioxidant capacity, DPPH, reducing power capacity (RPC), ferric reducing antioxidant power (FRAP), metal chelating capacity (MCC), nitric oxide (NO), and free radical scavenging activity, IC_{50} ranged 0.01-278.24 µg of ascorbic acid/mg of extract, 39.70-250.00%, 3.21-634.00%, 0.02-1817.88 µM Fe2SO4/100g, 22.29-210.43%, 0.02-70.50%, and 4.98-856.70 µg/g, respectively. Among the identified and quantified phenolic acids, leading examples were gallic acid (279.06 mg/100 g), vanilic acid (43.77 mg/100 g), Þ-courmaric acid (178.96 mg/100 g), ferulic acid (20.44 mg/100 g), and lutein (91.13 µg/100 g) in aonla, day fruit, elephant apple, and bilimbi. Moreover, all selected minor fruits are rich sources of bioactive, biochemical, and antioxidant compounds with potential for use in therapeutic applications.

Contribution/Originality: The study contributes the first logical analysis of selected minor indigenous fruits grown in Bangladesh in terms of phytochemical and antioxidant activities. The nutritional, phytochemical, and antioxidant profiles of these fruits may encourage their consumption rather than that of major and exotic fruits. Pharmaceutical researchers may apply this phytochemical and antioxidant profile for pharmaceutical research purposes.

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1. INTRODUCTION

Bangladesh is blessed with a great diversity of fruits and, in 2017, was placed sixth in world rankings for tropical fresh fruit production (FAOSTAT, 2019). A significant quantity of tropical fruits are underexploited – generally recognized as indigenous or minor fruits. These fruits are not commercially cultivated and are not frequently found in national or international markets. Usually these fruits are found in home yards, unused high lands, hill tracts, and roadsides. No extra care and farming management are needed to grow these fruits, and that is why people describe these as underutilized minor indigenous fruits in Bangladesh; year on year, these fruit trees are being destroyed. However, these minor fruits can be a good source of micronutrients and phytochemicals. Micronutrients are essential elements in the development of physical growth and help prevent various acute and chronic diseases. Low dietary intake of micronutrient-rich foods, as well as low absorption and lower bioavailability, are the leading causes of micronutrient deficiency. Phytochemicals with antioxidant capacity naturally present in fruits are of great interest due to their beneficial effects on human health, while regular consumption is associated with reduced risk of developing chronic diseases because they offer protection against oxidative deterioration (Paul et al., 2007). Nowadays, antioxidants are also considered as important as vitamins in the promotion of health and prevention of various diseases linked to reactive oxygen species (ROS), which have been linked to over 100 disorders (Halliwell & Gutteridge, 2000). Excess generation of ROS causes oxidative stress that damages cellular DNA, lipids, and proteins, leading to the pathogenesis of various diseases including cerebrovascular diseases. Among various antioxidants and phytochemicals, flavonoids, anthocyanin, phenolic acids, and other compounds are linked with natural protective agents, astringents, antibiotics, positive health effects, and antimicrobial properties (Osorio-Esquivel, Álvarez, Dorantes-Álvarez, & Giusti, 2011). In recent years, interest in antioxidant-rich fruits and their products has been growing in both domestic and international markets because of increasing appreciation of their role in the protection of human health. This has occurred because of their nutritional and antioxidant properties, and also because of the prospects offered to the agricultural and pharmaceutical industries and a promising future source of income for local inhabitants.

A nutrition database is of great importance in addressing the nutritional health benefits of both rural and urban communities. It is essential for the formulation of a national policy on food to have a database on nutritional composition. However, little information in nutrition databases is available for minor fruits. Several studies have been performed on a few fruits only, but these are indiscriminate and have not been scientifically documented or aimed at the consumer. Therefore, documentation, conservation, and revalorization of indigenous knowledge on minor fruits is urgently needed to promote nutritional health for both rural and urban inhabitants. With that in mind, the present research waas undertaken to analyze, document, and popularize the role of phytochemical content in highlighting the phenols, flavonoids, and carotenoids present in selected minor fruits in Bangladesh.

2. MATERIALS AND METHODS

2.1. Materials

Nine minor indigenous fruits were selected based on peoples' choice and production catchments in Bangladesh, and are shown in Table 1. Analytical grade chemicals and reagents used in this study were procured from Sigma-Aldrich (Steinheim, Germany).

Table 1. Selected minor indigenous fruits grown in Bangladesh.

Sl. no.	Bengali name	English name	Scientific name	Family
1	Aamlaki	Aonla	*Phyllunththus embelica* L.	Phyllanthaceae
2	Amra	Hog plum	*Spondias mombin* L.	Anacardiaceae
3	Bilimbi	Cucumber tree	*Averrohoa bilimbi* L.	Oxalidaceae
4	Chulta	Elephant apple	*Dillenia india* L.	Dilleniaceae
5	Day fall	Day fruit	–	–
6	Jara lebu	Citron	*Citrus medica*	Rutaceae
7	Satkara	Melanesian papeda	*Citrus macroptera*	Rutaceae
8	Sour kul	Ber	*Ziziphus mauritania*	Rhammaceace
9	Toikar	Toikar	*Garcinia pedunculata*	Clusiaceae

Source: Project Completion Report, Competitive Research Grant (ID: 328), Bangladesh Agricultural Research Council (BARC), Farmgate, Dhaka-1215, Bangladesh.

2.2. Methods

2.2.1. Sample Collection and Fruit Extraction

Fruits were collected from the Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Akbarpur, Moulvibazar, Bangladesh. After collection, the fruits were washed with potable water and surface dried. The fruits were then freeze-dried and milled to powder using a laboratory grinder. Fruit powder of known quantity was extracted in a thermostatic water bath (Vision Scientific Co. Ltd.) at 60°C for 1 h using methanol (80%, v/v) maintaining a fruit:solvent ratio of 1:20 (w/v). The fruit extract was filtered under vacuum, centrifuged at 4000×g for 10 min and the supernatant was collected and kept at -18°C until used for analysis.

2.2.2. Determination of Physicochemical Parameters

Physicochemical properties – moisture, protein, ash, total soluble solids (TSS), pH, and titrable acidity – were determined following the method of AOAC (2005). Ascorbic acid, starch, and total sugar content were determined based on the procedure of Ranganna (1995). Edible and non-edible portions of the fruits were measured using the gravimetric method.

2.2.3. Analysis of Minerals

Minerals analyzed included sodium, potassium, calcium, magnesium, phosphorus, sulphur, boron, copper, manganese, iron, and zinc. Before quantification, fruits were first wet ashed and then digested in nitric and perchloric acid solution at 320°C, cooled, diluted to an appropriate concentration, and filtered. This filtrate was then used as the stock solution for further analysis. Atomic absorption spectrophotometry (Model-AA-7000S, Shimadzu, Tokyo, Japan) was used to assess levels of sodium, iron, copper, zinc, boron, manganese, calcium, and magnesium. Potassium content was measured using flame photometry, while phosphorous and sulphur were assessed by spectrophotometry. Individual minerals were quantified by comparison to the corresponding standard mineral procured from Sigma Chemical Co., USA.

2.2.4. Determination of Phytochemicals

2.2.4.1. Total Phenolic Content

Twenty milligrams (0.02 g) of powder was dissolved in 1 ml of methanol to prepare a stock solution for experiments. A volume of 0.5 ml of the each extract (100 µg/ml) was mixed with 2 ml of Folin–Ciocalteu reagent (diluted 1:10 with deionized water) and neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. Absorbance of the colored solution was measured at 765 nm using double-beam UV-VIS spectrophotometry. Total phenolic content was determined from the linear equation of a standard curve prepared with gallic acid. Determination of total phenolic content in the extracts was determined according to the Folin–Ciocalteu method (Ough & Amerine, 1988), with gallic acid (GAE) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract (Aoshima, Hirata, & Ayabe, 2007).

2.2.4.2. Determination of Total Flavonoid Content

Total flavonoid content (TFC) of the fruit powder was determined by the aluminium chloride method (Chang, Yang, Wen, & Chern, 2002) with slight modifications: 0.5 ml of sample solution was mixed with 1.5 ml of methanol. To this mixture 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate were added. The final volume was made up to 5 ml by adding 2.8 ml of distilled water, and the reaction was left for 30 min at room temperature. Absorbance of the solution was measured at 415 nm and expressed as mg QE/g extract. TFC was calculated from the calibration curve of quercetin plotted (Figure 1). The curve obtained was found to be linear with equation $y = 0.0035 + 0.0056x$, and the correlation coefficient was found to be $R^2 = 0.9993$. TFC was expressed as mg quercetin equivalents per gram of extract (mg QE/g extract).

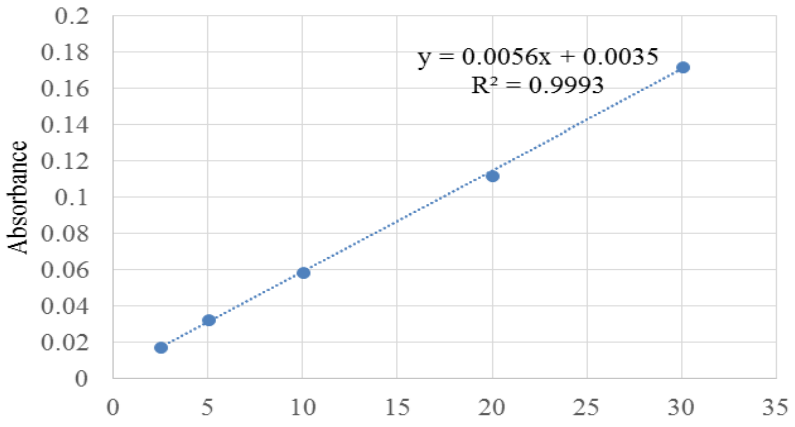

Figure-1. Calibration curve of quercetin plotted.

2.2.4.3. Determination of Total Anthocyanin (TA)

The method was adapted from Burgos et al. (2013), the concentration of TA being calculated using the molar extinction coefficient and molecular weight of malvidin-3-p-coumaroyl-glucoside for blue-violet pigments (545 nm, 3.02×10^4 l/mol/cm, 718.5 g/mol), pelargonidin-3-glucoside for red pigments (515 nm, 2.73×10^4 l/mol/cm, 486.5 g/mol), and cyanidin-3-glucoside for purple pigments (535 nm, 3.43×10^4 l/mol/cm, 449.2 g/mol). Results were expressed as mg/100 g DW.

2.2.4.4. Determination of Total Carotenoid Content

Total carotenoid was determined according to the method described by Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006). Absorbance was measured at λ 470 nm. Each extract was dissolved in n-hexane pro-analysis. ß-carotene solution in various concentrations was used as standard carotenoid compound and the standard curve. A linear regression equation of the standard curve was used for calculating total carotenoid content, and expressed as beta-carotene equivalents per 100 g of extract (mg/100 g).

2.2.4.5. Determination of ß-Carotene Content

β-Carotene content was determined using the procedure of Holden et al. (1999), with values expressed as µg/100g of fruit extract.

2.2.5. Determination of Antioxidant Activity

2.2.5.1. Total Atioxidant Activity

Total antioxidant activity was assessed using the phosphomolybdenum system according to the technique described by Prieto, Pineda, and Aguilar (1999), and results expressed as micrograms ascorbic acid (AA) per gram (µg/ml) of the sample.

2.2.5.2 Reducing Power Assay

The reducing power of the fruit extract was assessed using the approach of Guo, Saravanakumar, and Wang (2018). Ascorbic acid was used as the standard for the preparation of the calibration curve.

2.2.5.3. FRAP

FRAP activity was measured following the scheme outlined by Benzie and Strain (1996). A standard curve was made using ferrous sulphate aqueous solution (1–10 mM) and FRAP values were expressed as µM Fe (II)/100g of the sample.

2.2.5.4. DPPH Radical Scavenging Activity (DPPH-RSA) and IC_{50}

DPPH-RSA was evaluated by measuring the inhibition rate following the procedure described by Brand-Williams, Cuvelier, and Berset (1995), with modification. Exactly 0.1 ml of extract was placed in a Falcon tube and 1.4 ml of methanolic solution of DPPH added. The mix was left to rest for 30 min in the dark and absorbance at 517 nm was measured against a blank. The results are expressed as percentage radical scavenging activity:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_O - A_s}{A_o} \times 100$$

where A_o is absorbance of blank and A_s is absorbance of sample extract. The inhibition curves were then prepared and IC_{50} values calculated (Brand-Williams et al., 1995). BHT was the positive control.

2.2.5.5. MCC

MCC was determined based on Bahadori, Zengin, Bahadori, Dinparast, and Movahhedin (2018), with minor modification. Briefly, 2 ml of extract was placed in a glass tube to which 0.05 ml of ferrous chloride (2 mM), 3.7 ml of distilled water, and 0.2 ml of ferrozine (5 mM) were added. After 20 min of incubation under atmospheric conditions, absorbance was read at 562 nm against a blank. The following formula was applied to calculate MCC:

$$\text{Metal chelating capacity (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

2.2.5.6. Nitric Oxide (NO) Radical Scavenging Activity

Nitric oxide radical quenching activity was determined according to the procedure of Bogucka-Kocka, Zidorn, Kasprzycka, Szymczak, and Szewczyk (2018), with some modification. In a glass tube, 0.5 ml of extract was mixed with 2 ml of sodium nitroprusside (10 mM). This was followed by incubation under atmospheric conditions for 1 h. Thereafter, 0.5 ml of the incubated mixture was transferred to another centrifuge tube, Griess reagent (0.5 ml) was added, and the mixture rested for 30 min. Absorbance of the solution was read at 540 nm against a blank. The following formula was employed to calculate the result, expressed as percentage inhibition:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance (control)} - \text{Absorbance(sample)}}{\text{Absorbance (control)}} \times 100$$

2.2.6. Assessment of Phenolic Acids by HPLC

Phenolic compounds were assessed according to the method of Pandey and Negi (2018), with some adjustment, using high-performance liquid chromatography (HPLC; Shimadzu SPD-M10A) coupled with a photodiode array

detector and autosampler at 280 and 320 nm. Separation was achieved using a C18 column (250 × 4.6 mm²) of 5 µm particle size at room temperature. The mobile phase was 1% acetic acid (A) and 80% acetonitrile in A (B). The following gradient was used: 0.01–35 min, 0% of B; 35–40 min, 50% of B; 40–45 min, 100% of B; and 45–60 min, 0% of B. The flow rate was 1 ml/min and the injection capacity was 20 µl. Six phenolic standards (Þ-courmaric acid, gallic acid, vanilic acid, caffeic acid, ferulic acid, and lutein) were used for identification of respective phenolics, and quantification was accomplished using a standard curve.

3. STATISTICAL ANALYSIS

Data obtained for each analysis were expressed in duplicate as means ± standard deviation. Data were analyzed by one-way ANOVA with post hoc using Tukey's multiple comparisons test. Significance was defined at the 95% confidence level. Statistical analysis and data processing were performed using the software SPSS 17.0 (IBM Inc., New York).

4. RESULTS AND DISCUSSION

4.1. Biochemical Properties

4.1.1. Crude Protein

Analysis of crude protein content of selected minor fruits is shown in Table 2. It will be seen that in all fruits this is statistically significant, with levels of crude protein ranging from 2.39 to 4.10% (Table 2). The highest levels of protein were found in citron (4.10%) and satkara (4.06%). Total protein obtained from the Bangladeshi commercial mango fruits amrapali, chausa, fazlee, gopalbhog, himsagor, and langra was found to be 0.14, 0.26, 0.21, 0.07, 1.11, and 1.18 g/100 g, respectively (Ara, Motalab, Uddin, Fakhruddin, & Saha, 2014). The crude protein content of Indian minor fruits *Aegle marmalose* Correa, *Cordia myxa* L., *Zizipus mauritiana* Lam, *Averrhoa bilimbi* L., and *Grewia asiatica* Mast weas recorded as 2.2, 1.9, 1.04, 1.3, and 1.09 g/100 g, respectively (Mitra, Pathak, & Chakraborty, 2008). The crude protein content found in our minor indigenous fruits was higher than that found by both Mitra et al. (2008) and Ara et al. (2014).

4.1.2. Ash

The ash content of the nine selected minor fruits was statistically significant, ranging from 1.40 to 3.78%, the maximum (3.78%) being recorded in citron (Table 2). The highest ash content found in the present study is comparable to the findings of Shukla, Dubey, Jain, and Kumar (2001), who reported that *Adansonia digitata* contain 3.31% ash.

4.1.3. Total Sugar

Total sugar content of the nine selected minor fruit ranged from 2.83 to 5.64%. All values were statistically highly significant, with the maximum content recorded for hog plum (5.64 %) and the minimum for aonla (2.83%) (Table 2). Values for blilimbi, elephant apple, day fruit, citron, satkara, ber and toikar were 4.07, 4.13, 4.20, 4.27, 3.91, 4.35, and 4.02%, respectively. The results obtained from our study are supported by the findings of Ara et al. (2014), who reported that the total sugar content of commercial mango varieties ranged from 4.27 to 4.83%.

4.2. Starch

Starch is a major carbohydrate source of immense economic and nutritional value. It is essential that the food industry search for new starch sources to meet the requirements of both the food processing industry and consumers. Table 2 shows selected minor fruits containing higher starch levels and all statistically highly significant differences. The highest starch content (8.70%) was recorded for day fruit and the lowest for satkara (3.44%). The high total sugar content in day fruit may be due to enzymatic hydrolysis of starch to sugar (Nelson, 1944). The lower yield of starch in satkara is probably due to its increased respiration directly by genetical induced.

4.3. Moisture

The moisture content of the selected minor fruits varied. The data show that the moisture content of the selected minor fruits was statistically significant ($P < 0.05$), ranging from 76.62 to 80.73% – maximum in citron (80.73%) and minimum in aonla (76.62%) (Table 2). The results obtained from the study are comparable to those of Parveen and Khatkar (2015able 3), who reported aonla (variety Desi) with 81.26% moisture content.

4.4. Acidity

Acidity varied highly significantly among the selected minor fruits, ranging from 0.79 to 3.58% – the maximum was 3.58% in day fruit and the minimum in citron (Table 2). The results obtained from this study confirm that the acidity of the selected minor fruits is much higher than that found by Akhter et al. (2010) for langra (0.68%) and chausa (0.63%) commercial mango cultivars in Bangladesh. The reason for this discrepancy may be that most minor fruits are citrus fruits with variation in maturity stage, harvesting time, soil management, and cultural practices.

Table 2. Biochemical and mineral composition of selected minor fruits.

Parameter	Minor fruits								
	Aonla	**Hog plum**	**Bilimbi**	**Elephant apple**	**Day fruit**	**Citron**	**Satkara**	**Ber (sour)**	**Toikar**
Crude protein (%)	3.71± 0.03ab	3.80± 0.10ab	3.70± 0.20ab	3.26± 0.15bc	3.04± 0.01	4.10± 0.10a	4.06± 0.02a	3.18± 0.09bc	2.39± 0.57d
Ash (%)	1.92± 0.01f	2.23± 0.02e	3.33± 0.01bcd	3.45± 0.02bc	1.40± 0.10g	3.78± 0.02a	3.31± 0.10cd	3.21± 0.10d	3.49± 0.02b
Total sugars (%)	2.83± 0.01d	5.64± 0.03a	4.07± 0.06bc	4.13± 0.04bc	4.20± 0.10bc	4.27± 0.03bc	3.91± 0.06c	4.35± 0.40b	4.02± 0.02bc
Starch (%)	5.51± 0.27b	4.45± 0.08cd	4.13± 0.02de	4.71± 0.03cd	8.70± 0.20a	3.69± 0.58ef	3.44± 0.03f	4.15± 0.02de	4.85± 0.07c
Moisture (%)	76.62± 0.10g	78.44± 0.11ef	77.77± 0.10f	79.02± 0.13def	79.49± 0.54bcd	80.73± 0.25a	80.33± 0.09ab	78.61± 0.48def	79.82± 0.57abc
Acidity (%)	1.82± 0.02de	2.29± 0.02b	1.84± 0.02cde	1.92± 0.03cd	3.58± 0.03a	0.79± 0.02f	1.78± 0.10e	1.95± 0.05c	2.40± 0.02b
pH	2.65± 0.02e	2.94± 0.00cd	2.14± 0.02f	3.29± 0.02b	2.63± 0.10e	3.53± 0.03a	3.58± 0.09a	2.88± 0.04d	3.09± 0.02c
TSS (°B)	11.40± 0.02a	10.91± 0.01b	10.18± 0.02c	9.17± 0.00d	7.01± 0.01g	7.54± 0.25f	8.50± 0.10e	10.44± 0.05c	9.20± 0.02d
Edible (%)	84.62± 0.02d	47.59± 0.3f	98.00± 1.00a	79.03± 0.02e	90.95± 0.03b	79.77± 1.59e	44.70± 0.20g	88.37± 0.31c	84.01± 0.01d
Non-edible (%)	15.38± 0.02	50.50± 0.16a	2.00± 1.00f	18.02± 1.01c	8.01± 0.01e	20.05± 1.20b	50.01± 0.09a	9.30± 0.09e	15.33± 0.03d
Minerals									
Ca	0.25± 0.00e	0.27± 0.00e	0.58± 0.00cd	0.54± 0.00d	0.67± 0.02c	0.82± 0.05b	0.55± 0.00d	1.02± 0.05a	0.87± 0.06b
Mg	0.15± 0.01e	0.14± 0.00e	0.31± 0.00d	0.28± 0.00d	0.29± 0.02d	0.37± 0.02c	0.29± 0.00d	0.57± 0.03a	0.49± 0.03b
K	0.93± 0.00cd	0.93± 0.00cd	1.07± 0.01ab	1.08± 0.00a	0.40± 0.02f	0.64± 0.04e	0.99± 0.00bc	0.87± 0.03d	0.37± 0.04f
Na	0.32± 0.01a	0.16± 0.00de	0.18± 0.01cd	0.19± 0.00c	0.12± 0.00f	0.08± 0.01g	0.15± 0.00e	0.22± 0.00b	0.18± 0.00cd
P	0.42± 0.00a	0.42± 0.00a	0.11± 0.00ef	0.31± 0.01c	0.07± 0.01g	0.10± 0.00f	0.34± 0.00b	0.12± 0.00e	0.21± 0.00d
S	0.07± 0.00b	0.07± 0.00b	0.06± 0.00b	0.05± 0.01b	0.02± 0.00c	0.02± 0.00c	0.06± 0.00b	0.36± 0.00a	0.36± 0.00a
B	68.04± 2.16a	4.82± 0.02de	14.31± 0.02c	26.30± 0.14b	13.40± 3.51c	8.03± 0.30d	1.36± 1.01e	8.60± 0.26d	6.38± 0.40d
Cu	4.82± 0.02h	128.30± 0.10c	107.53± 0.97e	157.77± 0.24b	11.67± 0.30g	114.22± 5.02d	62.61± 0.02f	155.78± 1.31b	395.52± 1.77a

Fe	90.12± 0.02d	68.32± 0.06e	57.48± 0.21f	83.91± 0.05d	180.99± 5.86b	73.47± 2.98e	35.30± 0.03g	155.78± 1.31c	238.26± 1.07a
Mn	47.92± 0.01e	27.52± 0.01f	81.78± 0.03d	151.19± 0.99a	116.68± 6.38b	16.06± 0.60gh	11.22± 0.03h	93.84± 0.78c	19.99± 0.99g
Zn	18.84± 0.02ab	13.16± 0.15cd	20.00± 2.00ab	17.84± 0.28bc	23.34± 0.60a	11.20± 4.42d	10.14± 0.01d	10.73± 0.50d	22.33± 0.57ab

Note: Values are mean ± standard deviation ($n = 3$); Ca, Mg, K, Na, P, and S expressed as mg%; B, Cu, Fe, Mn, and Zn expressed as ppm. All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f, g, indicate significant result ($p<0.05$).

Table 3. Phytochemical and antioxidant properties of selected minor indigenous fruits.

Parameter	Minor fruits								
	Aonla	Hog plum	Bilimbi	Elephant apple	Day fruit	Citron	Satkara	Ber (sour)	Toikar
Phytochemical propertrties									
TPH (mg GAE/g)	2.10± 0.09	0.23± 0.02e	105.92± 0.14b	62.85± 0.05c	176.50± 2.42a	50.00± 0.08d	1.58± 0.08e	2.04± 0.06e	1.81± 0.07e
TF(mg QE/g)	45.04± 2.47b	2.84± 0.11d	150.02± 1.17a	26.16± 1.96c	27.00± 3.04c	27.85± 1.05c	23.38± 1.25c	2.26± 0.09d	18.33± 1.12
Total ß-carotene(µg/100 g)	6897.57 ±0.09a	1737.11 ±3.27d	39.33± 1.52f	41.95± 0.05f	1.41± 0.04g	9.58± 0.09g	2318.44± 5.60b	549.12± 6.42e	1815.88± 3.62c
TC (mg/100 g)	83.73± 0.39c	68.45± 0.12e	4.70± 0.30f	1.58± 0.09g	1.26± 0.23g	2.07± 0.11g	93.91± 0.80b	74.89± 1.12d	98.24± 0.09a
TA (mg/100 g)	15.53± 0.03b	5.11± 0.01d	47.46± 0.22a	1.74± 0.23e	1.15± 0.14f	1.19± 0.03f	6.25± 0.04c	1.66± 0.01e	6.24± 0.01c
AA (mg/100 g)	664.92± 1.00a	67.90± 0.05e	28.41± 0.09	40.29± 0.11f	16.67± 0.53i	24.28± 0.61h	210.43± 0.02b	160.12± 0.11c	142.81± 0.99d
Antioxidant activity									
TAC (µg AA/g)	0.02± 0.00f	0.01± 0.00f	108.06± 0.45c	85.90± 0.03d	122.32± 0.01b	278.24± 0.03a	0.01± 0.00f	0.02± 0.00f	1.14± 0.02e
DPPH (%)	250.00± 10.00b	314.00± 5.00a	73.27± 0.25d	68.40± 4.60d	87.70± 0.49c	70.20± 0.19d	39.70± 0.20e	41.66± 0.76e	45.00± 5.00e
RPA (µg AA/g)	490.50± 0.50b	634.00± 1.00a	18.71± 0.01e	5.89± 0.02f	3.21± 0.01f	3.85± 0.02f	93.00± 2.00d	105.66± 12.09d	130.00± 5.00c
FRAP (µM Fe_2SO_4/100 g)	38.01± 9.99e	4.87± 0.10f	1817.88± 3.62b	2325± 6.09a	1213.46± 0.47d	1247.50± 3.25c	0.35± 0.05f	1.86± 0.20f	0.02± 0.00f
MCC (%)	33.89± 0.02h	182.39± 0.06c	192.97± 1.01b	22.29± 0.09i	97.56± 0.10d	210.43± 0.02a	40.76± 0.09g	72.00± 1.00e	59.51± 0.01f
NO (%)	7.97± 0.10e	0.01± 0.00g	70.50± 1.00b	40.18± 0.03d	41.65± 0.02c	178.87± 0.02a	1.09± 0.06f	0.02± 0.00g	1.27± 0.06f
IC_{50} (µg/g)	255.20± 0.20d	856.70± 0.03a	30.36± 1.32g	4.98± 0.24i	49.57± 0.50f	11.26± 0.26h	362.00± 0.50c	242.78± 0.03e	504.00± 1.00b

Note: All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f, g,h, i indicate significant result ($p<0.05$).

4.5. pH

pH levels of the selected minor fruits are shown in Table 2; these ranged from 2.14 to 3.58. pH levels were highly significant, with maxima of 3.53 and 3.58 for citron and satkara, respectively, and a minimum of 2.14 for bilimbi (*Averrhoa bilimbi* L.). pH levels showed an inverse relationship to acidity, with a minimum of 0.79% for citron but a maximum of 3.53. A similar observation was also made by Ara et al. (2014) for major mango fruit commercial cultivars. Variation in pH among selected minor fruits may be due to variation in their internal metabolism and ripening behavior.

4.6. TSS

TSS include carbohydrates, organic acids, proteins, fats, and minerals. TSS denotes a fresh weight of 10–20%, and increases with fruit maturity in regard to the production of a less acidic and sweeter fruit. In our study, TSS of the selected fruits varied significantly among species with a maximum of 11.40°B for aonla and a minimum for day fruit (7.01°B). The results obtained from our study confirmed that there was an inverse relationship between TSS and acidity. The acidity of 1.82% in aonla was influenced by TSS (11.40°B) (Table 2). Likewise, another relationship was found between TSS and fruit maturity, with increase in TSS following fruit maturity (no maturity data are shown here). For example, in the initial stage, the taste of aonla appears to be astringent but, when fully matured, it becomes sweeter with increasing TSS.

4.7. Edible Percentage

Edible percentage differed significantly among the selected fruit cultivars, as shown in Table 2. The highest edible percentage was calculated at 98.0 in bilimbi (*Avverhoa bilimbi* L.); all of the fruit, except the pericarp, is used for consumption. The lowest edible percentage was recorded at 44.7 for satakara, due to removal of peel during preprocessing for consumption. However, the edible percentage of the selected minor fruits ranged from 44.7 to 98.0, being much higher than in major fruits (Ara et al., 2014).

4.8. Non-Edible Percentage

The non-edible percentage of selected minor fruits ranged from 2.0 to 50.5. The lowest (2.00%) non-edible percentage was found in bilimbi while the highest recorded were 50.5 and 50.0, in hog plum and satkara, resoectively, due to their natural fruit structure and peel, because most of the peel goes to waste during preprocessing.

4.9. Mineral Profiling

Minerals are the inorganic components present in foodstuff as ash when food is burned. Generally macro- and microminerals are present in foodstuffs and play important metabolic roles in body functions (Reilly, 2002), and contribute to our daily diet. In our study, 11 minerals were assessed from the selected minor fruits (Table 2). It will be seen that these contained a significant range of macrominerals including sodium (0.08-0.32 mg%), potassium (0.37–1.08 mg%), calcium (0.25–1.02 mg%), magnesium (0.14–0.57 mg%), and phosphorus (0.07–0.42 mg%); and considerable amounts of microminerals including sulphur (0.02–0.36 mg%), boron (1.36–68.04 ppm), copper (4.82–395.52 ppm), iron (35.30–238.26 ppm), manganese (11.22–151.19 ppm), and zinc (10.14–23.34 ppm). Our study indicates that sour ber is a rich source of Ca, Mg, and S; aonla is a rich source of Na and P, hog plum of P, and elephant apple of K and Mn. Toikar is a rich source of Cu and Fe, and day fruit of Zn. In comparison with Indian minor fruits, our selected minor fruits possessed lower amounts of various minerals. It is claimed in different studies that soil fertility, genetic factors, fertilizers, and geographical conditions play significant roles in the mineral content of fruits (Tiburski, Rosenthal, Deliza, de Oliveira Godoy, & Pacheco, 2011).

4.10. Phytochemical properties

Phytochemical components reputedly have the ability to lower the prevalence of various degenerative diseases such as cancer, heart attack, and cardiovascular disease, by blocking the activity of free radicals. The results recorded for phytochemical analyses of total phenolics, flavonoids, carotenoids, β-carotene, ascorbic acid, and anthocyanin are shown in Table 3.

4.10.1. Total Phenolic Content

Total phenolic content of the selected minor fruits ranged from 0.23 to 176.50 mg GAE/g (Table 3). The maximum was recorded for day fruit (176.50 mg GAE/g) and the minimum in hog plum (0.23 mg GAE/g). Guava, pineapple, soursop, passion, acerola camarinha, and mango recorded a polyphenol content of 83.1, 21.7, 84.3, 20.2, 580.1, 492.8, and 260.21 mg GAE/100g, respectively (Kuskoski, García Asuero, Morales Millán, & Fett, 2006; Tiburski et al., 2011).

4.10.2. TFC

Flavonoids are regarded as a low-molecular-weight substances in foodstuffs and boost antioxidant activity. Their content depends on the levels of polyphenols and geographical location. The value of total flavonoids found in the minor fruits studied ranged from 2.26 to 150.02 mg QE/g (Table 3). In our study, bilimbi ranked highest for flavonoid content (150.0259 mg QE/g) while the minimum was recorded as 2.26 mg QE/g in ber. This variation is somewhat similar to previous reports for exotic fruits: Osorio-Esquivel et al. (2011) found a TFC of 13.45–68.79 mg/100g in *Opuntia joconostle*. Since these fruits possessed a fair amount of flavonoids like other exotic fruits, its consumption would help to contribute to add antioxidants to our daily diet.

4.10.3. Total Carotenoid Content

It is evidenced from different studies that carotenoid plays a crucial role in human nutrition and health, and can lessen the risks of cancer and heart disease because of the activity of provitamin A (Tiburski et al., 2011). In this study, total carotenoid content of the selected fruits ranged from 1.26 to 98.24 mg/100g (Table 3). The total carotenoids contained in the selected fruit are comparable to reports published elsewhere: Shajib et al. (2013) reported carotenoid levels of 149 µg/100 g for wood apple, 161 µg/100 g for orboroi, and 218 µg/100 g for Burmese grape.

4.10.4. Total ß-Carotene Content

Total ß-carotene content of the selected minor fruits ranged from 1.41 to 6897.57 µg/100 g. The lowest ß-carotene content was calculated as 1.41 µg/100 g in day fruit and the maximum in aonla (6897.57 µg/100 g) (Table 3). Comparatively higher values for β-carotene were reported by Tiburski et al. (2011) for yellow mombin pulp (314 µg/100 g). It is evidenced that carotenoid content in fruits depends on various factors including soil conditions, maturity stage, enzymes, phenolic content, and genetics (Leong & Oey, 2012).

4.10.5. Ascorbic Acid Content

Ascorbic acid is an important bioactive compound and is considered the most powerful antioxidant in foodstuffs; regular intake lowers the risk of cancer in the human body (Almeida et al., 2011). In the selected fruits it ranged from 16.67 to 664.90 mg/100 g, with a maximum in aonla (664.90 mg/100 g) and a minimum in day fruit (16.67 mg/100 g) on a fresh weight basis (Table 3). These levels are much higher than in the report of Shajib et al. (2013) for monkey jack (14 mg/100 g), Burmese grape (12.1 mg/100 g), orboroi (20.8 mg/100 g), karanda (9.5 mg/100 g), mangosteem (14.4 mg/100 g), blackberry (25.7 mg/100 g), flacourtia (25.6 mg/100 g), rochelle (3.7 mg/100 g), and takituki (27.8 mg/100 g). According to Jukes (1974), the recommended daily intake of vitamin C (ascorbic acid) required to prevent scurvy in adults is about 10 mg, which indicates that the current study found a level of ascorbic acid in selected fruits exceeding that necessary to prevent scurvy, with a daily intake level of 100 g.

4.10.6. Anthocyanin Content

One of the key bioactive compounds in foodstuffs is anthocyanin, which shows potent antioxidant capacity. The anthocyanin content of our selected minor fruits ranged from 1.15 to 47.46 mg/100 g (Table 3). The maximum content noted was 47.46 mg/100 g in bilimbi (*Averrhoa bilimbi* L.) and the minimum in day fruit (1.15 mg/100 g). Moreover, comparatively similar results were found for some tropical fruits (red grapes, 27 mg/100 g; cherries, 112 mg/100g; strawberries, 21 mg/100g; and red raspberries, 92 mg/100g) (Wu et al., 2006).

4.11. Evaluation of Antioxidant Activity

Foodstuffs with high antioxidant properties play a crucial role in the inhibition of ROS-mediated diseases (Dutta & Ray, 2020).

4.11.1. Total Antioxidant Capacity

In this investigation, the antioxidant capacity of the selected minor fruits was assessed by an assortment of different tests. It can be seen that all fruits exhibited potent antioxidant capacity, ranging from 0.01 to 278.24 µg AA/g (Table 3). It is interesting that citron possessed a higher amount of antioxidant capacity (278.24 µg AA/g) than other fruits.

4.11.2. DPPH Radical Scavenging Activity

DPPH is a relatively stable free radical scavenger that donates hydrogen protons to unpaired electrons to convert them into paired ones. It is noted that DPPH radical scavenging activity of the selected minor fruits ranged from 39.7 to 314.0% (Table 3). The maximal DPPH was calculated as 314.0% in hog plum and the minimum in satkara (39.7%). However, all selected fruits in our study showed a strong capacity to scavenge free radicals.

4.11.3. RPA

The RPA of the selected minor fruits ranged from 3.21 to 634.00 µg AA/mg. The maximum value of the reducing power assay was calculated as 634.00 µg AA/mg in hog plum while the lowest was 3.21 µg AA/mg in day fruit (Table 3).

4.11.4. IC_{50}

The IC_{50} is a widely accepted method used to assess the antioxidant activity of foodstuffs, and its value is expected to be lower for higher free radical quenching ability (Sathyanarayanan, Chandran, Thankarajan, Abrahamse, & Thangaraj, 2018). In this study, IC_{50} values ranged from 4.98 to 504.0 µg/g while the maximum value was found in hog plum (856.7 µg/g) and the minimum in elephant apple (4.98 µg/g) (Table 3). Our results indicate that hog plum has potential antioxidant capacity due to its lower value of IC_{50} (4.98 ± 0.24 µg/g), which may be due to the presence of significant amounts of phenolics and flavonoids. This finding is also corroborated by the research of Sathyanarayanan et al. (2018).

4.11.5. MCC

MCC of the selected minor fruits ranged from 22.29 to 210.43% (Table 3), which indicates the ability of minor fruits to reduce different metallic ions and create a stable chemical bond to counteract free radicals. Recent studies have

reported that the redox properties of phenolic species enable them to work as reducing agents, by donating hydrogen and quenching singlet oxygen showing antioxidant activity and chelating metal ions (Elfalleh et al., 2011).

4.11.6. FRAP

The FRAP values found ranged from 0.35 to 2325.0 µM Fe_2SO_4/100 g (Table 3). Our results show a high FRAP value for elephant apple (2325.0 µM Fe_2SO_4/100 g), demonstrating that phenolic composites are among the leading contributors to the high antioxidant properties of this fruit. Previous reports evidenced that fruits with high phenolics can react with free radicals to form a stable product that blocks the radical chain reaction (Sathyanarayanan et al., 2018).

4.11.7. NO Radical Scavenging Activity

NO radical scavenging activity of the selected minor fruits ranged from 0.02 to 178.87%. The results revealed that citron is a rich source of NO radical scavenging activity, recording the highest value of 178.87%, the lowest value being 0.02% in sour ber (Table 3). Thus, our study reported high NO radical foraging activity (178.87 ± 0.02%), which may be due to the presence of different polyphenolic substances in citron.

4.12. Phenolic Compounds

HPLC was used for analysis of six major phenolic acids and matching to the respective standards. Phenolic acid levels in the selected minor fruits ranged 0.94–279.0 mg/100 g for gallic acid, 0.01–43.77 mg/100 g for vanillic acid, 0.16–178.96 mg/100 g for Þ–courmaric acid, 0.01–3.04 mg/100 g for caffeic acid, 0.01–20.44 mg/100 g for ferulic acid, and 12.03–97.35 µg/100 g for lutein. These results indicate that the leading phenolic compounds were gallic and ferulic acids for aonla; gallic acid for hog plum; gallic, Þ-courmaric, ferulic, and vanilic acids for bilimbi; gallic, Þ-courmaric, ferulic, and caffeic acids for elephant apple; gallic, caffeic, and Þ-courmaric acids for day fruit; Þ-courmaric and gallic acids for citron; gallic acid for satkara; Þ-courmaric and gallic acids for ber; and ferulic and caffeic acidc for toikar (Table 4).

Table 4. Phenolic acid content of selected minor indigenous fruits.

Parameter	Minor fruits								
	Aonla	Hog plum	Bilimbi	Elephant apple	Day fruit	Citron	Satkara	Ber (sour)	Toikar
Gallic acid (mg/100 g)	279.06 ±2.00a	58.33 ±0.33d	82.01 ±3.01b	38.14± 0.15e	3.62± 0.01g	0.94± 0.02g	66.00± 0.52c	26.50± 1.17f	62.74± 0.74c
Vanilic acid (mg/100 g)	43.77± 0.15b	42.02± 0.02b	70.50± 2.43a	0.01 ±0.00d	0.04± 0.01d	0.02± 0.01d	4.04± 0.04c	2.46± 0.05c	0.03± 0.01d
p-courmaric (mg/100 g)	1.06± 0.03e	0.96± 0.02e	13.16± 0.17d	11.51± 0.07d	178.96± 4.13a	19.26± 1.54c	3.00± 0.50e	38.14± 0.74b	0.16± 0.01e
Caffeic acid (mg/100 g)	0.09± 0.08c	0.01± 0.00c	0.05± 0.01c	3.04± 0.05a	0.45± 0.01b	0.01± 0.00c	0.10± 0.05c	0.02± 0.01c	0.02± 0.01c
Ferulic acid (mg/100 g)	5.54± 0.03c	0.21± 0.01d	9.59± 0.06b	20.44± 0.05a	0.01± 0.00e	0.01± 0.00e	0.01± 0.00e	0.20± 0.15d	0.21± 0.20d
Lutein acid (µg/100 g)	12.03± 0.04f	29.74± 0.11d	91.13± 3.80b	97.35± 0.35a	71.33± 0.33c	71.33± 0.33c	12.62± 0.12ef	14.85± 0.03ef	16.20± 0.10e

Note: All values are means of triplicate determinations ± SD. Means within columns with different letters a, b, c, d, e, f, g indicate significant result ($p<0.05$).

5. CONCLUSION

This study is the first to provide details regarding the biochemical properties, minerals, bioactive compounds, antioxidant activities, and phenolic compounds of nine key minor indigenous fruits grown in Bangladesh. The results indicate that aonla is a rich source of ascorbic acid (664.92 mg/100 g), total ß-carotene (6897.57 µg/100 g), and RPA (634.00 µAA/g); bilimbi is a rich source of total flavonoids (150.02 mg QE/g), total anthocyanin (47.46 mg/100 g), and FRAP (1817.88 µM Fe_2SO_4/100 g); citron is a rich source of TAC (278.24 µAA/g), MCC (210.43%), and NO (178.87%); hog plum is a rich source of DPPH (314.0%); and day fruit is a rich source of TPH (176.50 mg GAE/g). The findings suggest that all minor fruits studied possess levels of phytochemicals that may be applied as raw material for nutritional and pharmaceutical usage.

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