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NUTRIENT, MINERAL, ANTIOXIDANT, AND ANTHOCYANIN PROFILES OF DIFFERENT CULTIVARS OF *SYZYGIUM CUMINI* (JAMUN) AT DIFFERENT STAGES OF FRUIT MATURATION

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Abstract

The study reports profiles of nutrients, minerals, bioactive phytochemicals, and anthocyanins in four cultivars of *Syzygium cumini* (jamun) fruit at four maturation stages designated as D1 (light-green: very early stage of fruit-set), D2 (dark-green: full-size unripe stage), D3 (pink: semi-ripe stage), and D4 (deep-purple: fully ripe stage). No previous study has investigated profiles of these constituents in jamun at different fruit maturation stages. The most significant findings of this study relate with anthocyanins, not reported heretofore, the biosynthesis of which started as the aglycones of delphinidin and cyanidin at as early as D1 stage when sugars as their glycone components were not yet detected, and the first-time report of pelargonidin in jamun. Further, a total of five anthocyanins were identified, namely, cyanidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3-glucoside, malvidin 3-glucoside, and pelargonidin 3-glucoside. Yet another new information noted during the identification of anthocyanins was that all previous studies on jamun fruit had reported diglucosides as the major anthocyanins, whereas except for cyanidin diglucoside in the present study all other anthocyanins (also including cyanidin) were identified as monoglucosides. All cultivars showed high +90% DPPH scavenging ability, which may be attributed to the collective participation of several antioxidant components such as ascorbic acid, total phenols, and total anthocyanins, all present in high contents in the jamun fruit.

Key words: *Syzygium cumini*; Jamun; Fruit maturation stages; Anthocyanins; Pelargonidin; DPPH-inhibition; Minerals; Bioactive phytochemicals.

Introduction

Jamun, jambol, jambolan, jambolão, black plum, Indian blackberry, and Java plum are some of the names by which the large-sized evergreen tropical tree *Syzygium cumini* (L.) Skeels (syn. *Eugenia cumini*, *E. jambolana*, *Jambolifera chinensis*, *Myrtus cumini*, and *S. jambolanum*) is called in different regions of the world (Faria *et al*., 2011; eFloras, 2015). Native to the Indian subcontinent and adjoining regions of Southeast Asia (Bangladesh, Bhutan, China, India, Indonesia, Laos, Malaysia, Nepal, Pakistan, the Philippines, Sri Lanka, Thailand, and Vietnam), *S. cumini* is also grown as an introduced species in East-Africa (Madagascar), USA (Florida, Hawaii), and South America (Trinidad and Tobago, Surinam, Guyana, Brazil). The plant flowers in February-March or April-May, bearing in June-September, 1-3 cm ellipsoid to ovoid-shaped, one-seeded, deep-purple to bluish-black fruits when fully ripe containing fleshy deep purple-pink or grayish-white pulp, which has a mildly sour-sweet taste that colours the tongue purple (Veigas *et al.,* 2007; Faria *et al*., 2011). Different parts of jamun have been traditionally used for their nutraceutical nature, but it is the fruit that has been extensively used as an adjuvant therapy in type-2 diabetes, also having attributes of an antioxidant, free-radical scavenger, hepatoprotective, neuropsycho-pharmacological, diuretic, anti-HIV, antiallergic, antiarthritic, anti-inflammatory, antiulcer, nephroprotective, antihyperlipidaemic, antihelminthic, antimicrobial, antidiarrhoeal, stomachic, and astringent (Swami *et al*., 2012; Srivastava & Chandra, 2013). Jambolão fruit extract has been also reported to have antiproliferative and proapoptotic effects against breast cancer cells (Li *et al*., 2009a). These pharmaceutical properties are ascribed to the presence of bioactive polyphenolic constituents, due principally to their high antioxidant activity, such as carotenoids, gallic acid, quercetin, tannins, isoflavones, flavones, flavonoids, and anthocyanins (Faria *et al.,* 2011; Aqil *et al*., 2012). The intake of polyphenols is also correlated inversely to the incidence of several chronic diseases such as cardiovascular disease and several types of cancers, principally due to their increased antioxidant potential in plasma that is generally associated with vascular protection (Murtens-Talcott *et al*., 2008; Keen *et al.,* 2005).

Anthocyanins, among the polyphenolic constituents, are water-soluble pigments, produced as secondary plant metabolites, belonging to the flavonoid family of bioactive compounds that give fruits, vegetables, and flowers their characteristic red, blue and purple colours (Dixon & Pasinetti, 2010; Santos *et al.,* 2010). Though anthocyanins are more known to be associated with the specific colourful appearance they give to different plant parts, their most important role is their high antioxidant activity and protection against DNA damage, being able to scavenge free radicals such as superoxide (O_2^{-}), hydroxyl ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O^{-}$) – all chemical species that lead to lipid peroxidation of cell membranes (Kong *et al.,* 2003; Oancea & Oprean, 2011). Recent interest in anthocyanins is linked with its anti-inflammatory and anticarcinogenic properties, protection against heart disease and cancer, gastro-protective effects, anti-diarrheal/-viral/-fungal/-bacterial nature, and reduction in the risk of diabetes and cognitive function disorders (Costa *et al.,* 2013; Pojer *et al.,* 2013). Anthocyanins occur in nature as glycosides (sugar moiety) of anthocyanidins (aglycone). Whereas about 17 anthocyanidins are found in nature, only six (cyanidin, delphinidin, malvidin,

pelargonidin, peonidin, petunidin) are ubiquitous and of significance in the human diet (Miguel, 2011). The position and amount of hydroxylation and methoxylation in the basic anthocyanidin skeleton determine its colour and antioxidant activity (Jing *et al.*, 2014). Thus, comparative antioxidant activity (from highest to lowest) of the six anthocyanins is (Ghafoor & Al Juhaimi, 2014): delphinidin (three –OH groups) > petunidin (two –OH groups, one –OCH_3 group) > cyanidin (two –OH groups) = malvidin (one –OH group, two –OCH_3 groups) > peonidin (one –OH group, one –OCH_3 group) > pelargonidin (one –OH group). Likewise, if –OH groups predominate, then the colour is more towards the bluish shade; and if the –OCH_3 groups are more, then the colour will be reddish (Jing, 2006; Miguel, 2011). These qualitative-quantitative aspects in the jamun anthocyanin structure-function relationship present a unique opportunity to determine anthocyanin profile of the fruit at its different stages of maturation, which has never been considered heretofore but has the potential of use as an index of maturation-stage based nutritionally optimum antioxidant-anthocyanin classification. As the colour of jamun fruit changes from green to pink at the initial stages of fruit development through purple to bluish-black at maturity, contents of anthocyanin and chlorophyll, respectively, increase and decrease (Macheix *et al*., 1990). Further, total anthocyanins content is greatly influenced by cultivar type, level of fruit maturity, and environmental conditions (Green & Mazza, 1986). Considering the wide geographic distribution of jamun, anthocyanin studies are surprisingly limited to materials only from India and Brazil, and that too on fully ripe fruits, making no distinction between cultivars based on fruit size and fruit maturation stages (Veigas *et al*., 2007; Faria *et al*., 2011). Jamun has been also reported to be a rich source of minerals, nutrients, and other bioactive constituents (Swami *et al.*, 2012; Costa *et al.*, 2013), which have been reported to change a lot in the proximate composition and in the phytochemical constituents during the two-month process of its maturation (Baliga *et al*., 2011). These studies further noted that fruit composition in materials from different geographical regions (the Philippines, Honduras, India, Australia, and USA) varied significantly, which however made no distinction between the stages of fruit development and cultivars. The present study accordingly reports quantitative-qualitative profiles of anthocyanins, antioxidants, nutrients, and minerals in four cultivars of *Syzygium cumini* (jamun) growing in Pakistan at four fruit maturation stages distinguishable by their fruit size and colour, from the unripe light green to green, through semi-ripe pink, to ripe deep-purple.

Materials and Methods

Jamun fruit material: Jamun fruits of four *Syzygium cumini* cultivars, distinguishable on the basis of their fruit size at maturity, bearing on trees growing on the campus of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Lahore, Pakistan were harvested from late May until early August. Fruits were harvested onto dry ice at tree-side, sorted for their development stage over dry ice in the laboratory, classified into four stages of development, and immediately stored at –80 ^{o}C till further use. The jamun fruit pulp and peel (together called the pulp) were separated manually from the seed, blended in the frozen state in a small fruit processor, and immediately used for analysis of different constituents in accordance with their respective protocols as described below.

Chemicals and reagents: All the chemicals and standards used in the present study were high performance liquid chromatography (HPLC) grade having purity of above 98%, or of analytical grade. Chemicals used for different analyses were (Sigma-Aldrich, UK): the Folin-Ciocalteau reagent and gallic acid for total phenols determination; 2,2-diphenyl-1-picrylhydrazyl (DPPH) for total antioxidant activity; glucose, galactose, fructose, and mannose for sugar identification; cyanidin-3-O-glucoside, malvidin 3-O-glucoside; pelargonidin 3-O-glucoside and delphinidin 3-glucoside, cyanidin chloride, malvadin chloride, pelargonidin chloride, delphinidin chloride, and rutin trihydrate for anthocyanin identification.

Nutrient-chemical analysis

Total sugars: Total sugar content was determined according to the anthrone method (Anonymous, 2005), which briefly involved hydrolysis of 100 mg of the fruit pulp with 2.5 N HCl in boiling water bath, which was followed by the addition of 4.0 ml of anthrone reagent in 1.0 ml of the hydrolyzed fruit pulp extract. Absorbance of the green colour of the reaction mixture was measured at 620 nm (Shimadzu UV-1800, Japan). Total sugars were calculated against standard glucose solution plot.

Total acidity: Titratable acidity of the fruit pulp was determined according to the standard method (Anonymous, 2005). Briefly, 10 ml of the fruit pulp extract was titrated against 0.1 N NaOH using phenolphthalein as indicator. Total acidity was calculated against malic acid equivalent (0.0067 g) and expressed as malic acid percent.

Ascorbic acid (vitamin C): Ascorbic acid (vitamin C) was extracted from the fruit pulp using the modified method described by Abushita *et al*., (1997). Briefly, 10.0 g of fruit pulp was ground and mixed with 50 ml of 2% metaphosphoric acid followed by mechanical shaking in an orbital shaker in darkness for 15 min. The mixture was filtered through 0.2 µm filter disc and analyzed for vitamin C using HPLC (PerkinElmer Series 200 HPLC, USA) equipped with C18 column connected with UV detector at 225 nm wavelength. Vitamin C was eluted using mobile phase 0.1 M KH_2PO_4-methanol-tetrabutylammonium hydroxide (TBAOH), respectively, in the ratio of 97:3:0.05 at a flow rate of 1 ml/min. Vitamin C peaks were identified by comparing both the retention time and absorbance spectra with those of standard vitamin C solution.

Minerals composition: Macro minerals (Ca, Mg, Na, K, P) and micro minerals (Fe, Zn, Cu, Ni) content was determined by obtaining ash of 1.0 g of fruit pulp by incinerating at 550 ± 5 ^{o}C in muffle furnace in accordance with the standard method (Anonymous, 2005). The

mineral ash was dissolved in 6 N HCl and analyzed for mineral content by atomic absorption spectrophotometer (Unicam 969, UK).

Phytochemicals analysis

Extraction of phytochemicals: Extraction of phytochemicals was done by homogenizing three portions of 10 g fruit pulp in a blender to obtain identical triplicates. The homogenized pulp was mixed with 10 ml of methanol and centrifuged at 5000 rpm for 20 min. The supernatant was transferred to 50 ml volumetric flask. The residual pulp pellet was extracted three times with methanol in the same manner as the first extraction. The final volume of the fruit pulp extract was made up to 50 ml with methanol.

Total phenolic content: Total phenolic content was determined by the Folin-Ciocalteau method (Singleton *et al*., 1999). Briefly, 0.5 ml fruit pulp extract was mixed with 5 ml of Folin-Ciocalteau reagent (1:10) and the reaction mixture was incubated in darkness for 4 min at room temperature followed by the addition of 4 ml of 1 M Na_2CO_3 and again incubated in darkness for 120 min at room temperature. Absorbance of the reaction mixture was then recorded at 765 nm (Shimadzu UV-1800, Japan). A standard curve of gallic acid was plotted in the concentration range of 25-500 mg/L as the standard reference phenolic compound. Results were expressed as mg of gallic acid equivalents per 100 g fruit pulp (gallic acid equivalents per 100 g pulp weight; GAE/100 g PW).

Total anthocyanins: Total anthocyanins were quantified by pH-differential method (Denev *et al*., 2014). Briefly, 9.0 ml of buffers at pH 1.0 (KCl-HCl) and 4.5 (NaOAc-CH_3COOH) were added to 1.0 ml of fruit pulp extract, incubated in darkness for 20 min at room temperature. Absorbance of the reaction mixture was recorded at λ_{max} and 700 nm. Total anthocyanins were expressed as cyanidin-3glucoside equivalents (mg)/100 g pulp weight (CGE/100 g PW).

$$\text{Total anthocyanins (mg/L)} = \left(\frac{\Delta A \times MW \times DF \times 1000}{\varepsilon \times 1}\right) \quad (1)$$

where:
$\Delta A = (A_{max} - A_{700})\ pH_{1.0} - (A_{max} - A_{700})\ pH_{4.5}$; MW = molecular weight of cyanindin-3-glucoside (449.2 g/mol); DF = dilution factor; Ɛ = molar extinction coefficient (26900/M/cm); 1 = path of cuvette (cm).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity: Free radical scavenging capacity or antioxidant activity of the fruit pulp extract on the basis of DPPH$^{\bullet}$ free radical was determined in accordance with the method of Brand-Williams *et al*., (1995). Briefly, a known volume of the fruit pulp extract (200 µl) was mixed with 1 ml of 100 µM of DPPH$^{\bullet}$ solution in methanol. The reaction mixture was incubated in darkness at room temperature for 30 min. Absorbance of the reaction mixture (A_s) was measured at 517 nm and the percent inhibition of scavenging (antioxidant) activity was calculated as:

$$\text{Scavenging activity (\%)} = 1 - \left(\frac{A_s}{A_c}\right) \times 100 \quad (2)$$

where:
A_s and A_c = absorbance of the reaction mixture sample and control (without fruit pulp extract), respectively.

Antioxidant activity is defined as the amount of antioxidant required to decrease the initial concentration of DPPH$^{\bullet}$ by 50% (IC_{50}). IC_{50} was derived by plotting % inhibition versus the concentration plot (mg of the fruit pulp extract added to 1 ml of the DPPH$^{\bullet}$ solution).

HPLC identifications

Anthocyanin extraction and purification: Anthocyanin contents present in the four cultivars of jamun fruit at all the four stages of fruit development as classified in Table 1 were extracted in accordance with the protocol as described by Ozga *et al*., (2007). Briefly, 4 g of ground fruit pulp material was extracted in triplicates in 8 ml of acetone-methanol-water-formic acid solvent mixture (40:40:20:0.1; v/v/v/v), added with Celite 545 (Sigma-Ardrich), vortexed for 2 min and filtered through Whatman No. 4 filter paper. The fruit pulp residue was again washed with the solvent mixture. The first filtrate along with the washing were combined and evaporated to dryness under vacuum concentrator plus (Eppendorf, Germany). The dried residue was resolubilized in 10 ml of deionized water. One ml of the aliquot was then passed through methanol preconditioned Sep-Pak C18 cartridge (Sigma-Aldrich). The column was then washed with 5 ml of deionized water to remove impurities, such as organic acids and sugars from the C18 column. Pure anthocyanin and flavonoid fraction was eluted with 10 ml of 0.1% formic acid in methanol (v/v). The final fraction was again dried under vacuum using vacuum concentrator plus. The dried residue was redissolved in methanol for LC-MS and HPLC analyses.

b) HPLC anthocyanins identification: The fruit pulp extracts for anthocyanin analysis were chromatographed on 250x4.6 mm i.d. 5 µm Zorbax SB-C18 column (Agilent, USA) using HPLC equipped with UV/VIS detector (Perkin Elmer Series 200 HPLC, USA). 20 µl of the extracts were eluted at a flow rate of 1 ml/min using a linear gradient of 5% formic acid in water (solvent A) and methanol (solvent B), using a gradient elution programme: 0 to 25 min, 17% solvent B; 25 to 45 min, 23% solvent B; 45 to 56 min, 23-47% solvent B; 56 to 66 min, 47% solvent B; 66 to 67 min, 47-17% solvent B; 67 to 70 min, 17% solvent B and monitoring at 520 nm. Analytical standards of different anthocyanins were also run under similar conditions and used for the identification of anthocyanins as reference anthocyanidin aglycones.

Table 1. Classification of *Syzygium cumini* (jamun) into cultivars based on the size of fully mature fruit, and characterization of development stages of fruit maturity on the basis of fruit size and colour.

Fruit	Classification into cultivars	Characterization of development stages of the fruit		
		Average size range of fully mature fruits (cm)*	Colour at different development stages corresponding to D1, D2, D3, D4 stages of fruit maturity	Development stages of fruit maturity based on colour
Syzygium cumini (Jamun)	Cultivar-1	0.96 – 2.61	Light green; green; pink; deep purple	D1; D2; D3; D4
	Cultivar-2	1.12 – 2.60	Light green; green; pink; deep purple	D1; D2; D3; D4
	Cultivar-3	1.15 – 2.68	Light green; green; pink; deep purple	D1; D2; D3; D4
	Cultivar-4	1.36 – 2.89	Light green; green; pink; deep purple	D1; D2; D3; D4

* = Harvests of a single tree-representative of each designated cultivar; light green (D1) = Very early stage of fruit-setting; green (D2) = Nearly full size attainment; pink (D3) = Semi-ripe fruit; Deep purple (D4) = Fully ripe fruit

Aglycone-sugar identification: HPLC identification of aglycone anthocyanidins conjugated with sugar molecules was done by acid hydrolysis. Manually collected HPLC peaks were hydrolyzed in accordance with the method described by Lein *et al*., (1997). Briefly, the manually collected HPLC peaks were hydrolyzed with 250 µl of 2.4 N HCl at 110 ^{o}C for 30 min. The resulting mixture was split into two portions; one portion was rechromatographed for aglycone identification on HPLC, and the other portion was neutralized with 75 µl of ammonium hydroxide. The TLC of this fraction was performed for sugar identification using *n*-butanol: acetic acid: ethyl ether: water (9:6:3:1) as mobile phase and silica plates as the stationary phase (Hussain and Mahmood, 2011). Sugar spots on the TLC plate were developed using a spraying mixture of composition (5:5:1; v/v/v) of 5% diphenylamine in EtOH, 4% aniline in EtOH, and 85% phosphoric acid, respectively (Kumar *et al*., 2012).

MS identifications: Molecular masses of the identified anthocyanins/aglycone-anthocyanidins HPLC peaks of the fruit pulp extracts were characterized using the MS technique. Mass spectrophotometer LTQ XL (Thermo Fisher Scientific, USA) was operated on both positive and negative modes fitted with electron spray ionization (ESI) interface. One ml of the methanolic fruit pulp extract was analyzed at the flow rate of 10µl/sec and scanned within the atomic mass unit (amu) range of 150 amu to 2000 amu.

Results and Discussion

Fruit development stages and classification into cultivars: Based on their colour and size, *Syzygium cumini* (jamun) fruits were categorized into four development stages of maturation (D1, D2, D3, D4), respectively shown schematically in Fig. 1 as: (a) light green (very small at the early stage of fruit-setting); (b) green (unripe, near-full size attainment); pink (semi-ripe); and deep purple (fully ripe). Two varieties are differentiated in India-Pakistan as: (a) the more common early-ripening (June-July) table-variety 'Ra Jamun' bearing larger-sized (1-3 cm) juicy, sweet, oblong and dark-purple or bluish fruits; and (b) the lesser-liked late maturing (August) 'Kaatha Jamun' (used for vinegar-making) that bears in comparison with 'Ra Jamun' smaller-sized (1-1.5 cm), lesser juicy, slightly round, deep-purple or blackish, lesser sweet and more sour fruits with larger seeds (Morton, 1987). However, only 'Ra Jamun' variety was presently studied, which was classified into four morphotype cultivars based on morphological and organoleptic features, and average minimum and maximum fruit size at their fully ripe stage (Table 1). All cultivars followed similar pattern of fruit development, though showing differences in size at each stage of development.

Nutrient components

Total sugars: The pattern of increase in total sugars in the four cultivars at different stages of fruit development is shown in Table 2. With no sugars detected at stage D1, their contents from stage D2 continued to increase in all cultivars throughout the subsequent stages, attaining maximum at the fully ripe D4 stage, which in cultivars 1 and 3 was steep, respectively, culminating in 2.7-fold and 1.9-fold increase with total sugars of 1409 mg/100 g and 1312 mg/100 g, being modest in cultivars 2 and 4, reaching 551 mg/100 g and 582 mg/100 g at 2.2-fold and 2.6-fold. An observation of much significance is that total sugars in fully ripe fruits in cultivars 1 and 3 were between 2.25-2.55 times greater than in cultivars 2 and 4, thus making the former set of cultivars sweeter than the latter. No earlier study has reported on these aspects of total sugars and the pattern of their increase at various stages of fruit development in different cultivars of *Syzygium cumini* (jamun), which in addition to being of value to horticulturists and market vendors may be of use to those consuming this fruit as an adjunct therapy for the treatment of diabetes, in preferring cultivars bearing fruits with lesser-sugar contents. The relationship of increase in sugars as associated with decrease in organic acids during maturation has been extensively reported in different fruits, such as peach (Wu *et al*., 2005), blueberry (Ayaz *et al*., 2001), medlar (Glew *et al*., 2003), etc., also observing that as fruits ripen more sugars are synthesized and thus fruits become more sweet and less sour.

Total acidity (%): Total acidity of all cultivars was observed to follow a similar pattern of decrease in the four fruit maturation stages, with stage D1 showing the highest total acidity in all the four cultivars, remaining almost unchanged in stage D2 followed by a steep decrease in stage D3 (Table 2). Whereas no difference in total acidity was noted between D3 and D4 stages in cultivar-1, the decrease was little in cultivar-2 but significant in cultivars 3 and 4. The overall decrease in total acidity in the four cultivars ranged between 2.25-fold in cultivar-3 and 1.6-fold in cultivar-2. The fully ripe fruits of *Syzygium cumini* (jamun) of cultivars 2 and 3 were most sour, respectively, followed by cultivars 1 and 4. However, following the ratio of sugar/acidity in the four cultivars, their sweetness index may be rated in the decreasing order of more-sweet and lesser-sour as: cultivars 1, 3, 2, and 4. Though it is well

known that as fruits develop from unripe to ripe stages, organic acids degrade and sourness decreases (Wu *et al*., 2005), the pattern of decrease may differ in different fruits, such as: (a) maximum organic acid values at intermediate maturities of peaches (Wu *et al*., 2005); (b) first a decrease during passage through immature stages then a rise during mid-ripe stages and finally reduced acidity in ripe medlar fruit (Glew *et al.*, 2003); (c) initial increase in acidity followed by a decreased acidity in ripe wolfberry fruit (Zhao *et al*., 2015), (d) increase of citric acid in earlier stages and then its decrease in mango fruit (Léchaudel *et al*., 2005); and (e) unchanged levels in the first two immature stages followed by a steep decrease in the third semi-ripe stage and only a little further decrease in the fully ripe *Syzygium cumini* (jamun) fruit in the present study.

Ascorbic acid (vitamin C): Ascorbic acid was observed to steadily decrease throughout the four stages of maturation in a similar pattern in all cultivars as the fruit of *Syzygium cumini* (jamun) developed from immature to the ripe stage (Table 2). The minimum value of ascorbic acid (mg/100 g pulp) in fully ripe fruit was noted in cultivar-2 (19.63), whereas cultivars 1 (25.67) and 4 (25.63) had the highest values with cultivar-3 (23.67) only slightly behind these. Most reduction from D1 to D4 stage of 40.9% was also noted in cultivar-2, respectively, followed by 30.6%, 26.9% and 17.9% in cultivars 3, 1 and 4. Similar pattern of decrease of ascorbic acid throughout the stages of maturation process, as noted in the present study, was reported in medlar fruit, which reduced from (mg/100 g) 41.7 to 8.4 (Glew *et al*., 2003). Different values of ascorbic acid in *Syzygium cumini* (jamun) have been reported from diverse geographical regions, such as (mg/100 g edible portion): 5.7 from Honduras (Munsell *et al*., 1949); 14.3 from USA (Anonymous, 2015); 19.4 from Sindh, Pakistan (Shahnawz *et al*., 2009); and 30 from northern Bangladesh (Paul and Shaha, 2004). Ascorbic acid content is of significance due to its high antioxidant activity, immunity response against infection, health-promoting effects as related with certain types of cancer, coronary heart disease and cataract, and against several other health-distressing states (Iqbal *et al*., 2004; Angelo, 2013).

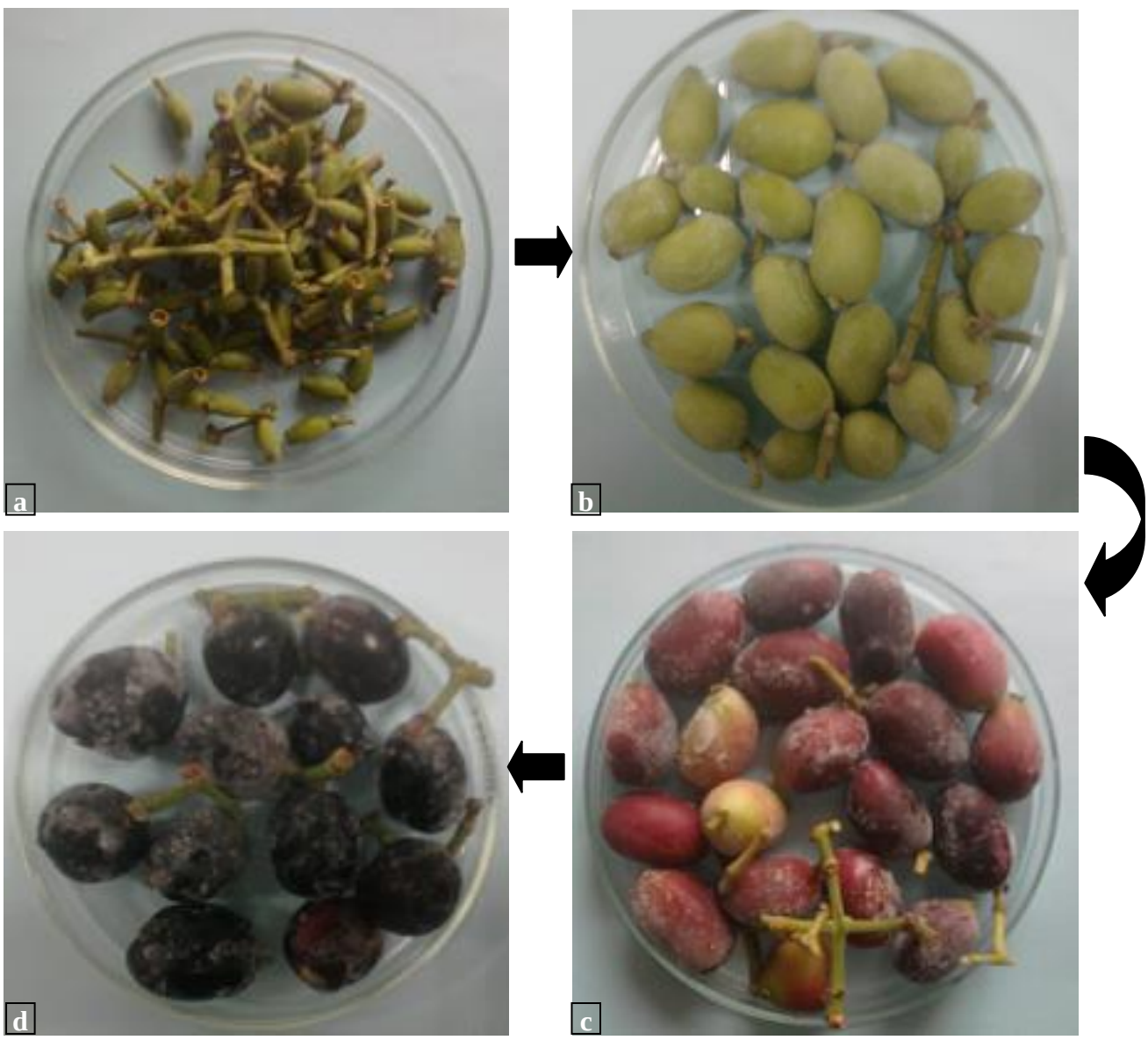


Fig. 1. Different fruit development stages, respectively, D1, D2, D3, and D4, recognized on the basis of changes in size and colour: (a) light-green (very small at the early stage of fruit-setting); (b) green (unripe, nearly full-size fruit attainment); (c) pink (semi-ripe fruit); and (d) deep purple (fully ripe fruit).

Table 2. Some important nutrient features and bioactive phytochemicals of four cultivars of *Syzygium cumini* (jamun) at different stages of fruit maturity.

Nutrient feature	Cultivar type	Fruit development stages			
		D1	D2	D3	D4
Total sugars (mg/100 g FW)	Cultivar-1	nd	519.55 ± 0.12	669.94 ± 43.38	1408.79 ± 18.95
	Cultivar-2	nd	247.72 ± 12.59	339.96 ± 5.73	551.37 ± 6.87
	Cultivar-3	nd	695.38 ± 1.52	705.39 ± 6.81	1311.79 ± 57.51
	Cultivar-4	nd	221.61 ± 6.57	450.46 ± 4.81	582.40 ± 25.70
Total acidity (%)	Cultivar-1	0.037 ± $<10^{-3}$	0.038 ± $<10^{-3}$	0.017 ± $<10^{-3}$	0.017 ± $<10^{-3}$
	Cultivar-2	0.032 ± $<10^{-3}$	0.032 ± $<10^{-3}$	0.021 ± $<10^{-3}$	0.020 ± $<10^{-3}$
	Cultivar-3	0.045 ± $<10^{-3}$	0.045 ± $<10^{-3}$	0.025 ± $<10^{-3}$	0.020 ± $<10^{-3}$
	Cultivar-4	0.033 ± $<10^{-3}$	0.032 ± $<10^{-3}$	0.020 ± $<10^{-3}$	0.016 ± $<10^{-3}$
Ascorbic acid (mg/100 g FW)	Cultivar-1	35.12 ± 1.11	32.17 ± 1.57	27.15 ± 1.32	25.67 ± 1.33
	Cultivar-2	33.21 ± 1.21	28.77 ± 1.11	21.67 ± 1.27	19.63 ± 1.11
	Cultivar-3	34.12 ± 1.21	29.13 ± 1.27	25.12 ± 1.07	23.67 ± 1.17
	Cultivar-4	31.22 ± 1.02	29.67 ± 1.08	27.12 ± 0.79	25.63 ± 1.77
Total phenols (mg GAE/100 g FW)	Cultivar-1	1590.10 ± 97.72	1994.32 ± 40.82	1720.64 ± 37.64	1808.08 ± 67.29
	Cultivar-2	1244.98 ± 74.58	1885.40 ± 69.01	1605.57 ± 19.03	1675.79 ± 56.38
	Cultivar-3	1181.62 ± 56.98	1648.81 ± 33.52	1580.34 ± 69.21	1283.59 ± 37.22
	Cultivar-4	1008.49 ± 7.05	1332.58 ± 2.63	1240.73 ± 16.14	1184.50 ± 1.51
DPPH (% inhibition)	Cultivar-1	-	-	-	90.41 ± 1.21
	Cultivar-2	-	-	-	90.97 ± 0.98
	Cultivar-3	-	-	-	90.24 ± 1.15
	Cultivar-4	-	-	-	90.46 ± 1.11
Total anthocyanins (mg/100 g FW)	Cultivar-1	15.69 ± 2.03	22.27 ±10.97	85.60 ±8.85	803.04 ± 28.85
	Cultivar-2	8.73 ± 1.27	23.45 ± 1.09	405.57 ± 9.21	1120.64 ± 16.14
	Cultivar-3	40.73 ± 6.40	80.34 ± 9.04	952.22 ± 9.04	1486.53 ± 19.18
	Cultivar-4	84.50 ± 7.29	683.59 ± 6.38	1675.79 ± 7.75	1808.08 ± 23.86

D1, D2, D3, D4 = Different fruit maturity stages; FW = Edible portion of *Syzygium cumini* fruit weight (= jamun fruit pulp and peel without the seed); nd = Not detected; GAE = Gallic acid equivalents; DPPH = (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

Mineral nutrients: Although minerals are not a source of energy, they are essential for several physiological processes, as cofactors for various enzymatic reactions, maintenance of cell structure and function, growth, and development of the human body (Murray *et al*., 2000; Soetan *et al*., 2010). Required in small amounts, minerals are categorized as macronutrients (Ca, Mg, P, K, Na needed >100 mg/day) and micronutrients (Fe, Cu, Zn, etc., needed <100 mg/day). Accordingly, dietary intakes of various minerals have been worked out as a system of nutrition recommendations by the U.S. Institute of Medicine (Higdon & Drake, 2013). Among various foods, fruits are recognized as a valuable source of minerals (Grosvenor & Smolin, 2002; Milton, 2003). Within this context, the pulp of *Syzygium cumini* (jamun) fruit is reported to be highly nutritive containing important minerals (Baliga *et al*., 2011), though the source studies lack data on the profile of minerals at different stages of development with no differentiation of varieties and cultivars. These aspects were duly considered in the present study (Table 3). It was noted that the fruit pulp of all cultivars was adequately rich in both macro- and micro-minerals, showing non-significant inter-cultivar differences in the D4 stage contents of most minerals (ignoring some erratic data), except Fe and K showing significant differences. All the minerals, except Fe, showed significant decreasing trends in their contents in all cultivars from the early D1 stage to the fully ripe D4 stage. Similar decreasing trends in all minerals have been reported in hazelnut varieties (Seyhan *et al*., 2007). The decreasing trends of different minerals commenced sharply at the D3 stage in most cases, which in Ca and Na occurred at the D2 stage. A critical view of the data presented in Table 3 reveals that the decreasing trends of minerals may be broadly grouped as high (Cu, Ca, Mg, K), moderate (P, Na), and low (Zn). Whereas shifts in the contents of biochemicals during various development stages may be explained in terms of metabolic transformations/ breakdowns of one form to another, the same is not plausible to minerals being non-changeable forms. Thus the trends of reduction of different minerals in the pulp component may be attributed to their relocation in the development of seed, the other integral part of the *Syzygium cumini* (jamun) fruit. Within this context the role of the group of high reduction minerals, particularly Ca is relevant being an essential element in cell division and fruit development (Bernadac *et al*., 1996). It may be observed from Table 3 that Fe contents rose sharply in all cultivars in the D3 and D4 stages, which also corresponded with a steep rise in anthocyanin contents at the same stages of fruit development (Table 2). It is evident that the rise in Fe contents is directly linked to the anthocyanin contents. The almost 4-fold increase in Fe contents in the D4 stage ripe fruits of all the four cultivars can be attributed to its potential involvement

with anthocyanins in metallopigmentation and copigmentation mechanisms as reported in several studies, such as: Z-chalcones in structurally simple glycosylated anthocyanins are perfectly sized for complexing Fe (Santos-Buelga *et al*., 2010); intense blue colour in fruits and flowers is due to complexing of delphinidin-based anthocyanins with Fe (Glover & Martin, 2012); and ferric cyanidin-3-glucoside formed very purple ferric complexes with an anthocyanin/Fe ratio of two (Cheng & Crisosto, 1997). Metallopigmentation/ copigmentation is a process in which metal ions interact with anthocyanins greatly enhancing its colour and also making the pigment complex slow to degrade (Welch *et al*., 2008). It is relevant to note that blue and purple are the predominant colours in *Syzygium cumini* (jamun) fruit. Elevated levels of Fe in the ripe fruit were further nutritionally beneficial being the principal factor in hemoglobin synthesis, particularly in iron deficiency anemia (Johnson-Wimbley & Graham, 2011). Another aspect of high nutrient worth noted in *Syzygium cumini* (jamun) fruit was the presence of potassium and sodium in the ideal ratio of above 2, being respectively 2.3, 2.4, 2.8, and 2.9 in cultivars 2, 4, 1, and 3. Fewer than 2% of adults in the U.S. meet the recommended K/Na ratio of above 2 (Rhodes *et al*., 2014). The health consequence of low K/Na ratio are severe, leading to high blood pressure, heart disease, and stroke, which has been associated with a higher risk of all-cause and cardiovascular disease mortality (Drewnowski *et al*., 2012). It is also to be noted that studies from different regions have reported significant variations in the contents of minerals from northern Bangladesh (Paul & Shaha, 2004), Honduras in Central America (Munsell *et al*., 1949), United States (Anonymous, 2015), southern and eastern India (Sehwag & Das, 2014), and southern Pakistan (Shahnawaz *et al*., 2009). Such variation may be linked to cultural practices, geo-climatic conditions, and varietal/cultivar differences (Seyhan *et al*., 2007; Soetan *et al*., 2010).

Table 3. Mineral nutrients in four cultivars of *Syzygium cumini* (jamun) at different stages of fruit development.

Mineral nutrient	Cultivar type	Fruit development stages				Decrease/increase (%↓/↑)
		D1	D2	D3	D4	
Zn (µg/100 g FW)	Cultivar-1	96.26 ± 2.71	80.25 ± 2.77	75.85 ± 3.35	60.29 ± 2.35	↓ 37.4
	Cultivar-2	99.76 ± 1.77	93.41 ± 2.76	64.56 ± 4.23	59.13 ± 3.71	↓40.7
	Cultivar-3	85.15 ± 2.72	74.19 ± 3.76	64.85 ± 1.23	57.47 ± 2.71	↓32.5
	Cultivar-4	57.18 ± 2.97	57.03 ± 1.68	56.04 ± 1.38	48.64 ± 1.57	↓14.9
Cu (µg/100 g FW)	Cultivar-1	27.34 ± 2.22	21.22 ± 2.17	19.07 ± 2.23	5.39 ± 1.21	↓80.3
	Cultivar-2	28.47 ± 2.10	24.77 ± 1.88	8.91 ± 1.57	6.96 ± 1.12	↓75.6
	Cultivar-3	24.89 ± 2.73	23.95 ± 2.37	8.58 ± 1.41	6.88 ± 2.44	↓72.4
	Cultivar-4	26.48 ± 2.31	25.88 ± 2.67	9.79 ± 2.33	5.45 ± 1.41	↓79.4
Fe (µg/100 g FW)	Cultivar-1	42.94 ± 3.37	44.57 ± 2.75	166.68 ± 4.23	194.23 ± 3.77	↑352.3
	Cultivar-2	43.39 ± 3.71	85.54 ± 4.32	184.61 ± 4.57	210.81 ± 5.12	↑385.8
	Cultivar-3	39.14 ± 2.74	45.48 ± 3.75	157.42 ± 5.71	165.08 ± 4.75	↑321.8
	Cultivar-4	41.45 ± 2.17	57.06 ± 2.33	169.07 ± 4.12	171.46 ± 3.78	↑313.7
Ca (mg/100 g FW)	Cultivar-1	146.58 ± 2.33	70.10 ± 2.78	41.88 ± 1.21	20.06 ± 2.57	↓86.3
	Cultivar-2	129.35 ± 3.22	51.76 ± 2.72	30.80 ± 2.78	23.84 ± 1.97	↓81.6
	Cultivar-3	98.16 ± 2.15	40.87 ± 2.11	23.21 ± 1.71	19.67 ± 1.57	↓80.0
	Cultivar-4	135.21 ± 2.57	49.77 ± 2.23	31.48 ± 1.35	17.67 ± 2.67	↓86.9
Mg (mg/100 g FW)	Cultivar-1	41.47 ± 2.57	30.92 ± 1.33	15.07 ± 2.22	9.59 ± 1.55	↓76.9
	Cultivar-2	45.76 ± 2.71	35.72 ± 3.31	17.77 ± 1.23	8.20 ± 1.11	↓82.1
	Cultivar-3	41.86 ± 1.15	36.93 ± 2.15	20.90 ± 2.77	8.78 ± 2.25	↓79.0
	Cultivar-4	49.99 ± 3.58	48.08 ± 3.75	21.37 ± 2.15	9.55 ± 2.33	↓80.9
P (mg/100 g FW)	Cultivar-1	55.12 ± 1.23	51.77 ± 2.22	50.23 ± 2.17	49.33 ± 2.57	↓10.5
	Cultivar-2	52.23 ± 2.11	45.67 ± 1.97	32.67 ± 1.67	23.11 ± 2.32	↓55.8
	Cultivar-3	48.66 ± 2.37	37.67± 2.12	22.17 ± 2.22	17.42 ± 1.77	↓64.2
	Cultivar-4	52.97 ± 2.32	44.77 ± 1.11	37.62 ± 2.34	24.67± 2.17	↓53.4
Na (mg/100 g FW)	Cultivar-1	195.45 ± 4.77	21.10 ± 3.37	62.82 ± 4.71	70.06 ± 3.67	↓64.2
	Cultivar-2	129.35 ± 3.77	83.52 ± 3.97	65.39 ± 1.99	47.68 ± 3.67	↓63.1
	Cultivar-3	98.16 ± 2.75	71.74 ± 2.72	52.82 ± 1.97	37.17 ± 3.21	↓62.1
	Cultivar-4	135.21 ± 3.74	72.31 ± 2.74	54.22 ± 1.78	40.71 ± 2.07	↓69.9
K (mg/100 g FW)	Cultivar-1	409.07 ± 3.71	380.47 ± 5.67	236.00 ± 3.77	195.51 ± 2.37	↓52.2
	Cultivar-2	367.93 ± 3.67	262.21 ± 4.71	147.39 ± 2.17	111.51 ± 3.57	↓69.7
	Cultivar-3	403.01 ± 4.71	351.99 ± 3.72	280.21 ± 2.71	107.17 ± 2.22	↓73.4
	Cultivar-4	377.32 ± 2.45	294.25 ± 4.72	123.05 ± 3.22	99.77 ± 2.14	↓73.6

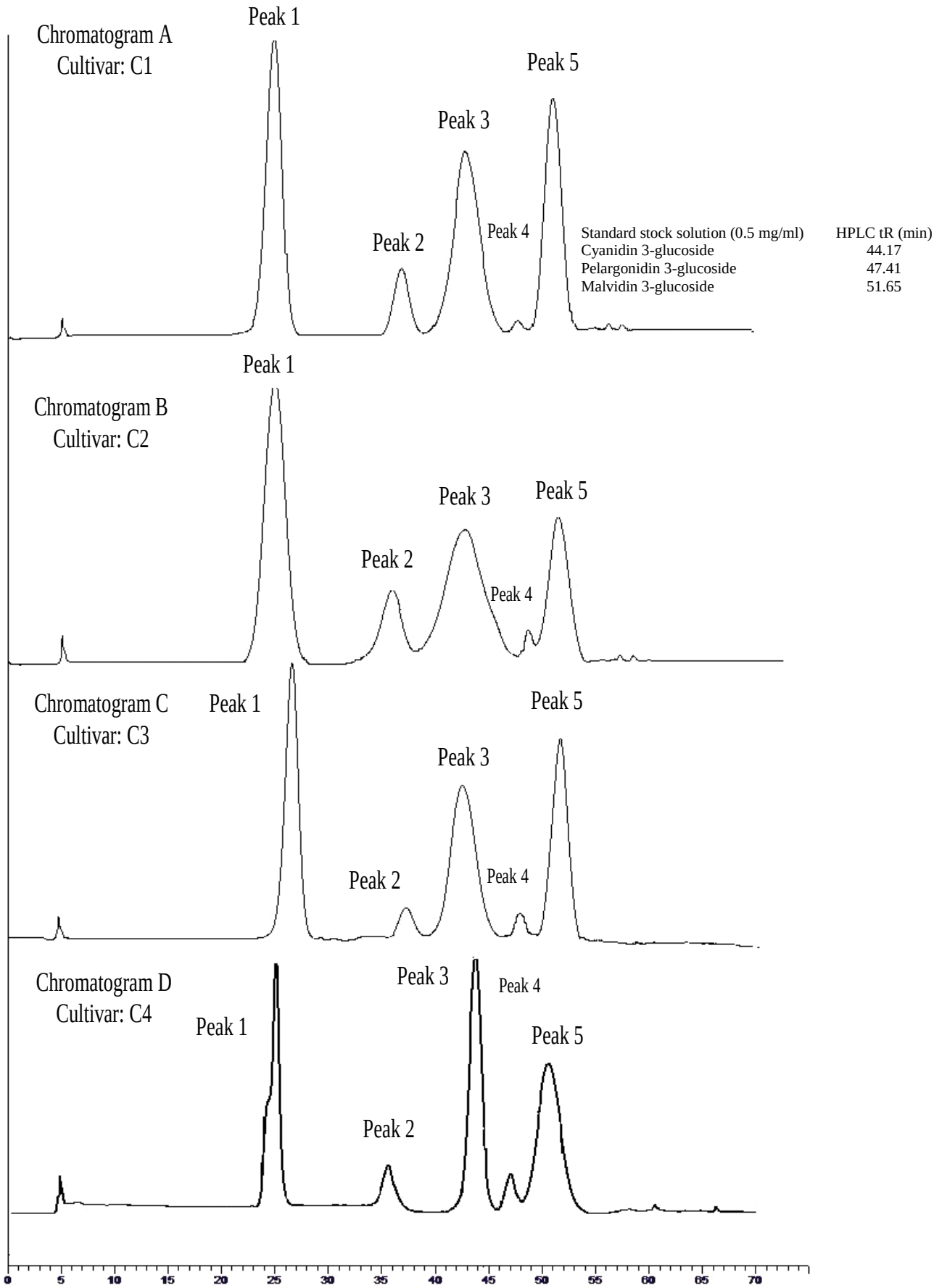
FW = Edible portion of *Syzygium cumini* fruit weight (jamun fruit pulp and peel without the seed); D1, D2, D3, D4 = Different fruit development stages; **%**↓ = Percent decrease between development stages D1 – D4; %↑ = Percent increase between development stages D1 – D4

Bioactive phytochemicals

Total phenols: Polyphenolic compounds are plant secondary metabolites produced to defend them against reactive oxygen species, UV-damage, and phytopathogens; however, much interest in them during recent decades is due to their beneficial antioxidant potential in human health (Panday & Rizvi, 2009). Antioxidants are compounds that prevent, inhibit or delay the harmful effects of oxidation of other molecules by hindering the production of free radicals that can start multiple chain reactions that eventually cause damage or death to the cell (Shebis *et al*., 2013). Data obtained on fourteen wild edible fruits from Burkina Faso showed that fruits with high phenolic contents had high antioxidant activities (Lamien-Meda *et al*., 2008), and the antioxidant activity of raspberry and blackberry was noted to be directly related to total phenolics (Sariburun *et al*., 2010). Further, though a positive and highly significant correlation was reported between total phenolics and antioxidant activity in a study on 28 plant products, the relationship between phenolics and antioxidant activity of anthocyanin-rich plants was exceptionally high, respectively, 88.9, 92.1, and 82.5 corresponding to 9747, 4180, and 2098 mg/100 g total phenols in purple sunflower husk, blueberry, and sweet cherry (Veliogu *et al*., 1998). It is also believed that several factors, such as biotic and abiotic, region of cultivation, climatic conditions, cultivar type, harvest time and the stage of fruit maturity, and extraction protocols influence the total phenol contents in fruits (Tlili *et al*., 2014). Total phenols in fruits of four cultivars of *Syzygium cumini* (jamun) at four different stages of development were thus determined (Table 2). In quantitative terms, total phenols in the four cultivars were significantly different with cultivar-1 showing the highest content (1808 mg gallic acid equivalents/100 g) successively followed by cultivars 2, 3, and 4 being the lowest in content (1184 mg gallic acid equivalents/100 g). The inter-cultivar pattern of changes in the total phenols was similar, though the D1 stage had the lowest contents rising to the highest in the D2 stage then declining in the D3 stage; however, cultivars 1 and 2 showed an increase, while cultivars 3 and 4 decrease in the D4 stage. Such variations in total phenols are in agreement with other studies reporting that the contents of phenolic compounds vary significantly between stages of fruit maturity (Sukrasno and Yeoman, 1993; Kondo *et al*., 2002; Tlili *et al*., 2014). Different quantities of total phenols in *Syzygium cumini* (jamun) from different geographical regions have been likewise reported, such as (mg gallic acid equivalents/100 g fresh fruit weight): 7185 in eastern (Assam) India, (Saikia *et al*., 2016); 560 in southern (Mysore) India (Veigas *et al*., 2007); and 148 in fruits from Brazil (Faria *et al*., 2011).

Total anthocyanins: Due to their pharmacological and biological attributes, flavonoids and anthocyanins, both phenolics by classification, have been linked with potential health benefits for humans (Harborne & Williams, 2000; Kong *et al*., 2003). It has been also reported that fruits containing anthocyanins as the major pigments in them have high total phenolic contents upon their maturity (Tanaka *et al*., 2008). Total anthocyanins content is greatly influenced by cultivar type, level of fruit maturity, and environmental conditions (Green & Mazza, 1986). As may be visualized from Fig. 1, fruit development stages 3 and 4 of *S. cumini* (jamun) in all the four cultivars were characterized by increasing anthocyanins content, with the semi-ripe (pink) stage D3 followed by the fully-ripe (deep purple) stage D4 having the highest. The increasing anthocyanins content corresponded with a decreasing shift of total phenols from D2 stage to the D3 and D4 stages of development (Table 2), thus indicating that some of the phenols may be involved in the synthesis of anthocyanins. Further, similar pattern of changes in the contents of anthocyanins in all the development stages of all four cultivars was noted. Starting with very low contents in the D1 stage, anthocyanin contents commenced to increase modestly at the D2 stage, but sharply at D3 and D4 stages, except in cultivar-4 which recorded sharp rise at D2 and D3 stages. As may be noted from Table 2, the increase in anthocyanins content was many-folds with the increasing level of maturity. Through the development stages from D1 to D4, increase in the anthocyanin contents in cultivars 1 to 4 was, respectively, 51.2-fold, 170.3-fold, 27.5-fold, and 21.4-fold. However, the highest anthocyanin content of 1808 mg/100 g at the fully ripe stage was observed in cultivar-4 followed by cultivars 2, 3, and 1. From the health benefit perspective of anthocyanins, cultivar-4 fruits of *Syzygium cumini* (jamun) may thus be rated as by the far the best followed by cultivar-2.

DPPH-based antioxidant activity assay: DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a dark-coloured crystalline powder composed of stable free radical molecules, the scavenging of which is the basis of an antioxidant assay (Sharma & Bhat, 2009). Accordingly, the DPPH-based free radical scavenging is most commonly used to determine the antioxidant capacity of fruits and other phenol-rich plant products (Sakanaka *et al*., 2005). The assay is based on the interaction of DPPH with antioxidants that involve either the transfer of hydrogen atom or electrons from the antioxidant to DPPH thus neutralizing its free radical nature (Naik *et al*., 2003). The DPPH free radical scavenging activity, expressed as its inhibition (%), of the fruits of four cultivars of *Syzygium cumini* (jamun) is given in Table 2. All cultivars showed high +90 % DPPH inhibition, which may be observed as strong and directly correlated to the presence of high total phenols in all the four cultivars (Table 2). More than 90% DPPH inhibition by Japanese persimmon extracts containing 112 mg/g total phenols was concluded as strong (Sakanaka *et al*., 2005). Black jamun (*Syzygium cumini*) pulp containing 7185 total phenols (mg gallic acid equivalents/100 g) showed 96.9 % DPPH inhibition, which was concluded as positive and significant correlation between total phenols and DPPH radical scavenging (Saikia *et al*., 2016). The high DPPH scavenging ability of the pulp of *Syzygium cumini* (jamun) may be attributed to the collective participation of several antioxidant components, such as ascorbic acid, total phenols, and total anthocyanins, all present in high contents in the fruit.



Standard stock solution (0.5 mg/ml)	HPLC tR (min)
Cyanidin 3-glucoside	44.17
Pelargonidin 3-glucoside	47.41
Malvidin 3-glucoside	51.65

Fig. 2. C18-HPLC profiles of anthocyanins at 520 nm where peaks 1-5, respectively, were: (1) cyanidin 3,5-diglucoside, (2) delphinidin 3-glucoside, (3) cyanidin 3-glucoside, (4) pelargonidin 3-glucoside, and (5) malvidin 3-glucoside; inset table shows HPLC retention times (tR) of standard stock solutions on anthocyanins.

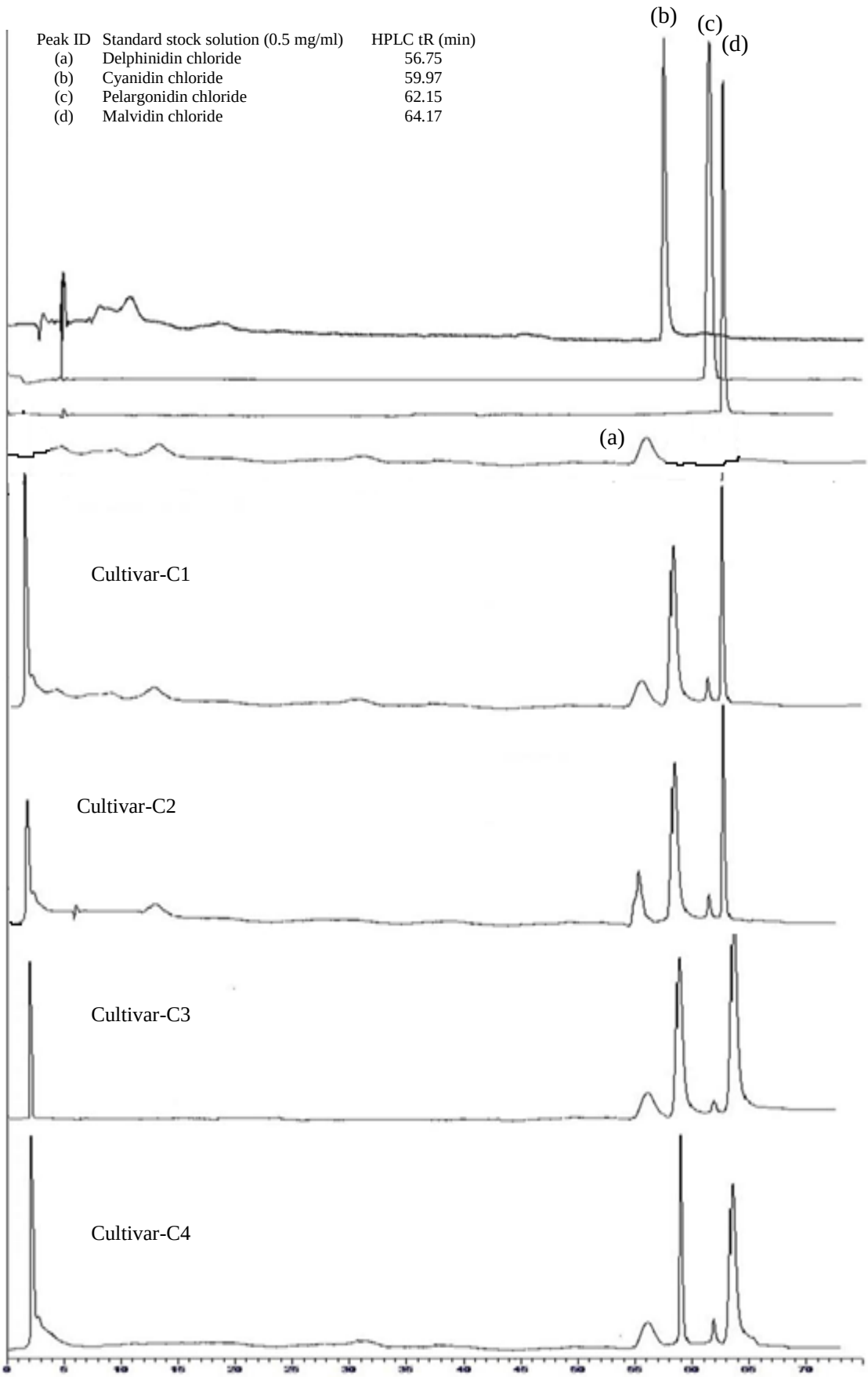


Fig. 3. Anthocyanin aglycone identification by C18-HPLC of peak fractions (1 – 5) collected at 520 nm after acid hydrolysis of D4 stage of fruit development of four cultivars of *Syzygium cumini* (jamun) as compared with the standard solutions of: (a) delphinidin; (b) cyanidin; (c) pelargonidin; and (d) malvidin as their chloride salts; Table 4 shows HPLC tR of standard stock solutions of anthocyanidin aglycones.

Table 4. HPLC chromatographic peaks and their retention time (tR), mass spectroscopic data of the HPLC peaks, anthocyanins identified in different cultivars of *Syzygium cumini* (jamun), in accordance with the HPLC tR data of standard stock solutions, also agreeing with the $[M]^+$, and MS/MS data of the respective anthocyanins reported in the literature.

Cultivar type	HPLC Peak #	HPLC tR (min)	$[M]^+$ (m/z)	MS/MS (m/z)	Anthocyanin identified
Cultivar-1	1	25.22	611	449, 287	Cyanidin 3,5-diglucoside
	2	36.17	465	303	Delphinidin 3-glucoside
	3	44.12	449	287	Cyanidin 3-glucoside
	4	47.50	433	271	Pelargonidin 3-glucoside
	5	51.77	493	331	Malvidin 3-glucoside
Cultivar-2	1	25.18	611	449, 287	Cyanidin 3,5-diglucoside
	2	36.21	465	303	Delphinidin 3-glucoside
	3	44.15	449	287	Cyanidin 3-glucoside
	4	47.47	433	271	Pelargonidin 3-glucoside
	5	51.57	493	331	Malvidin 3-glucoside
Cultivar-3	1	25.27	611	449, 287	Cyanidin 3,5-diglucoside
	2	36.29	465	303	Delphinidin 3-glucoside
	3	44.21	449	287	Cyanidin 3-glucoside
	4	47.22	433	271	Pelargonidin 3-glucoside
	5	51.60	493	331	Malvidin 3-glucoside
Cultivar-4	1	25.13	611	449, 287	Cyanidin 3,5-diglucoside
	2	36.19	465	303	Delphinidin 3-glucoside
	3	44.34	449	287	Cyanidin 3-glucoside
	4	47.37	433	271	Pelargonidin 3-glucoside
	5	51.67	493	331	Malvidin 3-glucoside

Anthocyanin appearance and identification: It was noted from the HPLC data that biosynthesis of the aglycones of anthocyanins, cyanidin and delphinidin anthocyanadins, commenced with the very initial fruit development stages D1 and D2. Further, the D1 aglycones specifically lacked the attachment of their glycone components in the making of a complete anthocyanin, as sugars were not detected at this stage of fruit maturation (Table 2). The HPLC profiles of anthocyanins at 520 nm of stage D4 of the four cultivars of jamun fruit are presented in Fig. 2, all of which had similar pattern of anthocyanin elution. A total of five anthocyanin peaks were identified. Peaks 3, 4, and 5 were, respectively, identified as cyanidin, pelargonidin, and malvidin. For the identification of aglycones of peaks 1 and 2, and reconfirmation of peaks 3, 4, and 5, by referring to their corresponding standard stock solutions (cf. Inset Tables Fig. 2 and Fig. 3), these peaks were manually collected, acid hydrolyzed, and rechromatographed under similar HPLC conditions. Peaks 1 and 2 were, respectively, identified as cyanidin and delphinidin, whereas peaks 3, 4 and 5 were reconfirmed as such using the hydrolyzed/ cleaved aglycones. As observed from the standard TLC run of glucose solution, the major sugar attached to these aglycones was identified as glucose. The HPLC peak fractions were further confirmed by their MS analysis. The MS data of these peaks are presented in Table 4. The molecular ion mass of 449 atomic mass unit (amu) with a fragment mass of 287 amu confirmed the aglycon cyanidin (287 amu) with an attached hexose sugar (287+162 = 449 amu), whereas the molecular ion mass of 611 amu indicated the presence of two hexose sugars (287+162+162 = 611 amu). Peak 1 of HPLC was, therefore, confirmed as cyanidin 3,5-diglucoside having molecular mass of 611 amu and the fragment mass of 449 amu and 287 amu (Jampani *et al*., 2014). Similarly, molecular ion mass of 465 amu with the fragment mass of 303 amu confirmed the identification of delphinidin 3-glucoside as peak 2 of HPLC (Jampani *et al*., 2014). HPLC peaks 3, 4, and 5 having molecular ion mass of 449 amu, 433 amu and 493 amu, with fragment mass of 287 amu, 271 amu, and 331 amu, respectively, were confirmed as cyanidin 3-glucoside, pelargonidin 3-glucoside, and malvidin 3-glucoside (Wu & Prior, 2005). Peak assignments also agree with the $[M]^+$ (m/z) and MS/MS (m/z) data reported for the diglucoside (Li *et al*., 2009a) and for monoglucosides (Kuskoski *et al*., 2003). Pelargonidin in jamun is being reported for the first time as none of the previous studies on this fruit from India (Aqil *et al*., 2012; Li *et al*., 2009b; Veigas *et al*., 2007) and Brazil (Faria *et al*., 2011) have reported this anthocyanin. Another significant observation is that of the five major anthocyanins identified in this study only one was the diglucoside, whereas the rest were monoglucosides unlike other listed studies that have reported major anthocyanins as diglucosides. This aspect of lesser units of glucose in the anthocyanin species may be of significance to those consuming *S. cumini* (jamun) fruit as adjunct therapy for diabetes.

Conclusion

The present study is the first on the changing quantitative profiles of nutrient and bioactive phytochemicals contents at different fruit development stages of *Syzygium cumini* (jamun). The study also recognizes four morphotype cultivars of *S. cumini* (jamun) based on morphological and organoleptic features of its fruit, which aspect has never been considered in any previous study. All cultivars, nevertheless, showed similar patterns of qualitative profiles of constituents at the four stages of fruit maturation, though the quantitative profiles varied considerably for contents among cultivars at different stages of fruit development. Based on the quantitative data obtained, it was concluded that for those consuming jamun fruit as adjuvant therapy for type-2 diabetes, fruits of cultivar-4 with very high DPPH scavenging ability, and contents of low sugar, lowest acidity, high ascorbic acid, and highest anthocyanins may be the best choice. It was noted that the fruit pulp of all cultivars was adequately rich in both macro- (Ca, Mg, K, Na, P) and micro-minerals (Cu, Fe, Zn), showing non-significant inter-cultivar differences in the D4 stage contents of most minerals, except Fe and K showing significant differences. All the minerals, except Fe, showed significant decreasing trends in their contents. It has been reported that complexing of delphinidin-based anthocyanins with Fe results in intense blue colour, whereas ferric cyanidin-3-glucoside forms very purple ferric complexes with an anthocyanin/Fe ratio of two in the metallopigmentation/copigmentation process thus greatly enhancing colour and also making the pigment complex slow to degrade, which may explain the persistent purple colour on tongue after jamun eating. It is relevant to note that blue and purple are the predominant colours in jamun fruit. Elevated levels of Fe in the ripe fruit were further nutritionally beneficial being the principal factor in hemoglobin synthesis, particularly so in iron deficiency anemia. Another aspect of high nutrient worth of minerals noted in jamun fruit was the presence of K and Na in the ideal ratio of above 2, the significance of which may be noted from reports that fewer than 2% of adults in the United States meet the recommended K/Na intake ratio of above 2. Most significant observations related with anthocyanins were the first-time detection of pelargonidin in jamun fruit, and the identification of five anthocyanins, which except cyanidin diglucoside were present as monoglucosides of delphinidin, malvidin, cyanidin, and pelargonidin. This latter aspect may be of significance to those consuming jamun fruit as the adjuvant therapy for type-2 diabetes desiring to consume fruit constituents with lesser sugar molecules. For the first time also being reported is the commencement of biosynthesis of aglycones delphinidin and cyanidin at the very early fruit maturation stage D1, the light-green fruit-set stage (Fig. 1), when even the presence of sugars was not detected.

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